Sulphate reduction rates and organic matter mineralization across a salinity gradient in sediments of the Mandovi Estuary, west coast of India.

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Abstract

The Mandovi Estuary, located at the tropical west coast of India, transforms from a freshwater-dominated body during the monsoon season to a tide-dominated system during post-monsoon season, with a salinity gradient developing from the freshwater end to the estuarine mouth (~0 to 33). Sulphate reduction (SR) rates were measured by the $^{35}$SO$_4^{2-}$ radiotracer method in estuarine sediments at three different sites. Porewater concentrations of hydrogen sulphide were negligible at all the sites and SR rates did not exhibit any clear trend along the salinity gradient. Pyrite content (3.45-12.9 mg g$^{-1}$) was the highest at the marine end and decreased towards the freshwater end. SR rate (15.71 mmol m$^{-2}$ d$^{-1}$) and total Fe content (14 – 20%) were highest in sediments of mid-estuary with intermediate salinity and variable porosity. Organic carbon (OC) mineralization rate through SR also did not show any trend along the salinity gradient with intermediate salinity site having higher values (31.83 mmol C m$^{-2}$ d$^{-1}$) compared to other sites (3.12-8.62 mmol C m$^{-2}$ d$^{-1}$). SR was responsible for ~8-50 % of sedimentary OC mineralization implying that OC mineralization through Fe (III) and Mn (IV) reduction possibly played major role owing to their high concentrations in estuarine sediments.

Keywords: Salinity gradient; Sulphate reduction; Mandovi Estuary; India
1. Introduction

Estuaries host complex and dynamic depositional regimes that are driven by mixing of freshwater with seawater, causing adsorption/desorption of solutes on/from particles and flocculation of suspended matter carried by the rivers. Tropical estuaries receive copious amounts of organic matter (OM) and experience high sedimentation of both allochthonous and autochthonous OM along with warm conditions that sustain elevated benthic respiration rates, making the estuarine sediments strongly reducing below a thin oxic surface layer (Hopkinson and Smith, 2005). In such organic-rich sediments, aerobic degradation of OM becomes less important (Canfield et al., 1993) resulting in the predominance of anaerobic modes of OM mineralization (Jørgensen, 1982; Sørensen et al., 1984; Kristensen et al., 2011). Anaerobic OM degradation depends on sedimentation rate, quality and quantity of OM, oxygen penetration depth and availability of electron acceptors such as nitrate (NO$_3^-$), oxides of iron (Fe (III)) and manganese (Mn (IV)), sulphate (SO$_4^{2-}$) and carbon dioxide (CO$_2$) in sediments (Froelich et al., 1979; Jørgensen, 1983; Canfield, 1994; Schubert et al., 2000). However, NO$_3^-$ reduction leading to production of elemental nitrogen (N$_2$) (denitrification) is limited to a thin oxygenated layer and thus does not play a major role in OM mineralization in coastal sediments (Jørgensen, 1983; Canfield et al., 1993). Sulphate reduction, Fe (III) and Mn (IV) reduction, and methanogenesis have been found to be the major pathways of anaerobic OM mineralization in marine and freshwater sediments (Jørgensen, 1982; Jørgensen et al., 1990; Canfield et al., 1993; Hoehler et al., 2001; Wilms et al., 2007). As SO$_4^{2-}$ is a major constituent of seawater, penetrating deep into sediments (Jørgensen, 1983; Jørgensen et al., 1990), SR strongly competes with aerobic decomposition that is confined to only a few mm thick top sediment layer (Revsbech et al., 1980; Sørensen and Jørgensen, 1987). It is estimated that SR and oxic decay make almost equal contributions to OC mineralization in coastal sediments (Jørgensen, 1982). The relative importance of SR is inversely proportional to water depth (Jørgensen, 1982) and directly proportional to sedimentation rate (Canfield, 1989). In contrast to the open ocean, in shallow marine systems, high sediment deposition and burial rates make more labile OC available for sulphate reducing bacteria (Canfield, 1989) making SR more important in OC mineralization (Sørensen and Jørgensen, 1987). Tropical macro-tidal estuaries such as the Mandovi Estuary, experience nearly marine conditions during the dry season and thus benthic metabolism during this period is expected to be dominated by SR. As the tropical estuarine sediments tend to be organic rich, the estuarine biogeochemistry can be largely controlled by the efficiency of benthic OM remineralization and consequent nutrient regeneration which sustains the ecosystem functioning through incessant feedbacks to pelagic realm.
Therefore, the relative importance of OM mineralization and burial in the sediments decides the status of the estuary as a source or a sink of CO$_2$.

The Mandovi Estuary located at the west coast of India exhibits extreme shifts in salinity regime between the monsoon (wet) and non-monsoon (dry) seasons. During the southwest (SW) monsoon season this estuary behaves like a freshwater body due to the high freshwater influx, while during the non-monsoon seasons this estuary remains tide dominated (Qasim and Gupta., 1981). Salinity within the estuary varies from ~1 (estuary head) to 34 (estuary mouth). Dense mangrove vegetation fringes along with human habitation and agricultural fields in the catchment area that supplies a large quantity of OM. The estuarine sediments are organic rich (up to ~10%; Araujo et al., 2018b). However, the quality and fate of OM is not well known and there is limited information on the rates of OM mineralization. As the oxygen penetration depth is shallow in estuarine sediments (~2mm; Pratihary et al., 2009), maximum OM mineralization is expected to occur anaerobically using other electron acceptors such as NO$_3^-$, Mn (IV), Fe (III) and SO$_4^{2-}$ (Thamdrup and Canfield, 1996). However, due to limited supply of NO$_3^-$, Mn (IV) and Fe (III) to deeper layers, and deeper penetration of SO$_4^{2-}$, the OM oxidation is expected to occur largely via the SR pathway (Alongi et al., 2000; Kristensen et al., 2000). The present study was aimed to investigate the variation of SR rates and its contribution to OM mineralization along the salinity gradient of the Mandovi Estuary using radiotracer technique.

2. Materials and methods

2.1 Study area

The Mandovi Estuary is ~75 km long, ~4 km wide and up to 10 m deep at its mouth (the Aguada Bay). It is a macro-tidal coastal plain estuary that gets completely flushed by freshwater during the SW monsoon (June-September). Freshwater is mainly derived from the Western Ghats and from a large catchment area (1150 km$^2$) at a rate reaching up to 175 m$^3$ s$^{-1}$ (Ram et al., 2003). Salt water intrudes up to ~55 km upstream during the dry period (November-May) causing the development of a prominent salinity gradient (from ~0 at the head to 34 at the mouth). The temperature of the estuarine water varies from 25 to 28°C during the SW monsoon but peaks to ~26-32°C during the dry seasons (Maya et al., 2011, Araujo et al., 2018a). During the premonsoon season, when the estuary experiences nearly marine condition, water column nutrient concentrations are low but the primary productivity is high (Krishna Kumari et al., 2002). By contrast, high turbidity during the SW monsoon seems to limit photosynthesis despite the availability of nutrients in moderately high concentrations (Maya et al., 2011). The estuary remains net autotrophic during the dry seasons and
net heterotrophic during the SW monsoon (Ram et al., 2003). A wind driven turbidity maximum zone (TMZ) occurs in the lower estuary throughout the year wherein the suspended particulate matter (SPM) concentration reaches up to 20 mg L\(^{-1}\) during the SW monsoon but decreases to 5 mg L\(^{-1}\) during the dry seasons (Kessarkar et al., 2009). The estuarine sediments are organic rich and silt-clay dominated (Alagarsamy, 1991; Prajith et al., 2015; Araujo et al., 2018b). Iron and manganese concentrations in the sediments and SPM are found to be very high (Alagarsamy, 2006; Kessarkar et al., 2013; Prajith et al., 2015) mainly due to weathering of the source rocks (Al and Fe laterites) in the Western Ghats as well as anthropogenic activities such as mining and transportation of mineral ores in the river channel.

2.2. Site selection and sample collection

Three sampling sites were selected representing three different salinity zones in the Mandovi Estuary. The sites were located close to the villages of Ganjem, Amona and Betim, representing near freshwater (upper estuary), brackish water (mid-estuary), and marine (lower estuary) zones, respectively (Fig.1). Sediment cores were collected at low tide from these locations during peak summer (late May-early June 2014, the pre-monsoon season). Immediately after collection, the cores were covered by aluminium foil and capped on both sides with rubber stoppers (to prevent contact with the atmosphere). Three cores were taken for the SR rate measurement in acrylic tubes (length 40 cm, ID 2.5 cm). Two of these served as experimental cores (duplicates) while the third was used as control. For injection of \(^{35}\)SO\(_4^{2-}\) radiotracer, holes had been drilled into the acrylic tubes at 1 cm intervals and filled with an opaque silicon sealant and covered with duct tape to prevent leakage of porewater and contamination by atmospheric oxygen. Four separate cores were also collected for measurements of porosity and granulometry, OC, pyrite, sedimentary Fe, Mn and Al, and porewater SO\(_4^{2-}\) and sulphide. Organic carbon, porosity, grain size and porewater SO\(_4^{2-}\) data are reported in Araujo et al. (2018b). Additional sediment cores were collected from the opposite bank of the estuary at the mid-salinity region to assess the spatial heterogeneity of SR rates and other parameters mentioned above.

2.3. Processing of cores and geochemical analyses

For measurements of pyrite and metal contents cores were sectioned at 1 cm intervals, transferred into air-tight plastic bags within a N\(_2\) flushed glove box and freeze-dried. For porewater sulphide measurement, porewater was extracted from a core using Rhizon tubings (Rhizosphere Research Products, Wageningen, The Netherlands) connected to helium flushed Hamilton glass syringes at every 1 cm interval.
2.3.1. Measurement of SR rates

The SR rates were determined using the radiotracer technique involving whole-core incubation (Jørgensen, 1978) as follows. The $^{35}\text{SO}_4^{2-}$ tracer (10 µL of $\sim$92.5 kBq $^{35}\text{SO}_4^{2-}$ mL$^{-1}$ in deionised water) was injected into the cores through the ports located at intervals of 1 cm and incubated in dark for 12-16 h. Further processing was done following the procedure of Kallmeyer et al. (2004) whereby the total reducible inorganic sulphur (TRIS) is distilled out and trapped as zinc sulphide. The detection limit for this method is <1 pmol SO$_4^{2-}$ cm$^{-3}$ d$^{-1}$ (Kallmeyer et al., 2004). Ultima gold XR scintillation cocktail was added to the samples and the final activity was counted on Wallac liquid scintillation counter with a counting window of 4 to 167 keV. The SR rates were calculated using the equation:

\[
\text{SR rates} = (\text{SO}_4^{2-}) \times P_{sed} \times \frac{a_{tris}}{a_{tris} + a_{tot}} \times \frac{1}{t} \times 1.06 \quad \text{(nmol cm}^{-3} \text{d}^{-1})
\]

Where, (SO$_4^{2-}$) is the porewater sulphate concentration (mM); $P_{sed}$ is the porosity of the sediment; $a_{tris}$ is the activity of total reducible inorganic sulphur (counts per minute, CPM/decays per minute, DPM); $a_{tot}$ is the total activity injected (CPM/DPM), $t$ is the incubation time (days). The factor 1.06 is the estimated fractionation factor between $^{35}$S and the natural isotope $^{32}$S (Jørgensen and Fenchel, 1974).

2.3.2. Pyrite analysis:

The reduced sulphur species (pyrite) were estimated following Kallmeyer et al. (2004); the liberated sulphide was measured spectrophotometrically (Cline, 1969). About 0.1-2 g of freeze dried sediment was used for this analysis and the precision based on triplicate measurements was ≤ 4%.

2.3.3. Major and trace metal analysis:

Iron, manganese and aluminium contents of sediments were also measured at all sampling sites. Sediment samples (50 mg) were weighed and transferred to Teflon vessels containing 10 ml of supra pure acid mixture (HF, HNO$_3$ and HClO$_4$ in the ratio 7:3:1). After keeping overnight at room temperature, the teflon vessels were heated at 190°C on a hot plate and dried completely. This step was repeated by adding 5 ml of acid mixture to ensure complete digestion of the sample. The digested samples were diluted to 100 ml and analysed on an inductively coupled plasma optical emission spectrometer (ICP OES, Agilent Technologies, 700 series). The detection limits for Fe, Mn and Al were 0.2, 0.05 and 0.9 parts per billion (ppb), respectively. Precisions of analyses based on duplicate measurements of samples were ±0.61, ±0.05 and ±0.32 parts per million (ppm) for Fe, Mn and Al, respectively. The Fe:Al ratios were also calculated at each depth for the three study sites.
These ratios represent the reactive iron pool of the sediments by nullifying the terrigenous effect (Lamy et al., 2000).

2.3.4. Porewater $H_2S$ analysis:

The dissolved $H_2S$ in extracted porewater (5 ml) was fixed immediately by adding 0.4 ml of mixed reagent (N,N-dimethyl-p-phenylenediamine sulphate and ferric chloride) and the absorbance of the colour developed was measured after 20 minutes at 670 nm using a Shimadzu, UV 1700 Spectrophotometer (Cline, 1969). The minimum detection limit for $H_2S$ measurement is ~1 µM. Duplicate analysis of a sulphide standard (10 µM) yielded a precision of ±0.14 µM.

2.3.5 C:N ratios

The C:N ratio, a proxy of the lability of OM (Kim et al., 2011), was calculated for each depth. Low C:N values (5-7) are typical of OM freshly produced by phytoplankton while values above 20 indicate the terrestrial origin; however, OM in its degraded phase may also yield values above 10 (Schubert et al., 2000).

3. Results

The SR rates at Ganjem were measureable only in the upper 10 cm where porewater $SO_4^{2-}$ concentrations were high. The rates were highest in the upper 2 cm and gradually decreased with depth, becoming insignificant at ~10 cm (Fig. 2a). Porewater $H_2S$ (maximum 0.93 µM) and pyrite (maximum 0.46 mg g$^{-1}$) concentrations were generally low at Ganjem, with the latter showing an increasing pattern below 18 cm (Fig. 2b). While the sedimentary Fe and Mn contents ranged from 7.12 to 8.56 % and 0.1 to 0.4 %, respectively, the Fe:Al ratios varied between 0.95 and 1.26 (Fig. 2c). The C:N ratios at this station varied between 14.9 and 22.2 (Table. 1, supplementary data). A different trend was observed at Amona where the SR rates were unexpectedly low at 0-2 cm, increased to ~50 nmol cm$^{-3}$ d$^{-1}$ at 3 cm and remained rather constant down to 18 cm depth. Below this depth, the rates increased to a maximum of ~350 nmol cm$^{-3}$ d$^{-1}$ (Fig. 3a). The pyrite content was higher, varying between 0.75 and 3.8 mg g$^{-1}$ from the surface to 18 cm depth, except for one near-zero value within the sandy band, and increasing thereafter to a maximum of 8.25 mg g$^{-1}$ at the bottom of the core. The $H_2S$ concentrations varied between 0 and 0.62 µM (Fig. 3b). Highest concentrations of Fe and Mn were recorded here (13.51- 21.12 % and 0.38- 1.05 %, respectively) with an increasing trend from surface to bottom of the core. The Fe:Al ratios were the highest, ranging from 2.63 to 4.14 (Fig. 3c). The C:N ratios in these sediments ranged between 13.6 and 18.9 (Table. 1). At Betim, the SR activity was observed throughout the core with the rates ranging between 3.08 and 58.61 nmol cm$^{-3}$ d$^{-1}$. The SR rates generally decreased with depth with minor
peaks located at 1-3 cm, and 5-6 cm depths (Fig. 4a). Pyrite concentrations showed a clear increasing trend with depth and ranged from 3.5 to 12.9 mg g\(^{-1}\). Thus, the pyrite content generally increased from the freshwater end to the marine end. The highest porewater H\(_2\)S concentration measured at this site was 0.89 µM (Fig. 4b). The Fe and Mn concentrations at this site were comparable to those at the freshwater site. While Fe and Mn concentrations ranged from 8.12 to 9.79 % and 0.16 to 0.27 %, respectively, Fe:Al ratios varied from 1.6 to 2.02 (Fig. 4c). The C:N ratios ranged between 12.8 and 17.3 (Table 1). The OC mineralization rates supported by SR were calculated (Table. 2) using the stoichiometry of equation (2) according to which for every mole of SO\(_4^{2-}\) respired, two moles of OC are oxidised (Lückge et al., 1999).

\[
\text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S} \quad \text{----------------} \quad (2)
\]

4. Discussion

4.1. Spatial variability of Sulphate reduction

Sedimentary SR can be controlled by several factors such as porewater SO\(_4^{2-}\) concentration, availability of labile OM, sediment porosity and grain size, and Fe (III) and Mn (IV) contents of the sediments (Froelich et al., 1979; Boudreau and Westrich., 1984; Canuel and Martens., 1996). We observed a significant variation in SR rates from Ganjem to Betim with the maximum rates occurring at Amona. The salinity gradient is expected to induce some degree of spatial heterogeneity in sediment biogeochemistry from the upper estuary to the lower estuary. Here we attempt to assess factors controlling SR rates in the three salinity zones of the estuary.

4.1.1. Upper estuary:

At Ganjem, due to low salinity (~1) of estuarine water, concentration of porewater SO\(_4^{2-}\) was the lowest (0-2.5 mM) especially below 9 cm (Araujo et al., 2018b). SR rates were the lowest here (0-50 nmol cm\(^{-3}\) d\(^{-1}\)) among all the sites and the SR profile corresponded well with the porewater SO\(_4^{2-}\) profile, indicating SR limitation by SO\(_4^{2-}\) availability. The SR rates in the upper sedimentary layer at Ganjem compared well with the rates (76-105 nmol cm\(^{-3}\) d\(^{-1}\)) reported from the freshwater zone of Colne estuary (Kondo et al., 2007). It has been reported that the SO\(_4^{2-}\) reducing activity is independent of porewater SO\(_4^{2-}\) concentration until it reaches very low levels (Boudreau and Westrich, 1984). This is substantiated by a strong and significant correlation (r= 0.88, p<0.001) of SR with porewater SO\(_4^{2-}\) concentration (Fig. 5a). Being a heterotrophic process, the SR activity is expected to be controlled by the availability of OC, particularly its labile fraction (Berner, 1971). However, SR showed a poor correlation with both OC (r= 0.10, p>0.05, not shown) and C:N (r= -0.14, p> 0.05) which indicated that OC availability did not limit SR in the Mandovi Estuary (Fig.
Thus, at the freshwater end of the estuary, the SR rates were mainly controlled by porewater SO\textsubscript{4}\textsuperscript{2−}.

### 4.1.2. Middle estuary:

SR rates varied widely (0-350 nmol cm\textsuperscript{3} d\textsuperscript{-1}) at Amona and the SR profile was unusual compared to the other two sites (Fig. 3a). The highest rates were observed not in the upper portion of the core, but at its lower end. Low or not-detectable SR occurred within the upper 3 cm; below this depth, the rates were modest – comparable to those observed in the upper parts of the cores from Ganjem and Betim – till 18 cm depth (i.e. just below the sandy band). The SR rates increased thereafter with depth to 350 nmol cm\textsuperscript{3} d\textsuperscript{-1} which was the highest rate recorded in this study. Similar observations (high SR rates in deeper sediments) have also been reported from sediments of Mtoni mangrove forest in Tanzania where the rates increased to 220 nmol cm\textsuperscript{3} d\textsuperscript{-1} below 14 cm depth (Kristensen et al., 2011). As the porewater SO\textsubscript{4}\textsuperscript{2−} concentration (14-17 mM) remained almost uniform throughout the core, limitation of SR by porewater SO\textsubscript{4}\textsuperscript{2−} is ruled out. Absence of SR or extremely low rates observed within the upper 3 cm could be due to greater availability of Fe and Mn in these sediments compared than at Ganjem and Amona (Fig. 3c) as OM mineralization through Fe (III) and Mn (IV) reduction is energetically more favourable than that coupled to SR. Since the OC content showed an opposite vertical trend i.e. decreasing downcore and the highest SR rates were associated with relatively low OC (~2%; Araujo et al., 2018b), apparently the SR rates were not regulated by OC at this site. In fact, the SR rate showed little or weak correlations with OC (r=-0.39, p<0.05, not shown), C:N ratio (r=-0.56, p<0.05) and porewater SO\textsubscript{4}\textsuperscript{2−} (r= 0.49, p<0.05) (Figs. 5b,e). The unusual pattern may be related to the sediment porosity to some extent which showed an irregular trend due to the presence of an intermediate ~6 cm thick layer of sand (Araujo et al., 2018b). Sandy (permeable) sediments may supply dissolved OM through diffusion and advection to deeper layers (Huettel and Rusch, 2000; D’Andrea et al., 2002). Moreover, this site was in proximity to mangrove forests which supply OM that may support high rates of SR (Alongi et al., 2000; Kristensen et al., 2011). Thus, it seems reasonable to conclude that the availability of OM in this mangrove dominated site (perhaps in the dissolved form) and other electron acceptors (Fe (III) and Mn (IV)) may principally control SR rates here. At this site, porosity and SR rates did not show significant spatial variability (p>0.05) between the two sites located at opposite sides of the estuary. However, the silt-clay fraction, OC and porewater SO\textsubscript{4}\textsuperscript{2−} concentration varied significantly (p<0.05) between the two sites which indicated spatial heterogeneity in the estuarine sediments despite both sites being mangrove dominated.

### 4.1.3. Lower estuary:
At Betim, significant SR activity occurred throughout the core (Fig. 4a) with intermediate rates (3-58 nmol cm$^{-3}$ d$^{-1}$) but no regular trend was observed in the SR rate profile. Similar SR rates have also been reported by Kondo et al. (2007) from the Alresford Creek (13.8-57.8 nmol cm$^{-3}$ d$^{-1}$) located at the marine end of Colne Estuary. The porewaters were uniformly replete with SO$_4^{2-}$ (21-24 mM) throughout the core and thus, SO$_4^{2-}$ concentration did not possibly control the observed SR rates. This is confirmed by a poor correlation of SR with porewater SO$_4^{2-}$ (r = 0.27, p>0.05) (Fig. 5c). Being close to estuary mouth, this site is expected to receive high flux of sediments due to flocculation and the presence of TMZ (Rao et al., 2011). Although, the SR rates were clearly lower below 10 cm than those above this depth, they remained well above detection limit (>1 pmol SO$_4^{2-}$ cm$^{-3}$ d$^{-1}$). Organic carbon content in this core was the lowest (1.6-3.2%) of all the three sites sampled and OC decreased below 10 cm and then remained almost uniform (Araujo et al., 2018b). The SR rates exhibited good correlation with the C:N ratio (r=0.83, p>0.01, Fig. 5f). The sedimentary SR rates at Betim, although lower than those recorded at Amona, were higher than those measured over the inner shelf off Goa having comparable OC and porewater SO$_4^{2-}$ concentrations (Naik et al., 2017). Therefore, at the marine end of the Mandovi Estuary, the availability of labile OM seems to be the predominant factor controlling sedimentary SR.

4.2. Interplay of reactive Fe and Mn, and SR

At the three study sites, apart from porewater SO$_4^{2-}$ and OC availability there could be other factors possibly controlling SR e.g. availability of Fe (III) and Mn (IV). Therefore considerable OM mineralization through Fe and Mn reduction is highly likely especially because of the copious amounts of Fe (~7-21%) in sediments of the Mandovi Estuary. Haematite, magnetite and goethite, abundantly found in the sediments of tis estuary, are products of weathering carried by the river (Prajith et al., 2015), with large-scale transportation of Fe and Mn ores by barges also making additional contribution. The Fe (14-20%) and Mn (0.4-1.1%) contents were the highest at Amona, about twice those recorded at Betim and Ganjem (Fig.4c). Reactive iron (as inferred from Fe:Al) was also higher than the crustal values (>0.44 - (Pattan et al., 2012) at all the sites with maximum value at Amona (2.6 – 4.1), which supports the proposed importance of Fe (III) reduction in OM mineralization. Importance of Fe (III) reduction is further evidenced by the extremely low H$_2$S concentrations (<1µM) in porewaters despite appreciably high SR rates in these sediments. Similarly low H$_2$S accumulation also observed in shelf sediments off Goa (Naik et al., 2017) has been ascribed to rapid removal of sulphide into the solid phase through precipitation as metal (mostly Fe) sulphides (Raiswell and Canfield, 1998). Presence of pyrite in the estuarine sediments (Figs. 2-4) and negative correlation, although statistically not significant (r= -0.13, p>0.05; supplementary figure) between
porewater H$_2$S and pyrite content, suggest that H$_2$S produced through SR is efficiently immobilized. This also corroborates significant Fe (III) reduction activity in sediments of the Mandovi Estuary. The highest pyrite content with a clear vertical trend was observed at Betim, where the SR rates were rather modest. Also, while the SR rates generally decreased with depth, the pyrite content increased downcore (Fig. 4a, b). But at Amona both the SR rate and pyrite content exhibited similar increasing trend with depth. Moreover, the pyrite content was relatively lower at this site despite higher SR rates and higher Fe concentrations. Apparently, other factors such as sediment resuspension, bioturbation and grain size of sediments that may facilitate oxidation and diffusion of H$_2$S may also contribute to its removal from porewaters and lower accumulation of pyrite (Aller, 1980; Berner and Westrich., 1985). At this site, traces of H$_2$S were present throughout the core (Fig. 2b). The middle portion of the core was devoid of pyrite, but the pyrite content increased steadily with depth below ~17 cm (Fig. 2b). The SR rates were very low at this site, and so the accumulation of pyrite might represent past SR activity or slow reduction over a long period of time. High build-up of methane (CH$_4$) was also observed below ~15 cm in the core (~10 mM; Araujo et al., 2018b). The low porewater SO$_4^{2-}$ concentration plausibly leads to shallow SO$_4^{2-}$ - CH$_4$ transition zone and vigorous methanogenesis (Brüchert et al., 2003; Thang et al., 2012). Hence the presence of pyrite at greater depths may be related to anaerobic methane oxidation by SO$_4^{2-}$, producing sulphide which further reacts with available Fe (II) to form pyrite (Fig. 2b).

The SR rate exhibited negative correlation with Fe:Al ratio at both the freshwater ($r= -0.33$, p>0.05) and marine ($r= -0.42$, p>0.05) ends of the estuary (Fig. 5g,i). By contrast, a positive correlation was observed between SR rates and Fe:Al at Amona. This positive relationship was apparently determined by the high SR rates (exceeding 100 nmol cm$^{-3}$ d$^{-1}$). At lower rates, SR showed a significant negative correlation with Fe:Al ($r= -0.83$, p<0.05; Fig. 5h) at Amona. Thus, while our results do not allow quantification of Fe (III) reduction and its contribution to total benthic respiration, they do underline its importance. Iron reduction has been reported to be an important anaerobic pathway in some other systems e.g. Satilla River estuary (Meiggs and Taillefert, 2011). Yet, in most other systems it is less important than SR. In the Indian Ocean region, high SR rates (2.5-16.1 mmol m$^{-2}$ d$^{-1}$) have been reported from the mangrove sediments of the Indus delta (Pakistan) by Kristensen et al. (1992). Even higher rates (18-53 mmol m$^{-2}$ d$^{-1}$) were recorded by Alongi et al. (2004) in a similar environment of Malaysia, where SR accounted for 51-75% of total CO$_2$ flux. Our results fall well within the range reported above.

4.3. Sulphate reduction, OM mineralization and implications for ecosystem
Our results suggest that SR is an important but not the dominant pathway of benthic respiration in Mandovi Estuary except for the mangrove-dominated brackish water zone (Amona; Table. 2). Nevertheless, SR rates within the Mandovi Estuary are significantly higher than those measured in the inner shelf sediments off Goa (Naik et al., 2017). One of the possible reasons for higher SR rates in estuarine sediments may be the higher sedimentation rates (Canfield, 1994) in the Mandovi Estuary (especially in the lower estuary off Panaji (~1-12.4 mm y⁻¹ - Prajith et al., 2015) as compared to those in the inner shelf region (~1.5 mm yr⁻¹ - Kurian et al., 2009). Higher sedimentation rates at the lower estuary may be related to the presence of TMZ during February – May (Kessarkar et al., 2009). Also, having a large catchment area comprising of tropical forests, agricultural fields, human settlements and mangrove vegetation, the Mandovi Estuary receives large quantities of OM and the shallow depth of the estuary is expected to result in greater deposition of OM on the seafloor as observed elsewhere (Jahnke, 1996). Another contributory reason may be the tropical location of this estuary, as higher temperatures (26-32° C) of the estuarine water (Maya et al., 2011; Araujo et al., 2018a) may elevate benthic respiration rates. The measured benthic respiration rates during this period were 38.4, 63.4 and 82.88 mmol C m⁻² d⁻¹ at upper, middle and lower parts of the estuary, respectively (Table. 2, Pratihary et al., unpublished data). The depth-integrated SR rates at the upper, middle and lower portions of the estuary were 1.56, 15.71 and 4.31 mmol m⁻² d⁻¹, respectively. Thus, SR would contribute about 8%, 50% and 10% of the OC mineralization at upper, middle and lower estuary respectively. Therefore, other anaerobic respiration pathways, especially Fe (III) reduction probably make important contributions to OC mineralization, as observed elsewhere by Lovley and Phillips (1987), Canfield et al. (1993) and Kraal et al. (2013).

5. Conclusions

Sedimentary SR rates in the Mandovi Estuary measured during the dry (pre-monsoon) season varied considerably along the salinity gradient being maximal in the mangrove-dominated mid estuary. These rates were 3-25 fold higher than the rates measured in the adjacent inner shelf sediments. The SR rates close to the river mouth were substantially lower, and even lower rates are recorded at the freshwater end of the estuary. Sulphate reduction was the dominant pathway of OC mineralization in the mid-salinity region, accounting for over half of the OC mineralization. At the other two sites, its contribution was relatively modest, implying that other oxidants such as Fe (III) and Mn (IV) were also important in anaerobic OM mineralization. Availability of labile OM appears to be the main limiting factor controlling SR at the marine end, but at the freshwater end the rate may be principally constrained by low SO_{4}^{2−} availability in porewaters. Factors responsible for very high SR activity in the mangrove dominated brackish water zone despite high Fe availability remain unclear, but
sediment characteristics and OC supply were perhaps important drivers. Very low porewater H$_2$S levels and moderately high solid-phase sulphide contents indicated immobilization of sulphide as metal (mostly Fe) sulphides. Further experimental study on the Fe (III) and Mn (IV) reduction would improve understanding of modes of OM mineralization and their roles in estuarine ecosystem functioning and sustenance.

6. Acknowledgements

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**Fig. 3**: Depth profiles of (a) SR rates (nmol cm$^{-3}$ d$^{-1}$) and porewater sulphate (mM) (Araujo et al., 2018b); (b) porewater H$_2$S (µM) and Pyrite (mg g$^{-1}$); and (c) sedimentary Fe, Mn (%) and Fe:Al ratios at Amona (mid estuary).

**Fig. 4**: Depth profiles of (a) SR rates (nmol cm$^{-3}$ d$^{-1}$) and porewater sulphate (mM) (Araujo et al., 2018b); (b) porewater H$_2$S (µM) and Pyrite (mg g$^{-1}$); and (c) sedimentary Fe, Mn (%) and Fe:Al ratios at Betim (lower estuary).

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Tables

**Table 1:** Geographical locations of study sites, salinity of overlying waters, C:N ratio, OC content (% dry wt.) and porosity of sediment samples.

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Salinity</th>
<th>C:N</th>
<th>OC (dry wt. %)*</th>
<th>Porosity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latitude</td>
<td>Longitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganjem</td>
<td>15°</td>
<td>28.133’N</td>
<td>05.596’E</td>
<td>14.9 - 22.2</td>
<td>4.8 – 9.9</td>
</tr>
<tr>
<td>Amona</td>
<td>15°</td>
<td>31.573’N</td>
<td>58.143’E</td>
<td>13.6 - 18.9</td>
<td>0.7 – 4.5</td>
</tr>
<tr>
<td>Betim</td>
<td>15°</td>
<td>30.326’N</td>
<td>49.796’E</td>
<td>12.8 - 17.3</td>
<td>1.56 – 3.42</td>
</tr>
</tbody>
</table>

*Araujo et al. 2018b

**Table 2:** Depth integrated SR rates (mmol m$^{-2}$ d$^{-1}$), OC mineralization rates (estimated through SR rates and total benthic respiration rates (mmol C m$^{-2}$ d$^{-1}$) and percent contribution of SR towards OC mineralization in the estuarine sediments.

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Depth integrated SR rates (mmol m$^{-2}$ d$^{-1}$)</th>
<th>OC mineralization rate (mmol C m$^{-2}$ d$^{-1}$)</th>
<th>Contribution of SR towards total OC mineralization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated through SR rates</td>
<td>Estimated through Benthic respiration*</td>
<td></td>
</tr>
<tr>
<td>Ganjem</td>
<td>1.56± 0.18</td>
<td>3.12± 0.37</td>
<td>38.6 (±4.04)</td>
</tr>
<tr>
<td>Amona</td>
<td>15.71 ±1.63</td>
<td>31.83 ± 3.27</td>
<td>63.4 (±3.28)</td>
</tr>
<tr>
<td>Betim</td>
<td>4.31± 0.61</td>
<td>8.62± 1.22</td>
<td>82.88 (±4.74)</td>
</tr>
</tbody>
</table>

*unpublished data (A. Pratihary et al.; manuscript in preparation)