

Carbon and nitrogen isotopic composition of suspended particulate organic matter in Zuari Estuary, west coast of India

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Abstract

The spatio-temporal variations of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of suspended particulate organic matter (SPOM), along with ancillary chemical and hydrographic parameters, have been examined monthly for one year (October 2010 to September 2011) at a total of seven stations to refine the understanding of SPOM sources and biogeochemical pathways in the tropical Zuari Estuary, west coast of India. The dynamic nature of C and N cycling in estuaries is reflected in the isotopic variation of SPOM as a function of space and time. The results exhibit marked seasonality, with the autochthonous component and the terrestrial component being the major SPOM sources during periods of low and high river discharges, respectively. Spatially, enrichments of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from the head ($\delta^{13}\text{C} = -29.6\text{‰}$, $\delta^{15}\text{N} = 1.78\text{‰}$, C/N = 7.09) to the mouth ($\delta^{13}\text{C} = -20.9\text{‰}$, $\delta^{15}\text{N} = 5.77\text{‰}$, C/N = 6.12) of the estuary indicate downstream reduction of terrigenous influence. The mean C:N ratios varied between 4 and 9 over the study period with low values during the monsoon, in spite of large terrestrial input, which can be attributed to microbial modification of terrestrial SPOM. A significant positive correlation of $\delta^{13}\text{C}$ with salinity suggests the reliability of $\delta^{13}\text{C}$ as a biogeochemical tracer for organic matter provenance. Carbon: chlorophyll plots reveal suppressed autochthonous production during the southwest monsoon which may be attributed to the rapid flushing time, light-limitation due to cloud cover and high water-column turbidity.

Keywords : Carbon isotope, Nitrogen isotope, C: N ratio, Particulate organic matter, Zuari, estuary

1. Introduction

Located at the confluence of freshwater and marine ecosystems, estuaries receive organic matter from multiple sources. Allochthonous sources comprise both marine and terrestrial inputs, with the latter including land runoff, domestic and industrial wastes, while the autochthonous supply comes through pelagic and benthic photosynthesis and chemoautotrophic production. Microzooplankton play a crucial role in transferring organic carbon from heterotrophic bacteria to higher trophic levels via the microbial loop. Alteration of particulate organic matter (POM) by the microbial community plays an important role in estuaries having long residence times. The estuarine POM serves as a significant source of nutrients to the adjacent coastal ecosystems (Rabalais *et al.*, 1996; Mayer *et al.*, 1998).

It is estimated that by 2025, about three-fourth the world's population will reside in coastal regions (Hinrichsen, 1997), making coastal ecosystems more vulnerable to human induced changes than they are today. It is essential to understand the origin and transformation pathways of organic matter in various estuarine and coastal systems in order to assess the current state of these ecosystems globally and predict the vulnerability of the coastal ocean to various potential scenarios of growth of anthropogenic drivers. Estuaries located in tropical regions with high human population density are expected to be most severely affected by human-induced changes. Despite their high vulnerability, relatively few studies have been conducted so far to understand the sources and transformation pathways of organic matter in such systems. (Cifuentes *et al.*, 1996; Andrews *et al.*, 1998; Maya *et al.*, 2011).

The continental shelf off western India is known to experience depletion of dissolved oxygen (DO) in the water column seasonally during the southwest (SW) monsoon when the low-oxygen subsurface waters are brought up through upwelling (Banse, 1959; Carruthers *et al.*, 1959). It has been suggested that this natural oxygen deficiency has intensified in the past few decades, possibly due to enhanced loading of nutrients and organic matter from land (Naqvi *et al.*, 2000, 2006). This loading is largely through numerous small rivers that originate in the Western Ghats. The Zuari is one such river that forms one of the largest estuaries along the Indian west coast. This tide-dominated estuary is extensively used for transportation of iron and manganese ores for overseas export from the Mormugao port, located at the mouth of the estuary, throughout the year. There are 129 industries and 10 large mines located in the river basin. Each mine discharges 1000-4000 tonnes of rejects per day which eventually end up in the river. In addition, the estuary also receives large quantities of, mostly untreated, sewage from municipal sources (<http://www.indiawaterportal.org/articles/mining->

[havoc-impact-mining-water-resources-go-a-article-dams-rivers-and-people](#)). Mining rejects act as a significant source of nitrate to the estuary (Desousa, 1999). Fishing is another important activity that goes on uninterrupted throughout the year, especially during the SW monsoon when fishing in the sea is temporarily suspended. Thus the estuary is subjected to considerable anthropogenic impact that is expected to alter the ecology and biogeochemical cycling in this water body.

Although several studies have been undertaken dealing with the biogeochemistry of the Zuari estuary (Shetye *et al.*, 2007), a detailed study on the sources of organic matter is lacking so far. Suspended sediment dynamics and geochemistry has been studied by Rao *et al* (2011) and Kessarkar *et al.* (2013) respectively in the Zuari and the neighbouring Mandovi estuaries, with the latter study focusing solely on the SW Monsoon season. In this paper, we examine the stable carbon and nitrogen isotopic composition of POM using the data collected for one year (from October 2010 to September 2011) in order to gain insights into sources of organic matter and intrinsic cycling of carbon and nitrogen in the Zuari Estuary.

2. Materials and Methods

2.1. Study Area

The Zuari Estuary is located in Goa between latitudes 15°20'N and 15°30'N, and longitudes 73°45'E and 74°10'E (Fig. 1). The Zuari river originates in the Western Ghats and flows nearly 70 km before meeting the Arabian Sea. The estuary is ~5.5 km wide at its mouth, rapidly narrowing down to ~0.5 km upstream. It is a shallow estuary, the depth varying from ~10 m at the mouth to ~3 m upstream. As a result, the estuary, which is under the influence of semi-diurnal tides (tidal range 1.5 m - 2.3 m), is vertically well-mixed during the dry season (October – May). During the SW Monsoon (June – September), the large freshwater influx due to heavy rainfall and land runoff (catchment area = 550 km²) results in partial stratification of the estuary which is more pronounced near the mouth.

As part of the Sustained Indian Ocean Biogeochemistry & Ecosystem Research (SIBER)-India programme, sampling was carried out at seven stations located along the length of the estuary (Fig. 1). Based on the salinity, we have classified the 7 stations into three zones. Moving upstream from the mouth, the first two stations were located in nearly marine Zone-I; the next two stations having intermediate salinity comprise Zone-II; and finally, the last three stations experiencing the lowest salinities form Zone-III.

2.2 Sampling and ancillary measurements

The observational period extended from October 2010 to September 2011. Sampling at the above mentioned stations was carried out once a month for the following measurements: water temperature and salinity, DO, nutrients (nitrate), chlorophyll-*a* and suspended particulate organic matter (SPOM) including its isotopic compositions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Salinity and temperature data were acquired using a portable Sea-Bird Electronics CTD (conductivity-temperature-depth) system. Niskin samplers (5 l) fixed on a polypropylene rope and equipped with reversing thermometers were used for water sampling. The conventional Winkler titrimetric method was followed for estimating DO (Grasshoff et al., 1983). Nutrient concentrations were measured using a SKALAR segmented flow analyzer following standard protocols (Grasshoff et al., 1983). Chlorophyll-*a* was estimated fluorometrically, after extraction with 90% acetone as per the JGOFS protocol (UNESCO, 1994).

2.3 Isotopic analyses:

Sub-samples for SPOM were taken in acid-washed plastic carboys and filtered through precombusted (450° C for 4 hours) GF/F filters (0.7 μm pore size). The filtration volume varied from 0.5 to 4 litres depending on the suspended load. The filters were exposed to acid-fumes (36% HCl) to remove carbonates and air dried in a clean laminar flow. Two aliquots each of 12 mm diameter were sub-sectioned from each filter and packed tightly in tin cups, for the analysis.

A EURO3000 Eurovector elemental analyzer coupled to a Thermo-Finnigan Delta V isotope ratio mass spectrometer in continuous-flow mode was used for isotopic analyses (Owens and Rees, 1989). Isotopic ratios are expressed in the standard delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) with respect to PDB and atmospheric nitrogen, respectively. The precision of nitrogen and carbon isotopic measurements was better than $\pm 0.2\%$ as determined from repeated measurements (after every 5 samples) of a working standard, ϵ -Amino-n-Caproic Acid or ACA ($\delta^{13}\text{C} = -25.3\%$ and $\delta^{15}\text{N} = 4.6\%$) and an in-house sediment standard COD ($\delta^{13}\text{C} = -21\%$ and $\delta^{15}\text{N} = 7.5\%$). A calibration curve made of ACA standards ranging from 1 to 4 μM for nitrogen and 6 to 24 μM for carbon was used to calculate carbon and nitrogen content in the SPOM.

3. Results:

The Zuari estuary experiences different hydrodynamic conditions throughout the year depending on the river runoff and tidal circulation. Hence, we have segregated the study period into three seasons: Pre-monsoon (February – May), SW Monsoon (June – September) and Post-monsoon (October –

January). Accordingly, the arithmetic means of the observations during the four months corresponding to each season (for surface and bottom depths, at each station) have been calculated to depict the seasonal variability. The standard deviations have been depicted as error bars in the figures.

3.1 Seasonal variability of water-column chemistry :

A) Pre-monsoon: The pre-monsoon season was characterized by the highest temperatures and salinities. While the amplitude of temperature variation (28.9°C – 30.8°C) was relatively small, salinity showed a large gradient among the three zones (varying from 33.9 psu at the mouth to 0.16 psu at the head). Water column was well-aerated (mean DO = 210.5 ± 32.7 µM, n = 56) and nitrate was present in low concentrations (mean NO₃⁻ = 1.23 ± 0.76 µM, n = 56) throughout the estuary. The Zone-III was the most oxygenated part of the estuary apparently due to the prevalence of fresher water conditions. Chl-*a* ranged from 2 to 7 µg l⁻¹ in Zones I and II, while higher concentrations reaching up to 16.3 µg l⁻¹ were observed at the two most upstream stations .

B) SW monsoon: The state of Goa receives ~90% of its annual rainfall from June to September and the large freshwater runoff passes through the estuaries. Water temperatures in the Zuari Estuary drop by 2-3°C from the pre-monsoon values. Salinity values also dip throughout the estuary to as low as 12.5 psu at the mouth. Zone-II experiences the maximum fluctuations in salinity and in Zone-III salinity is close to zero (Fig. 2b). DO concentrations were lower than those during the pre-monsoon season in all the three zones (mean DO = 161±45.4 µM, n = 56). The minimum concentration of 35 µM was recorded in the bottom waters at the mouth (St. Z1), coinciding with a high salinity value of 34.5 psu, the cause of which will be discussed later. Dissolved inorganic nutrients were copiously present (mean NO₃⁻ = 8.71 ±1.43 µM, n = 56) with the highest concentrations found in Zone-II (mean NO₃⁻ in Zone-II = 9.89 ±1.11 µM, n = 16) where the estuarine turbidity maximum is located. Zone –II also recorded relatively higher Chl-*a* (mean Chl-*a* in Zone-II = 4.58 ±1.13 µg l⁻¹) . For the estuary as a whole, the Chl-*a* levels (mean Chl-*a* = 3.4±1.28 µg l⁻¹) were lower than in the preceding pre-monsoon.

C) Post-monsoon: The freshwater flow recedes after the SW monsoon, leading to well-mixed conditions in the estuary during the post monsoon season (October-January). Temperature and salinity values increase again during this period. Temperature varies from 27.3°C to 28.3°C while salinity ranges from 33.8 psu at the mouth to 0.27 psu upstream. Nitrate concentrations drop (mean NO₃⁻ = 3.37 ±1.3 µM, n = 56), especially near the estuarine mouth, while DO levels increase (mean

DO = 179 ± 23.8 μM , n = 56) relative to the SW monsoon. Chl-*a* depicts a spatial trend similar to SW monsoon, albeit with slightly higher values (mean Chl-*a* = 3.72 ± 1.1 $\mu\text{g l}^{-1}$)

It is to be noted here that the anthropogenic impact on the Zuari estuary is not borne out by the nitrate concentrations in the water column. In fact these are several orders lower than, say, the German rivers Ems, Rhine, Elbe (nitrate upto 700 $\mu\text{mol/L}$, Johannsen *et al.*, 2008) or the Mississippi river in USA (nitrate upto 100 $\mu\text{mol/L}$, Dagg *et al.*, 2004). One explanation for this could be the generally lower nitrate concentration in tropical estuaries compared to the temperate ones (Eyre and Balls, 1999). In fact, the nitrate concentrations of other anthropogenically impacted systems along the west coast of India, the Mandovi estuary (upto 10 $\mu\text{mol/L}$, Maya *et al.*, 2011) and the Cochin estuary (upto 30 $\mu\text{mol/L}$, Martin *et al.*, 2013) are also in the range similar to the Zuari estuary. Our ongoing research indicates that there could be a huge dissolved inorganic nitrogen (DIN) sink on the land resulting in reduced concentrations in the coastal and estuarine waters (*S.W.A. Naqvi, personal communication*).

3.2 Temporal and spatial variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SPOM, POC and PON concentrations and C:N ratios

Irrespective of season, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the SPOM show a distinct trend of higher values in Zone-I, gradually decreasing through Zone-II to lowest values in Zone-III (Figs. 2 and 3). The results have been presented in Tables 1, 2 and 3.

A. Pre-monsoon

The $\delta^{13}\text{C}$ -SPOM ranged from -19.5‰ to -30.1‰ while $\delta^{15}\text{N}$ -SPOM varied between 7.26‰ and 1.35‰. The C:N ratio fluctuated over a narrow range, from 5 to 7, with no appreciable spatial variability (Fig. 4). The POC content ranged between 80-180 μM and the PON content varied from 12-25 μM . The POC distribution along the estuary roughly resembled a saw tooth-like pattern, with a slight increase at the interface of Zones I and II (Fig. 5a). PON concentrations also showed a similar pattern (Fig. 5b).

B. SW monsoon

During the SW monsoon, both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ exhibited markedly lower values (Fig. 3), consistent with data from the Mandovi Estuary collected during the SW monsoons in 2008-2009 (Maya *et al.*, 2011). The C:N ratios were higher (mean = 7.31 ± 0.9) than the pre-monsoon values

(mean= 6.35 ± 0.5). The highest values encountered during this season, were surprisingly within Zone-I, the marine end, instead of Zone-III, the freshwater end of the estuary.

The saw tooth pattern continued for POC although the values increased almost threefold (~ 350 - $450 \mu\text{M}$) in bottom waters of Zone-I (Fig. 5a). The trend of PON variability was very similar, with highest concentration reaching upto $\sim 55 \mu\text{M}$. (Fig. 5b)

C. Post-monsoon

After the retreat of the SW monsoon, the $\delta^{13}\text{C}$ -SPOM was characterized by higher values. However, there was no specific deviation from the preceding season in the distribution of $\delta^{15}\text{N}$ -SPOM. The $\delta^{15}\text{N}$ values dropped to less than 1‰ at the most upstream stations (Fig. 3). The C:N ratios varied in the 5-7 range, similar to the pre-monsoon season (Fig. 4). The POC concentrations were considerably lower during this season (between 40 - $130 \mu\text{M}$), being comparatively higher in Zone-II (Fig. 5a). There was considerable drop in the PON concentration as well (range = 8 - $15 \mu\text{M}$), which appeared to be evenly distributed throughout the estuary (Fig. 5b).

4. Discussion

4.1 Spatial variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SPOM in the Zuari Estuary

Spatially, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the SPOM exhibit a systematic co-variation with salinity (Fig.3). The $\delta^{13}\text{C}$ varied from a mean value of -29.6‰ (± 0.46 , $n=25$) at Z7, the innermost station, to -20.9‰ (± 1.08 , $n=25$) at Z1, located at the estuarine mouth. This trend is similar to that observed in other estuarine systems (e.g. in the Mississippi and Coatzacoalcos rivers by Eadie and Jeffrey, 1973), indicating inputs of the terrestrial organic matter by the rivers and also the upstream transport of marine POC by the tides which dominate the estuarine circulation during most parts of the year. The behavior of the $\delta^{15}\text{N}$ of SPOM was similar to that of $\delta^{13}\text{C}$ with higher values occurring at the mouth and decreasing upstream.

Plants have been classified into three groups according to their stable carbon isotopic composition (Smith and Epstein, 1971): (1) C-3 plants have $\delta^{13}\text{C}$ values ranging from -24‰ to -34‰ ; (2) C-4 plants have $\delta^{13}\text{C}$ values between -6‰ and -19‰ ; and (3) algae have intermediate $\delta^{13}\text{C}$ values (-12‰ to -23‰). In the case of nitrogen, while the $\delta^{15}\text{N}$ values of the terrestrial plants and soil organic matter vary from -5‰ to 18‰ , those of the marine organic matter fall in the range of 3‰ to 12‰ (Maya *et al.*, 2011, and references therein). Based on the above classifications, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

values for Zone-I fall within the range reported for marine phytoplankton and those from Zone-III indicate a strong influence by terrestrial organic matter; Zone-II is apparently a region of mixing between the two end-members.

4.2 Temporal variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SPOM in the Zuari Estuary

Pre-monsoon

The pre-monsoon is a season of low river discharge, and so the terrestrial carbon flux is expected to be quite limited and unimportant during this period. In Zone-I, the $\delta^{13}\text{C}$ was least negative (mean = -21.3‰) during the pre-monsoon. In Zone-III, the lowest values of the dataset, -30.12‰ and -30.14‰, apparently bearing signatures of the terrestrial C3 plants, coincided with the two highest concentrations of DO (274.5 and 270.5 μM) and also Chl-*a* (16.3 and 12.4 $\mu\text{g/L}$), the latter indicating high biological productivity.

The $\delta^{15}\text{N}$ was also found to be generally elevated during the pre-monsoon period than in the other two seasons. Presumably, this is in part due to lower inputs of terrestrial organic matter and also to generally low DIN concentrations. The mean nitrate concentration during this period was $1.23 \pm 0.76 \mu\text{M}$. An effective utilization of nutrients arising from high light intensity and water clarity would lead to little or no isotopic fractionation thereby contributing to higher $\delta^{15}\text{N}$ -SPOM.

SW Monsoon:

Depression of both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ occurred during the SW monsoon, a period of heavy rainfall in the coastal zone of western India. As a result of large freshwater inputs the estuarine water column becomes vertically stratified, and a salt wedge develops extending up to a distance of ~12 km from the river mouth (Qasim and Sen Gupta, 1981). Lower $\delta^{13}\text{C}$ values presumably arise primarily from inputs of terrigenous material. Previous studies have shown abundance of detrital organic matter in the estuary during this season (Verlencar and Qasim, 1985). The SW monsoon is also characterized by elevated DIN concentration and limited light availability in the water column due to cloud cover and high turbidity. Such conditions lead to partial nitrate utilization by phytoplankton and consequently significant isotopic fractionation between phytoplankton and nutrient source (Wada and Hattori, 1978; Wada, 1980; Mariotti *et al.*, 1984). This can, in part, account for the observed ^{15}N depletion in SPOM. The other potential contributor is the relatively lower $\delta^{15}\text{N}$ of the terrestrial organic matter as compared to the marine organic matter.

We observed a vertical thermal gradient of 2-3°C at the mouth during the period June-September, 2011. The cold subsurface water was also more saline and oxygen depleted. Coastal upwelling, during this period is known to cause seasonal hypoxic to anoxic conditions over the western continental shelf of India at very shallow depths (Naqvi et al., 2000; Jayakumar et al., 2001). Previously, based on observations during October-November 1980, Devassy (1983) had also observed presence of a ~6 m-thick cold subsurface layer near the mouth of the Zuari. This feature was reported to trigger blooms of diatoms and dinoflagellates in this area. Shenoy and Patil (2003) had also reported mixed blooms of diatoms and dinoflagellates during July which resulted in elevated DMS and DMSP concentrations. The authors attributed this bloom to a break in the southwest monsoon rhythm. The association of ^{13}C -enriched ($\delta^{13}\text{C}$ up to -21.2‰) high POC (356 μM) observed could be due to such a bloom formation. Similarly, the relatively higher $\delta^{15}\text{N}$ may be due to phytoplankton uptake of nitrate from the isotopically heavier pool in oxygen depleted waters that arises from denitrification (Wada et al., 1975; Liu and Kaplan, 1989; Brandes *et al.*, 1998).

Post-monsoon

The salt wedge formed during the SW monsoon gets diminished and the water column becomes more mixed during the post monsoon period (Qasim and Sen Gupta, 1981). Tides also regain the primary control on the estuarine circulation. Despite the prevalence of more marine conditions, some very low values of $\delta^{15}\text{N}$ were encountered during this season. While the low values could arise from N_2 fixation by diazotrophs (e.g. Maya *et al.*, 2011), this process is unlikely to occur in the presence of substantial nitrate (mean concentration = 3.37 μM). A more likely explanation is that the DIN taken up by phytoplankton is isotopically lighter. Thus, the lower $\delta^{15}\text{N}$ of NH_4^+ excreted by zooplankton (Sigman and Casciotti, 2001) or of NH_4^+ emanating from the sediments could lead to the production of organic matter depleted in ^{15}N . In the neighbouring Mandovi estuary, sedimentary flux of NH_4^+ has been found to be a major source of nutrient supply to phytoplankton (Pratihary *et al.*, 2009).

Also, in the estuaries along the Indian west coast, the zooplankton population is higher during the post monsoon period relative to the SW monsoon (Jyothibabu and Madhu, 2007). The $\delta^{13}\text{C}$ values during the post monsoon period are in general higher than those during the SW monsoon but lower than the pre-monsoon values. This could be attributed to higher respiration and zooplankton biomass that restricts the growing phytoplankton population, and thus results in intermediate $\delta^{13}\text{C}$ values.

5. Nature of organic matter

5.1 C:N ratio In dynamic systems such as the Zuari Estuary experiencing large seasonal shifts in hydrology and biogeochemistry, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data are often not sufficient for source identification of SPOM. In such cases, C:N ratio can be an important indicator of SPOM origin. As per the Redfield (1958) stoichiometry, a C:N atomic ratio of 6.6 is considered to apply for marine phytoplankton. Values ranging from 5 to 8 have been reported for freshwater phytoplankton (Harris, 1986; Kendall *et al.*, 2001). Bacteria usually have high protein content and their C:N ratio lies between 2.6 to 4.3 (Lee and Fuhrman, 1987). The ratio is much higher for terrestrial OM, varying between 12 and 200 (Hedges and Mann, 1979).

The mean C:N ratio of the SPOM during our study ranged between 5 and 8 (Fig. 4). The mean values for the pre-monsoon and post-monsoon periods were quite similar (6.35 ± 0.5 and 6.25 ± 0.7 , respectively) and well within the range reported for the marine plankton. This implies that in-situ production is a major source of organic matter in this estuary during the non-monsoon seasons.

The C:N ratios increased during the SW monsoon (mean 7.35 ± 0.9) apparently due to the large influx of terrestrial load, even though the observed values generally remained below the range reported for the land-derived OM: Out of the 54 samples analyzed between June to September, 2011, only 8 yielded C:N values between 9 and 11, which is still lower than the C:N ratios observed during high-discharge events in other estuaries such as the Tay Estuary in Scotland (Thornton and McManus, 1994), and the Lanyang-Hsi watershed in Taiwan (Kao and Liu, 2000). While it is not possible to pinpoint the exact cause of the low C:N values observed in the present study, several possible explanations may be offered: (1) According to Kendall *et al.* (2001), sites having C:N ratios between 8 and 15 during high flow events come under the influence of terrestrial soil. The high turbidity of the water column in the Zuari Estuary is indicative of the role of soil as an important contributor of POM. (2) Lower C:N ratios may reflect higher protein content of the organic matter. The particle-associated bacterial community the abundance of which increases during the SW monsoon in the Zuari Estuary (De Souza *et al.*, 2003) may be held responsible for this. Significantly, in the neighbouring Mandovi Estuary, Fernandes (2011) found organic matter to be N-enriched and attributed it to bacterial contribution. Thus, it is likely that this trend is common among all estuaries along the Indian west coast; (3) It is possible that phytoplankton contribution remains high even during the SW monsoon, but this is unlikely to be the case especially in turbid, freshwater dominated parts of the estuary.

5.2 Relationship between Chl-*a* and POC

In order to assess relative contribution of phytoplankton to total SPOM, we plotted POC versus Chl-*a* separately for the three seasons. Although there is considerable scatter of data, the slope of the POC:Chl-*a* regression line is much higher during the SW Monsoon than during other seasons (Fig. 6). This together with a high intercept reflects the large terrigenous particulate inputs during this season. On the other hand, the higher Chl-*a* concentration and lower POC:Chl-*a* slope during the pre-monsoon period point to the dominance of locally produced organic matter. The highest Chl-*a* concentrations were recorded in the upstream portion of the estuary (Fig. 7a; represented within the circle). As expected, these samples were also characterized by the lowest $\delta^{13}\text{C}$ values (Fig. 7b). Matching with the corresponding $\delta^{15}\text{N}$ and C:N values (Table 1), the autochthonous POM in the Zuari Estuary can be characterized by $\delta^{13}\text{C}$ values between -30 ‰ and -28‰, $\delta^{15}\text{N}$ between 1‰ and 4‰, and C: N ratio between 6 and 7. Thus, the major portion of the new POM is generated during the pre-monsoon in the low-salinity waters of the Zuari estuary. These results are in agreement with the observations of Matondkar *et al* (2007) who reported phytoplankton abundance being the highest in April (pre-monsoon) in the upstream part of the Zuari. These authors also attributed the low phytoplankton counts in September (SW monsoon) to rapid flushing of the estuary through monsoonal flow. Similarly lower phytoplankton growth has been also observed in other rapidly flushed estuaries such as Tweed Estuary (Uncles *et al*, 2000) and Danshuei Estuary (Liu *et al*, 2007). Thus, the low retention time (5-6 days, Qasim and Sen Gupta, 1981) of water in the Zuari Estuary during the SW monsoon seems to play a key role in the biogeochemistry in this estuarine system and probably also in other estuaries along the Indian west coast.

Large episodic increases in POC were observed during the SW monsoon, especially in Zone-I at the bottom depths where the salt-wedge was formed. The source of this POC is not clear. It could either be derived from local phytoplankton blooms, or may be caused by resuspension of fine, organic rich sediments. It may be possible that the SPOM generated and settled in the estuary during pre-monsoon might have been exported during the early phase of the monsoon. According to Kessarkar *et al.* (2013), the funnel-shaped estuarine mouth enhances turbulence and leads to resuspension of bottom sediments. As in other estuaries, an estuarine turbidity maximum may also be found in the Zuari estuary and the fine-grained material comprising this maximum may be enriched in organic matter.

7. Conclusion

Isotopic analysis of the SPOM in the Zuari Estuary reveals that the temporal and spatial variability of the SPOM is controlled by seasonal hydrodynamics. Precipitation, river discharge and tidal circulation account for much of the variation.

During the pre-monsoon period, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the SPOM progressively increase downstream and attain typical marine values at the mouth of the estuary. Spatial variability during this season arises from the influence of tides on one hand and enhanced in-situ production in the upstream part on the other.

During the SW monsoon season a large amount of allochthonous OM is added to the estuary, and, despite the presence of copious nutrients, primary production is suppressed by high turbidity, the rapid flushing of the estuary and light limited conditions. Spatial variability is significantly reduced during this time as the whole estuary experiences freshwater conditions. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the SPOM at the estuarine mouth show significant depletion of heavier isotopes. The estuarine system switches to net heterotrophy during this season (Ram *et al.*, 2003), also reflected by the C/N ratio which exhibits the signature of microbial N-enrichment in organic matter. Our data also provide evidence for incursion of oxygen depleted water upwelled at the estuarine mouth.

During the post-monsoon period the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ exhibited intermediate values presumably influenced by a high zooplankton biomass.

Phytoplankton growing in-situ and detrital (plankton and terrestrial) organic matter are the two major pools of organic matter in the Zuari estuary.

Acknowledgements

This research was carried out as a part of SIBER-INDIA project funded by the Ministry of Earth Sciences (MoES). P. Bardhan and A. Sarkar thank the Council of Scientific and Industrial Research (CSIR) for awarding them Junior and Senior Research Fellowships respectively. The authors are grateful to Mr. H. Dalvi, Mr. A. Methar, and Mr. B.R.Thorat for their assistance during field work. This is NIO Contribution no. XXXX.

References

1. Andrews, J. E., Greenaway, A. M., Dennis, P. F., 1998. Combined carbon isotope and C/N ratios as indicators of source and fate of organic matter in a poorly flushed, tropical estuary: Hunts Bay, Kingston Harbour, Jamaica. *Estuarine, Coastal and Shelf Science* 46, 743-756.
2. Banse, K., 1959. On upwelling and bottom-trawling off the southwest coast of India. *Journal of the Marine Biological Association of India* 1, 33-49.
3. Brandes J. A., Devol A. H., Yoshinari T., Jayakumar D. A., Naqvi S. W. A., 1998. Isotopic composition of nitrate in the central Arabian Sea and eastern tropical North Pacific: A tracer for mixing and nitrogen cycles. *Limnology and Oceanography* 43, 1680–1689.
4. Carruthers, J. N., Gogate, S. S., Naidu, J. R., & Laevastu, T., 1959. Shoreward upslope of the layer of minimum oxygen off Bombay: its influence on marine biology, especially fisheries. *Nature*, 183, 1084–1087.
5. Cifuentes, L. A., Coffin, R. B., Solorzano, L., Cardenas, W., Espinoza, J., Twilley, R. R., 1996. Isotopic and elemental variations of carbon and nitrogen in a mangrove estuary. *Estuarine, Coastal and Shelf Science* 43, 781-800.
6. Dagg, M., Benner, R., Lohrenz, S., Lawrence, D., 2004. Transformation of dissolved and particulate materials on continental shelves influenced by large rivers: plume processes. *Continental Shelf Research* 24, 833-858.
7. De Souza, M.J.B.D., Nair, S., Bharathi, P.A.L., Chandramohan, D., 2003. Particle-associated bacterial dynamics in a tropical tidal plain (Zuari estuary, India). *Aquatic Microbial Ecology* 33, 29–40.
8. Desousa, S. N., 1999. Effect of mining rejects on the nutrient chemistry of Mandovi estuary, Goa. *Indian journal of marine sciences*, 28, 355-359.
9. Devassy, V. P., 1983. Plankton production associated with cold water incursion into the estuarine environment. *Mahasagar* 16, 221–233.
10. Eadie, B. J., Jeffrey, L. M., 1973. $\delta^{13}\text{C}$ analyses of oceanic particulate organic matter. *Marine Chemistry* 1, 199-209.
11. Fernandes L., 2011. Origin and biochemical cycling of particulate nitrogen in the Mandovi estuary. *Estuarine, Coastal and Shelf Science* 94,291–298.
12. Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, 419 pp.
13. Harris, G.P., 1986. *Phytoplankton Ecology: Structure, Function, and Fluctuation*. Chapman and Hall.
14. Hedges, J.I., Mann, D.C., 1979. Lignin Geochemistry of marine-sediments from the southern Washington coast. *Geochimica et Cosmochimica Acta* 43, 1809–1818.
15. Hinrichsen, D., 1997. *Coastal waters of the world: trends, threats, and strategies*. Island Press.

16. Jayakumar, D.A., Naqvi, S.W.A., Narvekar, P.V., George, M.D., 2001. Methane in coastal and offshore waters of the Arabian Sea. *Marine Chemistry* 74, 1–13.
17. Johannsen, A., Dahnke, K., Emeis, K., 2008. Isotopic composition of nitrate in five German rivers discharging into the North Sea. *Organic Geochemistry* 39, 1678–1689.
18. Jyothibabu, R., Madhu, N.V., 2007. Zooplankton in the Mandovi and Zuari estuary. In: Shetye, S.R., Dileep kumar, M., Shankar, D. (Eds.), *Mandovi and Zuari Estuaries*, pp. 83–90.
19. Kao, S.J., Liu, K.K., 2000. Stable carbon and nitrogen isotope systematics in a human-disturbed watershed (Lanyang-Hsi) in Taiwan and the estimation of biogenic particulate organic carbon and nitrogen fluxes. *Global Biogeochemical Cycles* 14, 189–198.
20. Kendall C., Silva S. R., Kelly V. J., 2001 Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrol. Proc.* 15, 1301–1346.
21. Kessarkar, P.M., Shynu, R., Rao, V.P., Chong, Feng, Narvekar, T., Zhang, J., 2013. Geochemistry of the suspended sediment in the estuaries of the Mandovi and Zuari Rivers, Central west coast of India. *Environmental Monitoring and Assessment* 185, 4461–4480.
22. Lee, S., Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Applied and Environmental Microbiology* 53, 1298–1303.
23. Liu, K.K., Kaplan, I.R., 1989. The eastern tropical pacific as a source of ^{15}N -enriched nitrate in seawater off southern California. *Limnology and Oceanography* 34, 820–830.
24. Liu, K.-K., Kao, S.-J., Wen, L.-S., Chen, K.-L., 2007. Carbon and nitrogen isotopic compositions of particulate organic matter and biogeochemical processes in the eutrophic Danshuei Estuary in northern Taiwan. *Science of the Total Environment* 382, 103–120.
25. Mariotti, A., Lancelot, C., Billen, G., 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended matter in the Scheldt estuary. *Geochimica et Cosmochimica Acta* 48, 549–555.
26. Martin, G. D., Jyothibabu, R., Madhu, N. V., Balachandran, K. K., Nair, Maheswari, Muraleedharan, K. R., Arun, P. K., Haridevi, C. K., Revichandran, C., 2013. Impact of eutrophication on the occurrence of *Trichodesmium* in the Cochin backwaters, the largest estuary along the west coast of India. *Environmental monitoring and assessment* 185, 1237–1253.
27. Matondkar, S.G.P., Gomes, H. do R., Parab, S.G., Pednekar, S., Goes, J.I., 2007. Phytoplankton diversity, biomass, and production. In: Shetye, S.R., Kumar, M.D., Shankar, D. (Eds.), *The Mandovi and Zuari Estuaries*. National Institute of Oceanography, Dona Paula, Goa, India, pp. 67–81.
28. Maya, M. V., Soares, M. A., Agnihotri, R., Pratihary, A. K., Karapurkar, S., Naik, H., Naqvi, S. W. A., 2011. Variations in some environmental characteristics including C and N stable isotopic composition of suspended organic matter in the Mandovi estuary. *Environmental monitoring and assessment* 175, 501–517.

29. Mayer, L. M., Keil, R. G., Macko, S. A., Joye, S. B., Ruttenger, K. C., Aller, R. C., 1998. Importance of suspended particulates in riverine delivery of bioavailable nitrogen to coastal zones. *Global Biogeochemical Cycles* 12(4), 573-579.
30. Naqvi, S. W. A., Jayakumar, D. A., Narvekar, P. V., Naik, H., Sarma, V. V. S. S., D'souza, W., Joseph, S. George, M. D. (2000). Increased marine production of N₂O due to intensifying anoxia on the Indian continental shelf. *Nature* 408, 346-349.
31. Naqvi, S. W. A., Naik, H., Jayakumar, D. A., Shailaja, M. S., Narvekar, P. V., 2006. Seasonal oxygen deficiency over the western continental shelf of India. In *Past and Present Water Column Anoxia* (pp. 195-224). Springer Netherlands.
32. Owens, N. J., Rees, A. P., 1989. Determination of nitrogen-15 at sub-microgram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometry. *Analyst* 114, 1655-1657.
33. Pratihary, A.K., Naqvi, S.W.A., Naik, H., Thorat, B.R., Narvekar, G., Manjunatha, B.R., Rao, V.P., 2009. Benthic fluxes in a tropical estuary and their role in ecosystem. *Estuar. Coast. Shelf Sci.* 85, 387-398.
34. Qasim, S.Z., SenGupta, R., 1981. Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. *Estuarine, Coastal and Shelf Science* 13, 557-578.
35. Rabalais, N. N., Turner, R. E., Justic, D., Dortch, Q., Wiseman, W. J., Gupta, B. K. S., 1996. Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries* 19, 386-407.
36. Ram, A.S.P., Nair, S., Chandramohan, D., 2003. Seasonal shift in net ecosystem production in a tropical estuary. *Limnology and Oceanography* 48, 1601-1607.
37. Rao, V.P., Shynu, R., Kessarkar, P.M., Sundar, D., Michael, G.S., Narvekar, T., Blossom, V., Mehra, P., 2011. Suspended sediment dynamics on a seasonal scale in the Mandovi and Zuari estuaries, central west coast of India. *Estuarine, Coastal and Shelf Science* 91, 78-86.
38. Redfield, A.C., 1958. The biological control of chemical factors in the environment. *American Scientist* 46, 205-222.
39. Shenoy, D.M., Patil, J.S., 2003. Temporal variations in dimethylsulphoniopropionate and dimethyl sulphide in the Zuari estuary, Goa (India). *Marine Environmental Research* 56, 387-402.
40. Shetye, S. R., Shankar, D., Neetu, S., Suprit, K., Michael, G. S., Chandramohan, P., 2007. The environment that conditions the Mandovi and Zuari estuaries.
41. Sigman, D.M., Casciotti, K.L., 2001. Nitrogen isotopes in the ocean. In: Thorpe, S., Threlkoff, K. (Eds.), *Encyclopedia of Ocean Sciences*. Elsevier Verlag, New York, pp. 1884-1894.
42. Smith, B.N., Epstein, S., 1971. Two categories of ¹³C/¹²C ratios for higher plants. *Plant Physiology* 47, 380-384.

43. Thornton, S.F., McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine system: evidence of the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science* 42, 219-233.
44. Uncles, R.J., Bloomer, N.J., Frickers, P.E., Griffiths, M.L., Harris, C., Howland, R.J.M., Morris, A.W., Plummer, D.H., Tappin, A.D., 2000. Seasonal variability of salinity, temperature, turbidity and suspended chlorophyll in the Tweed Estuary. *Science of the Total Environment* 251, 115-124.
45. UNESCO. 1994. Protocols for the Joint Global Ocean Flux Study (JGOFS). Core Measurements, IOC Manual and Guides 29, 170pp.
46. Verleacar, X.N., Qasim, S.Z., 1985. Particulate organic matter in the coastal and estuarine waters of Goa and its relationship with phytoplankton production. *Estuarine, Coastal and Shelf Science* 21, 235-243.
47. Wada, E., Kadonaga, T., Matsuo, S., 1975. ^{15}N abundance in nitrogen of naturally occurring substances and global assessment of denitrification from isotopic viewpoint. *Geochemical Journal* 9, 139-148.
48. Wada, E., Hattori, A., 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds by marine diatoms. *Geomicrobiology Journal* 1, 85-101.
49. Wada, E., 1980. Nitrogen isotope fractionation and its significance in biogeochemical processes occurring in marine environments. *Isotope Marine Chemistry*, 375-398.
50. Web Reference:

<http://www.indiawaterportal.org/articles/mining-havoc-impact-mining-water-resources-go-article-dams-rivers-and-people>

Table 1. Isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), elemental C and N concentrations (μM) and C:N ratios of SPOM during pre-monsoon season in the Zuari Estuary. Note that S and B indicate surface and bottom waters respectively.

Station	Depth	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	POC	PON	C/N
Z1	S	-19.52	7.26	94.57	17.51	5.40
Z1	B	-20.64	6.48	79.91	12.48	6.40
Z2	S	-23.20	5.57	127.79	18.99	6.73
Z2	B	-21.82	6.27	171.94	24.41	7.04
Z3	S	-26.35	5.61	120.88	18.57	6.51
Z3	B	-25.21	7.08	130.74	20.76	6.30
Z4	S	-28.49	4.75	82.23	14.73	5.58
Z4	B	-27.05	4.64	110.08	17.27	6.37
Z5	S	-29.45	5.08	69.69	12.14	5.74
Z5	B	-27.92	4.69	104.39	15.26	6.84
Z6	S	-28.69	2.98	92.61	15.09	6.14
Z6	B	-29.20	3.48	95.65	14.71	6.50
Z7	S	-30.12	3.60	139.60	20.65	6.76
Z7	B	-30.14	1.35	139.24	21.11	6.60

Table 2. Isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), elemental C and N concentrations (μM) and C:N ratios of SPOM during SW Monsoon season in the Zuari estuary. Note that S and B indicate surface and bottom waters respectively.

Station	Depth	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	POC	PON	C/N
Z1	S	-22.84	5.24	80.84	13.76	5.88
Z1	B	-21.20	6.50	356.33	44.75	7.96
Z2	S	-25.43	5.41	124.23	20.64	6.02
Z2	B	-23.43	5.03	427.08	54.78	7.80
Z3	S	-27.16	3.63	98.07	16.45	5.96
Z3	B	-25.45	3.01	224.51	29.42	7.63
Z4	S	-28.78	2.97	84.08	12.99	6.47
Z4	B	-27.99	2.94	99.45	12.30	8.08
Z5	S	-29.72	3.27	114.19	16.92	6.75
Z5	B	-28.99	1.41	120.47	14.39	8.37
Z6	S	-29.34	1.66	67.06	8.97	7.48
Z6	B	-29.53	1.09	63.28	8.23	7.69
Z7	S	-29.25	1.95	86.73	9.99	8.68
Z7	B	-29.02	2.20	66.11	8.73	7.57

Figures captions:

Fig.1: Location of sampling sites in the Zuari Estuary, Goa, India.

Fig.2: Variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SPOM during pre-monsoon, south-west monsoon and post-monsoon in the Zuari Estuary. Note that S and B indicate surface and bottom waters respectively.

Fig.3: Season-wise variations in mean $\delta^{13}\text{C}$ (■) and $\delta^{15}\text{N}$ (◆) of SPOM with the salinity in the Zuari estuary.

Fig. 4: Variations in C:N ratio of SPOM during pre-monsoon, South-West monsoon and post-monsoon in the Zuari Estuary. Note that S and B indicate surface and bottom waters respectively.

Fig. 5: POC concentrations (a) and PON concentrations (b) of SPOM in the Zuari Estuary during pre-monsoon, SW Monsoon, and post-monsoon. Note that S and B indicate surface and bottom waters respectively.

Fig. 6: Linear correlations of POC vs. Chl-*a* during pre-monsoon, South-West monsoon and post-monsoon.

Fig. 7(A) Variation of Chl-*a* against salinity and 7(B) variation of Chl-*a* against $\delta^{13}\text{C}$ during pre-monsoon season, representing autochthonous production.

Fig.1

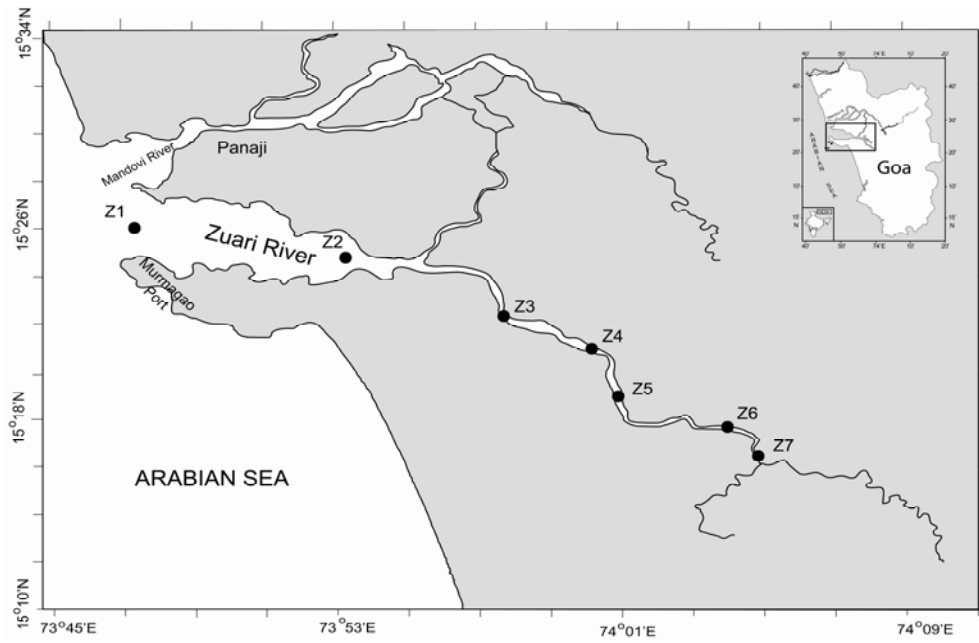


Fig. 2

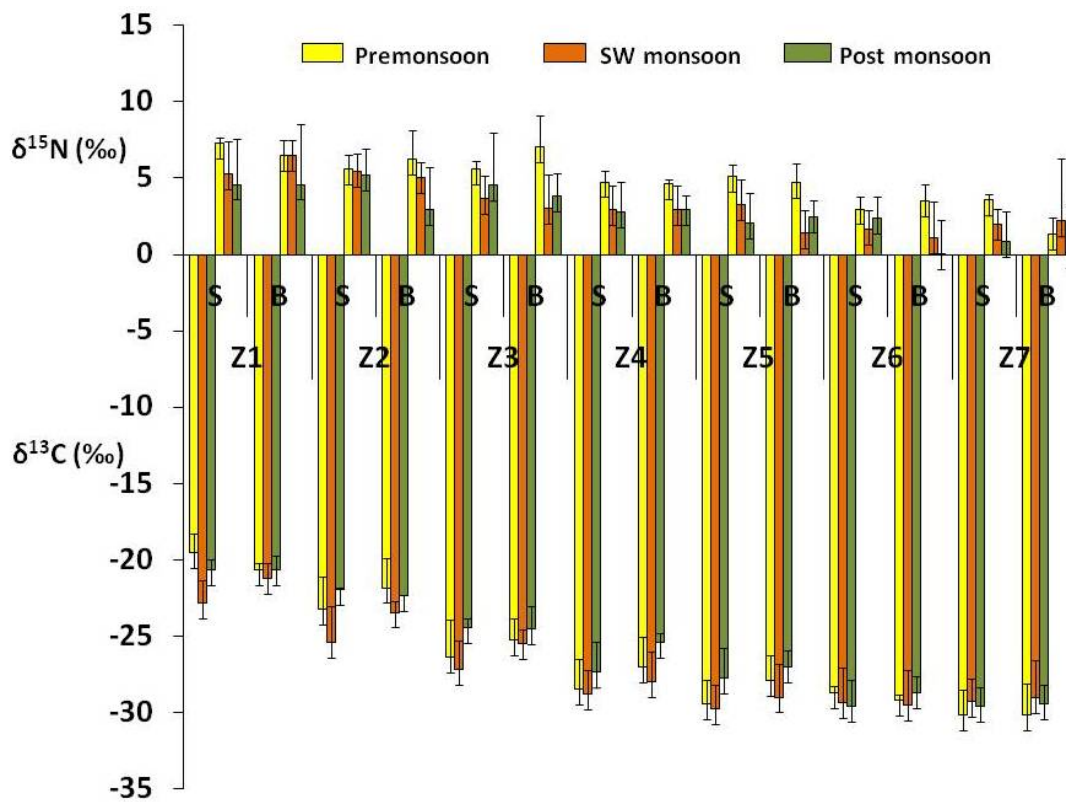


Fig. 3

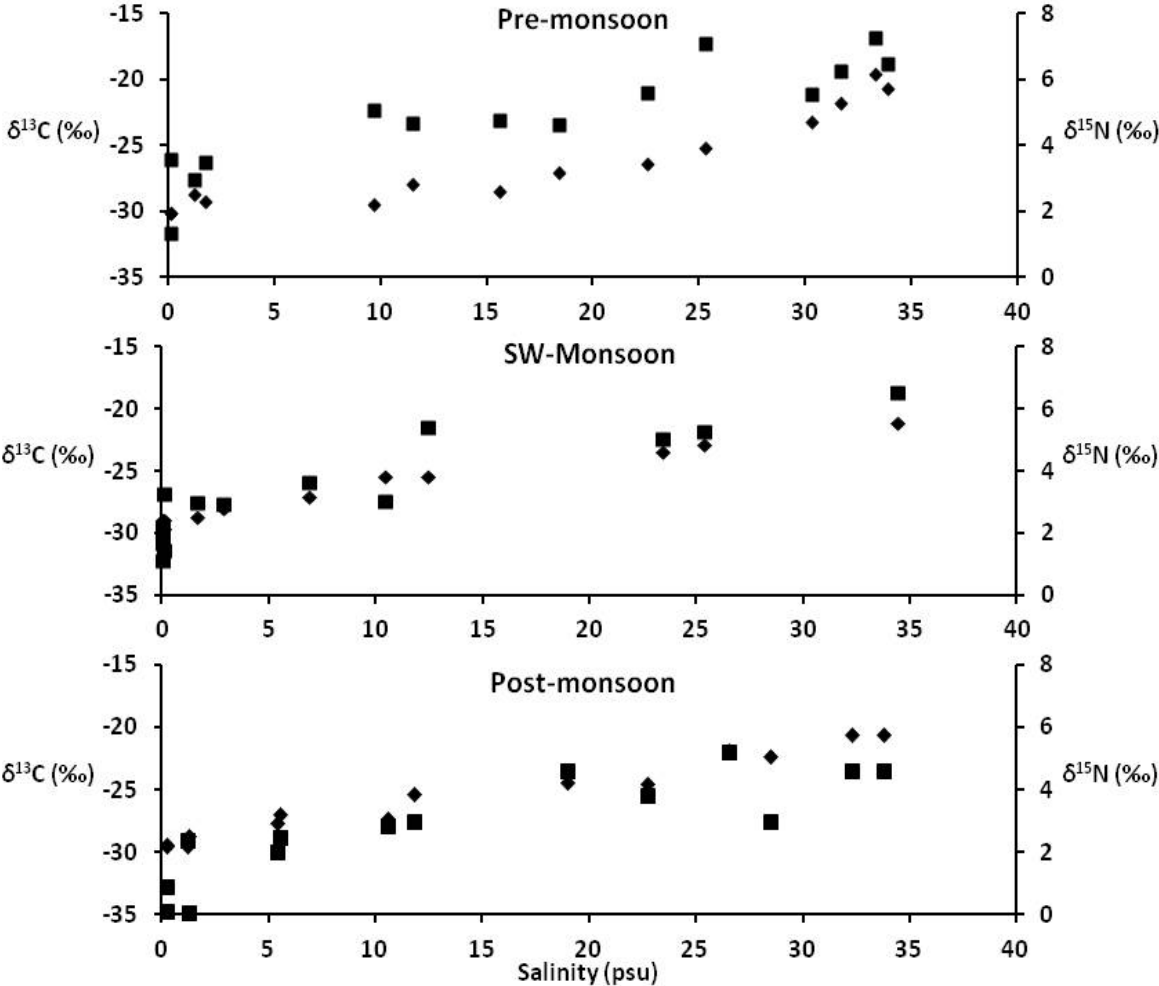


Fig. 4

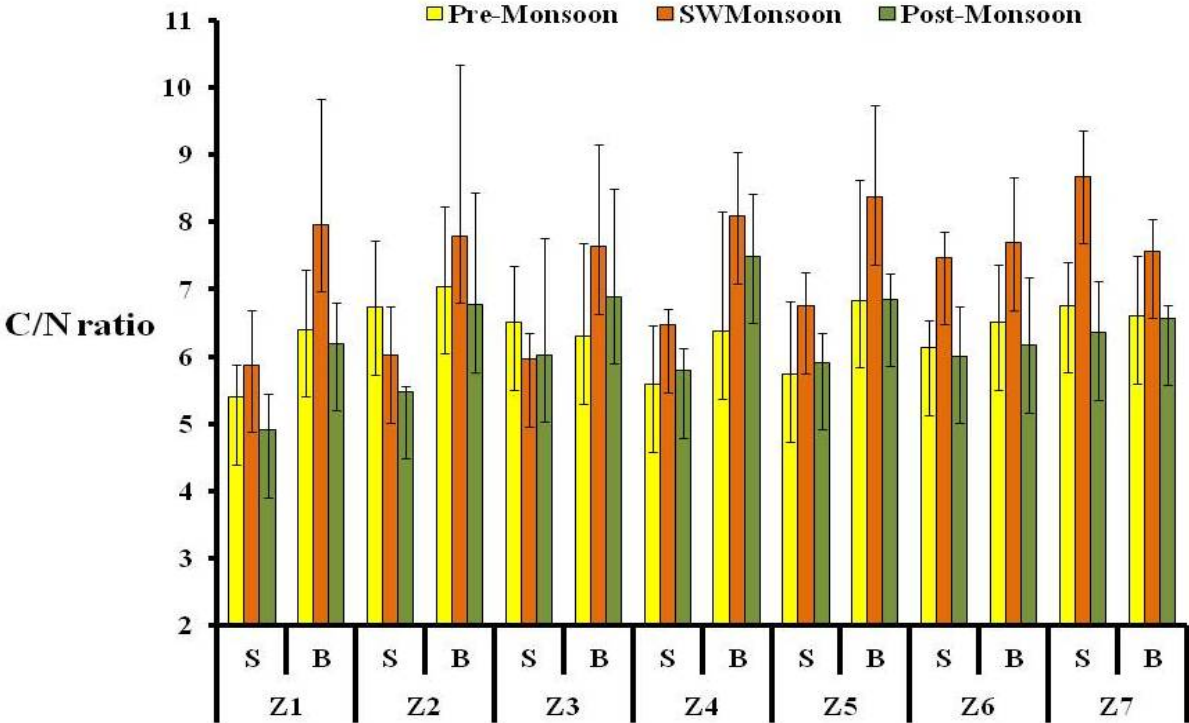


Fig. 5 (a)

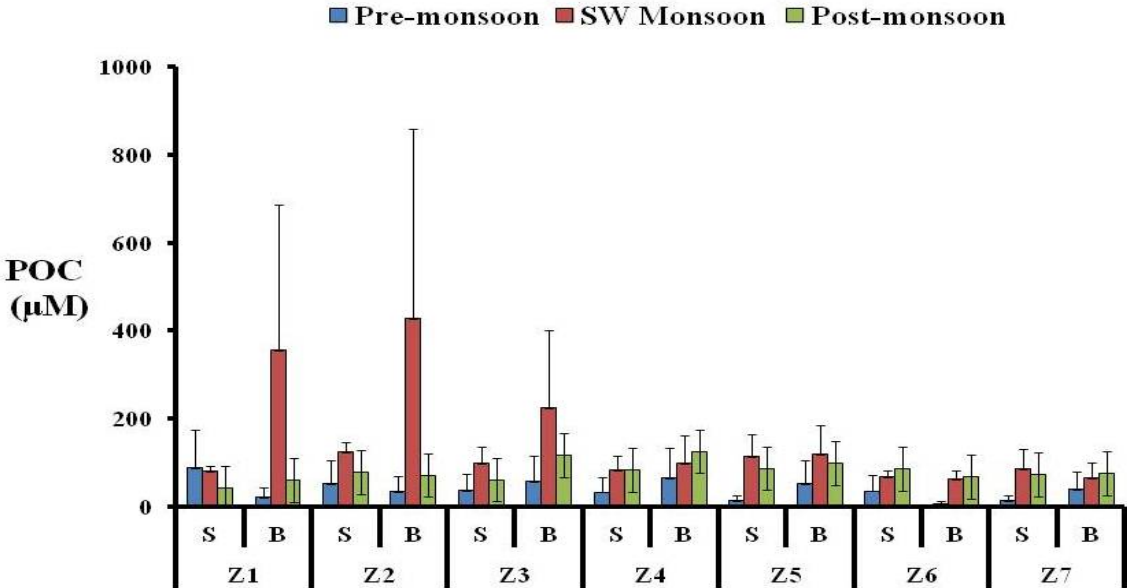


Fig. 5(b)

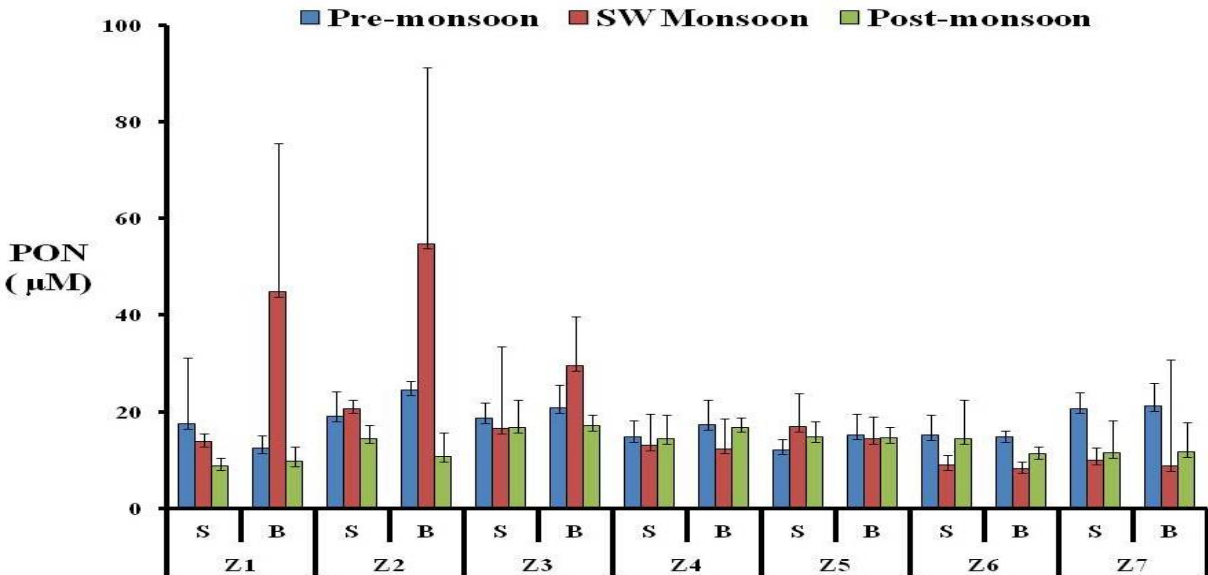


Fig (6)

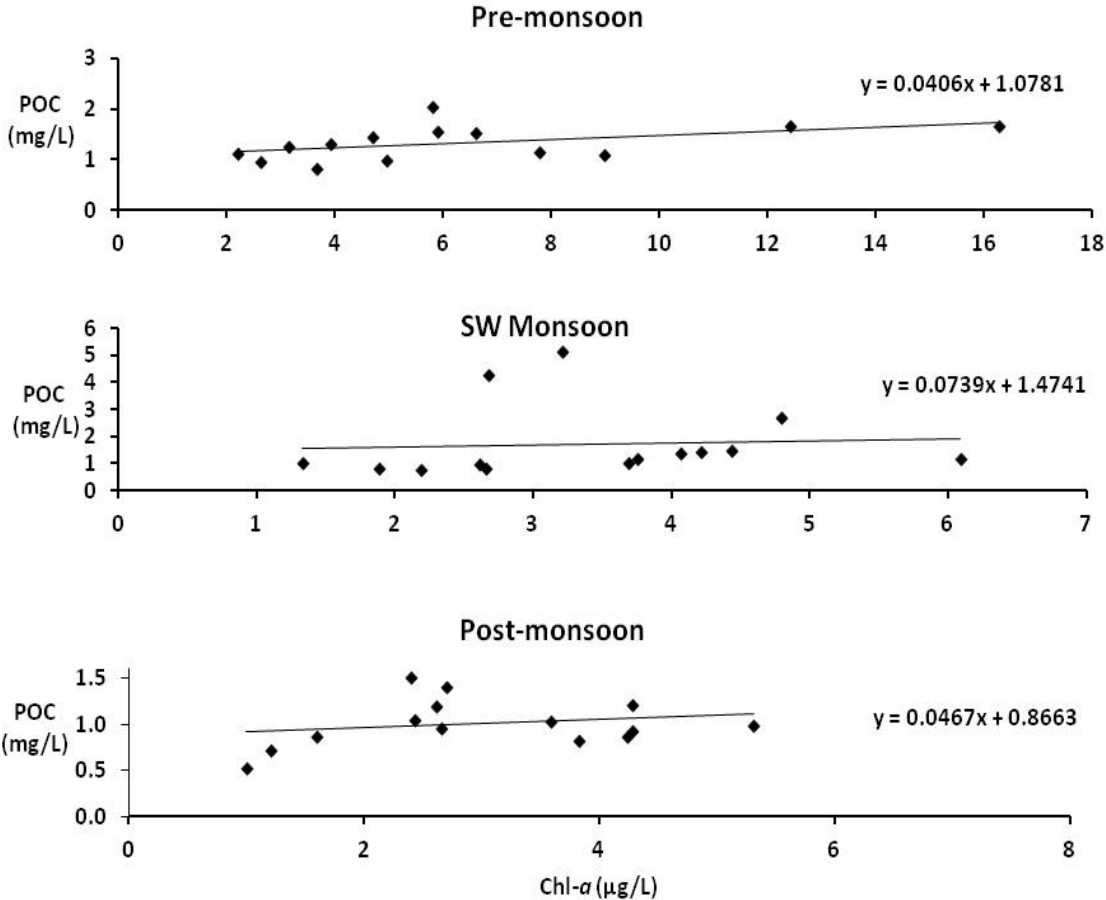


Fig (7)

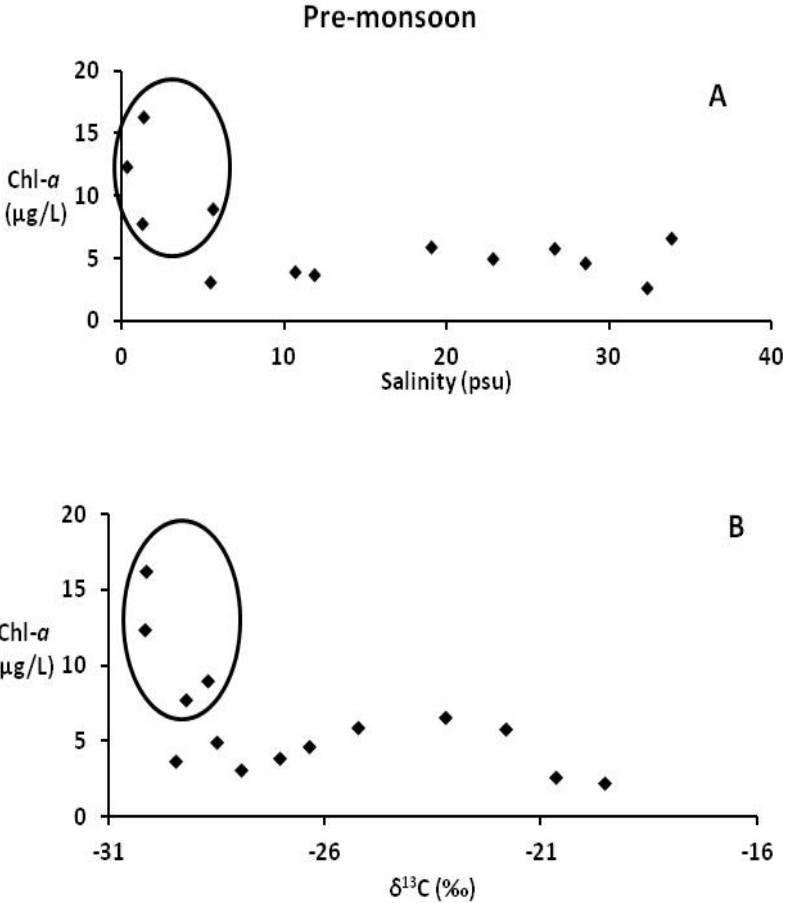


Table 3. Isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), elemental C and N concentrations (μM) and C:N ratios of SPOM during Post Monsoon season in the Zuari estuary. Note that S and B indicate surface and bottom waters respectively.

Station	Depth	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	POC	PON	C/N
Z1	S	-20.68	4.59	43.54	8.87	4.91
Z1	B	-20.68	4.59	60.13	9.71	6.20
Z2	S	-21.93	5.22	79.47	14.49	5.48
Z2	B	-22.34	2.96	72.14	10.65	6.78
Z3	S	-24.47	4.57	100.59	16.69	6.03
Z3	B	-24.51	3.80	117.80	17.10	6.89
Z4	S	-27.36	2.81	82.93	14.32	5.79
Z4	B	-25.42	2.97	126.09	16.82	7.50
Z5	S	-27.73	2.02	87.53	14.80	5.91
Z5	B	-27.02	2.48	99.54	14.53	6.85
Z6	S	-29.56	2.38	86.52	14.40	6.01
Z6	B	-28.68	0.74	69.23	11.22	6.17
Z7	S	-29.59	0.87	72.67	11.42	6.36
Z7	B	-29.45	0.69	76.78	11.68	6.57