

Assessing seasonal nitrogen transformations in the Godavari estuary, India

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Abstract

Indian estuaries are hypothesized to export a significant amount of nitrogen nutrients to the coastal waters due to the high use of fertilizers. To examine the possible sources of nitrogen nutrients and their modifications within the estuary, a study was conducted by collecting samples at monthly intervals for a period of a year. The river Godavari is the largest monsoonal estuary in India and is fed by monsoon rainfall. Hence the entire estuary is filled with fresh water from July to September (the wet period) whereas saline water is dominant during the dry period. An order of magnitude increase in nitrate concentrations was observed during the wet due to a peak in discharge compared to the dry period. The isotopic composition of nitrogen in nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$) was lower during the wet than the dry period and displayed a significant linear relationship with Chlorophyll-a (Chl-a), particulate organic nitrogen (PON) and carbon (POC) indicating that biological process modified their composition. The lower $\delta^{15}\text{N}_{\text{NO}_3}$ and higher $\delta^{18}\text{O}_{\text{NO}_3}$ values were noticed during the wet than during the dry period. The $\delta^{15}\text{N}_{\text{NO}_3}$ versus $\delta^{18}\text{O}_{\text{NO}_3}$ plot suggests a possible source of nitrate through the nitrification of soil/sewage-borne nitrogen. The higher $\delta^{18}\text{O}_{\text{NO}_3}/\delta^{15}\text{N}_{\text{NO}_3}$ ratios (>1) were observed during August and September than in other months indicating the occurrence of denitrification during the former months. In contrast, an insignificant change in $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ within the estuary suggested that denitrification occurred upstream and not within the estuarine zone. River discharge brought residual nitrate to the estuary leading to less input of nitrate to the coastal Bay of Bengal than hitherto hypothesized.

Keywords: Nitrate, stable isotopes of N and O, river discharge; denitrification; Godavari estuary

1. Introduction

For the past several decades, anthropogenic activities enhanced nitrogen levels leading to eutrophication in estuaries and coastal oceans resulting in the degradation of habitat (Smith, 2003; Camargo and Alonso, 2006; Wang et al., 2018). Among the several nutrients, reactive nitrogen (Nr) has received significant attention as it regulates primary production and, therefore, the estuarine ecosystem (Nixon, 1983; Maguer et al., 2004; Howarth and Marino, 2006). The estuary is an efficient biogeochemical reactor as it receives a significant amount of carbon, nitrogen and phosphorus through river discharge and these nutrients are modified within the estuary before being discharged to the coastal ocean. The input of nutrients through the estuary to the ocean is increasing in many coastal regions due to the rapid increase in human population and industrial activities (Cloern, 2001; Shen et al., 2015; Sinha et al., 2017; Qin et al., 2019). Identifying its source and cycling in the estuary is highly important to manage fertilizer utilization and sewage treatment before it is delivered to the river.

India ranks as the second-largest fertilizer-utilizing country in the world and it is hypothesized that the excess nitrogen may be transported to the coastal ocean eventually leading to seasonal eutrophication (Naqvi et al., 2000). Indian rivers are mostly dammed to conserve freshwater during the dry period. The Godavari River is the largest monsoonal river in the Indian subcontinent, which is a heavily dammed river. The freshwater entering the estuary peaks from July to September with almost nil discharge from January to May (Sarma et al., 2009; 2010; 2014). The nitrate concentrations during the peak discharge period are 10-15 times higher than that during the dry period (Sarma et al., 2010). Despite high nutrients, the phytoplankton biomass is minimal during the peak discharge period due to a decrease in light penetration caused by a high suspended matter load. The increase in biomass by freshwater algae is associated with moderate discharge ($<2000 \text{ m}^3 \text{ s}^{-1}$), in contrast, saline water dominates during the dry period with dominant marine species (Sridevi et al., 2015; Bharathi et al., 2018; Venkataramana et al., 2021).

Sarma et al. (2010) reported river discharge is one of the major sources of nutrients to the estuary during the wet period whereas submarine groundwater discharge and benthic exchange may play a significant role during the dry period. Several investigations suggested that the source of organic carbon and nitrogen in the estuary is dominated by terrestrial plant debris during the wet period and in situ production during the dry period in the Godavari estuary (Krishna et al., 2015; Sarma et al., 2014; Gawade et al. 2017). With the high amount of fertilizers per hectare (173-245 kg/hectare) utilized to grow three crops per year along the bank of the river, the excess fertilizer nitrogen may be flushed to the estuary and then to the coastal Bay of Bengal (Bristow et al., 2017). In contrast, Rao and Sarma (2016) observed that the mean concentration of nutrients in the Indian estuaries is much lower than

that in Europe and USA estuaries due to the dilution of riverine waters by monsoonal rainfall. Sarma et al. (2013) reported the signatures of riverine nutrients up to a maximum of 10 km from the coast. Such a low supply of nitrogen delivery to the Bay of Bengal may be attributed to weak tidal mixing, dilution by river discharge and removal of nitrogen as a gas through intensive denitrification. The balance among the sources, internal cycling and removal processes may govern nutrient flux to the coastal ocean. The objective of this study is to identify the sources of nitrogen and examine the possible modifications in the estuary on a monthly time scale.

2. Material and methods

2.1. Study region

The Godavari is the largest monsoonal river in the Indian subcontinent and originates in the Western Ghats and flows to the East into the Bay of Bengal after transecting ~1480 km. The basin receives an annual rainfall of 1512 mm/y, of which >75% is received during summer (June-September; CPCB, 1995) and the remaining during winter (November- February). More than 350 dams and barrages were built in the river basin and it was the highest among river basins in India. The last dam in eastern India, close to the estuary, is at Dowleiswaram, ~80 km west of the mouth (Fig. 1). Godavari River is surrounded by villages and small cities with several industries. The soil along the banks of the Godavari estuary is highly fertile. As a result, this region is agricultural active growing three crop yields per year (Bhattacharyya and Kumar 2015). Since agriculture is depending on the availability of freshwater, the crops change from low-water consumption plants during dry seasons, such as leguminous plants, to high water-consuming crops during wet (such as paddy) seasons.

2.2. Sampling

Monthly time-series observations were conducted at 10 stations (Fig. 1) between January and November 2019, which includes dry (January to June) and wet periods (July to November), along the Godavari estuary covering the upper to the lower estuary. However, the isotopic composition of N and O of nitrate was measured during March, August, September, October and November months only. Due to low concentrations of nitrate during the dry period (January to May), only three samples were been measured in March. Water samples from the surface and bottom, where water depth exceeds 3 m, were collected in 5-litre Niskin bottles. The temperature and salinity of the water column were measured using a CTD sensor (19 Plus, Sea-Bird Electronics, USA), which has a precision of 0.001°C and 0.001 respectively.

2.3 Analytical Procedures

2.3.1. Analysis of hydrographic properties

About 100 ml of water samples were filtered through GF/F (0.7 μm) filter and stored in plastic bottles for nutrients and stored at low temperatures until analysis. Water samples of 1-2 liters were filtered through a pre-weighed 0.22 μm polycarbonate filter (Millipore) and dried at 60°C for 24 h and re-weighed. The difference in weight was taken as suspended particulate matter (SPM). In the case of the peak discharge period (July to September), only 100-300 ml of water was filtered due to the high suspended load. Phytoplankton pigments were collected by passing 1 to 3 litres of the water through GF/F (0.7 μm) filter which was stored in liquid nitrogen for later phytoplankton pigment analysis by High-Performance Liquid Chromatography (HPLC).

Ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were determined with an Eco-Lab autoanalyzer (EnviroNtech, USA) with colourimetric assay (Grasshoff et al. 1983). The analytical precision varied between 0.03 to 0.05 μM for all the concentrations and was calculated on repeated measurements of the same sample (n=10). The Chl-a retained on the GF/F filter was first extracted into 90% acetone and injected into the High-Pressure Liquid Chromatography (HPLC) for quantitative estimation, following Roy et al. (2015).

2.3.2 Isotope Ratio Measurements

About 100 ml of water samples were filtered through a GF/C (0.45 μm) filter into the plastic bottle and stored at room temperature. We checked the preservation methods of keeping samples at freezing temperature (-20°C) and room temperature and did not find significant differences within the measurement errors. Nitrogen and oxygen isotope ratios of nitrate and nitrite were determined separately following a modified protocol of Mcilvin and Altabet (2005) (Semaoune et al., 2012; Sebilo et al., 2019). This method consists of the chemical reduction of NO_3^- into N_2O . The isotope compositions of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N_2O were measured using an isotope ratio mass spectrometer (IRMS, Delta Vplus, Thermo Scientific, Bremen, Germany) in a purge-and-trap system coupled with a Finnigan Gas Bench II system (Thermo Scientific, Bremen, Germany). Results are reported in the typical delta notation in ‰ with respect to the standards air for $\delta^{15}\text{N}$ and Vienna Standard Mean Ocean Water (V-SMOW) for $\delta^{18}\text{O}$, respectively (eq. 1).

$$\delta_{\text{sample}} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where R is the isotopic ratio $^{15}\text{N}/^{14}\text{N}$ or $^{18}\text{O}/^{16}\text{O}$.

Nitrate-certified reference materials subject to the same analytical procedures were used to calibrate the isotopic composition of N_2O (USGS34, $\delta^{15}\text{N} = 1.8\text{‰}$, $\delta^{18}\text{O} = -27.9\text{‰}$, USGS35, $\delta^{15}\text{N} = +2.7$

‰, $\delta^{18}\text{O} = +57.5\text{‰}$ and USGS32, $\delta^{15}\text{N} = +180\text{‰}$, $\delta^{18}\text{O} = +25.7\text{‰}$ for nitrate standards. The precision and accuracy were $\pm 0.3\text{‰}$ and $\pm 0.5\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ respectively.

2.3.2. Measurements of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$

$\delta^{18}\text{O}$ values of water were measured on Picarro CRDS L2130 and L2120 with an average reproducibility and accuracy better than $\pm 0.05\text{‰}$ as detailed in Reverdin et al. (2022).

2.4 Conservative mixing line computation

The two endmembers (riverine and seawater) conservative mixing line for nitrate was computed based on Middelburg and Nieuwenhuize (2001):

$$\text{NO}_{3\text{mix}} = q \times \text{NO}_{3\text{river}} + (1-q) \times \text{NO}_{3\text{sea}} \quad \text{---(1)}$$

Where $\text{NO}_{3\text{mix}}$, $\text{NO}_{3\text{river}}$ and $\text{NO}_{3\text{sea}}$ are nitrate concentrations due to theoretical mixing of the two river water and seawater endmembers respectively. q is the fraction of fresh water and it is calculated as:

$$q = (\text{S}_{\text{sea}} - \text{S}_{\text{estuary}}) / (\text{S}_{\text{sea}} - \text{S}_{\text{river}}) \quad \text{--- (2)}$$

where S_{sea} , $\text{S}_{\text{estuary}}$ and S_{river} are salinity in the seawater, estuary and river water endmembers respectively.

Similarly, the conservative mixing line for isotope values of the two endmembers is computed as:

$$\delta_{\text{mix}} = [q \times \text{NO}_{3\text{river}} \times \delta_{\text{river}} + (1-q) \times \text{NO}_{3\text{sea}} \times \delta_{\text{sea}}] / \text{NO}_{3\text{mix}} \quad \text{--- (3)}$$

Where δ_{river} and δ_{sea} are the isotope values of the end members for freshwater and seawater respectively and δ_{mix} is the isotopic values of the mixture of river water and seawater at a given location in the estuary. Details of the freshwater and seawater end members are given in Table 1. The upstream river values, measured in this study, were taken as freshwater endmembers whereas sewage (Jiang et al., 2021; Aravena et al., 1993; Ye et al., 2015; Archana et al., 2016) and precipitation (Yang et al., 2014) were taken from literature. The marine signatures were obtained from the surface waters of the Bay of Bengal (Bristow et al., 2017; Table 1).

3. Results

3.1. Temporal variability in river discharge

The river discharge in 2019 to the Godavari estuary displayed strong temporal variability with almost no discharge from January to May and a weak discharge ($150\text{-}320 \text{ m}^3 \text{ s}^{-1}$) was noticed during May (Fig. 2). The peak in discharge was initiated in July (up to $60,000 \text{ m}^3 \text{ s}^{-1}$) and decreased to $\sim 20,000 \text{ m}^3$

s⁻¹ in September. The pulse of high discharge for only three days occurred during September and decreased rapidly to near zero by the end of December.

3.2. *Horizontal distribution of hydrographic properties*

The salinity of the estuarine waters varied between 0 and 32, increasing from the upper to the lower estuary during March (dry period) (Fig. 3a). The salinity at the upper estuary (Alamuru) was always zero (freshwater) but significant monthly variability in salinity was noticed from Kotipalli onwards to the lower estuary during March (Fig.3a). The salinity in the entire estuary varied between 0 and 2 from August to November (Fig. 3a) associated with high discharge (Fig. 2) with an almost insignificant gradient between the upper and lower estuary.

The concentration of SPM varied between 10 and 200 mg/l with the highest concentrations during August and September and the lowest during March (Fig. 3b). The increase in the concentration of SPM during August-September was associated with high river discharge (Fig. 2). Enhanced SPM values in the middle of the estuary to the lower estuary were observed during all studied months. The concentration of Chl-a varied between 0.5 and ~40 mg m⁻³ during the entire study period with the lowest during August to September and the highest during November (Fig. 3c). The low Chl-a concentration during August and September were associated with high SPM concentrations (Fig 3b). A significant increase in Chl-a concentration in the entire estuary was observed (>10 mg m⁻³) during October and November (>25 mg m⁻³; Fig. 3c).

Uniformly higher nitrate concentrations were observed during August (47.2±3 µM) and September (61.6±4 µM). The concentration of nitrate was low during March (<0.4 µM) with increased concentration in the middle of the estuary (>10 µM). The concentration of nitrate varied between 20 and 40 µM during October and November whereas higher concentrations of >60 µM were observed during September (Fig. 4a). The concentration of nitrate decreased from the upper to lower estuary during all months (Fig. 4a).

The concentration of nitrite varied between 0.1 to 1.5 µM with higher concentrations (>1 µM) during September and October and lower (<0.5 µM) during other sampled months (Fig. 4b). The concentration of nitrite was mostly <5% of nitrate during the entire study period. The concentration of nitrite did not show significant spatial variability from the upper to the lower estuary during all months sampled (Fig. 4b).

3.3. *Spatial and temporal variations in the isotopic composition of N and O in nitrate*

$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate varied from 3.7 to 11‰ and 0 to 9.5‰ respectively in the Godavari estuary with large spatial and temporal variability (Fig. 4c, d). Relatively enriched values of $\delta^{15}\text{N}_{\text{NO}_3}$ were observed in the entire estuary during March, October and November compared to August and September (Fig. 4c). The $\delta^{15}\text{N}_{\text{NO}_3}$ decreased from the upper to lower estuary during all sampling periods, except at the middle of the estuary (30 km) during March, August and September (Fig. 4c). The $\delta^{18}\text{O}_{\text{NO}_3}$ displayed a similar spatial and temporal distribution pattern to $\delta^{15}\text{N}_{\text{NO}_3}$ (Fig. 4d) with a significant decreasing trend from upper to lower estuary during all months sampled with lower value during March (Fig. 4d).

3.4. Relationship of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ with hydrographic parameters

The $\delta^{15}\text{N}_{\text{NO}_3}$ displayed a significant linear relationship with temperature, Chl-a, NH_4 , NO_3 , DON, TN, $\delta^{15}\text{N}_{\text{PON}}$ and $\delta^{13}\text{C}_{\text{POC}}$ in the Godavari estuary whereas an inverse relationship was observed with dissolved oxygen and SPM concentrations (Table 2). The $\delta^{18}\text{O}_{\text{NO}_3}$ displayed a significant linear relationship with Chl-a, NO_3 , DOC, DON, and TN whereas inverse relation with NH_4 , and $\delta^{13}\text{C}_{\text{POC}}$ in the Godavari estuary (Table 2).

4. Discussion

4.1. Nitrogen concentrations in response to hydrological conditions

The freshwater discharge into the Godavari estuary is dam-controlled and it is released during the monsoon period (July to November) when the dam reservoir is filled (Fig. 2). In contrast, the dam gates are completely closed during the dry period to conserve freshwater for domestic and industrial needs. The concentration of NO_3^- and NO_2^- was an order of magnitude higher during the wet period (August- November) than the dry period (March; Fig. 4) confirming that river discharge brings terrestrial nutrients to the estuary whereas regenerated nutrients may be a significant source during the dry period (Sarma et al., 2010). Despite high nutrients during August-September, phytoplankton biomass (in terms of Chl-a) was low (Fig. 3c) probably due to high SPM associated with discharge (Fig. 3b) that can decrease the euphotic layer to a few centimeters (Sarma et al. 2009). The increase in SPM during the discharge period was partly caused by the disturbance of upper sediment due to high flow leading to the appearance of benthic organisms (polychaetes) in the water column (Venkataramana et al., 2021) and also surface runoff from the soil. The decrease in flow leads to stability of the water column (Sridevi et al., 2015) and a decrease in SPM concentrations (Fig. 3b) created the conducive condition for phytoplankton to grow during October and November (Fig. 3c). Sarma et al. (2009) reported that SPM controls the phytoplankton bloom during the wet period when a sufficient amount of nutrients is available.

A decrease in the concentration of nitrate was observed from the upper to lower estuary from August to November associated with a slight decrease in $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ (Fig. 4a and 4c) suggesting that either biological uptake of nitrate, and/or dilution by marine nitrate may be a possible reason (Sebilo et al., 2006). The significant linear relationship of Chl-a to $\delta^{15}\text{N}_{\text{NO}_3}$, PON to $\delta^{15}\text{N}_{\text{NO}_3}$ and POC to $\delta^{15}\text{N}_{\text{NO}_3}$ (Table 2) strongly indicates that enhanced biological production may enrich $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3 . The inverse relationship of SPM with $\delta^{15}\text{N}_{\text{NO}_3}$ suggests that enhanced SPM decreased light availability and therefore the low rate of phytoplankton uptake of nitrate. The low phytoplankton biomass decreases the fractionation of $\delta^{15}\text{N}_{\text{NO}_3}$ during uptake. This suggests that modification of nitrate concentrations through primary production may be insignificant from August to September. Little variations in $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ were observed from the upper to lower estuary during August and September suggesting less modification within the estuary due to high river discharge and low residence time (Sridevi et al., 2015). Insignificant modifications of dissolved organic carbon and $\delta^{13}\text{C}_{\text{DOC}}$ were reported in the Godavari estuary during the peak discharge period (Kumar et al., 2022).

4.2. Sources of nitrate to the Godavari estuary

The estuary receives nitrogen nutrients from various sources such as river discharge from upstream, sewage/manure, synthetic fertilizers, atmospheric deposition and marine nitrogen.

India is the second-largest fertilizer-consuming country in the world with a high rate of increase in consumption rates from 12.4 kg/hectare in 1969 to 175 kg/hectare in 2018-2019 (Source: Ministry of Agriculture: rbi.org.in/Scripts/PublicationsView.aspx?id=20070). The excess fertilizer is expected to land in the river that may be carried to the estuary during the wet period. We measured the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3 in the fertilizer and it was found to vary between -1.0 to 3.2 (1.0 \pm 0.5‰) and 17.2 to 28.3 (23.3 \pm 2‰) respectively.

It is now recognized that the isotopic composition of nitrate from the sub-root zone, which will seep through the unsaturated zone to the groundwater, does not directly reflect the balance of the primary nitrogen sources from which it is derived (Sebilo et al., 2013; Lorette et al., 2022). The part of nitrogen in fertilizers, and atmospheric deposition is not immediately used by plants and will be assimilated into the pool of soil organic matter. The turnover of this organic matter and subsequent mineralization will produce "new" nitrates whose isotopic composition does not directly reflect that of the exogenous nitrogen input. Thus, the $\delta^{15}\text{N}$ value of nitrate is closely related to that of the soil. The associated $\delta^{18}\text{O}$ value of nitrate is a nitrate produced by nitrification from the mineralization of organic matter. During nitrification processes, two molecules of oxygen come from water and one molecule from dissolved

oxygen to form NO₃ (Andersson and Hooper, 1983). Hence the expected $\delta^{18}\text{O}_{\text{NO}_3}$ can be computed from $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^{18}\text{O}_{\text{O}_2}$ using the following equation:

$$\delta^{18}\text{O}_{\text{NO}_3 (\text{exp})} = 2/3 \delta^{18}\text{O}_{\text{H}_2\text{O}} + 1/3 \delta^{18}\text{O}_{\text{O}_2}$$

where $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ is the measured value in the water and $\delta^{18}\text{O}_{\text{O}_2}$ is the atmospheric oxygen value which is taken as +23.5‰ (Aravena and Mayer, 2009). Taking into account that the $\delta^{18}\text{O}$ values of H₂O varied in this study between -3.5 and -0.33‰, the expected $\delta^{18}\text{O}$ value of nitrate produced by nitrification without any other process should vary between 5.5 and 7.6‰ (Fig. 5). All the $\delta^{18}\text{O}_{\text{NO}_3}$ points during August fell within this range suggesting that nitrate is produced through nitrification. In contrast, $\delta^{18}\text{O}_{\text{NO}_3}$ values during March were lower than expected from nitrification suggesting other processes or sources may be important.

The $\delta^{15}\text{N}$ versus $\delta^{18}\text{O}$ of nitrate plot is useful for the determination of the sources of nitrogen. The $\delta^{15}\text{N}$ of nitrate in fertilizers and atmospheric deposition depends on the isotopic composition of the air and varies around 0‰. However, the $\delta^{18}\text{O}$ value differs strongly between atmospheric deposition and fertilizer. The $\delta^{18}\text{O}$ of nitrate in fertilizer depends on the $\delta^{18}\text{O}$ of atmospheric O₂ ($\delta=23.5\text{‰}$) whereas the $\delta^{18}\text{O}$ values of nitrate in atmospheric deposition are more enriched. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of fertilizer used in the Godavari region varied between -0.4 to 3.2‰ (mean of $1.0 \pm 1.8\text{‰}$) and 17.2 to 28.3‰ (mean of $23.3 \pm 5\text{‰}$) respectively (Fig. 6). The $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ data of the Godavari during different months differed significantly from these sources of nitrogen but were more associated with the nitrification of nitrogen in soil and sewage (Fig. 6) suggesting that nitrification is the major source of nitrate in the study region. The relationship between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO₃ did not display any spatial trends between the upper and lower estuary during the sampling period (Fig. 6b) suggesting that denitrification did not occur within the estuary.

4.3. Transformation of nitrogen in the Godavari estuary

Transformations of nitrogen occur through biogeochemical processes such as volatilization, nitrification and denitrification in the estuaries. According to the Rayleigh equation, a negative relation between $\delta^{15}\text{N}_{\text{NO}_3}$ and $\ln(\text{NO}_3)$ suggests biological nitrogen removal (Wankel et al., 2009). The observed inverse relationship between $\ln(\text{NO}_3)$ and $\delta^{15}\text{N}_{\text{NO}_3}$ during March and September indicates the possible removal of nitrate through biological uptake (Fig. 7). In contrast, an insignificant relationship was observed between $\ln(\text{NO}_3)$ and $\delta^{15}\text{N}_{\text{NO}_3}$ during August, October and November indicating weak modification. During August, there is no uniform systematic changes in $\delta^{15}\text{N}_{\text{NO}_3}$ between the upper and lower estuary (Fig. 4c). On the other hand, a decrease in concentrations of

nitrate associated with the depletion of $\delta^{15}\text{N}_{\text{NO}_3}$ from the upper to lower estuary was noticed during October and November suggesting dilution with marine waters (Fig. 4c). Nevertheless, variable relationships during different months indicate that multiple biogeochemical processes may be responsible for the measured NO_3 concentrations in the Godavari estuary.

Several studies suggested that the denitrification process changes both nitrogen and oxygen isotopic composition (Sebilo et al., 2006; Xue et al., 2009; Yi et al., 2017). The occurrence of denitrification can be identified based on the ratio of $\delta^{18}\text{O}_{\text{NO}_3}/\delta^{15}\text{N}_{\text{NO}_3}$, which varies between 0.5 and 1.2 in the case of denitrification and a lower ratio suggests mineralization and nitrification. Several investigators found $\delta^{18}\text{O}_{\text{NO}_3}/\delta^{15}\text{N}_{\text{NO}_3}$ ratio of 0.5 based on the NO_3 removal (Böttcher et al, 1990, Kendall et al., 2007; Xuan et al., 2020). Conversely, laboratory culture assays investigating respiratory denitrification and nitrate assimilation have consistently found an $\delta^{18}\text{O}:\delta^{15}\text{N}$ slope ≈ 1 (Rohde et al., 2015; Granger et al., 2010; Treibergs and Granger 2017; Granger et al., 2008; Karsh et al., 2012). The strong coherence among a wide range of microorganism cultures (eukaryotes, algae, cyanobacteria, etc.) regarding isotopic fractionation of nitrate consumption processes has been systematically presented, implicating the emergent 1:1 ratio is a result of enzymatic reduction by nitrate reductase (Granger et al., 2010). The relation between $\delta^{15}\text{N}_{\text{NO}_3}$ and $\ln(\text{NO}_3)$ and the concentration of dissolved oxygen also indicates the possible occurrence of nitrification and denitrification (Chen et al., 2006; Xue et al., 2009; Ding et al., 2014). The $\delta^{18}\text{O}_{\text{NO}_3}/\delta^{15}\text{N}_{\text{NO}_3}$ ratio varied between 0.2 and 1.3 during the entire study period with a lower value (0.3) during March and October (0.6) than in other months (1.1) indicating the occurrence of denitrification during August, September and November months. Several studies suggested that denitrification may occur when dissolved oxygen concentrations are <2 mg/l (Chen et al., 2006; Fu et al., 2019). In contrast, >4 mg l^{-1} of dissolved oxygen concentrations was observed during the entire study period in the Godavari estuary suggesting that denitrification did not occur within the estuary but may be possible at the catchment area and brought the denitrified nitrate to the estuary through river discharge.

Summary and conclusion

To examine the possible sources of nitrate and modifications in the estuary, monthly observations were conducted in the Godavari estuary. A significant salinity gradient was observed between the upper and lower estuary during the dry period, in contrast, the entire estuary was filled with fresh water during the wet period. The concentration of nitrate was low (1.5 μM) during the dry period and significantly increased during the wet period (>45 μM). The isotopic composition of nitrogen in nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$) varied between 4.3 and 11.0‰ in the Godavari estuary. The linear relationship of Chl-a to $\delta^{15}\text{N}_{\text{NO}_3}$,

PON to $\delta^{15}\text{N}_{\text{NO}_3}$ and POC to $\delta^{15}\text{N}_{\text{NO}_3}$ (Table 2) strongly indicates that enhanced biological production enriched $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3 . The $\delta^{15}\text{N}_{\text{NO}_3}$ values were lower during August and September ($5.3\pm 0.6\text{‰}$) compared to March ($7.5\pm 0.3\text{‰}$) in contrast, higher $\delta^{18}\text{O}_{\text{NO}_3}$ during the former than the latter indicating that anthropogenic nitrogen may have a significant contribution during the wet period. Though the $\delta^{18}\text{O}_{\text{NO}_3}/\delta^{15}\text{N}_{\text{NO}_3}$ ratio varied between 0.3 and 1.2 with higher values (>1) during August and September than in other months, indicating denitrification during the former two months. On the other hand, insignificant modifications in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ were noticed between the upper and lower estuary suggesting that denitrification might have occurred in the catchment area and denitrified nitrate is brought to the estuary through river discharge. As a result, less nitrate is effluxed to the coastal Bay of Bengal than hitherto hypothesized that a high concentration of riverine nitrate responsible for the formation of coastal eutrophication and deoxygenation along the Indian coast (Naqvi et al., 2000; Bristow et al., 2017).

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Table 1: Endmembers for the potential N sources used in this study.

Sources	$\delta^{15}\text{N}_{\text{NO}_3}$ (‰) Air	$\delta^{18}\text{O}_{\text{NO}_3}$ (‰) SMOW	Reference
Precipitation	-2.1±5	58.8±30	Yang et al., 2014
Sewage	15.0±3.0	3.5±1.4	Jiang et al., 2021; Aravena et al., 1993; Ye et al., 2015; Archana et al., 2016
Marine	4.5±0.5	3.6±0.6	Bristow et al., 2017
River discharge	5.3±1.7	6.0±1.8	This study
Fertilizer	1.0±1.8	23.2±5	This study

Table 2: The relationship of water column properties with $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ in the Godavari estuary

Relation	r^2 ; p-value	Relation	r^2 ; p-value
Temperature vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.37 (0.001)	Temperature vs $\delta^{18}\text{O}_{\text{NO}_3}$	n.s
Chl-a vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.43 (0.001)	Chl-a vs $\delta^{18}\text{O}_{\text{NO}_3}$	+0.20 (0.05)
DO vs $\delta^{15}\text{N}_{\text{NO}_3}$	-0.26 (0.001)	DO vs $\delta^{18}\text{O}_{\text{NO}_3}$	n.s
SPM vs $\delta^{15}\text{N}_{\text{NO}_3}$	-0.40 (0.001)	SPM vs $\delta^{18}\text{O}_{\text{NO}_3}$	n.s
NH_4 vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.60 (0.001)	NH_4 vs $\delta^{18}\text{O}_{\text{NO}_3}$	-0.38 (0.001)
NO_3 vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.30 (0.001)	NO_3 vs $\delta^{18}\text{O}_{\text{NO}_3}$	+0.18 (0.05)
DOC vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.10 (0.05)	DOC vs $\delta^{18}\text{O}_{\text{NO}_3}$	+0.63 (0.001)
DON vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.38 (0.001)	DON vs $\delta^{18}\text{O}_{\text{NO}_3}$	+0.37 (0.001)
TN vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.27 (0.001)	TN vs $\delta^{18}\text{O}_{\text{NO}_3}$	+0.50 (0.001)
$\delta^{15}\text{N}_{\text{PON}}$ vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.20 (0.05)	$\delta^{15}\text{N}_{\text{PON}}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$	n.s
PN vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.25 (0.001)	PN vs $\delta^{18}\text{O}_{\text{NO}_3}$	n.s
$\delta^{13}\text{C}_{\text{POC}}$ vs $\delta^{15}\text{N}_{\text{NO}_3}$	+0.32 (0.001)	$\delta^{13}\text{C}_{\text{POC}}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$	-0.24 (0.05)

Figure 1: The map shows the location of the stations in the Godavari estuary. The region of the Dowleiswaram dam is also shown. The distance of each location from the upper estuarine sampling location is given in the bracket.

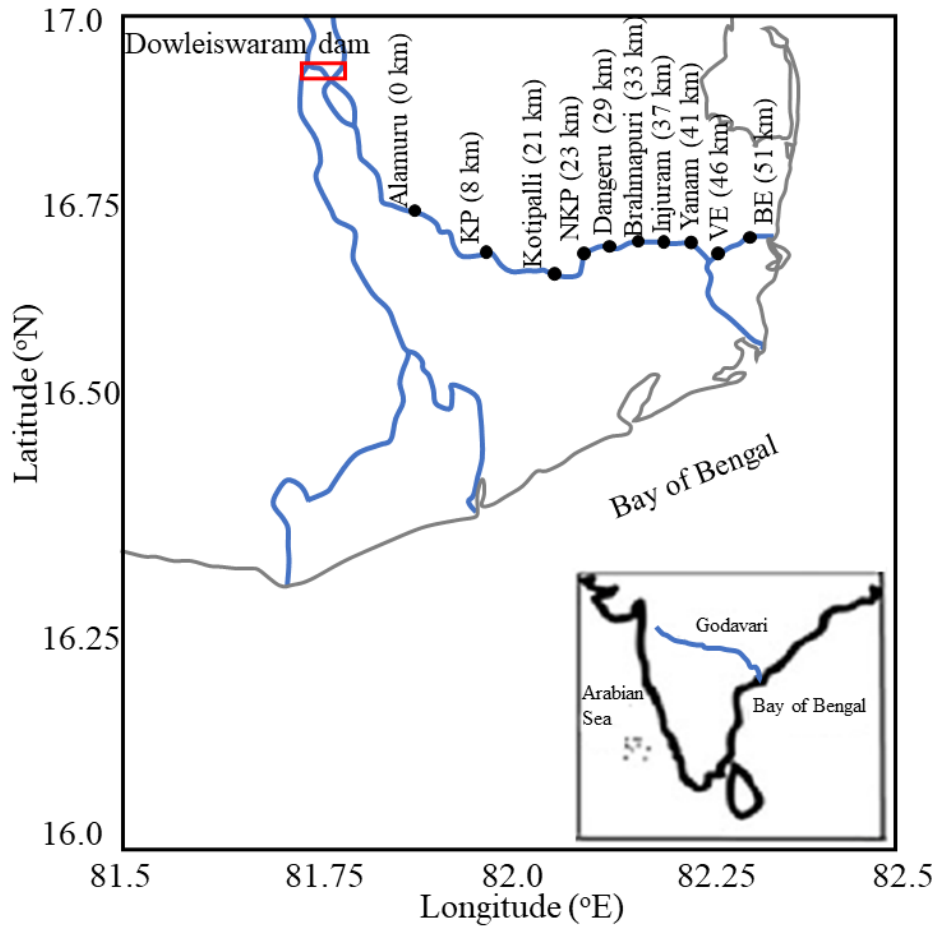


Figure 2: The daily mean river discharge from the Dowleiswaram dam to the Godavari estuary during the study period. The date of sampling during each month is shown as a dashed line. The discharge from May to July is shown in the inset.

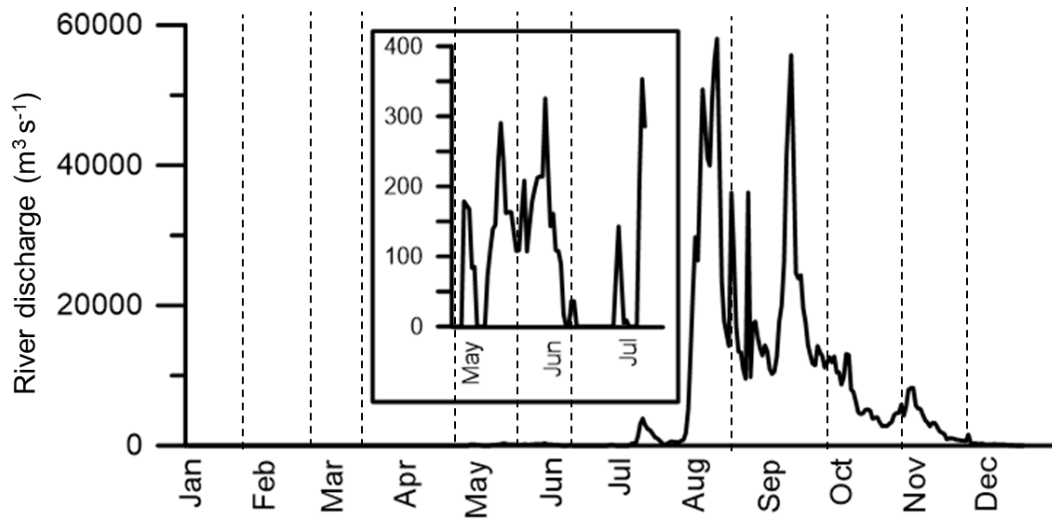


Figure 3: The temporal and spatial variability in (a) salinity, (b) SPM (mg/l) and (c) Chl-a (mg/m³) in the Godavari estuary during March, and August-November, 2019. The open black circles denote the bottom samples during March (Mar-Bot).

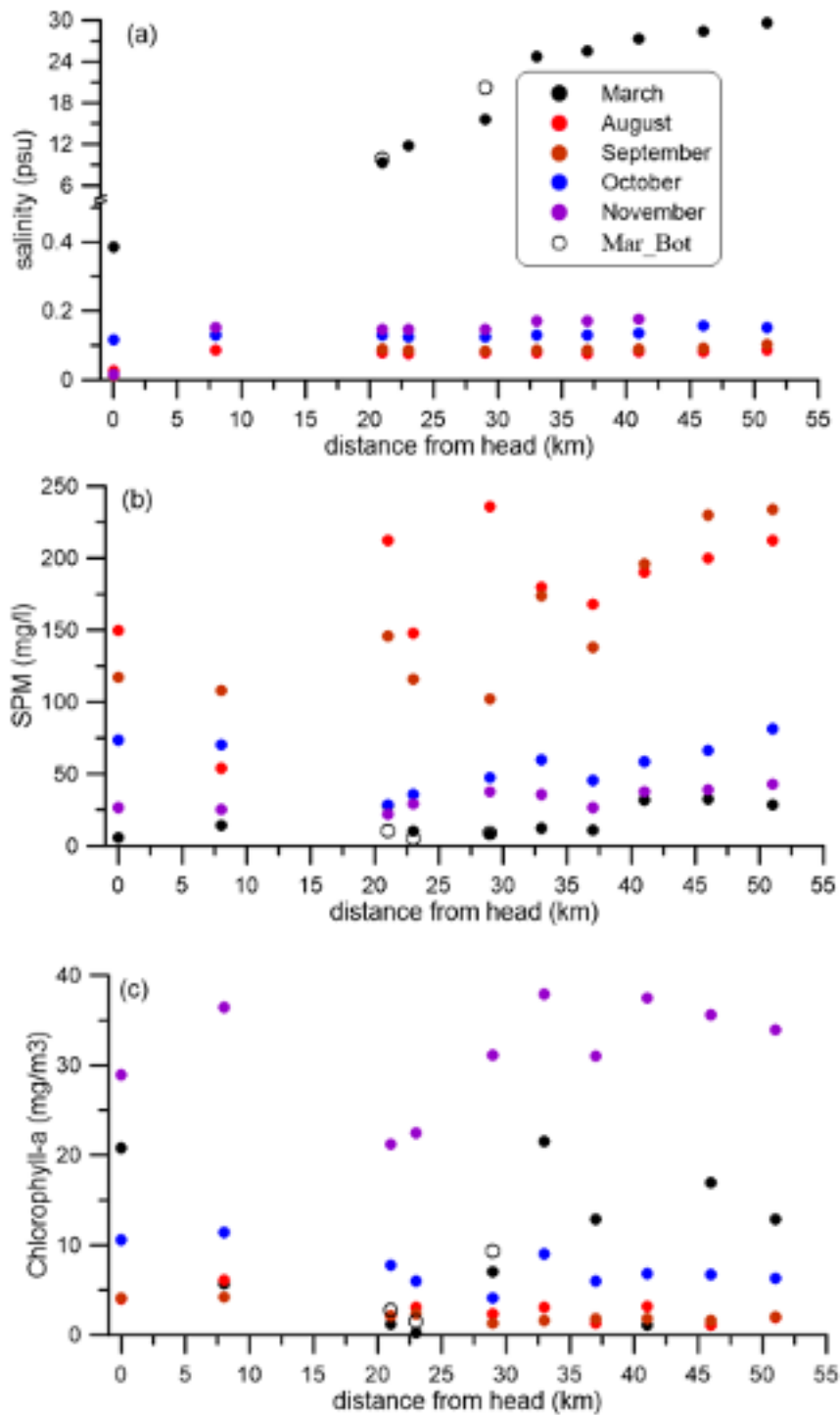


Figure 4: The temporal and spatial variability in (a) nitrite (μM), (b) nitrate (μM), (c) $\delta^{15}\text{N}_{\text{NO}_3}$ and (d) $\delta^{18}\text{O}_{\text{NO}_3}$ in the Godavari estuary during March, and August-November, 2019. The open black circles denote bottom samples during March

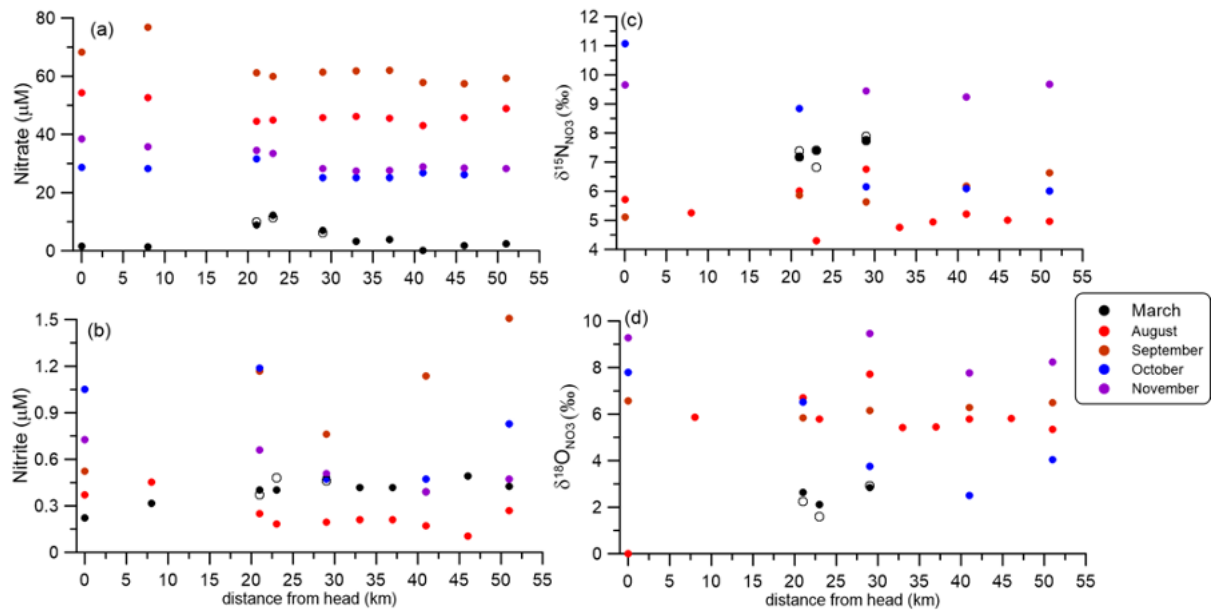


Figure 5: The plot of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ versus $\delta^{18}\text{O}_{\text{NO}_3}$, where the dashed line (black) represents the limit of the nitrification process and the straight line (black) shows the limit of exchange with H_2O . The blue dashed line was drawn for the data during March.

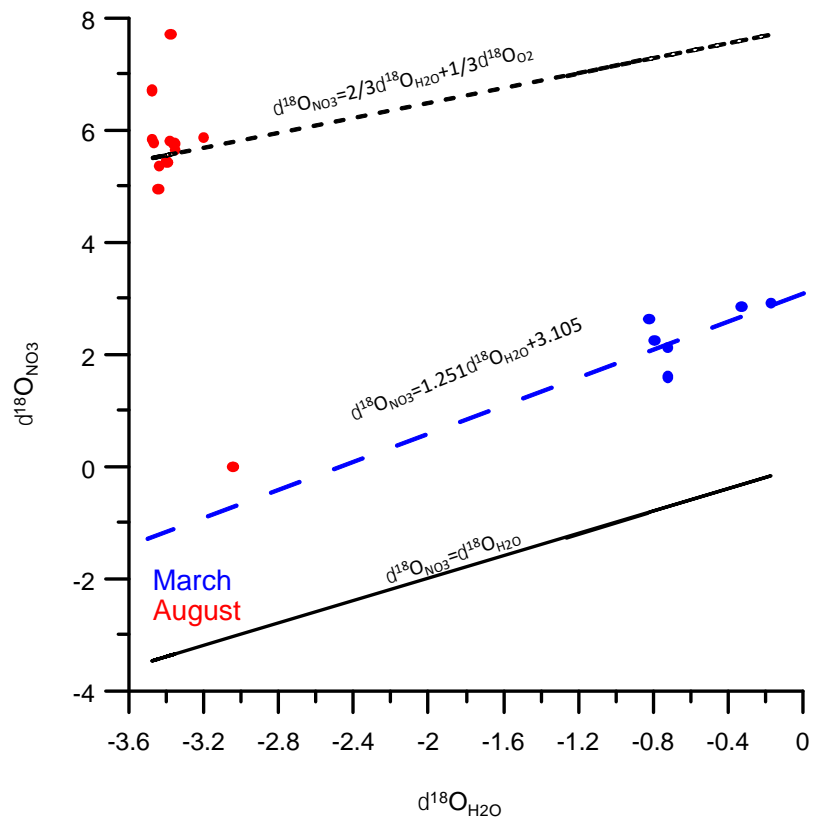


Figure 6: a) The plot of $\delta^{15}\text{N}_{\text{NO}_3}$ versus $\delta^{18}\text{O}_{\text{NO}_3}$ during the study period and the ranges of atmospheric nitrogen deposition, nitrification of soil and sewage nitrogen and fertilizer nitrogen are given. b) The data points that fall within the nitrification and soil/sewage were expanded to examine spatial trends within the estuary. The distance from the upper estuary (km) were posted for each point.

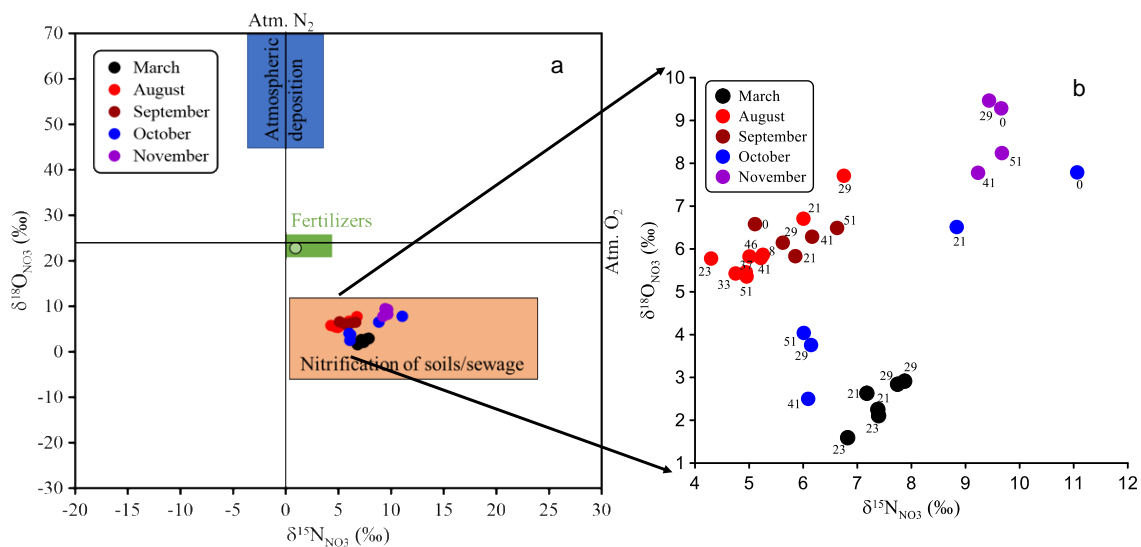


Figure 7: The relationship between $\ln(\text{NO}_3)$ and $\delta^{15}\text{N}_{\text{NO}_3}$ in the Godavari estuary. The regression line and equation are shown for March and September.

