

Abstract

In order to test the hypothesis that the ambient iron concentrations could regulate sulfate reducing activity (SRA), 10 cm cores were examined from test and reference sites. The test site at Diwar mangrove ecosystem is highly influenced by iron released by the movement of barges carrying iron ore during the non-monsoon seasons and the reference site at Tuvem is relatively pristine. The average iron concentrations were 17.9% (± 8.06) at Diwar and 6.3% (± 1.5) at Tuvem. Sulfate reducing rates (SRR) ranged from 50.21 to 698.66 $\text{nM cm}^{-3}\text{d}^{-1}$ at Tuvem, and from 23.32 to 294.49 $\text{nM cm}^{-3}\text{d}^{-1}$ in Diwar. Pearson's correlation coefficient between SRR and environmental parameters showed interesting relationships. At Tuvem, the SRR was controlled by SO_4^{2-} ($r= 0.498$, $p<0.001$, $n=60$) more than organic carbon ($r=0.316$ $p<0.05$, $n=60$). At Diwar, the SRR was governed by the iron concentrations at a r -value of -0.761 ($p<0.001$, $n=60$), suggesting that about 58% of the variation in SRR was influenced negatively by variations in ambient iron concentrations. This influence was more than the positive influence of TOC ($r=0.615$, $p<0.001$, $n=60$). Laboratory experiments to check the influence of iron on SRR also support our field observations. At an amendment of 50 ppm Fe^{3+} there was an increase in SRR but at 100 ppm amendment onwards inhibitory effect was observed. At 1000 ppm Fe^{3+} there was a decrease in the SRR upto 93% of control. Thus, our study showed that ambient iron concentrations influence SRR negatively at Diwar and counters the positive influence of organic carbon. Consequently, the influence could cascade to other biogeochemical processes in these mangrove swamps, especially the mineralization of organic matter to carbon dioxide by sulfate respiration.

Keywords: iron; sulfate; organic matter; sulfate reduction; mangroves;

1. Introduction

Mangroves are one of the most productive ecosystems in the world and occupy considerable part of the tropical coastline, ca 200 sq. km along Goa alone. The decomposition of rich mangrove detritus is essentially a microbially mediated process. It is the POM (Particulate Organic Matter) that either gets degraded or recycled in the sediments or is exported to adjacent areas (Woodroffe 1985; Kristensen et al., 1988). In estuarine and shallow sea ecosystems, sulfate reduction has been reported to be the most active process. It comprises about 20 to 40% of the global sulfate reduction (Skyring, 1987). Dissimilatory sulfate reduction may contribute > 50% of the organic matter mineralization in continental shelf sediments (Jørgensen 1982; Skyring 1987; Canfield et al., 1993). However, Kristensen, et al., (1991) have shown that sulfate reduction may account for up to 100% of total sediment metabolism (measured as CO₂ efflux) in mangrove sediments.

A number of environmental factors are known to influence the sulfate reduction rates (SRR) including bacterial abundance, availability of sulfate and lability of organic carbon, temperature, salinity, pH, (Connell and Patrick Jr, 1968; Roychoudhury et al., 1998; Brandt et al., 2001; Kostka et al., 2002; Meier et al., 2005; Pallud and Van Cappellen, 2006) availability of water (Jones et al., 1989) and redox conditions (Hamilton, 1998; Okabe et al., 1999). Inhibition of sulfate reduction and methane production by addition of Fe³⁺ has been reported by Lovley and Phillips (1987) and Van Bodegom et al. (2004). Recently, Krishnan and Loka Bharathi, (2009) have shown that iron modulates even nitrification rates in these mangrove sediments.

The quantitative relevance of the different physical, chemical and biological factors (eg. frequency and duration of inundation, freshwater input, seasonality of precipitation and temperature, storms, bioturbation, etc) make these environments very complex in terms of their geochemistry (Hines, 1991; Luther et al., 1991; Otero and Macias, 2002). Factors controlling microbial sulfate reduction and their spatial and temporal dynamics in intertidal sediments may also depend on site specific factors which are still not fully understood (Hubas et al., 2006). The results obtained in one region therefore cannot be extrapolated to other areas (Kristensen et al., 1988, 1991, 1992). Hence, it is necessary to carry out detailed studies on the spatial and temporal patterns of geomicrobial processes in the mangrove sediments (Kristensen et al., 1992).

Sporadic attempts have been made to quantify SRR in tropical mangroves (Kristensen et al., 1988. 1992, 2000. Alongi et al., 2000. 2001. 2004) but seasonal monitoring is rarely reported. Even though some cyclic studies have been outlined in temperate intertidal surface sediment (Al-Raei et al., 2009) and salt pans (Kerkar and Loka Bharathi., 2007), lack of seasonal statistics in mangroves has hampered our detailed research on these prolific coastal marine ecosystems. This is unfortunate because such information is needed to ameliorate uncertainty in SRR particularly in the intertidal habitats that may be greatly affected by changes in sea level as a result of climate change (Alongi et al., 2001). Sanders et al., (2010) have also stated that mangrove ecosystems would respond to such specific changes in climate.

The present study was undertaken to elucidate the factors controlling SRR coupled to organic mineralization as a function of time and space in mangrove sediments of the south west coast of India. It tests the hypothesis that ambient iron concentrations regulate SRR. The test site at Diwar mangrove ecosystem is influenced by iron released by the movement of barges carrying iron ore during the non-monsoon seasons and the reference site at Tuvem is relatively free from such influence.

2. Materials and methods

2.1. Site description

Sediment samples were collected from Tuvem and Diwar mangroves. The sampling site at Tuvem (15⁰ 38.28'N and 73⁰ 47.71'E) is on the Chapora estuary and that at Diwar (15⁰ 30.42' N, 73⁰52.28'E) on the Mandovi estuary. The Diwar mangroves are separated from the main land by the river Mandovi. The Mandovi river is heavily used for transportation of iron ore from mines located upstream. Besides, the iron ore beneficiation plants situated on the river bank, discharge effluents directly into the estuary. However, Tuvem is considerably free of such activity. Both the sites are free from human habitation and agricultural practices. Similar mangroves such as *Rhizophora* sp., *Sonneratia* sp., *Avicennia* sp. and *Excoecaria* sp. vegetate both the sites. Though the drainage is more at Diwar at 3,580 million cubic meters than at Tuvem at 588 million cubic meters, the basin area more at Diwar at 1,580 sq. kms than at Tuvem at 255 sq. kms, the ratio of basin area to drainage is almost equal at both the sites~ 2.3. (www.goenvvis.nic.in).

[Fig. 1]

2.2. Sampling methods

Seasonal sampling was carried out in October to January, representing the post monsoon season, February to May, the pre-monsoon and June to September, the monsoon season. Sediment samples were collected in triplicates during the low tide using PVC hand held corers of 15cm length and 3.68 cms diameter. Subsequent to sample collection, the corers were sealed at both the ends with sterile core caps to prevent contact with air and transported to the laboratory in an ice box and analyzed within 1 hr of collection. Eh and pH were measured immediately in triplicate at every 2 cm interval with one set of core as per the instructions given by the manufacturer (Thermo Orion model 420A, USA). Another set were subsequently sectioned at 2 cm intervals for all analysis. Sediment density was determined from the weight and volume of wet sediment sample. Total organic carbon (TOC) was determined in triplicate by using Allen et al., (1976). In brief, an amount of 0.5gm of dried (60°C) sediment in a 500 ml flask, 10 ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ and 20 ml of acid mixture (2.5 gm of Ag_2SO_4 in Conc. H_2SO_4) was added and shaken for 1 minute and allowed to stand for 30 minutes. About 200 ml distilled water, 10 ml of 85% H_3PO_4 , 0.2gm of NAF and 0.5ml diphenylamine indicator were added and titrated with Mohr's salt till end point with glucose as standard.

The moisture content was estimated as loss in weight of wet sediment after drying at 80°C for 24 hrs. The dried sediment was used for determination of C:N ratio with a CHN analyzer (Thermo Finningan, Flash EA1112). Sub samples for metal analysis were dried at $60^{\circ}\text{C}(\pm 2)$ for 48 hrs and disaggregated in an agate mortar before chemical treatment for iron analysis. The detailed procedure for sediment digestion was carried out as in Balaram et al. (1995). In brief, for each sample, a known quantity (0.2g) of sediment was digested in a Teflon vessel with a solution (10 ml) of concentrated HF, HNO_3 , and HClO_4 in ratio of 7:3:1. The digested sediment was then dried on a hot plate in a fume hood chamber. The procedure was repeated with 5ml of acid mixture. Further, 2 ml of concentrated HCl was added followed by 10 ml of HNO_3 . This residue was then warmed and transferred to a clean, dry flask and the final volume of 50 ml was made up with double distilled water. Iron concentrations in this digest were measured using a flame

atomic absorption spectrophotometer (PerkinElmer, Model 5000). All the acids used, were procured from Merck.

2.3. Pore water analysis: sulfide, sulfate and salinity

Sediment from the cores were extruded at 2cm intervals and centrifuged at 5000 rpm for 10 minutes at 4°C for collecting pore water. The supernatant was carefully drawn with a 5 ml syringe for the analysis of salinity, sulfate and sulfide. For sulfide, samples (0.5 ml) were fixed in 10 ml of zinc acetate (2% wt/vol) and later analyzed spectrophotometrically (Cline, 1969). For sulfate analysis, samples (0.5ml) were fixed in a vial containing concentrated HCl (0.01ml) and the parameter was determined turbidometrically by BaSO₄ precipitation (Cleseri et al., 1998). Salinity was measured using a hand refractometer (S/MILLE, ATAGO, Co. Ltd, Japan).

2.4. Sulfate reduction rates

Sulfate reduction rates were measured in duplicate using King's assay (2001). Mini-cores (2ml) with 1 cm diameter from the above intact cores were processed corresponding to the depths (0-2, 2-4, 4-6, 6-8 and 8-10) using a cut-off syringe. Radioactive sodium sulfate 10 µl (³⁵SO₄²⁻, specific activity, 74KBq from BARC, Mumbai) was injected into the section to distribute the label evenly and the activity was arrested at the end of 8 hours by adding 5ml (5% wt/vol) zinc acetate and frozen at -20⁰ C till further analysis. Radiotracer assay in the sediment was carried out using a single step chromium reduction assay (King, 2001). Radioactivity was measured with a liquid scintillation counter (Perkin Elmer Wallace 1409 DSA) and sulfate reduction rates (SRR) were calculated using the equation:

$$SRR = (H_2^{35}S / ^{35}SO_4^{2-}) \times ^{32}SO_4^{-2} \times IDF / T$$

Where SRR =Sulfate reduction rates, H₂³⁵S=radioactivity of reduced sulfur in DPM, ³⁵SO₄⁻² = radioactivity of sulfate at the beginning of incubation, ³²SO₄⁻²= pore water sulfate concentration in µM SO₄⁻²cm⁻³, IDF (Isotopic discrimination factor) = 1.06, T = time of incubation in hours.

2.5. Effect of iron on sulfate reduction rates: slurry experiment

Slurry experiments were carried out with Tuvem sediments. Such experiments could be conducted only with reference site where background concentration of iron was low at 3.66%. Aliquots of sections were prepared as mentioned before. Approximately 2ml of this aliquots sediment were put into 5 ml vials in triplicate and then amended with 2ml of water soluble ferric chloride stock solutions to give final concentrations of 50, 100, 200, 500 and 1000 ppm. Controls without amendments were also included. All the vials were flushed with oxygen free nitrogen gas. The resulting sample slurries were acclimatized for 1 hr and supplemented with 10 μ l of radioactive sodium sulfate ($^{35}\text{SO}_4^{2-}$, specific activity, 74KBq) and incubated at room temperature for 8 hrs. Samples were then fixed and analyzed using King's assay as described earlier.

2.6. Statistical analysis

Statistical analyses have been carried out using Statistica 6.0 software. One way ANOVA was used to test for difference between factors time, site and depth. Pearson's correlation coefficient and linear regression analysis were used to establish the relation among different parameters. Parametric statistics have been used and normality and homogeneity of variances have been tested using one way Kolmogorov-Smirnov (K-S) test.

3. Results

3.1. Physico-chemical properties of sediments- Eh, pH and water content

The redox conditions were more negative at Tuvem during all the three season. Eh values decreased significantly with depth in all studied profiles. Max values 219.6 \pm 131 and 187.7 \pm 117 mV were observed during the pre-monsoon season at Diwar and Tuvem respectively (Table 1). Upper sediment layer (0-2, 2-4 cm) was relatively oxic (Eh >145 mV) at both the sites. Minimum (-100 \pm 160 mV) redox potential was observed at Tuvem during post monsoon season at 8-10 cm depth. The pH values varied from 6.7 to 7.3 at both the site.

[Table 1]

At Diwar, the sediment water content varied from 32-42 % during non- monsoon seasons and 50-54% during monsoon season. However, at Tuvem an opposite trend was observed. The water content was high during pre-monsoon and post monsoon seasons ~ 50% with only ~ 41% during monsoon season (Table 1).

3.2. Pore water chemistry- salinity, sulfate and sulfide

The salinity at Diwar varied from 6.25(\pm 2.99) to 19.25(\pm 2.99) and at Tuvem from 6.00(\pm 1.41) to 16.50(\pm 2.89) (Table 1). The sulfate concentrations for two stations varied significantly (ANOVA, $F=9.15$, $p<0.01$, $df=1$). The average sulfate concentration at Diwar was 9.55 mM and maximum values 19.50 mM were observed during pre-monsoon. At Tuvem, average sulfate concentration was 7.38 mM with a maximum value 14.41 mM during pre-monsoon (Fig. 2). At both sites, seasonal variability in sulfate concentrations were significant [ANOVA, (Diwar, $F=75.75$, $p<0.001$, $df=2$) and Tuvem (ANOVA, $F=31.36$, $p<0.001$, $df=2$)]. Down core variability was insignificant except during the post-monsoon (ANOVA, $F=3.3$, $p<0.05$, $df=4$) at Diwar. Between the two sites the concentration of this parameter showed a significant variation (ANOVA, $F=9.14$, $p<0.01$, $df=1$).

The sulfide concentration for two stations varied significantly (ANOVA, $F=316.33$, $p<0.001$, $df=1$). At Diwar, average sulfide concentration was 0.073 mM. In contrast at Tuvem, sulfide values were higher during all three seasons and average values were 1.17 mM. (Fig. 2).

[Fig. 2]

3.3. Total organic carbon (TOC) of sediments

Lowest values of 2.5% (\pm 1.25) and 1.48% (\pm 0.73) were recorded during the monsoon season at Tuvem and post-monsoon season at Diwar respectively. Highest accumulation of TOC was observed at Tuvem during the pre-monsoon season at 3.64% (\pm 0.97) and in the monsoon season at Diwar with values of 3.28% (\pm 0.79) (Fig. 3). At both the sites, down core variability in TOC was insignificant [ANOVA, Diwar, $F=0.09$, $p>0.05$, $df=4$) and Tuvem ($F=1.72$, $p>0.05$, $df=4$)]. The seasonal variability in TOC at Diwar (ANOVA, $F=38.58$, $p<0.001$, $df=2$) and Tuvem (ANOVA, $F=6.71$, $p<0.01$, $df=2$) was significant.

3.4. C:N ratio

At Diwar a low C:N ratio 3.83 was observed during pre-monsoon season. The monsoon values were 13.86 irrespective of depth (Fig. 4). During the post monsoon, values of 7.20 were observed and the seasonal variability in C:N ratio was significant (ANOVA, $p < 0.01$, $df = 2$). At Tuvem, C:N values were 10.71, 10.26 and 12.82 during the pre-monsoon, post monsoon and monsoon season respectively.

[Fig.3]

[Fig.4]

3.5. Iron

Average values of iron at Tuvem ranged from 5.03% to 7.03% for the entire study period. There was neither inter-monthly nor inter-seasonal down core variability at Tuvem. In contrast, high, inter seasonal variability was observed at Diwar (ANOVA, $F = 160.56$, $p < 0.001$, $df = 2$). During the pre-monsoon season, the highest accumulation of Fe ($26.93\% \pm 2.58$) was noticed, followed by post monsoon ($16.06\% \pm 3.11$) and monsoon season ($10.08\% \pm 6.04$) respectively. The variability in iron concentrations between the two sites were significant (ANOVA, $F = 120.22$, $p < 0.001$, $df = 1$).

3.6. Sulfate reduction rate (SRR)

Variability in SRR between the two sites was significant for the whole year (ANOVA, $F = 23.06$, $p < 0.001$, $df = 1$). SRRs at Diwar were higher during the monsoon than non monsoon seasons (ANOVA, $F = 87.26$, $p < 0.001$, $df = 1$). Rates were $75.46(\pm 29.46)$, $216.14(\pm 47.06)$ and $123.78(\pm 26.68)$ $\text{nM cm}^{-3} \text{d}^{-1}$ during pre-monsoon, monsoon and post monsoon respectively. At Tuvem the highest activity of $295.9(\pm 177.50)$ $\text{nM cm}^{-3} \text{d}^{-1}$ was observed during post monsoon and comparable values of $289.09(\pm 173.43)$ $\text{nM cm}^{-3} \text{d}^{-1}$ were noted during the pre-monsoon season. Down core variability was insignificant at both the site. Seasonal variability was noteworthy at Diwar (ANOVA, $F = 87.26$, $p < 0.001$, $df = 2$) and Tuvem (ANOVA, $F = 5.30$, $p < 0.01$, $df = 2$).

The depth integrated SRR (Σ SRR, 0-10 cm) (Fig. 5) showed that at Diwar, the monsoon rates were ca.3 times higher than pre-monsoon and two times higher than the post monsoon season. At Tuvem during the monsoon season, the integrated SRR values were approximately half of the pre-monsoon and post monsoon season.

[Fig.5]

Laboratory experiments

3.7. Effect of iron concentration on the SRR using sediment slurry

Average SRR decreased with increasing iron concentrations. A 14% increase in SRR was observed with 50 ppm amendment of iron to an ambient iron concentration of 3.66%. Further increase in concentration lead to a gradual decrease in SRR with a 25% decrease with 100 ppm and finally a 93% decrease at with 1000 ppm of iron concentrations (Fig. 6).

[Fig.6]

3.9. Interaction between microbial and environmental parameters

All variables have normal distribution at both the sites as checked by one way Kolmogorov-Smirnov (K-S) test. At Diwar, SRR showed a negative correlation with Fe ($r^2=0.578$, $y=-6.40X+253.0$, $p<0.001$, $n=60$) and sulfate ($r^2=0.592$, $y=-11.02X+245.51$, $p<0.001$, $n=60$) and a positive correlation with TOC ($r^2=0.378$, $y=38.40X+56.81$, $p<0.001$, $n=60$), water content ($r^2=0.407$, $y=392.81X-29.03$, $p<0.001$, $n=60$) and sulfide ($r^2=0.227$, $y=834.05X+77.45$, $p<0.001$, $n=60$).

At Tuvem, SRR showed a positive correlation with sulfate ($r^2=0.248$, $y=26.51X+51.83$, $p<0.001$, $n=60$), TOC ($r^2=0.100$, $y=45.54X+102.78$, $p=0.014$, $n=60$) and water content ($r^2=0.180$, $y=6.228X-45.92$, $p=0.001$, $n=60$).

4. Discussion

Sulfate reduction is an important mineralization process in organic rich coastal sediments. Seasonal monitoring in the mangrove swamps, could improve our understanding of these

productive coastal marine ecosystems which are vulnerable to human impacts. Integrated SRR measured in the present study in the mangrove of Diwar and Tuvem in Goa range from 6.28 to 63.4 mM m⁻² d⁻¹ which are comparable to SRR reported by Alongi et al., (2004) in the Malaysian mangroves and the range of 0.002–65.75 mM m⁻² d⁻¹ by Kerkar and Loka Bharathi (2007) in salt pans of Goa at Ribandar. The present rates are slightly higher than those reported by Kristensen et al. (1992) in the mangrove sediments of Pukhet Thailand and lower than the salt marshes in Germany (Al-Raei et al., 2009 ; Schubert et al., 2000). The SRR in the present study are lower than those reported by Alongi et al., (2005) at 19-281 mM m⁻² d⁻¹ in mangrove sediments of China (Table 2).

[Table. 2]

Sulfate reducing bacteria (SRB) and SRR are highly influenced by environmental parameters notably the ambient organic carbon and sulfate content. The activity greatly reduces the Eh to the negative potential and increases the pH of the sediments. While Eh generally increases with depth, pH does not have a pattern.

Another important factor that affects the process is the water content of the sediments as it helps in transportation of nutrients. A positive correlation ($p < 0.001$, $n = 60$) between SRR and water content at both the sites were observed, suggesting the strong role of this factor in influencing this process. Such influence has also been observed by Jones et al., (1989), Musslewhite et al., (2007) in deep terrestrial subsurface sediments.

Equally important in this study has been the influence of sulfate ion. Though sulfate occurs at a concentration of 28 mM in the sea water and is not known to limit SRR in marine and estuarine sediments, we have noticed that it has negative influence on SRR at Diwar. About 63% of the variation in SRR is apparently negatively correlated with sulfate concentration ($r = -0.796$, $p < 0.001$, $n = 60$) (Fig. 7b). However, this influence may not directly be due to sulfate but to the iron that co-varies ($r = 0.867$, $p < 0.001$, $n = 60$) (Fig. 7a). This co-variation is more due to the anthropogenic influence of movement of iron barges that are active during the non monsoon seasons. On the contrary, sulfate has a positive influence on SRR at Tuvem suggesting that sulfate is limiting in this station ($r = 0.498$, $p < 0.001$, $n = 60$) (Fig. 7d). Lowest level of this parameter measured at Tuvem was 2.79 mM. Sulfate is known to limit SRR in limnetic

environments. For example, studies of SRR in sediments of Little Rock Lake established that SRR in the intact sediments is limited by sulfate rather than organic matter (Urban et al., 1994). Roychoudhury et al., (2006) also showed that increase in sulfate concentration (>15mM) in coastal aquifer, enhanced the SRR.

The higher concentration of sulfide in the pore water of Tuvem than Diwar is suggestive of the existence of a limited source of reactive Fe available for acid volatile sulfide and pyrite precipitation. Previous studies have shown that in marine sediments the rapid reaction between H₂S and reactive form of Fe maintains low concentrations of dissolved sulfide in pore water (Canfield, 1989). In the present study the sulfide measured could have been underestimated. Certain amount of oxidation of dissolved sulfide during the centrifugation and analysis could not have been ruled out. Attempt has been made to compare our values with those measured by microelectrodes in salt marshes of Portugal by Sundby B., et al (2005). Their values ranged from non-detectable to 3µm. However, our lowest values were six times higher than the highest value reported by them and ranged from 18 µM to 2450 µM. Al-Raei et al., (2009) have reported H₂S values upto 25 mM in temperate intertidal sediments by following Cline method.

At Tuvem and Diwar there exists a positive relationship between SRR and TOC ($r=0.319$, $r^2=0.378$, $y=38.40X+56.81$, $p<0.05$, $n=60$ and $r=0.615$, $r^2=0.10$, $y=45.54X+102.78$, $p<0.001$, $n=60$ respectively), suggesting TOC as a limiting factor. Surprisingly, at Diwar, a negative correlation between SRR and TOC ($r= -0.545$, $p<0.01$, $n=20$) was evident during pre-monsoon. This relationship is probably indirectly due to iron which is known to inhibit SRR at higher concentrations.

SRR could also be influenced by C:N ratio. As an initial characterization of the organic material C:N ratio was measured. In general, low C:N values of 5-7 are characteristics for marine organic material (Redfield et al., 1963). Values higher than 20 indicate a terrestrial source of organic matter (Scheffer and Schachtschabel, 1984). At Diwar, a high average C:N ratio signature (13.85) of sediments, points to a relatively higher terrestrial source of organic matter during the monsoon season, which is considered to be less labile. However, high SRR of 216.14 nM cm⁻³ d⁻¹ during this season, suggest that lability of organic matter plays relatively little role in dictating the SRR in this system. The average C:N molar ratio during the pre-monsoon (3.82) and post

monsoon (7.20) are much lower. Hence, the organic carbon is comparatively more labile, compared to the monsoon season. However, the average SRR during the pre-monsoon ($75.46 \text{ nM cm}^{-3} \text{ d}^{-1}$) and post monsoon ($123.78 \text{ nM cm}^{-3} \text{ d}^{-1}$) are considerably lower than those measured during monsoon. Lower SRR in spite of high lability of substrate could be due to the inhibitory effect of excess iron in the system.

Iron plays both direct and indirect role in the modulation of SRR. Our results showed that during the pre-monsoon season at Diwar, the average concentration of iron was 2.67 times higher at 26.94% than the at 10.08% during monsoon season. The average pre-monsoon SRR at $75.46 \text{ nM cm}^{-3} \text{ d}^{-1}$ was 0.35 times the monsoon rates at $216.14 \text{ nM cm}^{-3} \text{ d}^{-1}$. During the post monsoon, the rates ($123.78 \text{ nM cm}^{-3} \text{ d}^{-1}$) were 0.57 times the monsoon season. Pearson's correlation coefficient between the total iron concentration and SRR ($r = -0.761$, $p < 0.001$, $n = 60$) (Fig. 7a) showed that the total iron concentration inhibited the SRR in the mangrove sediments at Diwar, while at Tuvem iron played an insignificant role. (Fig.7c). Few studies have covered the impact of iron enrichment and subsequently, the effect of these iron concentrations on the sulfate reduction rates. The inhibitory effects of iron (Fe^{+2}) at 8.5 mM concentrations on the SRR of an anaerobic sludge reactor has been reported by Gonzalez-Silva et al., (2009). Iron additions ($0.7 \text{ mol Fe m}^{-2}$) to organic enriched sediments and organic poor sediments in Mediterranean sea grass meadows demonstrated that iron addition suppresses the SRR at organic rich sites (Holmer et al., 2005). The suppressed sulfate reduction was due to a shift in bacterial metabolism to microbial iron reduction. Impact of Fe^{3+} addition in biofilms in sewers was put forth by Zhang et al., (2009), showing inhibitory activity upto 39 to 60% in sulfate reducing bacteria. Lovley and Phillips, (1987), revealed that SRR in the sediment was reduced by 86-100% through the addition of ferric oxy-hydroxide. Inhibitory effect of metallic ions on pure strains and mixed culture of SRB was also put forth by Utgikar et al., (2001; 2002). Sulfate reduction could be suppressed due to preference for iron reduction over SRR (Thamdrup, 2000). Alternatively iron could directly inhibit SRR (Gonzalez- Silva et al., 2009).

[Fig.7]

In the present study, slurry experiments with sediments elucidate some of the aspects of iron inhibition of SRR. Slurry experiments have generally been criticized for not truly representing

in-situ condition due to change in solid to solution ratio affecting substrate transport. Possible change in microbial consortia and buildup reaction by-products over time may impede reaction rates or pathways (Roychoudhury et al., 1998). Nevertheless, in the present study the effect of iron (Fe^{+3}) on the SRR of mangrove sediments of Tuvem was carried out in the laboratory to complement field observations. The background concentration of total iron in sediment used for slurry experiment was 3.66%. Supplementing with 50 ppm of Fe^{+3} had a stimulatory effect (14%) on the SRR. However, increasing Fe^{+3} to 100 ppm and above, reduced SRR. Further increase in iron concentrations up to 1000 ppm brought about a decrease in SRR up to 93% (Fig. 6). These results show that Fe^{3+} had a strong inhibitory effect on the SRR at concentrations above 100 ppm in mangrove sediments.

The possible mechanism for inhibition of SRA in mangrove sediments by iron may be attributed to the presence of metal sulfide precipitates in the system. Active SRB culture produce bisulfide (HS^-) that react with the dissolved metal and form insoluble metal sulfide. The fine metal (including iron) sulfide precipitate could concentrate in the vicinity of SRB and effectively blanket the cells and may reduce the access to reactants like sulfate and volatile fatty acids to the necessary enzymes (Zhang et al., 2009). The other possibility could be that heavy metal could deactivate the enzyme of SRB by reacting with their functional groups. It could denature the protein of microorganisms and compete with the essential compounds utilized by SRB. (Mazidji et al. 1992; Atlas et al. 1998).

Though the foregoing studies clearly bring out the negative influence of high iron concentrations in sediment on SRA in the mangrove swamps of Goa, the results could be applicable to any other sedimentary systems under the influence of this element particularly the mangrove ecosystems from other tropical regions. It is probable that other metals could also influence the activity to varying degrees. Such studies would give further insights into stimulatory and inhibitory effect of ambient metal concentrations on SRR.

5. Conclusion

Our results conclude that in Diwar, the concentration of iron has a control over SRR. SRR oscillated seasonally with a change in the total iron concentrations at Diwar. Both the field and laboratory experiments deduce that iron had an inhibitory effect on SRR. Concentration of Fe in

Diwar sediment was more inhibitory than stimulatory to this process. However, at Tuvem, the factors controlling this activity were sulfate concentrations and TOC. Even though iron was the key controlling factor for SRR at Diwar, the sulfate concentration, water content and total organic carbon, may have synergistically played a role in controlling this process at both the sites. The upstream ferromanganese transport is apparently the main contributing source of excess of Fe to the Mandovi estuary, thus possibly affecting the biogeochemical process of carbon mineralization in these mangrove swamps. These finding could have implications on the flux of greenhouse gases like CO₂ from the mangrove sediments.

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7. References

- Allen, S. E., Grimshaw H. M., Parkinson J. A., Quarmby C., Roberts J. D., 1976. Chemical analysis in methods in plant ecology. Chapman SB, Editor. Oxford; Blackwell Scientific Publications, chapter 13, pp 411–466.
- Al-Raei, A.M., Bosselmann, K., Bottcher, M.E., Hespeneide, B., Tauber, F., 2009. Seasonal dynamics of microbial sulfate reduction in temperate intertidal surface sediments: controls by temperature and organic matter. *Ocean Dynamics* 59, 351–370.
- Alongi, D.M., Pfitzner, J., Trott, L.A., Tirendi, F., Dixon, P., Klumpp, D.W., 2005. Rapid sediment accumulation and microbial mineralization in forests of the mangrove *Kandelia candel* in the Jiulongjiang Estuary, China. *Estuarine, Coastal and Shelf Science* 63, 605–618.
- Alongi, D.M., Sasekumar, A., Chong, V.C., Pfitzner, J., Trott, L.A., Tirendi, F., Dixon, P., Brunskill, G. J., 2004. Sediment accumulation and organic material flux in a managed mangrove ecosystem: estimates of land-ocean-atmosphere exchange in peninsular Malaysia. *Marine Geology* 208, 383–402.
- Alongi, D.M., Wattayakorn, G., Pfitzner, J., Trott, L.A., Tirendi, F., Zajorskis, I., Brunskill, G.J., Davidson, A., Clough, B.F., 2001. Organic carbon accumulation and metabolic pathways in sediments of mangrove forests in southern Thailand. *Marine Geology* 179, 85–103.
- Alongi, D.M., Tirendi, F., Trott, L.A., Xuan, T.T., 2000. Benthic decomposition rates and pathways in plantations of mangrove *Rhizophora apiculata* in the Mekong delta, Vietnam. *Marine Ecology Progress Series* 194, 87–101.

- Atlas, R.M., Bartha, R., 1998. *Microbial Ecology: Fundamentals and Applications*, fourth ed. Benjamin/Cummings, Menlo Park, California.
- Balaram, V., Ramesh, S.L., Anjaiah, K.V., 1995. Comparative study of the sample decomposition procedures in the determination of trace and rare earth elements in anorthosites and related rocks by ICP-MS. *Fresenius Journal of Analytical Chemistry* 353, 176–182.
- Brandt, K.K., Vester, F., Jensen, A.N., Ingvorsen, K., 2001. Sulfate Reduction Dynamics and Enumeration of Sulfate-Reducing Bacteria in Hypersaline Sediments of the Great Salt Lake (Utah, USA). *Microbial Ecology* 41, 1–11.
- Canfield, D.E., 1989. Reactive iron in marine sediments. *Geochimica Cosmochimica Acta* 53, 619–632.
- Canfield, D.E., 1993. Organic matter oxidation in marine sediments. In: Wollast, R., Mackenzie, F.T., Chou, L. (Eds.), *Interactions of C, N, P and S Biogeochemical Cycles and Global Change*. Springer, Berlin, pp. 333–363.
- Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard methods for the examination of waste and wastewater. 20th edition, American Public Health Association . Handbook of water and waste water treatment plant operations. Virginia, 119–126.
- Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* 14, 454–458.
- Connell, W.E., Patrick Jr, W.H., 1968. Sulfate reduction in soil: effects of redox potential and pH. *Science* 159, 86–87.
- Gonzalez-Silva, B.M., Briones-Gallardo, R., Razo-Flores, E., Celis, L.B., 2009. Inhibition of sulfate reduction by iron, cadmium and sulfide in granular sludge. *Journal of Hazardous Material* 172, 400–407.
- Hamilton, W.A., 1998. Sulfate-reducing bacteria: Physiology determines their environmental impact. *Geomicrobiology Journal* 15, 19–28.
- Hines, M.E., 1991. The role of certain infauna and vascular plants in the mediation of redox reactions in marine sediments. (Eds.), *Diversity of environmental biogeochemistry*. Elsevier, Amsterdam, pp. 275–285.
- Holmer, M., Duarte, C.M., Marba, N., 2005. Iron additions reduce sulfate reduction rates and improve seagrass growth on organic-enriched carbonate sediments. *Ecosystems* 8, 721–730.
- Hubas, C., Davoult, D., Cariou, T., Artigas, L.F., 2006. Factors controlling benthic metabolism during low tide along a granulometric gradient in an intertidal bay (Roscoff Aber Bay, France). *Marine Ecology-Progress Series* 316, 53–68.

- Hyun, J.H., Mok, J.S., Cho, H.Y., Kim, S.H., Lee, K.S., Kostka, J.E., 2009. Rapid organic matter mineralization coupled to iron cycling in intertidal mud flats of the Han River estuary, Yellow Sea. *Biogeochemistry* 92, 231–245.
- Jones, R.E., Beeman, R.E., Suflita, J.M., 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. *Geomicrobiology Journal* 7, 117–130.
- Jørgensen, B.B., 1977. Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. *Marine Biology* 41, 7–17.
- Jørgensen, B.B., 1982. Mineralization of organic matter in the sea bed—the role of sulphate reduction. *Nature* 296, 643–645.
- Kerkar, S., Loka Bharathi, P.A., 2007. Stimulation of sulfate-reducing activity at salt saturation in the salterns of Ribandar, Goa, India. *Geomicrobiology Journal* 24, 101–110.
- King, G.M., 2001. Radiotracer assays (^{35}S) of Sulfate Reduction Rates in Marine And Freshwater Sediments. *Methods in Microbiology* 30, 489–500.
- Kostka, J.E., Roychoudhury, A.N., Van Cappellen, P., 2002. Rates and controls of anaerobic microbial respiration across spatial and temporal gradients in saltmarsh sediments. *Biogeochemistry* 60, 49–76.
- Krishnan, K.P., Loka Bharathi, P.A., 2009. Organic carbon and iron modulate nitrification rates in mangrove swamps of Goa, south west coast of India. *Estuarine Coastal and Shelf Science* 84, 419–426.
- Kristensen, E., Andersen, F., Holmboe, N., Holmer, M., Thongtham, N., 2000. Carbon and nitrogen mineralization in sediments of the Bangrong mangrove area, Phuket, Thailand. *Aquatic Microbial Ecology* 22, 199–213.
- Kristensen, E., Andersen, F.O., Kofoed, L.H., 1988. Preliminary assessment of benthic community metabolism in a south-east Asian mangrove swamp. *Marine Ecology Progress Series* 48, 137–145.
- Kristensen, E., Devol, A.H., Ahmed, S.I., Saleem, M., 1992. Preliminary study of benthic metabolism and sulfate reduction in a mangrove swamp of the Indus Delta, Pakistan. *Marine Ecology Progress Series* 90, 287–297.
- Kristensen, E., Holmer, M., Bussarawit, N., 1991. Benthic metabolism and sulfate reduction in a southeast Asian mangrove swamp. *Marine Ecology Progress Series* 73, 93–103.
- Lovley, D.R., Phillips, E.J., 1987. Competitive Mechanisms for Inhibition of Sulfate Reduction and Methane Production in the Zone of Ferric Iron Reduction in Sediments. *Applied and Environmental Microbiology* 53, 2636–2641.

- Luther, G.W., Ferdelman, T.G., Kostka, J.E., Tsamakis, E.J., Church, T.M., 1991. Temporal and spatial variability of reduced sulfur species (pyrite, thiosulfate) and pore water parameters in salt marsh sediment. *Biogeochemistry* 14, 57–88.
- Mazidji, C.N., Koopman, B., Bitton, G., Neita, D., 1992. Distinction between heavy-metal and organic toxicity using edta chelation and microbial assays. *Environmental Toxicology and Water Quality* 7, 339–353.
- Meier, J., Costa, R., Smalla, K., Boehrer, B., Wendt-Potthoff, K., 2005. Temperature dependence of Fe (III) and sulfate reduction rates and its effect on growth and composition of bacterial enrichments from an acidic pit lake neutralization experiment. *Geobiology* 3, 261–274.
- Musslewhite, C.L., Swit, D., Glipen, J., McInerney, M.J., 2007. Spatial variability of sulfate reduction in shallow aquifer. *Environmental Microbiology* 9, 2810–2819.
- Okabe, S., Itoh, T., Satoh, H., Watanabe, Y., 1999. Analyses of spatial distributions of sulfate-reducing bacteria and their activity in aerobic wastewater biofilms. *Applied and Environmental Microbiology* 65, 5107–5116.
- Otero, X.I., Macias, F., 2002. Variation with depth and season in metal sulfides in salt marshes soils. *Biogeochemistry* 61, 247–268.
- Pallud, C., Van Cappellen, P., 2006. Kinetics of microbial sulfate reduction in estuarine sediments. *Geochimica Cosmochimica Acta* 70, 1148–1162.
- Redfield, A.C., Ketchum, B.H., Richards, FA, 1963. The influence of organisms on the composition of sea water. *The Sea* 2, 26–77.
- Roychoudhury, A.N., Viollier, E., Van Cappellen, P., 1998. A plug flow-through reactor for studying biogeochemical reactions in undisturbed aquatic sediments. *Applied Geochemistry* 13, 269–280.
- Roychoudhury, A.N., McCormick, D., 2006. Kinetics of sulfate reduction in a coastal aquifer contaminated with petroleum hydrocarbons. *Biogeochemistry* 81, 17–31.
- Scheffer, F., Schachtschabel, P., 1984. (11th Eds.). *Lehrbuch der Bodenkunde*. Enke Verlag, Stuttgart, 442 pp.
- Schubert, C.J., Ferdelman, T.G., Strotmann, B., 2000. Organic matter composition and sulfate reduction rates in sediments off Chile. *Organic Geochemistry* 31, 351–361.
- Skyring, G.W., 1987. Sulfate reduction in coastal ecosystems. *Geomicrobiology Journal* 5, 295–374.
- Sundby, B., Caetano, M., Vale, C., Gobell, C., Luther, G.W., Nuzzio, D.B., 2005. Root induced cycling of lead in salt marsh sediments. *Environmental Science and Technology* 39, 2080–2086.

- Thamdrup, B., 2000. Bacterial manganese and iron reduction in aquatic sediments. In: Schink, B., Eds. *Advances in microbial ecology* 16. Kluwer Academic/Plenum Publishers, New York, pp. 41–84.
- Urban, N.R., Brezonik, P.L., Baker, L.A., Sherman, L.A., 1994. Sulfate Reduction and Diffusion in Sediments of Little Rock Lake, Wisconsin, *Limnology and Oceanography* 39, 797–815.
- Utgikar, V.P., Chen, B.Y., Chaudhary, N., Tabak, H.H., Haines, J.R., Govind, R., 2001. Acute toxicity of heavy metals to acetate-utilizing mixed cultures of sulfate-reducing bacteria: EC100 and EC50. *Environmental Toxicology and Chemistry* 20, 2662–2669.
- Utgikar, V.P., Harmon, S.M., Chaudhary, N., Tabak, H.H., Govind, R., Haines, J.R., 2002. Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. *Environmental Toxicology* 17, 40–48.
- Van Bodegom, P.M., Scholten, J.C.M., Stams, A.J.M., 2004. Direct inhibition of methanogenesis by ferric iron. *FEMS Microbiology Ecology* 49, 261–268.
- Woodroffe, C. D., 1985. Studies of a mangrove basin. Tuff Crater, New Zealand: 111. The flux of organic and inorganic particulate matter *Estuarine Coastal and Shelf Science* 20, 447–461.
- Zhang, L., Keller, J., Yuan, Z., 2009. Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. *Water Research* 43, 4123–4132.
- www.goaenvis.nic.in (ENVIS Goa). ENVIS node under the project "Environmental information system". Set up by ministry of Environment and forest, government of India under its world bank assisted Environmental Management Capacity Building Technical Assistance Project (EMCBTAP). (last accessed on 15 July 2011).

Legend to figure

Fig.1. Map with sampling locations.

Fig.2. Seasonal down-core variation in S^{2-} and SO_4^{2-} concentration. Diwar (●); Tuvem (◆).

Fig.3. Seasonal down-core variation in TOC (%). Pre-monsoon (●); Monsoon (◆); Post-monsoon (▲).

Fig.4. Seasonal variation in C: N ratio at Diwar and Tuvem: Bars indicate the standard deviation.

Fig.5. Integrated sulfate reduction (Σ SRR) in upper 10 cm sediment core. Diwar (●); Tuvem (◆).

Fig.6. Effect of increase in iron concentration on SRR in slurry experiments. Bar indicates the standard deviation.

Fig.7. SRR v/s Fe and SO_4^{-2} at Diwar (a & b) and Tuvem (c & d). [Monthly average values were represented since significant down core variability was not significant]. Inset show the extent of relatedness between the parameter.

Fig.1.

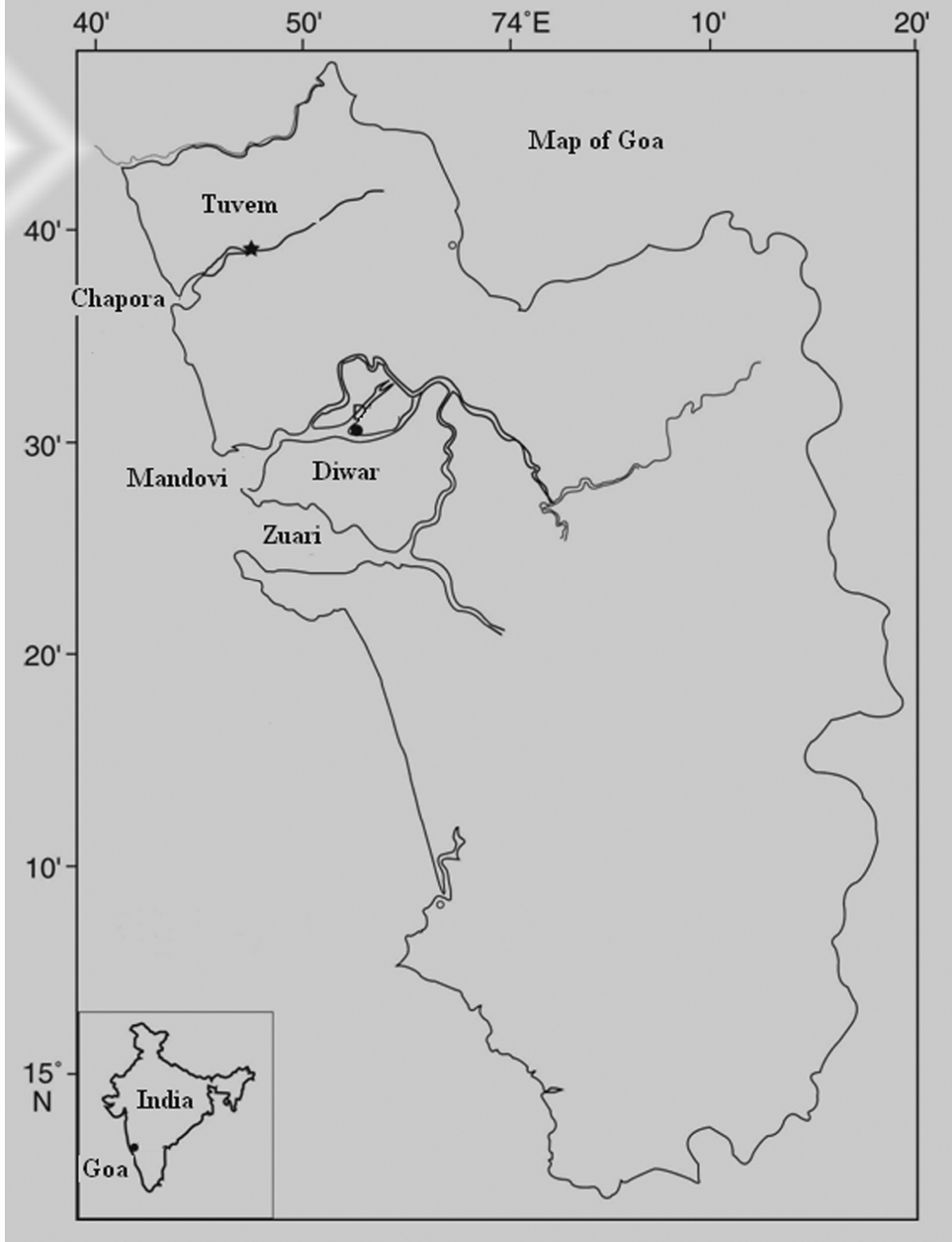


Fig.2

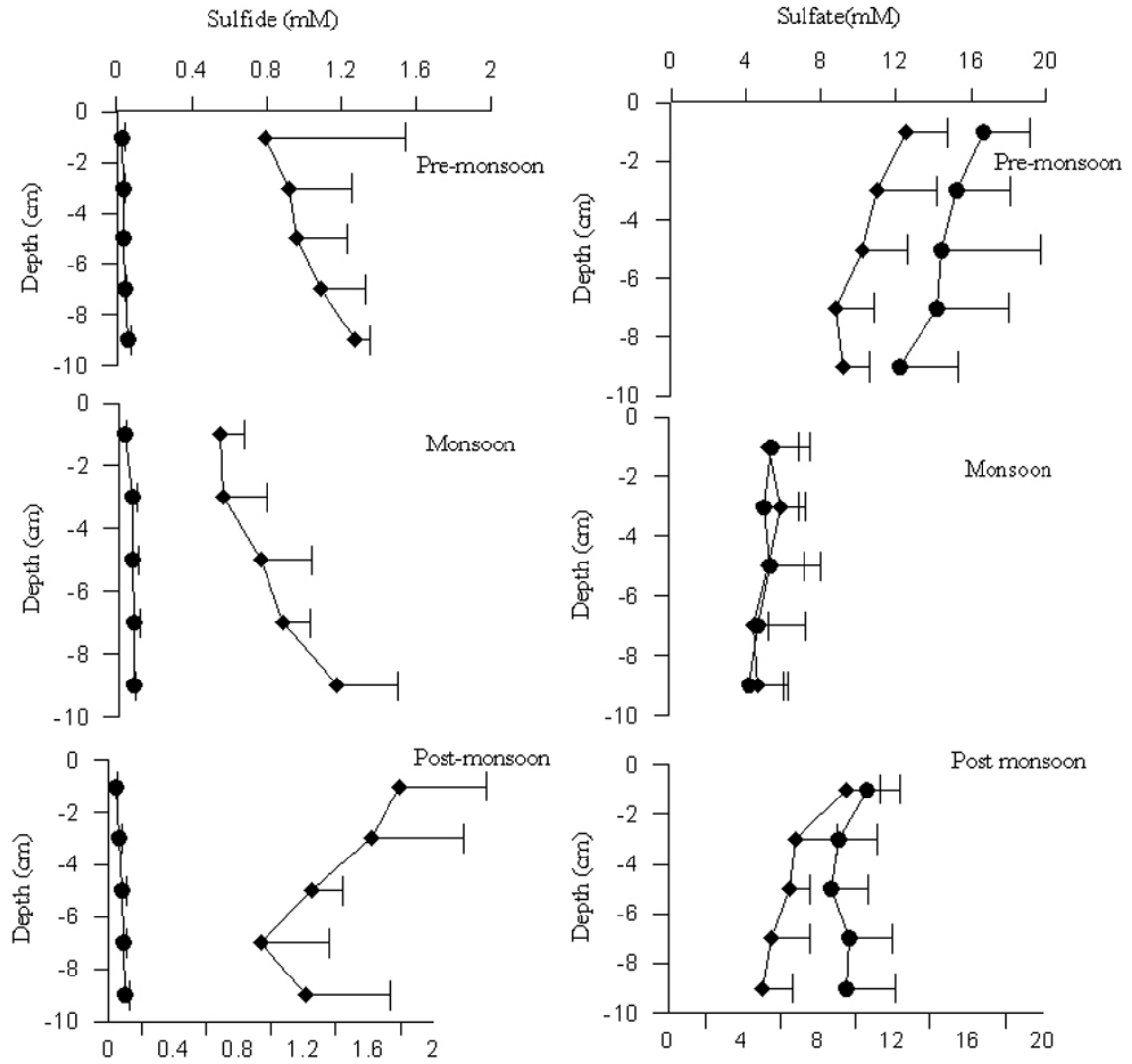


Fig.3.

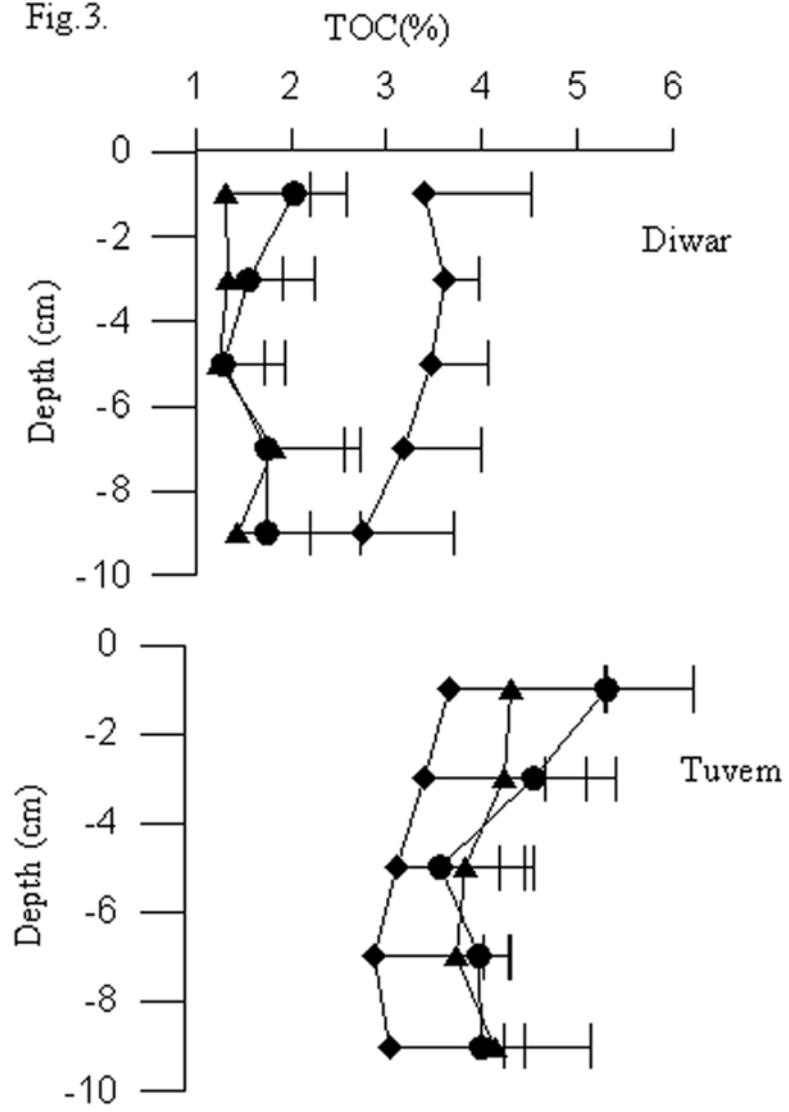


Fig.4.

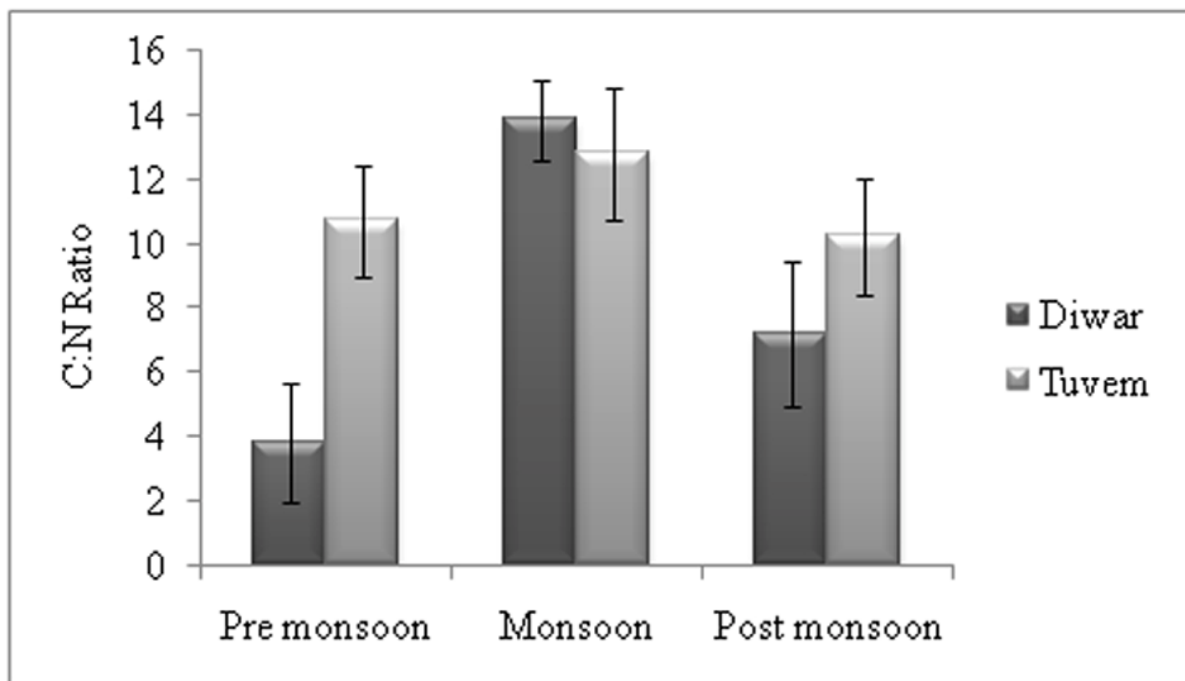


Fig.5.

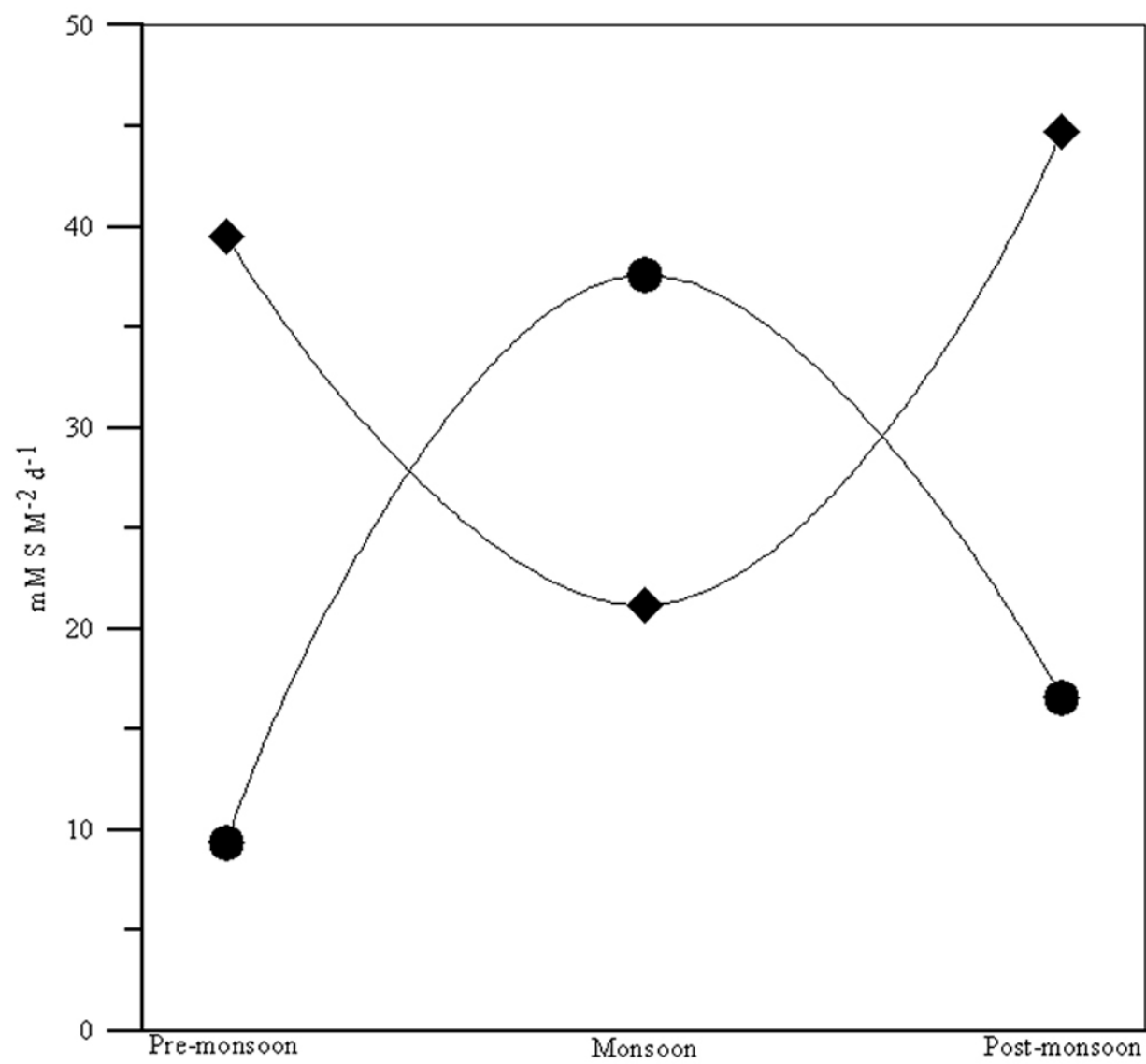


Fig.6.

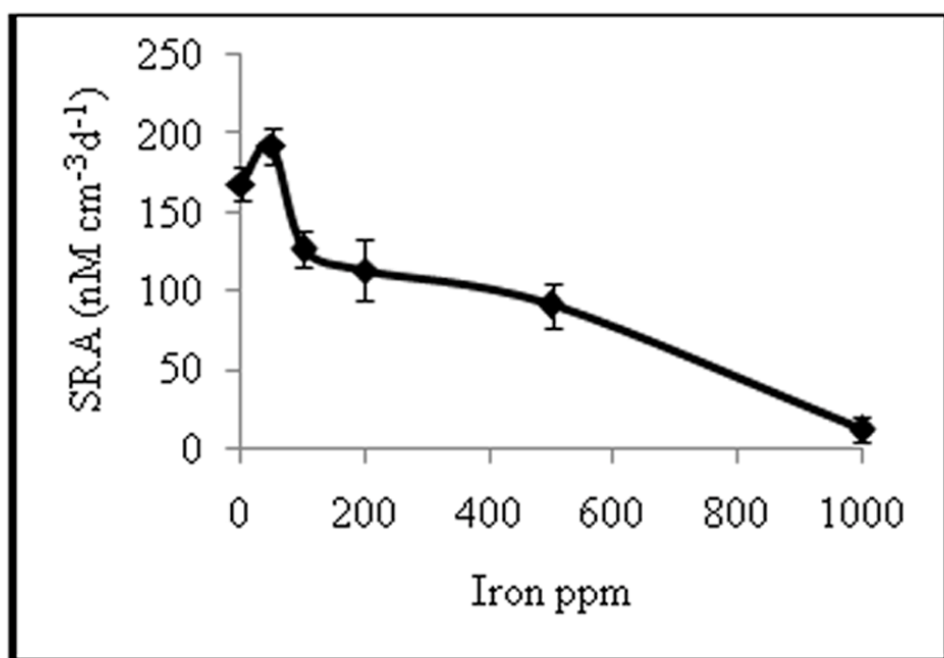


Fig. 7.

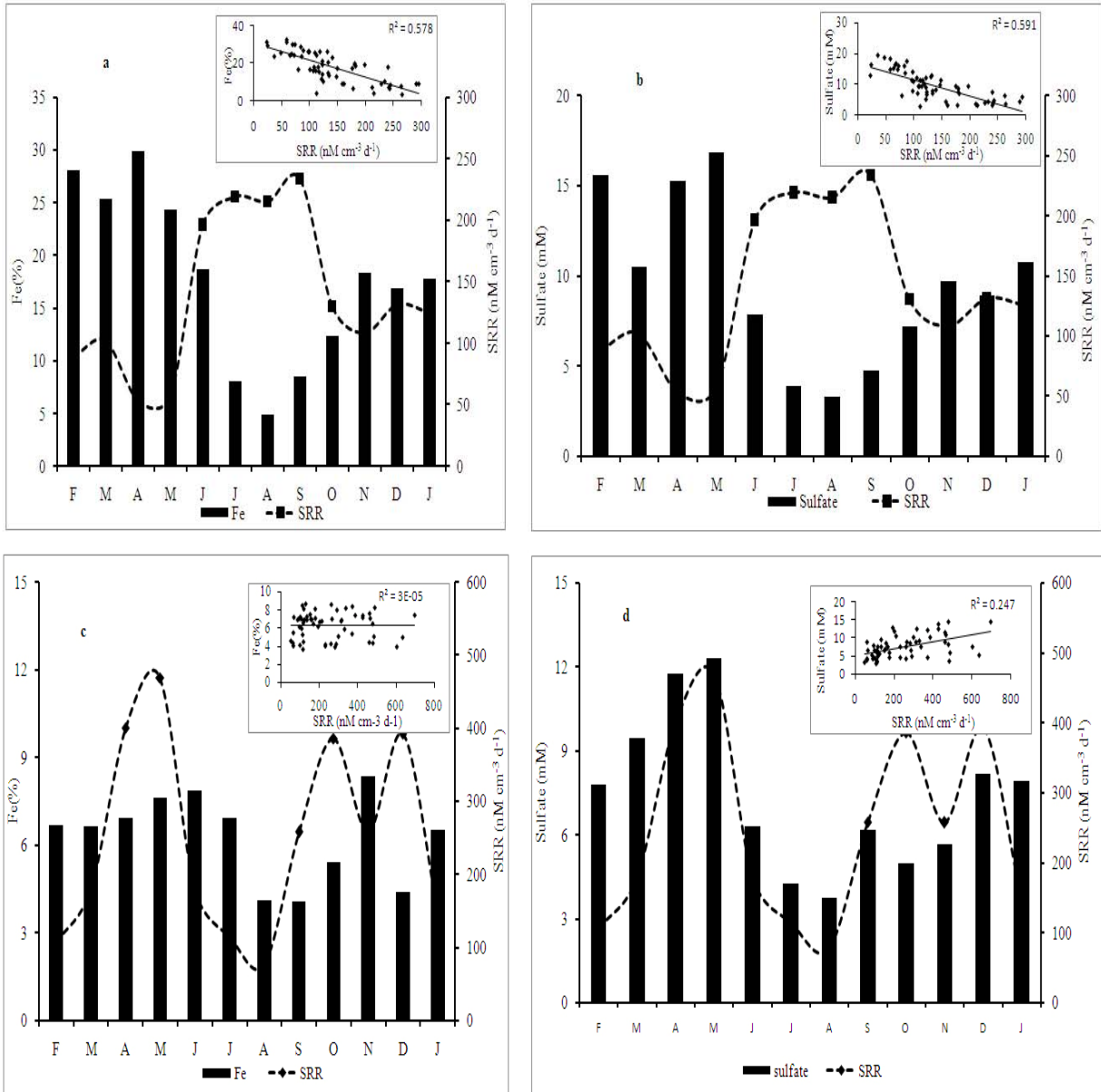


Table 1: Comparison of physico- chemical parameters between Diwar and Tuvem

Season	Depth (cm)	Eh (mV)		pH		water Content (%)		Salinity	
		D	T	D	T	D	T	D	T
Pre- monsoon	0-2	219.6 (131)*	187.5 (117)	7.1 (0.14)	6.95 (0.05)	39 (12)	55 (13)	19.25 (2.99)	16.50 (2.88)
	2-4	178.1 (106)	117.3 (81.80)	6.95 (0.1)	6.88 (0.09)	34 (03)	53 (8.5)	14.00 (2.16)	14.5 (3.32)
	4-6	83.43 (92)	8.8 (103)	6.8 (2.4)	6.82 (0.09)	32 (11)	45 (17)	15.25 (3.59)	13.75 (2.50)
	6-8	47.4 (63)	-30.3 (122)	6.72 (0.09)	6.80 (0.08)	37 (09)	48 (07)	16.00 (4.55)	10.75 (1.75)
	8-10	32.03 (46)	-71.28 (140.6)	6.8 (0.08)	6.80 (0.14)	40 (09)	49 (01)	11.5 (3.510)	10.75 (0.96)
.Monsoon	0-2	237.6 (29)	149.7 (98.8)	7.05 (0.06)	7.1 (0.06)	54 (06)	45 (14)	7.00 (1.41)	7.50 (0.58)
	2-4	156.4 (93)	119.8 (93.5)	7.03 (0.05)	7.0 (0)	55 (03)	44 (20)	7.25 (2.21)	7.50 (0.58)
	4-6	54.32 (46)	48.5 (61.29)	6.9 (0.05)	7.0 (0.06)	55 (03)	39 (12)	7.00 (3.36)	7.25 (2.22)
	6-8	-28.4 (38)	-2.4 (11.6)	7.0 (0.08)	7.0 (0.06)	52 (05)	37 (12)	6.75 (2.36)	6.25 (1.26)
	8-10	-58.7 (62)	-30.2 (20.65)	6.93 (0.05)	6.9 (0.10)	50 (07)	39 (11)	6.25 (2.99)	6.00 (1.41)
Post-monsoon	0-2	178.3 (118)	143.39 (117.4)	7.05 (0.1)	7.03 (0.05)	35 (14)	56 (11)	16.25 (2.5)	11.75 (2.06)
	2-4	184 (127)	123.85 (102.6)	6.95 (0.19)	7.03 (0.05)	36 (12)	54 (06)	12.75 (2.22)	9.50 (3.70)
	4-6	36.09 (38)	-26.85 (135.8)	6.85 (0.05)	6.9 (0.05)	37 (10)	48 (07)	12.00 (2.16)	9.50 (1.91)
	6-8	6.8 (25)	-68.93 (144)	6.8 (0.08)	6.8 (0.05)	42 (06)	48 (06)	11.00 (2.45)	8.00 (2.94)
	8-10	-21.2 (6.05)	-100.7 (160.3)	6.8 (0.08)	6.93 (0.05)	38 (06)	45 (11)	9.00 (1.83)	6.50 (1.30)

(D= DIWAR, T=TUVEM), *= ± sd. Standard deviation

Table. 2. SRA in the study compared with published rates.

S.NO	places	sulfate reduction rates	reference
1	Mangrove, China	19-281 mM M ⁻² d ⁻¹	Alongi, et al.,(2005)
2	Mangrove, Malaysia	19-53 mM M ⁻² d ⁻¹	Alongi, et al., (2004)
3	Mangrove, Southern Thailand	0.6-16.9 mM M ⁻² d ⁻¹	Alongi, et al., (2001)
4	Mangrove, Vietnam	0.2-13 mM M ⁻² d ⁻¹	Alongi et al., (2001)
5	Mangrove, Pakistan	2.5-16.1mM M ⁻² d ⁻¹	Kristensen, et al.,(1992)
6	Salt marshes, Germany	0.9-106 mM M ⁻² d ⁻¹	AL-Raie, et al., (2009)
7	Ribandier salt pan, Goa- India	0.002-66mM M ⁻² d ⁻¹	Kerkar, et al., (2007)
8	Sediment Off Chile, Germany	40-430 nM cm ⁻³ d ⁻¹	Carsten .J. et al., (2000)
9	Mangrove, Pukhet, Thailand	5-140 nM cm ⁻³ d ⁻¹	Kristensen, et al., (2000)
10	Intertidal mud flat, Korea	226-1516nM cm ⁻³ d ⁻¹	Hyun.j.et al., (2009)
11	Present study		Integrated values
	Diwar Pre-monsoon	23.32-131.94 nM cm ⁻³ d ⁻¹	6.2-13.6 mMM ⁻² d ⁻¹
	Monsoon	112.6-294.49 nM cm ⁻³ d ⁻¹	24.5-61.4mMM ⁻² d ⁻¹
	Post monsoon	79.22-147.31nM cm ⁻³ d ⁻¹	14.8-33.4 mMM ⁻² d ⁻¹
	Tuvem Pre-monsoon	62.55-698.66nM cm ⁻³ d ⁻¹	15.5- 63.4 mMM ⁻² d ⁻¹
	Monsoon	50.21-338.14 nM cm ⁻³ d ⁻¹	10.43-34.0 mMM ⁻² d ⁻¹
	Post monsoon	99.88-635.86 nM cm ⁻³ d ⁻¹	20.41-61.2mMM ⁻² d ⁻¹