

**Sulfate reducing bacteria and their activity in
mangrove swamps of Goa**

A Thesis Submitted to Goa University for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

BIOTECHNOLOGY

**By
Kuldeep Attri**

**Goa University,
Taleigao Goa
2012**

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Kuldeep Attri

Research Guide

Dr. Savita Kerkar

Goa University,
Taleigao Goa
2012

Dedicated to my family.....

DECLARATION

I hereby declare that the thesis entitled "**Sulfate reducing bacteria and their activity in mangrove swamps of Goa**", submitted for the Degree of **Doctor of Philosophy in Biotechnology** to the Goa University, has been carried out by me at Department of Biotechnology, Goa University under the supervision of **Dr. Savita Kerkar** (Research Guide).

The work is original and has not been submitted in part or full by me for any other degree or diploma to any other university/institute. Materials obtained from other sources have been duly acknowledged in the thesis.

Place:

Kuldeep Attri

Date:

(Research Scholar)

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled “ **Sulfate reducing bacteria and their activity in mangrove swamps of Goa**” submitted by **Kuldeep Attri** was carried out under my supervision at the Department of Biotechnology, Goa University, Goa.

The thesis submitted is a record of research work done by the student during the period of study and it has not previously formed the basis for the award to the student for any degree, diploma, associateship, fellowship or other similar titles. The thesis represents independent work on the part of the student.

(Dr. Savita Kerkar)
Research Guide

ACKNOWLEDGMENT

This thesis has been compiled after five and half years of research that has been carried out since I came to Department of Biotechnology, Goa University in January 2007. By that time, I have worked with a number of people whose contribution in assorted ways to the research and the making of this thesis deserves special mention. It is a pleasure to convey my gratitude to them all in my humble acknowledgment.

In the first place I would like to record my gratitude to Dr. Savita Kerkar for her supervision, advice and guidance from the very early stage of this research as well as giving me extraordinary experiences throughout the work. Her true scientific intuition has made her as a constant oasis of ideas and her passion in science, which exceptionally inspired and enriched my growth as a student, researcher and a scientist.

I gratefully acknowledge Dr. P.A. Loka Bharathi for her advice, supervision and crucial contribution, which was crucial for this thesis. Her involvement with her originality has triggered and nourished my intellectual maturity that I will benefit from this for a long time.

I express my sincere thanks to Drs. S.R. Shetye (Director, NIO), Rasik Ravindra (Former Director, NCAOR), M.A. Atmanand (Director NIOT) and S.Rajan (Acting Director, NCAOR) for their support, facilitation and continuous encouragement. I am thankful to Prof. G.N.Nayak, Dean, Life Sciences, Goa University for his constant support and encouragement. I acknowledge the support rendered by Drs Sanjeev Ghadi and Usha Murlidharan (HOD, Biotechnology Goa University) for all their timely actions. I gratefully acknowledge the help rendered by Drs. Urmila Barros and U.M.X. Sangodkar.

My sincere thanks to Drs. Shanta Achuthankutty and K.P. Krishnan for their support and encouragement. I am thankful to Dr. Judith for her suggestion and kind consideration to be the VC's nominee and member of the faculty research committee. I express my sincere thanks to Drs. M.P. Tapaswi (NIO) and Gopakumar (Goa University librarian) for their efficient support in procuring the best literature and journals. The financial support from MoES and CSIR is greatly appreciated for providing me the opportunity to work at Goa University. I thank Tonima, Flory, Renosh, Milind, Shivraj, Kirti, Bhaskar, Shuvankar, Surya, Nuncio, Sheryl, Christy, Ravi, Sudheer, Lillian and other friends whose names I might have left out.

It is a pleasure to express my gratitude wholeheartedly to Mr. Ulhas, Martin, Serrao, Mrs. Sanjana and Mrs. Ruby for their help throughout my research work.

Where would I be without my family? My parents deserve special mention for their inseparable support and prayers. My Father, Mr. Jaipal Singh, in the first place is the person who put the fundamental of my learning character, showing me the joy of intellectual pursuit ever since I was a child. My Mother, Mrs. Jashveeri Devi, is the one who sincerely raised me with her caring and gently love. Sincere thanks to Mr. Sanjeev Attri, for being supportive and a caring sibling.

Words fail me to express my appreciation to my wife Mrs. Sangeeta whose dedication, love and persistent confidence in me, has taken the load off my shoulder. I owe her for being unselfish and letting her intelligence, passions, and ambitions collide with mine. Finally, I would like to thank everybody who was important to the successful realization of my thesis, as well as expressing my apology to those whom I could not mention personally one by one.

Kuldeep Attri

Acronyms

Ae	Aerobic
An	Anaerobic
ANOVA	Analysis of variance
AODC	Acridine orange direct count
APS	Adenosine phosphosulfate
ATP	Adenosine triphosphate
BARC	Bhabha atomic research centre
CARD	Catalysed reporter deposition
CFU	Colony forming unit
cm	Centimetre
Co	Cobalt
°C	Degrees centigrade
d⁻¹	Per day
df	Degree of freedom
DMS	Dimethyl sulphide
DMSP	Dimethyl sulfopropionate
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DSR	Dissimilatory sulphate reduction
d/w	Distilled water
FISH	Fluorescence in situ hybridization
g	Gram
LOM	Labile organic matter

mg	Milligram
mM	Milli mole
MMPA	Methyl mercapto propionate
mV	Milli volt
NA	Nutrient agar
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide hydrogen
ng	Nano gram
nM	Nano mole
nm	Nanometre
PAPS	Phosphoadenosine phosphosulfate
PSU	Practical salinity units
RNA	Ribonucleic acid
SD	Standard deviation
SOD	Superoxide dismutase
SRA	Sulphate reducing activity
SRB	Sulphate reducing bacteria
SRP	Sulphate reducing prokaryote
SRR	Sulfate reduction rate
TC	Total count
TOC	Total organic carbon
Tg	Trillion gram
μg	Microgram
μM	Micromole
y⁻¹	Per year

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Chapter 1

Introduction

Mangroves are commonly referred to as the tidal forests of coastal wetlands, existing in the intertidal zones of sheltered shores, estuaries, tidal creeks, backwaters, lagoons, marshes and mud-flats of the tropical and sub-tropical regions. Though, mangroves generally prefer shallow sheltered intertidal swampy regions, they are capable of establishing and growing in the shallow sheltered sandy and rocky coasts. They form an important ecological asset and economic resource of the coastal environment. The mangroves are the most productive and ecologically sensitive ecosystems, which can efficiently fertilize the sea and potentially protect the coastal zone and thus act as the buffer zone. Mangrove regions are rich in detritus (organic matter) and serve as a natural nursery and feeding ground for a variety of fishes and shellfish and hence used for aquaculture practices (McIntosh, 1982; Achuthankutty and Nair, 1983). The mangroves exist under very hostile and inhospitable conditions like fluctuating salinity, tidal extremes, wind velocity, high temperature and muddy anaerobic soil. The plants have peculiar adaptations such as support roots, viviparous germination, salt-excreting leaves, breathing roots, knee roots etc. by which the plants are well-adapted to water-logged and anaerobic saline soils of the coastal environment. The mangrove flora can also adapt to climatic changes (precipitation and temperature), sea level rises and to the incidence of solar ultraviolet-B radiation (Rahaman, 1990; Swaminathan, 1991; Moorthy, 1995; Moorthy and Kathiresan, 1996). They play a significant role in sedimentation, helping in land building process, and also contribute in reducing erosion with the help of their specialized root network.

The mangrove area in Asia equals more than 5.8 million hectares and accounts for about 38% of global mangrove area, representing the highest percentage of mangroves worldwide. Indonesia has the largest extent of mangroves in the region (and in the world), accounting for about half the regional extent of mangrove area. Other Asian countries (in order of mangrove area) are Malaysia, Myanmar, Bangladesh and India, which, together with Indonesia, account for more than 80 percent of total Asian mangrove area. Asia has the largest mangrove area among the continents, and these systems though fragile are biodiversity hot spots. The edaphic and coastal features of South and Southeast Asian countries, together with the high rainfall and significant riverine inputs, are particularly favorable to the development of well-structured mangrove forests. Some of the largest mangrove forests in the world are found in Asia, the best known being the Sundarbans, a transboundary forest covering approximately 1 million hectares in Bangladesh and India.

Distribution and ecology of mangroves in Goa (south-west coast of India):

Within the broad geographic range, mangroves grow in the environmental setting ranging from high humid to extremely arid conditions. Mangroves form the dominant intertidal vegetation in the tropical and sub-tropical regions (Blasco, 1984). The presence of mangrove have been observed to correlate with the areas where the water temperature of the warmest month exceeds 24 °C. Mangroves are restricted to the lower latitude 32° S - 38 °N in the tropical regions of which the maximum diversity and area cover lies between 25° S - 25° N. Indian mangroves are distributed in about 6,740 Km² (Krishnamurthy et al. 1975) which constitute 7%

of the total Indian coastline (Untawale, 1987). Along the central west coast, approximately 21,000 hectares of mangrove area have been estimated, while along Goa it is estimated to be ~ 2,000 hectares (Jagtap, 1985; Jagtap et al. 1993, 1994). The Goan coastline is approximately 110 Km long and within the latitude $15^{\circ} 00'N$ - $15^{\circ} 52'N$ and longitude $73^{\circ} 30'E$ - $74^{\circ} 44'E$. The inter-tidal zones of two major (Mandovi and Zuari) and seven minor estuaries in Goa are mostly flanked on both sides by rocky cliffs formed with silty-sand and silty-clay along with copious amounts of organic matter. Mandovi and Zuari are the two major estuaries that flow over an area of 2500 Km² that is about 68% of the total geographical area and are important for the economy of the territory. They flow through the mining areas and are heavily used for transporting ferromanganese ores to the Marmugao harbor (Goa). About two-third of the total ferromanganese ores of Goa come from the mines located in the basins and watersheds of these two estuaries. Cobalt is also known to combine with hydroxides of iron and manganese as well as silty minerals (Baralkiewicz & Siepak, 1999). In fact, 90% of ferromanganese ores are transported through these estuaries in barges (Nair et al. 2003). The mangroves grow luxuriantly in alluvial soil substrate, which are fine textured, with loose mud or silt and rich in humus and sulfides (Rao, 1987). Their distribution is limited by temperature (Duke, 1992) and they prefer moist atmosphere and freshwater inflow, which brings in abundant nutrients and silt from terrestrial sources. Repeatedly flooded but well-drained soils support good growth of mangroves, but impeded drainage is detrimental (Gopal and Krishnamurthy, 1993). The Indian mangrove flora is comprised of more than 60 species belonging to 41 genera and 29 different

families of which 50% are reported from the west coast (Deshmukh, 1991). About 25 species reported from east coast are not found along the west coast. Similarly, about 8 species that characterize the west coast are absent on the east coast. *Rhizophora*, *Sonneratia*, *Avicennia*, *Excoecaria* etc. are some of the dominant mangrove genus found along the Mandovi and Zuari estuaries, while *Bruguiera*, *Acanthus*, *Derris*, *Clerodendrum* etc. are less abundant.

Sulfate Reducing Bacteria:

Sulfur is among the most abundant elements on the Earth. It is mainly present as pyrite (FeS_2) or gypsum (CaSO_4) in rocks and sediments and as sulfate in seawater. The sulfur cycle is closely linked to other element cycles, such as the carbon and nitrogen cycles. Microorganisms play an important part in sulfur transformations (Figure. 1a & b). Sulfate is the second most abundant ion next to chloride in sea water. The amount of sulfate in the oceans is controlled by three major processes (Turchyn, 2005), input from rivers, sulfate reduction and sulfide re-oxidation on continental shelves and slopes and burial of anhydrite and pyrite in the oceanic crust. Sulfate is taken up as a nutrient in the assimilatory cycle and reduced to sulfide, which is then incorporated into sulfur-containing amino acids and enzymes. Oxidation and reduction reactions for the generation of metabolic energy are also important, such as sulfide oxidation by chemolithotrophic sulfur bacteria and dissimilatory sulfate reduction by sulfate-reducing bacteria (SRB). Bacteria and Archaea use sulfate as a terminal electron acceptor and hence known as sulfate-reducing prokaryotes (SRP) or sulfate-reducing microorganisms. SRB are anaerobic microorganisms that are widespread in anoxic habitats, where

they use sulfate as a terminal electron acceptor for the degradation of organic compounds, resulting in the production of sulfide. SRB are considered to be the foci of the complex anaerobic ecology within the sediments.

Sulfate reducing activity:

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 to more than 50% of the organic matter mineralization in continental-shelf sediments (Jørgensen, 1982; Skyring, 1987; Canfield et al. 1993). However, Kristensen, et al. (1991) has shown that sulfate reduction may account for up to 100% of total sediment metabolism (measured as CO₂ efflux) in mangrove sediments. The sulfate reduction is controlled by biotic and abiotic factors. The quantitative relevance of different physical, chemical and biological factors 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). It is therefore necessary to carry out detailed studies of the spatial and temporal patterns of sediment processes in the mangrove forests (Kristensen et al. 1992).

Sporadic attempts have been made to quantify the sulfate reducing activity as rates SRR (sulfate reducing rates) 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; Laverman et al. 2012) and salt pans (Kerkar and LokaBharathi, 2007), lack of seasonal statistics in mangroves has hampered our detailed studies 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).

Sulfate reduction, though a key reaction in the environment is influenced by an array of factors. Some have positive influence while some have negative influence. Hence, the health and sustainability of the 'Mangrove-Buffer Zones', and there by coastal environments needs to be studied from the perspective of sulfur cycling and sulfate reduction in particular.

Sulfate reduction can be applied beneficially to biotechnology, in the removal of heavy metals from groundwater and waste water. This application takes advantage of differences in the chemical properties of metal sulfates and sulfides (Hulshoff-Pol et al. 1998; Lens et al. 2007). Metal sulfates (cadmium, cobalt, copper, iron, nickel and zinc) are highly soluble, but the corresponding metal sulfides have low solubility. With sulfate reduction, metals can be precipitated, recovered and reused.

Aims and Objectives of the present study:

The aim of the present study was to understand the principle factors influencing sulfate reduction rates in mangrove ecosystems and to delineate the taxonomy of the sulfate reducing bacterial community therein. We have also explored their potential in harnessing metals from the metal salt. This study has been conducted with the following objectives:

- To quantify the abundance of sulfate reducing bacteria.
- To identify the sulfate reducing bacteria
- To quantify the sulfate reducing activity in sediments and understand the influence of environmental factors
- Bioremediation of heavy metal using SRB to immobilize Co.

Significance:

Mangrove forests that once covered more than 200,000 km² of sheltered tropical and subtropical coastlines is disappearing worldwide at a rate of 1 to 2% per year. This is greater than the loss in coral reefs or tropical rainforests. Most of this happens in the developing countries where >90% of the world's mangroves are located. As mangrove areas are reducing or getting fragmented, their long-term survival is at a great risk, and essential ecosystem services may be lost. Therefore, any further decline in the mangrove area is likely to be followed by accelerated functional losses. Mangroves act as a CO₂ sink as well as an essential source of oceanic carbon.

The decline also affects mangrove-dependent fauna, as well as physical benefits like the buffering of sea-grass beds and coral reefs against the impacts of river borne siltation, protection of coastal communities from sea-level rise, storm surges, and tsunamis. It has been estimated that sulfate reduction can account for more than 50% of the organic carbon mineralization in marine sediments (Jorgensen, 1982), which indicates the importance of sulfate reducers in both the sulfur and carbon cycles. It is therefore important to study anaerobic processes like SRR in the mangrove sediments.

This study is probably the first of its kind in India addressing the sulfate reduction on a monthly basis in mangroves along with the bacterial flora mediating this process. This work adds a new dimension to ecological management in coastal zones by demonstrating the elements functioning and governing sulfate reduction in these environments. Moreover, the SRB isolated from these environments could be used for bioremediation of metals in different environments.

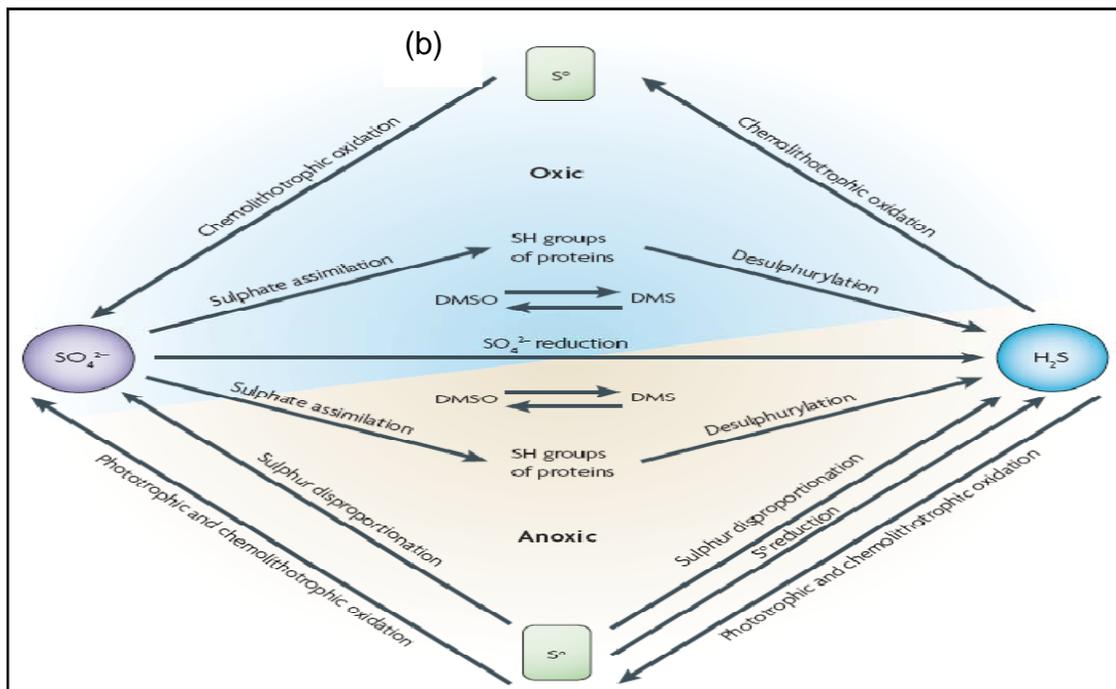
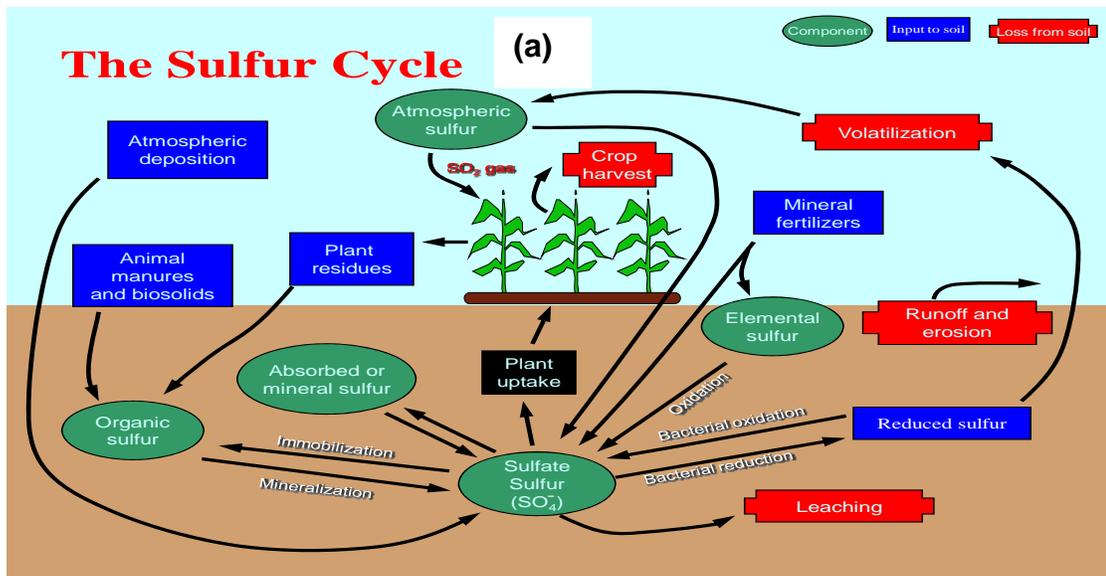


Figure: 1. **(a)** Overview of sulfur cycle **(b)** Sulphur transformations. Sulfate-reducing bacteria have a key role in the sulfur cycle. They use sulfate (SO_4^{2-}) as a terminal electron acceptor in the degradation of organic matter, which results in the production of hydrogen sulphide (H_2S). Subsequently, the sulfide can be oxidized aerobically by chemolithotrophic sulfur-oxidizing bacteria (for example, *Thiobacillus* or *Beggiatoa* spp.) or anaerobically by phototrophic sulfur bacteria (for example, *Chlorobium* spp.) to elemental sulphur (S^0) and SO_4^{2-} . Other transformations, which are carried out by specialized groups of microorganisms, result in sulfur reduction (for example, *Desulfuromonas* spp.) and sulfur disproportionation (*Desulfovibrio sulfodismutans*). Organic sulphur compounds, such as dimethylsulphoxide (DMSO) can be transformed into dimethylsulphide (DMS) and vice versa by several groups of microorganisms. SH (sulfhydryl). From Madigan & Martinko (2006). *Brock — Biology of Microorganisms* 11th edn Pearson Education, London.

2.1. Definition and Distribution of Mangroves:

Mangrove ecosystems normally exist in intertidal zones and play an important role in protecting the coastal environment from natural calamities. “Mangroves” refers to plants and the forests. According to Macnae, (1968) “Mangal” refers to the habitat or the forest community and “Mangroves” to the plant species. Duke, (1992) also reported the use of “Mangrove” or “mangrove tree” or “mangrove fauna”. The origin of the word “mangrove” could be traced to the Portuguese word ‘mangue’ (= a type of trees) and the English word ‘groves’ (= a group of trees). In French, the word ‘manglier’ is similar to ‘mangue’. Probably all these words originated from the Malay word, ‘Manggi-manggi’ (Macnae, 1968). The mangrove forests are also referred to as “tidal forests”, “oceanic rain forests” and “coastal woodlands”. Changing wind velocity and wind patterns, tides, fluctuating temperature and salinity gradients have resulted in the evolution of varied adaptive strategies in the natural mangrove flora and fauna. So far there are no reports of any terrestrial plant that can survive these adverse conditions (Kathiresan, 1991; Kathiresan and Bingham, 2001). 'Mangals' are constantly exposed to water-logged and anaerobic saline soils. Occurrence of support roots, viviparous germination, salt-excreting leaves, breathing roots, knee roots, etc. makes them well-adapted to coastal environment. Other aspects like change in climate (precipitation and temperature), sea levels rise and incidence of solar ultraviolet–B radiation also pose ecological challenges to the coastal flora and fauna (Rahaman, 1990; Swaminathan, 1991; Moorthy, 1995; Moorthy and Kathiresan, 1996). Alluvial soil is an excellent substrate facilitating successful growth of

mangroves. These substrates are fine textured, loose mud or silt, rich in humus and sulfides (Rao, 1987). They develop in low lying and broad coastal plains where the topographic gradients are very small and the tidal amplitude is large. Their distribution is governed by temperature (Duke, 1992) and they prefer moist atmosphere and freshwater inflow, which brings in abundant nutrients and silt from terrestrial sources. Unsheltered shores pose a potential threat to mangrove seedlings from waves and currents. Periodically flooded but well-drained soils support good growth of mangroves. Improper drainage is detrimental for mangrove vegetation (Gopal and Krishnamurthy, 1993). Indian mangroves are distributed in about 6,740 sq. km (Krishnamurthy et al. 1987) which constitute 7% of the total Indian coastline (Untawale, 1987). In general, there are three different types of mangroves in India viz. deltaic, backwater-estuarine and insular. The backwater-estuarine type is characterized by funnel-shaped estuaries of major rivers (Indus, Narmada and Tapti) or backwaters, creeks, and neritic inlets. The insular mangroves can be found in Andaman and Nicobar islands where many tidal estuaries, small rivers, neritic islets, and Lagoons supporting rich mangrove flora (Gopal and Krishnamurthy, 1993). Majority (70%) of the mangrove vegetation is encountered in the east coast while the west coast accounts for only 12%. The rest is found along the bay islands of Andaman and Nicobar (Krishnamurthy et al. 1987; Kathiresan, 1995).

Natural regeneration of mangroves in Goa have been studied (Kumar, 2000) and various methods of regeneration of mangroves have been described (Kumar, 1999). In Maharashtra and Goa, mangroves exist mainly as large patches

along the Mandovi, the Vasishta, the Savithri and the Kundalika estuaries, the Dharamtar, the Panvel, the Vasai, the Thane and the Vaitarana creeks (RSAM, 1992). The mangroves occur over an area of 2538 ha in Goa. Mangroves in the Mandovi estuary of Goa spread to an area of 700 ha with distinct zones, which differ in environment, species composition and growth (FSI, 1997). Mascarenhas and Chauhan (1998) have reported that Goa once had a luxuriant mangrove swamp of around 20 km inland from the open sea coast during the recent geological past, when the sea level was 1 to 3 m lower than present.

2.2. Ecology of Mangroves:

2.2.1. Microbiology of Indian mangroves

Mangroves have been ecologically well-studied (Gopal and Krishnamurthy, 1993) along the Sundarbans (Naskar and Guha Bakshi, 1989), the Andaman-Nicobar Islands (Singh et al. 1986, 1987; Ellis, 1987; Dagar, 1987; Rao and Chakrabarti, 1987), the Mahanadi delta (Banerjee and Choudhury, 1987), the Krishna estuary (Prasad, 1992), the Cauvery delta (Kathiresan, 2000) and the Mumbai coasts (Ghosh et al. 1994). Mangroves provide a unique ecological niche to a variety of microorganisms (Agate, 1991) and about 125 species of microorganisms (bacteria, fungi, algae) have been identified (Kathirvel, 1996). The photosynthetic microorganisms behave like heterotrophs in the mangrove environment. Cyanobacteria and photosynthetic bacteria survive in low light or partially dark conditions by utilizing the suspended organic matter, which are available abundantly in the mangrove waters (Rao and Krishnamurthy, 1994). This unique heterotrophic adaptation of photoautotrophs, is a mechanism of survival in

hostile coastal anaerobic conditions of mangrove habitat (Rao and Krishnamurthy, 1994). Hydrocarbonoclastic bacterial isolates have been reported from mangals of Andaman (Shome et al. 1996). Sulfate reducing bacteria have been isolated from the mangrove swamps of Goa (Saxena et al. 1988; Lokabharathi et al. 1991). Purple photosynthetic bacteria have been reported by Vethanayagam (1991) and isolated from Pichavaram mangrove sediments: two major groups viz. purple sulfur bacteria (family- Chromatiaceae, strains belonging to *Chromatium* sp.) and purple non-sulfur bacteria (family- Rhodospirillaceae, strains belonging to *Rhodopseudomonas* spp.). Besides sulfur bacteria, the iron oxidizing and iron reducing bacteria do exist in mangrove habitat. This type of bacteria is higher in mining areas of Goa than in non-mining mangroves areas of Konkan (Panchanadikar, 1993). Methanogenic bacteria have been studied for the first time for their distribution and ecology in mangrove sediments of Pichavaram (Ramamurthy et al. 1990). The bacterial counts are generally high during the post-monsoon months as compared to fungal the counts and actinomycetes, which are maximum during the monsoon months (Mini Raman and Chandrika, 1993).

2.2.2. Mangrove and metals:

Estuarine and intertidal zones are important sinks for many persistent pollutants which accrue in organisms and bottom sediments (Szefer et al. 1995). With increasing urbanization and industrialization, many regions have been subjected to considerable environmental stress (Agoramoorthy and Hsu, 2005; Hsu et al. 2006). Near shore marine ecosystems are especially prone to anthropogenic inputs due to their proximity to the coast. In tropical zones, the

most dominant intertidal areas are characterized by mangrove fringes (Tomlinson, 1994). Mangroves ecosystems play an important role in regional climate change (Forster et al. 2007) and represent one of the major biodiversity hotspots. These ecosystems serve both a source and sink for nutrients and sediments for other inshore marine habitats including sea grass beds and coral reefs (Dorenbosch et al. 2004; Duke et al. 2007). Mangrove sediments also operate as a biogeochemical sinks for trace metals (Harbison, 1986) mainly due to the high concentrations of organic matter and sulfides under permanently reducing conditions (Harbison, 1981; Lacerda and Abrao, 1984; Lacerda et al. 1988; Silva, 1988). According to Lacerda et al. (1988) marine suspended matter with elevated metal load is actively trapped within mangrove environment.

Mangroves are often exposed to heavy metal pollution due to a variety of anthropogenic activities ranging from shipping, post development and manufacturing of metals and sewage and storm-water discharge (Mackey and Mackay, 1996). Due to their toxicity and bio-accumulation potential, the persistence and cycling of heavy metals is of serious concern in mangrove environments (Lacerda et al. 1988; Mackey and Hodgkinson, 1995; Clark et al. 1998; Tam and Wong, 2000). Currently, studies assessing the potential problems related to heavy metal accumulation from the Indian mangroves regions are limited (Thomas et al. 1997; Ray et al. 2006; Krishnan et al. 2007). Ferromanganese ore mining is an important industry in Goa. The estuarine channel of Mandovi river is crucial for the economy of the state since it is heavily used to transport large quantities of ore to the Marmagoa harbour. Lush mangrove vegetation, fringes this

estuarine system. The mining activity upstream in the watershed may influence the biological and geochemical conditions of the estuarine waters. Although the impacts of iron-ore processing on the surface sediments of the Mandovi estuary (Alagarsamy, 2006), and adjacent salt pans (Pereira et al. 2012) have been documented, their influence on the surrounding mangrove ecosystem is sparsely addressed.

2.2.3. Significance of mangrove ecosystems:

The value of mangrove is a measure of its importance to society. Which can be considered in three hierarchical levels: population, ecosystem and global. At the population level, mangrove-dependent fish, shellfish, animals and timber provide important and valuable harvests and recreational fishing and hunting. At the level of the whole ecosystem, mangroves have value to the public for flood mitigation, storm abatement, aquifer recharge and water quality improvement, aesthetic and general subsistence. At the regional and global level, mangroves contribute to the stability of available nitrogen, atmospheric sulfur, carbon dioxide and methane (Mitsch and Gosselink, 2000). As most tropical mangroves are the receivers of fertilizer- enriched agricultural runoff and are an ideal environment for denitrification, they are likely to be important to the world's available nitrogen balance. Also, ammonia for fertilizer production is manufactured from nitrogen gas at more than double the rate of all natural fixations. Wetlands in general have been recommended as a key ecosystem in providing a solution to this eutrophication (Mitsch and Gosselink, 2000). Mangrove ecosystems provide habitats for numerous animals and micro-organisms (Cannicci et al.

2008; Nagelkerken et al. 2008), live in close interaction with the mangrove vegetation (Bouillon et al. 2004; Kristensen et al. 2008). Mangrove forests provide essential functions and services to coastal populations, such as protection of the coastal zone (e.g. Badola and Hussain, 2005; Dadough-Guebas et al. 2005b; Barbier et al. 2008; Kaplan et al. 2009) and a variety of timber and non-timber forest products (Bandaranayake, 1998, 2002; Walters et al. 2008). The sulfate reduction by SRB in the mangrove ecosystem plays an important role in remineralization of organic carbon and precipitate the metals as metal sulfides and entrap them in fine sediments, and so act as the buffer zone between the fresh water and the open ocean.

2.3. Sulfate Reduction:

2.3.1. Assimilatory sulfate reduction:

Sulfate-reducing activity (SRA) that takes place for the incorporation of sulfide radical for biosynthetic cycle is referred to as assimilatory sulfate reduction (ASR). Sulfate is reduced to sulfide in the assimilatory cycle which combines with serine to form cysteine. This in turn can be converted to methionine (Figure 2).

These two amino acids are the main constituents of sulfur-containing molecules in the cells. Sulfur content can vary from 0.3% in eel grass to 3.3% in marine algae. As the N:S ratio in land plants is only 30:1, the reductive assimilation of sulfate is less important than nitrate. Assimilatory reduction is common among organisms and does not lead to the production of sulfide. The eight-electron reduction of sulfate to sulfide proceeds in different stages. As the ion is stable it needs to be activated with ATP. The enzyme ATP sulfurylase

catalyzes the attachment of sulfate ion to phosphate of ATP to form adenosine phosphosulfate (APS). Another P is added to APS to form phosphoadenosine phosphosulfate (PAPS) before it gets reduced to form sulfite.

2.3.2. Dissimilatory sulfate reduction:

The SRA that takes place in anaerobic respiration is termed as dissimilatory sulfate reduction (DSR). Here sulfate is used as terminal electron acceptor leading to the production of sulfide. Here too the sulfate ion is activated by ATP to form APS. However, in this case, the sulfate moiety of APS is reduced directly to form sulfite with the release of AMP. Thus the first product of ASR and DSR is sulfite (Figure 2).

Dissimilatory SRB act as agents of synergy in sulfur cycle and bring about syntrophic associations. The end products from organic substrate oxidation and sulfate reduction, lead to the formation of sulfide and carbon dioxide. SRA can account for nearly 80% of organic carbon mineralization in marine environment, especially in coastal regimes where nearly 5×10^{12} kg yr⁻¹ of sulfate gets reduced

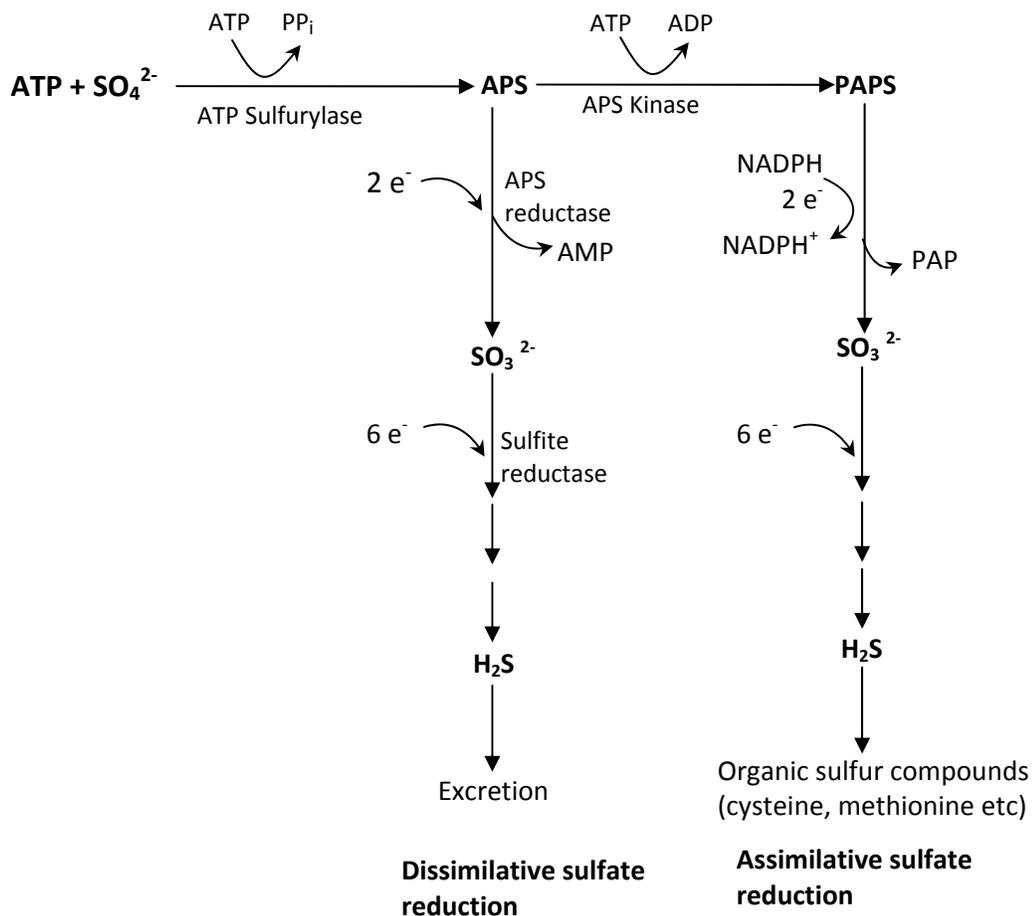


Figure.2. Schemes of assimilative and dissimilative sulfate reduction

2.4. States of Sulfur:

Sulfur and its species are important geochemical agents. While the elemental sulfur is the fourteenth most abundant element on Earth, sulfate is the second most abundant ion next only to chloride in seawater and carbonate in freshwater. Elemental sulfur is produced hydrothermally and also by oxidation of sulfide by weathering. Sulfur transformations govern the compositions of the oceans, and the redox balance on the Earth's surface. It is complex due to a

variety of oxidation states. Besides, some transformations occur at significant rates both bacteriologically as well as chemically. Sulfur appears in both oxidized and reduced states. The sulfur cycle involves eight electron oxidation/ reduction reactions between the most reduced H_2S (-2) to the most oxidized SO_4^{2-} (+6). It acts as either electron donor or acceptor in many bacterially mediated reactions.

2.5. Distribution of SRB:

SRB are not only versatile in their use of various electron acceptors and electron donors, they can also thrive in a range of different environmental conditions. They are ubiquitous and can be found in many natural and engineered environments where sulfate is present. SRB have been detected or isolated from marine sediments (Boschker et al. 1998; Ravensschlag et al. 2000; Mussmann et al. 2005; Webster et al. 2006), hydrothermal vents (Jeanthon et al. 2002), hydrocarbon seeps (Knittel et al. 2003; Kniemeyer et al. 2007) and mud volcanoes (Stadnitskaia et al. 2005), and are abundantly present in hypersaline microbial mats, even at saturating oxygen concentrations (Rissati et al. 1994; Minz et al. 1999). They have been detected in habitats with extreme pH values, such as acid-mine drainage sites, where the pH can be as low as 2 (Sen, 2001) and in soda lakes, where the pH can be as high as 10 (Geets et al. 2006). SRB have been detected and isolated from oil fields (Nilsen et al. 1996), as well as from the deep sub-surface (Kovacik, 2006). They are also present in freshwater sediments (Sass et al. 1998), in the rhizosphere of plants (Hines et al. 1999; Bahr et al. 2005), in aquifers and in engineered systems, such as anaerobic waste-water treatment plants (Ramsing et al. 1993; Wawer et al. 1997; Oude et al. 1994; Dar et al. 2005;

Ben-Dove et al. 2007). Most SRB are free-living, but some are present in consortia with other microorganisms, such as methanotrophic archaea (Boetius et al. 2000), or even in a more intimate relationship, for example, together with sulfur-oxidizing Gammaproteobacteria as endosymbionts in the marine worm *Olavius algarvensis* (Dubilier et al. 2001), thereby providing the host with nutrients (Woyke et al. 2006).

2.6. Diversity of Sulfate Reducing Bacteria:

Metabolically sulfate reducing prokaryotes (SRP) are highly diverse, and have been shown to be abundant in marine sediments (Devereux et al. 1992; Ravensschlag et al. 2000). They share the capability to reduce sulfate, but may also reduce alternative electron acceptors. Sulfate reducing prokaryotes are also phylogenetically diverse and are found in two Archaeal and four Bacterial phyla (Rabus et al. 2000) (Figure 3). The diversity of sulfate reducing bacteria in marine sediments has been investigated by clone libraries of the 16S rRNA gene (Devereux and Mundfrom, 1994; Bowman and McCuaig, 2003; Purdy et al. 2003a; Guan et al. 2012) or a more powerful approach for the detection of SRB is the use of so-called functional genes which encode enzymes that play an important part in the sulfate-reduction pathway, such as *dsrAB*, which encodes the dissimilatory sulphite reductase (Wagner et al. 1998; Guan et al. 2012), or *aprBA*, which encodes the dissimilatory adenosine-5'-phosphosulfate reductase (Meyer et al. 2007). Cloning or denaturing gradient gel electrophoresis of PCR amplified 16S rRNA (Dhillon et al. 2003; Dar et al. 2005), *dsr* (Minz et al. 1999; Geets et al. 2006; Dar et al. 2007) or *aprA* (Meyer et al. 2007) gene fragments has been used to

determine the diversity of SRB in many different habitats. Recently, a DNA microarray, the SRP-PhyloChip (Loy et al. 2002) has been used to detect SRB in natural samples, such as acidic soils (Loy et al. 2004). However, these methods have the disadvantage that they provide little or no information on the number of SRB cells that are present. Using different probes between 2% and up-to 21% of prokaryotic DNA in marine sediments could be assigned to this group (Rooney-Varga et al. 1997; Sahm et al. 1999a; Ravensschlag et al. 2000). This group includes metabolically versatile organisms, e.g. the genera *Desulfosarcina* and *Desulfococcus*, and species with 16S rRNA sequences that are up-to 18% divergent (Aeckaersberg et al. 1991; Widdel and Bak, 1992; Harms et al. 1999).

Quantitative real-time PCR is a highly sensitive technique that can be used to quantify the number of SRB, and has been used, for example, to determine the number of SRB in rice field soils (Stubner, 2002; 2004), soda lakes (Foti et al. 2007) and industrial waste water (Ben-Dov et al. 2007). Moreover, this technique can also be used to study the expression of functional genes, such as *dsrAB* (Neretin et al. 2003). Another technique that can be used to quantify the number of SRB is fluorescence *in situ* hybridization (FISH), which also allows their spatial distribution to be visualized (Dar et al. 2007). Many different probes have been developed to target the rRNA of different taxonomic groups of SRB (Stahl et al. 2007). Mussmann et al. (2005) used a combination of FISH with catalysed reporter deposition (CARD-FISH) to study the vertical distribution of SRB in intertidal mud-flat samples. They found that up to 11% of all cells were SRB and

that organisms related to the genera *Desulfosarcina* and *Desulfobulbaceae* dominated the surface layer of the sediment.

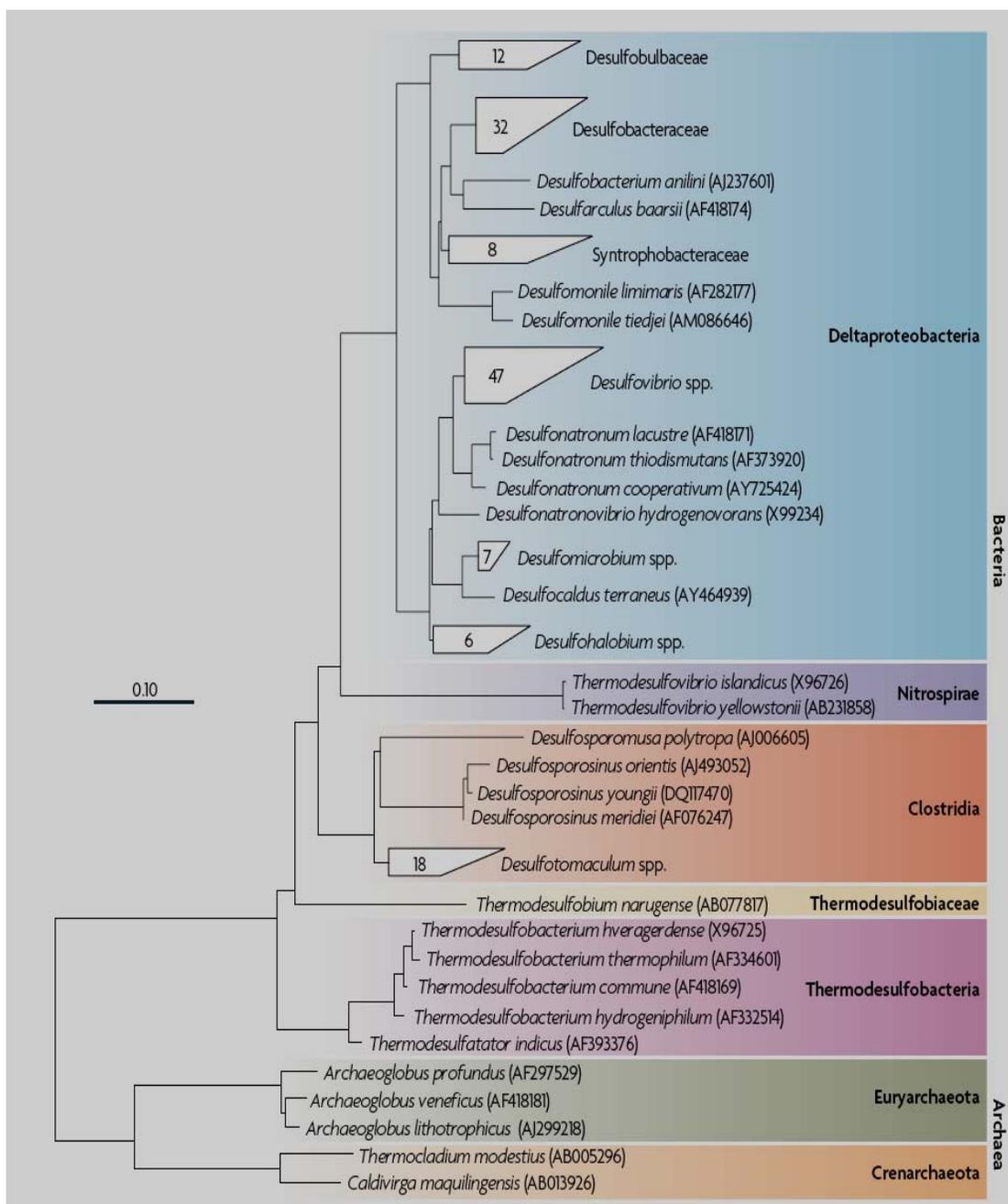


Figure: 3. Phylogenetic tree based on nearly complete 16S ribosomal RNA (rRNA) sequences of described sulphate-reducing bacterial species. (Muyzer & Stams, 2008).

2.7. Factors Governing Sulfate Reduction Rates:

The benthic compartment together with the overlying pelagic system forms an important avenue for a plethora of reactions in the sulfur cycle. Knowledge of the role of SO_4^{2-} reduction in sediments has increased greatly since the introduction of the use of ^{35}S as a tracer for determining sulfate reduction rates (Sorokin, 1962; Jørgensen and Fenchel, 1974). Methods now routinely include a reduction step using reduced chromium under acidic conditions to incorporate all major reduced inorganic sulfur species that may form during SO_4^{2-} reduction (Westrich, 1983; Fossing and Jørgensen, 1989; Meier et al. 2000). With increase in depth increases reduced sulfur compounds and the abundance of SO_4^{2-} reducing bacteria are useful as comparative indicators of the SO_4^{2-} reduction process, but they are poor indicators of actual rates of activity. The rate of organic matter input (i.e. sedimentation) and the availability of SO_4^{2-} controls the rate of SO_4^{2-} reduction in sediments. Sulfate is rarely limiting in marine systems except in brackish estuarine waters and with depth in sediments where SO_4^{2-} has been depleted. Sulfate reduction rates in sediments can span several orders of magnitude, but typical near-shore rates in the upper 5–10 cm of marine sediments are often 50–500 $\text{nmol cm}^{-3} \text{ d}^{-1}$ (Skyring, 1987). SRR in sediment with unusually rapid sedimentation rates can be reach 2,000 $\text{nmol ml}^{-1} \text{ d}^{-1}$ (Crill and Martens, 1987). Sulfate reduction in salt marshes and microbial mats can reach rates as high as 4,000 $\text{nmol ml}^{-1} \text{ d}^{-1}$ and 14,000 $\text{nmol ml}^{-1} \text{ d}^{-1}$ respectively (Canfield and Des Marais, 1991; Hines et al. 1999). Anaerobic CH_4 oxidation supports a large fraction of the SO_4^{2-} reduction in some marine sediment. The process may be

carried out by a single organism that couples methanotrophy to SO_4^{2-} reduction. Anaerobic methane oxidation coupled to sulfate reduction is thermodynamically favorable under conditions found in marine sediments (Martens and Berner, 1977):



2.7.1. Temperature:

Sulfate reduction occurs over a wide range (0–110 °C) of temperatures (Jørgensen et al. 1992; Stetter et al. 1993; Knoblauch and Jørgensen, 1999; Kostka et al. 1999b; Castro et al. 2000; Elsgaard et al. 1994; Sievert and Kuever, 2000). Above, 115 °C, SO_4^{2-} reduction is believed to occur only by thermochemical reactions (Machel, 2001). Like other biological processes, biological SO_4^{2-} reduction is affected strongly by temperature. Seasonal changes in SO_4^{2-} reduction activity often follow temperature well, except for lags in activity due to the time required to remove oxygen and other competing electron acceptors (Jørgensen, 1977; Hines et al. 1982; Crill and Martens, 1987). In general, rates of activity can vary by factors of 5 to 30 between winter and summer, and these changes have profound influence on redox conditions and the accumulation of reduced species (Jørgensen, 1977).

2.7.2. Carbon:

Sulfate reduction is controlled strongly by the quantity and quality of organic matter present. In marine sediments, there is a strong relationship between organic sedimentation rate and SO_4^{2-} reduction rate (Goldhaber and Kaplan, 1975) and it is generally held that SO_4^{2-} reduction is limited by organic matter availability

except when SO_4^{2-} concentration is quite low (Boudreau and Westrich, 1984; Westrich and Berner, 1984; Dornblaser et al. 1994). In general, there is a 2:1 molar relationship between labile carbons deposited into the SO_4^{2-} reduction zone and SO_4^{2-} reduced (Thamdrup and Canfield, 1996). The quantity of nitrogen and phosphorus mineralized during SO_4^{2-} reduction can be predicted using C/N/P ratios of organic matter and SO_4^{2-} reduction stoichiometry (Martens et al. 1978; Hines and Lyons, 1982). Sulfate reduction activity responds rapidly to increased inputs of organic material with the seasonal deposition of spring phytoplankton blooms resulting in significant increases in activity in lake (Hadas and Pinkas, 1995) and ocean sediments (Boetius et al. 2000a). Although it is clear that low-molecular-weight fatty acids are important substrates for SO_4^{2-} reducing bacteria, different groups of SO_4^{2-} reducers may consume different acids (Boschker et al. 2001). Some electron donors are more readily metabolized by methanogens than SO_4^{2-} reducers and these “noncompetitive” substrates can allow methanogenesis to occur in the presence of active SO_4^{2-} reduction (Oremland et al. 1982). Examples of these types of substrates include C1 compounds like methylated amines, methylated sulfur compounds (e.g. dimethylsulfide and methane thiol), and methanol. Methylated nitrogen and sulfur compounds are common degradation products of osmoregulating compounds found in certain marine algae and salt marsh grasses, and their use by methanogens partially explains the occurrence of significant concentrations of CH_4 in SO_4^{2-} containing estuarine and salt marsh sediments (Dacey et al. 1987; Kiene, 1996b).

2.7.3. Sulfate and molecular oxygen:

The SO_4^{2-} concentration in sediments affects its reduction only when SO_4^{2-} concentrations are low. The reduction of SO_4^{2-} in marine sediments appears to be zero-order at 2 mM SO_4^{2-} concentrations (Goldhaber and Kaplan, 1974; Boudreau and Westrich, 1984). Freshwater contains very little SO_4^{2-} compared to seawater. The importance of SO_4^{2-} reduction in sediments increases in an estuary as the salinity increases (Capone and Kiene, 1988). In freshwaters, SO_4^{2-} concentrations must be much lower before they limit SO_4^{2-} reduction (Lovley and Klug, 1983b; Bak and Pfennig, 1991; Sinke et al. 1992). The vertical extent of the SO_4^{2-} reduction zone increases substantially as more SO_4^{2-} becomes available, while the methanogenic zone is “pushed” deeper into the sediment and its contribution to carbon mineralization decreases in importance. Sulfate-reducing bacteria are generally anaerobic, but recent studies have shown that some SRB are tolerant to O_2 and are able to withstand several hours of full aeration. This is observed with SRB which are common inhabitants of the oxic regions of microbial mats (Krekeler et al. 1997; Cypionka, 2000). Sulfate reducers withstand O_2 stress better in the presence of co-metabolizing bacteria that presumably consume O_2 (Gottschalk and Szwezyk, 1985). However, studies of co-cultures of a SO_4^{2-} reducer and a facultative anaerobe suggested the occurrence of O_2 -dependent growth by the SO_4^{2-} reducer in the absence of SO_4^{2-} (Sigalevich et al. 2000a, b; Sigalevich and Cohen, 2000). Sulfate-reducing populations within active sediments, like microbial mats, can exhibit a bimodal distribution with two distinct maxima of differing populations, one within surficial oxic layers and another in deeper anoxic

sediments (Ramsing et al. 1993; Risatti et al. 1994; Sass et al. 1997; Minz et al. 1999a). Sulfate reducers within or near oxic regions can benefit from labile organic compounds released by photosynthetic bacteria (Canfield and Des Marais, 1991; Visscher et al. 1992; Teske et al. 1998) or vascular plants (Blaabjerg and Finster, 1998; Hines et al. 1999; Scheid and Stubner, 2001). Indeed, rates of SO_4^{2-} reduction measured in oxic regions of microbial mats can be equal or exceed those noted in deeper anoxic lamina (Canfield and Des Marais, 1991; Frund and Cohen, 1992; Visscher et al. 1992). The complete oxidizing and gliding SO_4^{2-} reducer, *Desulfonema*, is a common inhabitant of the oxic–anoxic interface in microbial mats (Risatti et al. 1994; Teske et al. 1998; Fukui et al. 1999), and is also abundant within the partially oxygenated rhizosphere of salt marsh plants (Rooney-Varga et al. 1997). Filamentous morphology, aggregate formation, and diurnal migration are all adaptations that allow this species to thrive in rapidly changing conditions.

A number of other environmental factors are known to influence the sulfate reduction rates (SRR) including bacterial abundance, lability of organic carbon, 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 Fe^{3+} addition has been reported by Lovley and Phillips, (1987) and Van Bodegom et al. (2004). Influence of electron donor on the growth and activity of sulfate reducing bacteria has been studied by Cao et al. (2012)

Despite the potential importance of sulfate reduction, only a few studies have explored the factors regulating this process in mangroves (e.g. Kristensen et al. 2000; Alongi et al. 2005), and no single set of factors has emerged consistently as the regulator of sulfate reduction rates.

2.8. Application of Sulfate Reducing Bacteria:

2.8.1. Corrosion of metals

The activity of SRB could be deleterious to all underground constructions because of their involvement in corrosion. The sulfide they produce is responsible for anodic corrosion, and their propensity to scavenge hydrogen generated in underwater metal structures could cause cathodic corrosion. However, some of these activities could be used in metal recovery from wastewater treatment as metal sulfides. The synergy existing between SRB and other microbes could be effectively used in bioremediation and ecosystem management.

2.8.2. Degradation of aromatic and other compounds:

SRB can also degrade other compounds like benzene. Other mono-aromatic hydrocarbon such as toluene and xylene are known to be degraded under sulfate reducing conditions (Beller et al. 1992 a; Edwards and Galic, 1992; Lovely et al. 1995). Sulfate reducing consortia have been successfully used for reductive dechlorination of halogenated phenols (Hagblom, 1998). Exopolysaccharides produced by SRB have shown to be modified by the presence of carbon steel, which may increase its metal binding capacity (Zinkevich et al. 1996).

2.8.3 Metal tolerance in SRB:

Studies on metal microbe interactions are generally restricted to aerobic bacteria (Foster, 1983), anaerobic consortia (White and Gadd, 1996) and sometimes to mesophilic SRB (Loka Bharathi et al. 1990). Harithsa et al. (2002) have studied the heavy metal tolerance and response in terms of growth and activity (SRA) using HgCl_2 and $\text{Pb}(\text{NO}_3)_2$ at the final concentrations of 50, 100 and 200 ppm and 100, 200 and 500 ppm respectively.

2.8.4. Bioremediation of heavy metals:

The removal of toxic metals from industrial wastewaters has been practiced for several decades; the cost-effectiveness of the commonly used treatment technologies such as oxidation–reduction, filtration, electrochemical treatment, evaporation, ion exchange and reverse osmosis processes is limited. High reagent requirement and unpredictable metal removal are disadvantages of these methods. Further, strong and contaminating reagents are used for desorption, resulting in toxic sludge and secondary environmental pollution. Bioremediation, which involves the use of microbes to detoxify and degrade environmental contaminants, has received increasing attention in recent times to clean up a polluted environment (Malik, 2004). Bioremediation, being in situ treatment, offers several advantages over the conventional chemical and physical treatment technologies, especially for diluted and widely spread contaminants (Eccles, 1995). The recent advances in treatment of metal containing wastewaters with SRB are a promising alternative over chemical methods (Christensen et al. 1996). The major application of SRB to wastewater treatment is based on their ability to reduce

sulfate to sulfide, which then reacts with most metals to form insoluble sulfides (Lyew and Sheppard, 2001). The potential advantages of metal sulfide precipitation include production of lower sludge volume and lower solubility products as compared to hydroxide precipitation (Peters et al. 1985; Venkatachalam, 1998). In addition, valuable metals can be recovered from metal sulfide sludges (Boonstra et al. 1999). SRB have been used for the bioremediation of metals i.e. Cd, Cr, Cu, Mn, Ni and Zn, by White and Gadd (1997); Cr (IV), (Chardin et al. 2002); Zn, (Radhika et al. 2006); As and Se, (Luo et al. 2008); As, Cd, Cr, Cu, and Zn, (Viggi et al. 2009); and Uranium, (Boonchayaanant et al. 2010). While the attempts for bioremediation of Cobalt using the SRB is rare (Krumholz et al. 2003).

Chapter 3

Materials and Methods

3.1. Study Area:

3.1.1. Location and description of the mangroves:

Mandovi and Chapora are two tropical estuaries lying in close geographic proximity on the west coast of India. The hydrological characteristics of these two estuarine systems are governed by the monsoon regime. The physical characteristics of the Mandovi and the Chapora estuaries have been described by Varma and Rao, 1975; Varma and Cherian, 1975; Murthy et al. 1976. Based on the environmental characteristics, the Mandovi estuarine system is classified as a tide dominated coastal plain estuary and geo-morphologically identified as drowned river valley estuaries (Murthy et al. 1976). The estuarine channel of the Mandovi is used to transport large quantities of ferromanganese ores from mines located upstream to the Mormugao harbor (Arabian Sea), while Chapora is free from such activities. Lush mangrove vegetation fringes both the estuarine systems. Distribution of mangrove in Indian subcontinent is given in Figure 4.

To study the influence of physico-chemical, biological and anthropogenic factors on sulfate reduction, two sites were selected. The control site which is relatively free and less affected from anthropogenic effects is located at Tuvem in the Chapora estuary at 15°38.28' N and 73°47.71' E (Figure 5 a). The Chapora river runs 30 km in length covering approximately 700 hectares of water area. Tidal influence is felt upto 18-20 km of river mouth (Untawale et al. 1982). Mangroves cover about 218.5 hectares of the total river area. The experimental site which is exposed to enrichment of metal ores is located at Diwar in the Mandovi estuary at 15°30.42' N and 73° 52.28' E (Figure 5 b). Comparatively the Mandovi is longer

than the Chapora estuary. The river has its origin from the Parwa Ghat of Sahayadri hills of Karnataka and joins the Arabian Sea through Aguada Bay, after traversing a stretch of about 70 km. In both the estuaries, the pre and post-monsoon flow is regulated by the semi-diurnal tides. The position of the stations was fixed using Global Positioning System (GPS) (Magellan GPS NAV 5000TM, USA).

Goa houses 14 mangrove tree species found on the west coast of India and has one of the best mangrove forests of the west coast. The species found includes *Rhizophora mucronata*, *R. apiculata*, *Avicennia officinalis*, *A. marina*, *A. alba*, *Kandelia candel*, *Ceriops tagal*, *Sonneratia alba*, *S. caseolaris*, *Bruguiera gymnorhiza*, *B. cylindrica*, *Aegiceras corniculatum*, *Excoecaria agallocha*, *Acanthus illicifolius* and *Xylocarpus spp* (Singh et al. 2004). Almost all species are found in most of the river estuaries. *Kandelia*, a rare species on the west coast, is found in plenty in the Mandovi and Zuari estuaries. However, *Avicennia* species is dominant in most regions with high salinity and *Sonneratia* species is dominant in low salinity areas. The Mandovi river houses most of the species found in Goa. The Chapora estuary is 30 km long, having *Sonneratia alba*, *Aegiceras corniculatum*, *Avicennia officinalis*, *Avicennia marina*, *Acrostichum aureum*, *Acanthus illicifolius*, *Bruguiera gymnorhiza*, *Excoecaria agallocha*, *Kandelia candel*, *Thespesia populnea* (a mangrove associate), *Rhizophora mucronata*, and *Acacia intsia*. Tall grasses were also observed along the estuary. *Cocos nucifera* and *Thespesia populnea* are commonly found along the banks (Singh et al. 2004).

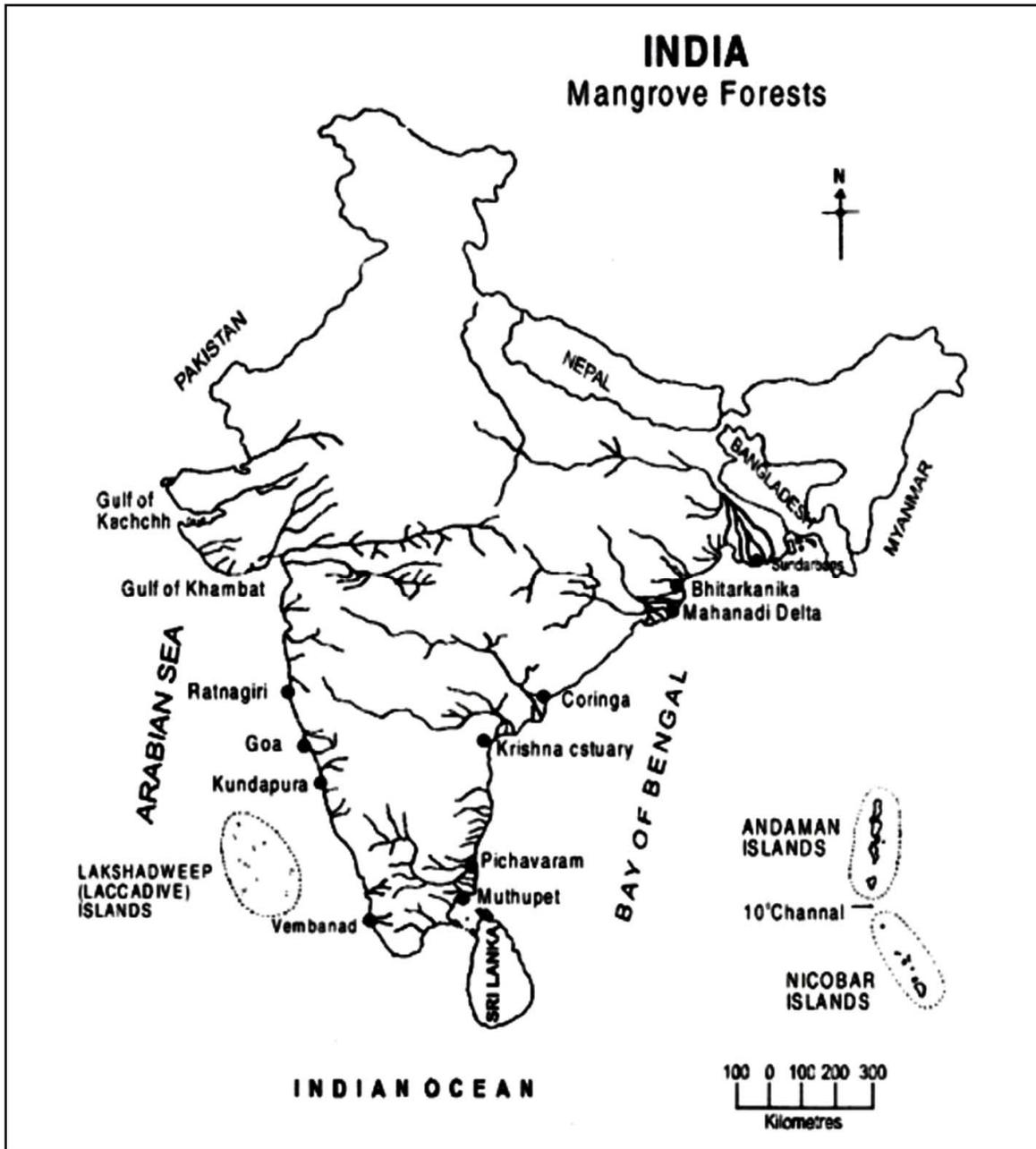


Figure: 4. Distribution of mangrove (•) in Indian subcontinent
 Source: (Kathiresan and Qasim, 2005)

3.2. Sampling Period:

Monthly samples were analyzed for a period of one year i.e. September 2007 to August 2008. Data obtained from the observations made, was subsequently grouped to tally with three seasonal periods viz. pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January). The sediments samples were analyzed for a period of pre-monsoon (April), monsoon (July) and post monsoon (December) from both the sites for the metals i.e Mn, Co, Cu, Cr and Pb.

3.3. Sample collection:

Sediment cores (0-10 cm) from Tuvem and Diwar were collected in triplicates using a 1.5 inch diameter and 15 cm long graduated PVC pipe along with overlying water samples. The cores were sealed at both ends with sterile core caps to prevent direct contact with air and transported to the laboratory in an icebox for further physico-chemical and microbiological analyses. Water samples were collected in sterile glass bottles. All collections were carried out during the low tide.

3.4. Measurement of Physico-chemical Parameters:

The physico-chemical parameters of the selected sites were monitored. For the mangrove water, the parameters observed and estimated were salinity and dissolved oxygen. Sediment characteristics include temperature pH, Eh, trace metals, total organic carbon, labile organic matter, C/N ratio, moisture content,

density and grain size. Sediment pore water was collected by slow centrifugation (5000 rpm) and analyzed for sulfate, sulfide and salinity.

3.4.1. Hydrological Parameters:

3.4.1.1. Salinity:

Salinity was measured using a hand refractometer (S/Mill-E, ATAGO Co. Ltd., Japan) calibrated to zero with distilled water.

3.4.1.2. Dissolved oxygen (DO):

The dissolved oxygen (DO) concentration in the water samples was estimated using Winkler's titrimetric method (Carpenter, 1965). Water samples were collected in 125 ml acid washed (10% HCl) glass stopper bottles and fixed immediately with 1 ml each of Winkler's A & B (Composition in appendix). The samples were mixed and the precipitate was allowed to settle. The sample was acidified with 1 ml of (10 N) sulfuric acid and then titrated in the laboratory with 0.01 N sodium thio-sulfate using starch as indicator. The procedure was standardized using potassium iodate. The concentration of DO was expressed as ml per liter.

3.4.2. Bulk sediment parameters:

3.4.2.1. Temperature:

Temperature was measured with a field thermometer (76 mm immersion, ZEAL, England).

3.4.2.2. *Hydrogen Ion concentration (pH):*

pH was measured using a digital pH meter (Thermo Orion model 420 A, USA) after calibrating it with the standard buffers of pH 4, 7 and 10.01 respectively.

3.4.2.3. *Moisture content:*

The moisture content of the sediment was estimated by taking 1 gm each of wet sediment and drying it overnight at 90 °C in the oven and weighed. The difference in weight of dry and wet sample was expressed as the moisture content in percentage (%) dry weight of sediment.

3.4.2.4. *Density:*

Sediment density was determined from the weight and volume of wet sediment sample. A known volume of sediment (1ml) collected with a plastic syringe without the luer tip (mini corer) was weighed and density was calculated.

3.4.2.5. *Redox potential (Eh):*

Combination of redox electrode (Orion Research cat No. 967800) was used to check the redox potential of sediment cores. The redox electrode was calibrated with standards of solution A and solution B (Composition in appendix) as per Orion Manual. Solution A was transferred to a beaker and redox electrode placed in the solution. Once the reading stabilized the potential was verified to be around 192 ± 2 mV. The electrode was rinsed and the measurements were repeated with solution B and verified to be around 258 ± 5 mV (i.e. approximately 66 mV greater than A).

The sealed anoxic graduated core was opened and the redox potential was checked immediately by introducing the electrode at specific points in the core i.e. 0-2, 2-4, 4-6, 6-8 and 8-10 cm as described in Orion instruction manual.

3.4.2.6. Estimation of metal concentration in sediments:

Sediment samples were analyzed for major and trace metals as described by Balaram et al, (1995). The sediment digestion was carried out in a sealed Teflon vessel (digestion bombs). Powdered dried sediment (0.2 g) was transferred into a Teflon (P.T.F.E., polytetrafluoroethylene) vessel and digested with a solution (10 ml) of concentrated HF, HNO₃, and HClO₄ in a ratio of 7:3:1. The above mixture was then dried on a hot plate in a fume hood chamber. The residue was further treated with 5 ml of acid mixture. Subsequently 2 ml of concentrated HCl was added followed by 10 ml of HNO₃. The residue was warmed and transferred to a clean, dry flask to make a final volume of 50 ml with double d/w. The concentrations of iron and other metals (i.e Mn, Co, Zn, Pb and Cr) in digested sediments were analyzed with an atomic absorption spectrophotometer (AAS; GBC 932 AA model) equipped with deuterium background corrections. Blank corrections were applied wherever necessary and the accuracy was tested using standard reference material MAG-1 (United Geological Survey) and GR-1 (Green River sediment). All the acids used were procured from Merck. Concentrations of metals were expressed in ppm except iron was expressed in percentage.

3.4.2.7. Total organic carbon (TOC):

TOC was estimated by titrimetry according to Allen et al. (1976) method that involves complete wet oxidation of organic matter in the sample with chromic acid. Sediment samples were dried overnight at 70 °C in the oven and ground to a fine powder using a mortar and pestle. Sediment sample (0.5 g) was weighed in a conical flask with 25 ml of acid dichromate and incubated in a water bath at 60 °C for one hour. Distilled water (100 ml) was added and titrated against ferrous ammonium (Mohr's salt) using diphenylamine as the indicator (0.5 ml or 15 drops). The end point was noted as the change in color from dark blue to green. Blanks were processed without sediments (values which were subsequently subtracted). Glucose (0.01g) was used as standard. The TOC was expressed as percentage.

3.4.2.8. Labile organic matter (LOM):

Labile organic matter (LOM) was calculated as the sum total of the protein, carbohydrate and lipid fraction of the dried sediments.

3.4.2.8.1. Protein:

Protein was estimated with Lowry et al. (1951) method. A known amount of dry sediment (0.1gm) was weighed in a centrifuge tube and 2 ml of NaOH was added to the tube, vortexed and mixed properly. Tubes were kept in boiling water bath for 5 minutes and allowed to cool at room temperature ($28 \pm 2^{\circ}\text{C}$). Centrifuged at 5000 rpm for 5 minutes till the appearance of a clear supernatant. An aliquot supernatant of 0.5 ml in triplicates was used for protein estimation. Distilled water (0.5 ml) was added to 0.5 ml supernatant and mixed. Reagent C (5ml)

(composition in appendix) was added, vortexed and kept in the dark for 10 minutes. Folin Ciocalteu reagent (1:1 dilution) (0.5 ml) was added and vortexed and incubated in the dark for 20 minutes. Absorbance was recorded at 750 nm using a Shimadzu spectrophotometer. For calibration, 50 mg of bovine serum albumin (BSA) dissolved in 50 ml of 1N NaOH was used, and a working stock was prepared using distilled water. A reagent blank was used as the control. The protein concentration was expressed as percentage (% , g/100g).

3.4.2.8.2. Carbohydrate:

Carbohydrate was estimated according to Dubois et al. (1956) method. A known amount of sediment (0.1 gm) was weighed in a centrifuge tube and 2 ml of 5% TCA was added to each tube for extraction. Tubes were kept in boiling water bath for 3 hrs at 80 to 90 °C. Tubes were allowed to cool at room temperature and centrifuged at 5000 rpm for 5 minutes to obtain a clear supernatant. The supernatant (0.5 ml) was used for the carbohydrate estimation. It was mixed with 0.5 ml distilled water and 1 ml of phenol reagent (composition in appendix). Concentrated H₂SO₄ (5 ml) was added from the burette and mixed during addition and incubated for one hr. The absorbance was recorded at 490 nm. A standard curve was prepared using D-glucose as a standard. Reagent blank with 1 ml distilled water was used as a control. The values were expressed as percentage (% , gm/100gm).

3.4.2.8.3. Lipids:

Lipid content was estimated by the acid dichromate method outlined by Parsons et al. (1984). Sediment (0.1gm) was weighed in a centrifuge tube. Organic solvent (8 ml) (composition in appendix) was added for extraction of lipids and vortexed for one minute. Tubes were centrifuged at 5000 rpm for 5 minutes to obtain a clear supernatant. The supernatant was transferred to a separating funnel. CHCl_3 , (2ml) and 2 ml of distilled water were added, mixed thoroughly and allowed to stand. The lower layer was collected in an evaporating flask and evaporated to dryness in a rotary vacuum (Equitron Roteva). The dried lipid was transferred to a tube and 2 ml of 0.15% acid dichromate (composition in appendix) was added and kept in a boiling water bath for 15 minutes. The tubes were allowed to cool at room temperature and 4.5 ml d/w was added, mixed and again cooled. Absorbance was recorded at 440 nm. Stearic acid was used as the standard. Reagent blank was used as a control. Values were expressed as percentage (% , gm/100gm).

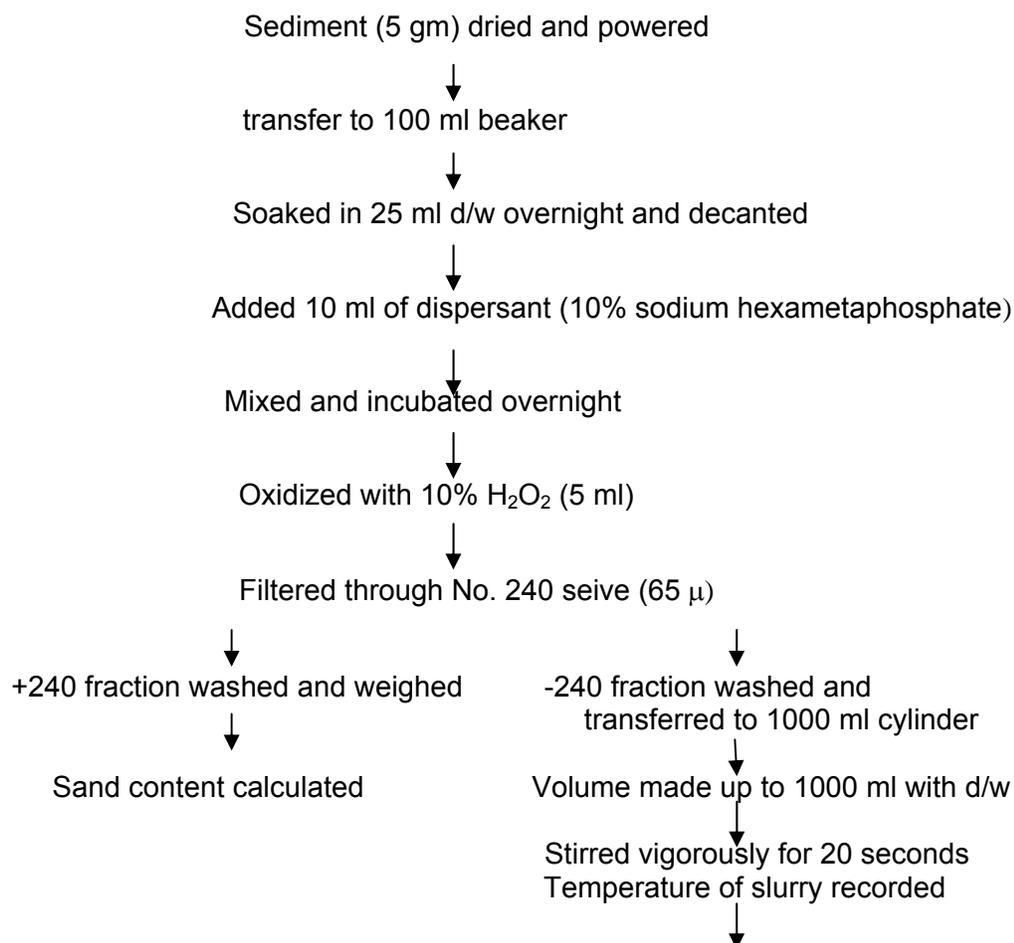
3.4.2.9. C/N Ratio:

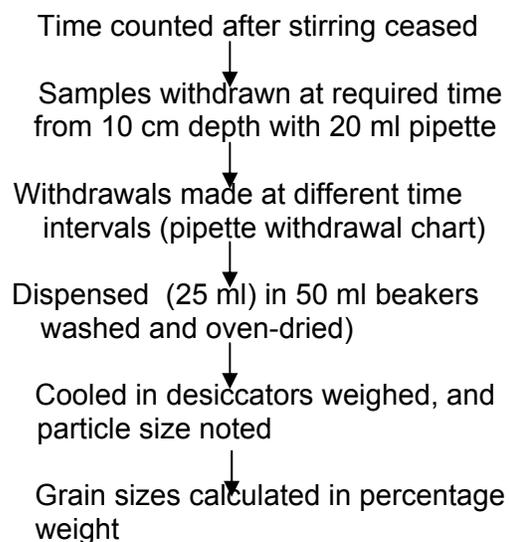
The sediments used to estimate organic carbon and nitrogen content were dried at $60(\pm 2)^\circ\text{C}$ for 48 h. Samples were placed either in an automatic grinder for homogenization or homogenized manually with mortar and pestle. Once homogenization was complete, a small aliquot (0.1 g) was taken in a beaker. To this aliquot 1-2 ml of 1N HCl was added and observed for effervescence. The procedure was repeated till no effervescence was seen and allowed to settle

overnight. The supernatant was discarded and sediment was washed with milli-Q water and dried in an oven, ground with a mortar and pestle and used for analyses. The samples were analyzed using an Elemental Analyzer (Thermo Finningan, Flash EA1112) with L-cystine as a standard. Precision of analyses was checked against NIST 1941b.

3.4.2.10. *Grain Size analysis:*

The quantity of sand, silt and clay fractions in of mangrove samples at five different depths were determined using the wet sieving method for sand and the pipette method for silt and clay analyses (Day, 1965; Carver, 1971).





The particle size was noted based on the pipette withdrawal as given below (Table 1) and the grain size analyses was calculated in terms of percentage of sand, silt and clay.

Table: 1. Time table for pipette withdrawal

Diameter of Particle (mm)	< .625	< .031	< .016	< .008	< .004	< .002	< .0005
Depth of Withdrawal (cm)	10	10	10	10	5	5	3
Time of Withdrawal	seconds	min'/sec"	min'/sec"	min'/sec"	min'/sec"	hour:/min'	hour/min
Temperature (Celsius)							
20	29	1' 55"	7' 40"	30' 40"	61" 19"	4: 05'	37: 21'
21	28	1' 52"	7' 29"	29' 58"	59' 50"	4: 00'	
22	27	1' 50"	7' 18"	29' 13"	58' 22"	3: 54'	
23	27	1' 47"	7' 08"	28' 34"	57' 05"	3: 48'	
24	26	1' 45"	6' 58"	27' 52"	55' 41"	3: 43'	33: 56'
25	25	1' 42"	6' 48"	27' 14"	54' 25"	3: 38'	
26	25	1' 40"	6' 39"	26' 38"	53' 12"	3: 33'	
27	24	1' 38"	6' 31"	26' 02"	52' 02"	3: 28'	
28	24	1' 35"	6' 22"	25' 28"	50' 52"	3: 24'	31: 00'
29	23	1' 33"	6' 13"	24' 53"	49' 42"	3: 10'	
30	23	1' 31"	6' 06"	24' 22"	48' 42"	3: 05'	

3.4.3. Ambient Sulfate, Sulfide and Salinity:

3.4.3.1. *Pore-water extraction:*

Extraction of interstitial waters is usually done with pressure-operated squeezes or centrifugation. In the first method, the sediment core is crushed mechanically under high pressure to expel the water (Manheim, 1966; Kriukov and Manheim, 1982; Zimmermann et al. 1978; Bender et al. 1987; Nath et al. 1988). Alternatively the pressure is generated by passing an inert gas through the core that displaces the pore water (Reeburg, 1967). In the centrifugation method, the sediment core is placed in a tube and centrifuged at 5000 rpm to expel the pore water, which then is siphoned off. High vacuum suction has also been used to recover pore waters (Manheim, 1966). An advantage of these methods is that since the sections of the core are well defined, it would be possible to obtain profiles of distribution of elements. In the present study pore water was collected by centrifugation method at 5000 rpm. Cores were sectioned at 2 cm interval, loaded separately into centrifuge tubes. The tubes were spun at low R.P.M (5000) at 4 °C for 10 minutes (REMI Cooling Centrifuge). The water was then carefully siphoned out into a pre-cleaned 100 ml polyethylene bottle. Further, the pore water was filtered on GF/F and then subsequently filtered on 0.22 μ membrane filter. The filtrate was analyzed for sulfate, sulfide and salinity.

Spinning at low temperature and RPM ensured minimal disturbance to any benthic organisms in the sediments, which on lysis could change the pore water chemistry. The advantage of this technique is enhancement of the possibility of profiling without compromising on changes arising during handling. Thus the

measurements of parameters in interstitial waters or the rates of sulfate and sulfide were least affected by external factors associated with handling.

3.4.3.2. Sulfate (SO_4^{2-}):

SO_4^{2-} was estimated by turbidometry (Standard methods, 1981). (APHA). Water (1ml and 2 ml) was transferred into screw cap tubes and acidified to pH 1 with 4 N HCl. The acidified samples were neutralized stepwise using 10 N NaOH, 1 N NaOH and 0.1 N NaOH so as to bring to pH 5, 6 and finally to 7 respectively. The volume is made up to 25 ml with distilled water. Conditioning solution (1.25 ml) is added agitating continuously with a magnetic piece. 2 ml BaCl_2 of (30%) was added, stirring continuously for 1 minute. The sulfate ions are precipitated in such a manner that uniform size precipitate of barium sulfate is produced. The optical density is measured at 365 nm against a blank in a spectrophotometer model (Milton Roy Spectronic – 1201) after 10 minutes of incubation. For each set of estimations, a standard with ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$, (40 mg/L) was prepared (composition in appendix).

3.4.3.3. Sulfide (S^{2-}):

Water (1ml) was fixed in 10 ml of 2% zinc acetate in screw capped sterile test tubes for determining the sulfide concentration (Cline, 1969). These contents were transferred into a volumetric flask to which 5 ml dimethyl-para-phenylenediamine sulfate (DMPD) was added, followed by 0.5 ml of iron III ammonium sulfate (FAS), mixed and allowed to stand for 10 minutes. The volume was made upto 50 ml with d/w. The sulfide ion reacts with N, N-dimethyl-p-

phenyle-nediamine and produces a methylene blue colour, which is spectrophotometrically measured at 670 nm against a blank. The amount of sulfide present in the sample was calculated from the standard curve of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$.

3.4.3.4. Salinity:

Salinity was measured using a hand refractometer (S/Mill-E, ATAGO Co. Ltd., Japan) calibrated to zero with distilled water.

3.5. SRA: ^{35}S isotope method:

3.5.1. Preparation of samples:

Cores were purged with oxygen free nitrogen gas and stored at 4 °C till analyses. Sub-samples were taken with 2 ml disposable syringes from the core at 0-2: 2-4, 4-6, 6-8 and 8-10 cms sections. The sediment samples were transferred into glass vials and supplemented with autoclaved seawater and thoroughly mixed. Nitrogen gas was purged in 2 sets of duplicates in glass vials. 10 μl (i.e. 74 K Bq) of $^{35}\text{SO}_4^{-2}$ (Sodium sulfate) was injected along the length of the 2 cm sub-core slurry to distribute the label evenly and was incubated for 6 hrs in dark.

3.5.2. Distillation, trapping and assay of reduced ^{35}S end products:

Radiotracer assay in sediments was carried out using single step chromium reduction assay (King, 2001). Here the reduction of non sulfate ^{35}S sulfur and distillation of ^{35}S sulfides was carried out in 3 necked round-bottom boiling flasks (20 ml quantity) heated on a heating mantle. Schematic of a distillation apparatus for trapping the chromium reducible sulfur is given in Figure 6. The flask was

connected to a condenser through which cooling water flowed at a moderate rate. The condenser outlet was constructed of a length of teflon tubing that connected a series of traps (3 glass vials) containing a solution of 5% zinc acetate (5 ml per trap). The glass vials were capped with butyl rubber stoppers sealed with aluminum caps, one neck of the flask had a gas inlet to control a flow of nitrogen (30 ml. min^{-1}) to the boiling flask. The unused port was sealed with a ground glass plug that could be opened to introduce samples and chromous chloride. Ten ml of 5% zinc acetate was transferred into

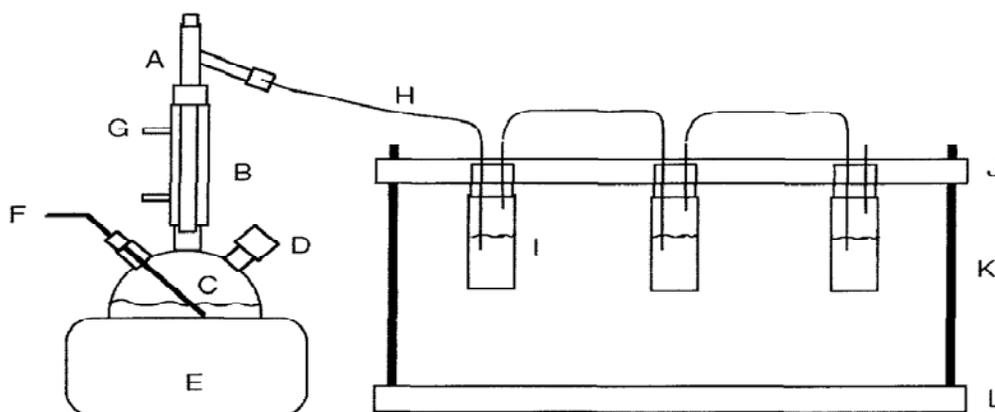


Figure: 6. Modified from Kings (2001). Schematic of a distillation apparatus for trapping chromium reducible sulfur. T-connector (A) leading from condenser (B) to a length of Teflon tubing (H); inlet and outlet for condenser cooling water indicated by G (note: several condensers may be connected in series). 100 or 200 ml three-neck boiling flask (C) with closure (D) on one port for adding sample and chromous chloride. Inlet (F) for a stream of nitrogen from a valve and flow regulator. Heating mantle (E) for bringing chromous chloride to boiling. Scintillation vial (I) with zinc acetate mounted in an acrylic board (J) with vial caps recessed into the board. Threaded rod (K) mounted onto acrylic base or plywood base coated with polyurethane to facilitate cleaning.

the round bottom flask and the sub-core (2 cm^3) with the radio labeled sulfides was added and gently swirled to mix and 10 ml chromous chloride was added to the flask (preparation of chromous chloride in appendix). The mixture was boiled for ca. one hour. The glass vials were removed and pooled together (total 15 ml). 1 ml

was transferred into scintillation vials with 5 ml of cocktail W, SRL 03200117 (Sisco research lab, Bombay) in triplicates. Blank was 5 ml cocktail + 1 ml zinc acetate + 10 μ l of the radiolabeled ^{35}S . The samples were kept in scintillation vials for 24 hours for stabilization and then analyzed using a Perkin Elmer Wallac 1409 DSA liquid scintillation counter. The sulfate reduction rate was calculated using the formula:

$$\text{SRR} = (\text{H}_2^{35}\text{S}/^{35}\text{SO}_4^{2-}) \times ^{32}\text{SO}_4^{2-} \times \text{IDF}/\text{T}$$

SRR = reduction rates

H_2^{35}S = radioactivity of reduced sulfur (DPM)

$^{35}\text{SO}_4^{2-}$ = radioactivity of at the beginning of incubation

$^{32}\text{SO}_4^{2-}$ = pore water concentration ($\mu\text{M SO}_4^{2-} \text{ cm}^{-3}$)

Where IDF (Isotopic discrimination factor) = 1.06

T = time of incubation in hours.

SRR was expressed as $\text{nM cm}^{-3} \text{ d}^{-1}$

Sub-samples of chromous chloride sediment slurries (100 μ l) for estimating the unreduced ^{35}S -sulfate were also fixed in 5 ml of the cocktail and analyzed.

3.5.3. Mesocosm Experiments- Factors influencing sulfate reduction:

Slurry experiments were carried out with Tuvem sediments. Such experiments could be conducted only with reference site where background concentrations of iron were low at 3.66%. Aliquots of sections were prepared as mentioned before. Approximately 2 ml of aliquots of sediments were put into 5 ml

vials in triplicates and then amended with 2 ml of water soluble ferric chloride and manganese chloride stock solutions to give final concentrations of 50, 100, 200, 500 and 1000 ppm of ferric chloride and manganese chloride respectively in two sets of vials. Separate experiments were also carried out to study the impact of sulfate amendments upto 20mM and carbon upto 30mM. Controls without amendments were included. All the vials were flushed with oxygen free nitrogen gas. The resulting sample slurries were acclimatized for 1 hr and subsequently 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 in the dark. Samples were then fixed and analyzed using King's assay as described above (section 3.5.2).

3.7. Bacteriological Studies:

3.7.1. Total Bacterial Counts (TC):

Sediment cores were brought to the laboratory and sectioned at 2 cm intervals up to a depth of 10 cm aseptically. The subsamples were serially diluted to 10^{-2} with mangrove water and were fixed immediately with buffered formalin for total bacterial counts. Bacterial abundance was determined by acridine orange direct count (AODC) method (Hobbie et al. 1977). Sediment dilutions (2 ml) preserved with 2% (final concentration) buffered formalin were stained with acridine orange (Hi-Media, Mumbai) (final concentration 0.01% w/v) for five minutes and then filtered on to black stained nucleopore filter with a pore size of 0.22 μ m. Samples were counted with Olympus (BH) epifluorescence microscope, using a 515 nm barrier filter and at least 10 fields of >30 bacteria field⁻¹ were counted. Bacterial abundance was expressed as numbers per g wet weight of the sediment. Flowchart is given below

3.7.2. Plate counts:

Sub samples (10 g) sediment from each of 2 cm sediment core were sampled using sterile syringe cores. The samples were transferred to 90 ml of full strength sterile seawater (10^{-1} dilution). Tween80 (50 μ l) was added and the mixture was sonicated at 40 mHz for 10 seconds. Serial dilutions of the sediment samples were made in autoclaved seawater to yield dilutions from 10^{-1} to 10^{-7} . Heterotrophic bacteria were isolated on nutrient strength of 100% + 2% agar (A concentration of 100% corresponds to 8 g nutrient broth HiMedia Laboratories Pvt. Ltd., Bombay, India) (Composition in appendix) prepared in seawater. 100 μ l from each dilution in triplicates were plated on nutrient agar plates. Bacterial counts in the form of colony forming units (CFU) formed on the medium were recorded after a 5 day incubation period at 28 ± 2 °C. Bacterial colonies were enumerated and expressed as CFU per g wet weight of sediment.

3.7.3. SRB:

3.7.3.1. Agar shake counts:

The agar shake tube method involves exclusion of part of the oxygen from the medium and is affected by growing the organisms inside the culture medium, this being semi solid media. Here growth is similar as in shake culture in a tube. The media used for isolation, enrichment and enumeration of SRB was a modification of Hatchikian's medium (1972), which was prepared in sea water from the mangroves.

For the enumeration of colony forming unit (CFU) mangrove sea water (10 ml) and sediments from 0-2, 2-4, 4-6, 6-8 and 8-10 cm (ca 10 gms) were serially diluted in autoclaved sea water upto 10^{-7} dilution. 2.5 ml of dilutions from 10^{-2} , 10^{-3} and 10^{-4} were inoculated into screw cap test tubes containing modified Hatchikian's agar media (Appendix), which was supplemented with one of the substrates viz. sodium lactate (0.75% v/v), sodium acetate (0.2% w/v). After gently tilting the tube up-side down 1-2 times, the tubes were placed in cold water and solidified agar tubes were overlaid with a sterile paraffin oil/paraffin wax (2:1) mixture to prevent entry of air. Black colony forming units were counted within 8 days of incubation at room temperature (28 ± 2 °C) in the dark until the number showed no further increase. The colony numbers were expressed as CFU per g wet weight of sediment. Assays were carried out in triplicates and averages \pm SD were expressed.

3.7.3.2. MPN method:

Sulfate reducing bacteria (SRB) were also enumerated by the most probable number (MPN) on Hatchikian medium (1979) by using both lactate (1 mM) and acetate (1 mM) as carbon sources. One ml of each dilution (10^{-1} to 10^{-7}) was inoculated in triplicates in both the lactate and acetate media until the inoculation from the highest dilution yielded negative results. The culture tubes were incubated in the dark for a period about 30 days at $28(\pm 1)$ °C. After incubation, the tubes were tested for the presence of sulfide. The combinations of positive and negative tubes were scored and MPN was assessed from McCready's table (Rodina, 1972).

3.7.4. Identification:

3.7.4.1. Isolation:

Pure cultures of SRB were obtained by isolating colonies from highest agar dilutions. The purified cultures were maintained in Hatchikian's liquid medium prepared in mangrove sea water. Precautions were taken to grow the SRB under low and non-detectable levels of oxygen by bubbling nitrogen gas when necessary and resazurine (0.5mg/L) was used intermittently as an indicator of anaerobic conditions. SRB, in large volumes were cultured in 125 ml reagent bottles. For smaller volumes and pure culture maintenance, 15 ml screw-cap test tubes were used.

3.7.4.2. Classical Taxonomy:

The tests for identification of SRB upto the genus level was carried out for 88 pure isolates. Standard protocols were followed for SRB identification. Classical taxonomy was carried out on the basis of morphological, physiological and biochemical characteristics as described in Bergey's Manual of Systematic Bacteriology 1984, 1986, and 1989 and The Prokaryotes (1991).

3.7.4.2.1. Motility:

Hanging drop preparation was used to ascertain motility.

3.7.4.2.2. Gram staining:

The Gram staining of cultures was performed as discussed by Hans Christian Gram (1884).

3.7.4.2.3. Cytochrome identification:

To check the presence of cytochrome a, b and c, spectra of air oxidized and sulfide reduced suspension (1-2 drops of saturated solution of Na₂S) of whole cells were determined using a scanning spectrophotometer UV-1601 UV-visible spectrophotometer, Shimadzu. Scanning was carried out from 500 nm to 700 nm to check for appearance of absorption peak at 605, 565 and 505 nm for Cytochrome a, b and c respectively. The culture supernatant with Na₂S was used as a blank (Postgate, 1979).

3.7.4.2.4. Desulfovirdin test:

A dense suspension of cells from liquid culture was treated with 1 to 2 drops of 2 M NaOH and was examined for fluorescence with a spectrofluorimeter Shimadzu RF5300. The excitation was fixed at 365 nm and emission was scanned from 400 to 900 nm (Postgate, 1979).

3.7.4.2.5. Catalase Test:

0.1 ml of SRB from liquid culture was removed with a pasteur pipette on clean dry slides and exposed to air for 30 minutes. Later a drop of 3% hydrogen peroxide was added. The slide was observed after 5 minutes for bubbles either macroscopically or with a low-power microscope.

3.7.4.2.6. Oxidase Test:

An aqueous solution of 1% tetra methyl-p-phenylenediamine was freshly prepared. Sterile filter paper strips 1 cm x 2.2 cm was soaked with the above

solution. The tube with the SRB culture was vortexed and 0.1 ml of the culture suspension was smeared on the moistened paper. A positive test was indicated by a purple blue coloration within 30 seconds.

3.7.4.2.7. *NADH Oxidase:*

The NADH oxidase activity of SRB extracts were assayed spectrophotometrically at 340 nm by following the rates of aerobic disappearance of NADH.

5 ml of the SRB culture suspension was sonicated using an ultrasonicator (Vibra cell) with a continuous pulse for 90 seconds. The sonicated suspension was centrifuged using an Eltek Research Centrifuge TC 4100D USA. 1 ml of this supernatant was taken and 500 μ M of the Tris HCl (pH 7.0) and 10 μ M NaDH was added. The volume was made upto 2 ml. Absorbance was measured at 340 nm in a Milton Roy Spectronic 1201 Spectrophotometer. Decrease in the OD showed the presence of the enzyme NADH oxidase (O'Brien and Morris, 1971).

3.7.4.2.8. *Substrate utilization:*

SRB isolates were checked for growth on other substrates besides the one they were isolated on. Substrate utilization was tested in SRB liquid media with the following substrates (final concentration): Sodium acetate (0.2% w/v), propionate (0.07% w/v), butyrate (0.08% w/v), benzoate (0.05% w/v), sodium pyruvate (0.22% w/v), formate (0.01% w/v), palmitate (0.05% w/v), ethanol (1% v/v), sodium lactate (0.6% w/v). Growth, sulfide production, cell counts and pH of the final media after 10 days of incubation were noted. To check the utilization of

glucose 0.2 ml of each of the 88 SRB isolates were inoculated in liquid SRB media containing 1% of glucose. The tubes were incubated for 10 days. Increased in sulfide was measured as a signature of positive growth as described earlier.

3.7.4.2.9. NaCl tolerance:

SRB isolates were inoculated (0.2 ml) in Hatchikian's liquid media prepared by adding 0, 2, 4% NaCl as a final concentration. The growth of the isolates was noted positive based on the sulfide produced in the media.

3.8. Bioremediation of cobalt using SRB:

3.8.1. Media Used:

Pure cultures (SRB) grown with lactate (1 mM) as a carbon source in Hatchikian's media with 0.8 % agar were used for the present study. Bioremediation experiments were conducted by isolating pure cultures in liquid Hatchikian's media.

3.8.2. Screening of SRB for Cobalt Tolerance:

To isolate the potential isolates for bioremediation 88 isolates were screened for cobalt tolerance. Cobalt being the highest contamination factor in both Diwar and Tuvem throughout all the three seasons in the mangrove sediments was selected as the metal for bioremediation. The isolates (88) were grown in liquid Hatchikian's media with lactate as a carbon source in screw cap tubes. Cobalt was added in final concentrations of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 mg/L. Resazurin was used as a redox

indicator (0.5 mg/L). Resazurin is colorless in a medium at pH 7 that has a value of $E_h \leq -100$ mV (Twigg, 1945).

An aliquot of the SRB (master culture) was washed thoroughly by suspending it in autoclaved 0.89% saline and centrifuged at 500g for 10 minutes (thrice) to ensure the removal of traces of dissolved sulfide prior to its use as inoculum for the cobalt tolerance study. One ml of cell suspension was used as inoculum in the above prepared media tubes and incubated in dark for 25 days. All the tests were carried in duplicates. Growth of the individual culture was monitored by measurement of sulfide. Hatchikian's media without SRB and cobalt and Hatchikian's media without cobalt with SRB (duplicate tubes) were maintained as two controls.

3.8.3. Bioadsorption and Bioremediation studies:

3.8.3.1. Bioadsorption

Isolate SKKL8 was chosen as the best candidate due to its tolerance to 600 ppm of cobalt. The bacterial cell pellet (SKKL8) tolerating 500 mg/L of cobalt was dispersed in 100 mL of Hatchikian's media supplemented with lactate with 500 mg/L cobalt solution in Erlenmeyer flasks and placed in a Remi orbital shaker at 200 rpm at 35 °C, incubated for 30 days. The residual cobalt (2 ml) in the solution was filtered through a 0.22 µm membrane filter. The cobalt in the filtrate (2 ml) was analyzed using an atomic absorption spectrophotometer. The amount of cobalt bioadsorbed was obtained by taking the difference between the initial and final concentrations of cobalt in the filtrate. The bacterial cell was used solely as a surface for bioadsorption.

3.8.3.2. Bioremediation using active cells of SKKL8 and Co at 500 ppm.

Bioremediation was carried out in screw cap tubes (16 ml) using the pure isolate (1ml) in Hatchikian's medium devoid of iron in the presence of 500 mg/L of cobalt in triplicates. The initial cell protein concentration in 1 ml of cell suspension was measured. The media- pH, Eh, sulfide, cobalt concentration and cell protein were monitored every three days. Samples for cobalt analysis were filtered through 0.2 μm membrane filter and analyzed. Hatchikian's media without SRB and cobalt (negative control) and Hatchikian's media without cobalt with SRB (positive control) were maintained as two controls. The above parameters were analyzed as mentioned in section 3.8.6.

3.8.3.3. Bioremediation using hydrogen sulfide stripped of bacteria and Co at 5000 ppm:

Isolate SKKL8 wherein the bacterial growth and sulfide precipitation of the cobalt ion take place simultaneously, a set-up (Figure 7) was fabricated to carry out the bioremediation process. A serum bottle was fitted with an inlet and an outlet connected to two separate tubes. The outlet tube was opened into an Erlenmeyer flask containing the desired metal solution of known concentration to be precipitated. The flask was sealed with a cotton plug. The serum bottle containing a fully grown Isolate SKKL8 in Hatchikian's medium devoid of iron was purged with nitrogen gas through the inlet tube, which displaced the bacterially produced hydrogen sulfide gas through the outlet tube into the Erlenmeyer flask. The copious evolution of hydrogen sulfide gas resulted in the precipitation of cobalt

added (5000 ppm) as its sulfide (BPH-CoS) in the flask. The diagram of the experimental set-up is shown in Figure

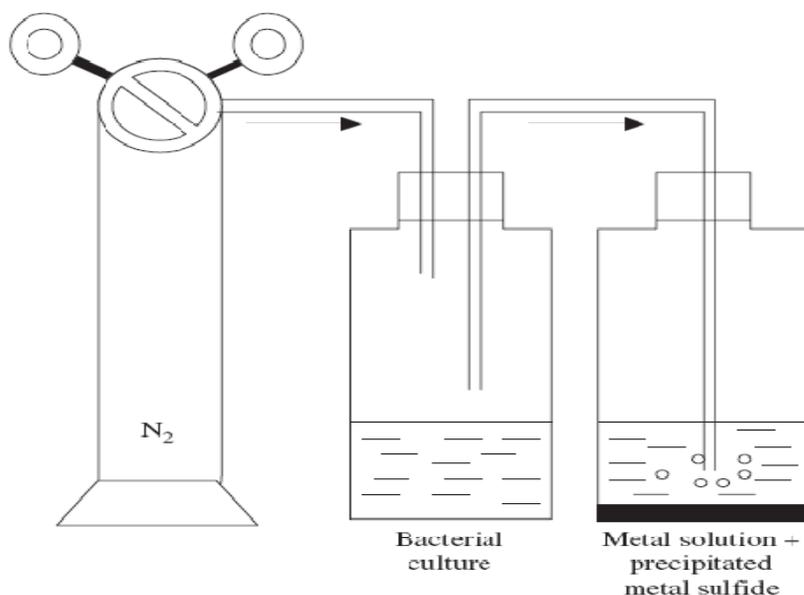


Figure: 7. Schematic diagram of the experimental set-up for Co bioremediation.

3.8.4. Analytical Techniques:

Eh and pH were measured by Thermo Orion instrument supplied with separate Eh and pH electrode. Total cell protein was determined using quantitative colorimetric Coomassie assay (Bradford, 1976). SRB (0.5 ml) samples from Hatchikian's media were withdrawn from the tubes and placed in test tubes with 0.5 ml of 1 N NaOH. Each tube was covered with a steel cap and incubated in an oven at 99 °C for 10 minutes. The tubes were left for 15 minutes at room temperature (28±2 °C) to cool. After 15 minutes 0.1 ml of 6 N HCl was added to each tube and vortexed. Coomassie reagent (1ml) was added to each tube, vortexed and left for 15 minutes. The absorbance of each solution was measured at 595 nm and compared to a standard curve generated for bovine

serum albumin (0 µg/ml to 200 µg/ml). Samples (2 ml) Hatchikian's media with SKKL8 were filtered using a 0.22 µm membrane filter. The filtrate was diluted with 3% HNO₃ and aqueous cobalt concentrations were measured using an atomic absorption spectrophotometer (GBC 932 AA model). Filtrate (1 ml) was fixed with zinc acetate (5%w/v) and sulfide concentrations were measured spectrophotometrically using the methylene blue method (Cline, 1969). For sulfate concentrations, a known amount 0.5 ml and 1 ml samples were fixed with one drop of concentrated HCl and analyzed by turbidometric method (Clesceri, 1969).

3.8.5. Characterization of metal precipitate:

Scanning electron microscopy coupled with energy dispersive X-ray spectrometry (JEOL JSM-5800LV, USA) was done to examine the chemical nature of the precipitate produced by SSKL8 and in control. The dried precipitate was placed on a metal stub and coated with gold prior to analysis using SEM-EDX (Nadagouda et al. 2009).

3.8.6. Characterization of the isolate:

3.8.6.1. Morphological and biochemical characteristics of the isolate:

The SKKL8 was identified using standard microbiological methods. Morphology, vegetative cell and spore characters were observed under a phase contrast microscope (100X objective) with a 4 day old culture. The physiological and biochemical characters viz. oxidase, catalase, NADH oxidase, desulfovirdinn, cytochrome identification and salt tolerance were checked in liquid Hatchikian's media. Electron donor utilization by isolate SKKL8 for lactate, acetate, benzoate,

butyrate, formate, propionate, pyruvate, ethanol, and glucose was checked. The concentration for all the substrates were used same as mentioned in section 3.7.4.2.8.

3.8.6.2. 16S rRNA gene sequencing

Phylogenetic analysis of bacterial strain SKKL8 was carried out by sequencing the 16S rDNA sequence amplified using eubacterial universal 16S rDNA primers (Brosius, 1978). The primer set (forward 5'GGATTAGATACCCTGGTA-3' and reverse 5' GACTACCAGGGTATCTAATC-3') were used to amplify the 16S rDNA. Sequencing of amplified PCR products was carried out with ABI 3100 (Applied Biosystems, Foster City, USA). The sequence was later compared to sequences of type strains from Ribosomal Database Project (RDP) (<http://rdp.cme.msu.edu>). These sequences were downloaded from the NCBI (National Centre for Biotechnology Information) database and phylogenetic trees were constructed using PHYLIP (Felsenstein, 2006).

3.8.6.3. Calculation for carbon mineralization via sulfate reduction

Carbon mineralization has been calculated based on Redfield's stoichiometry for organic matter oxidation by sulfate reduction $C_{org}: SO_4^{-2} = 2.1$ (Volkov et al, 1998) and on the basis that on an average 2 moles of organic carbon (C) is oxidized for every mole of sulfate reduced (Jorgensen, 1977). Calculation details are given in appendix.

Chapter 4

Results

4.1. Hydrological parameters

4.1.1. Salinity:

Salinity was the most widely varying parameter during the study period in Tuvem as well as Diwar (Figure 8 a & 9 a). The average salinity of the mangrove water in Tuvem and Diwar was 17.58 and 14.06 psu respectively on a monthly basis throughout the year. The variation in salinity between Tuvem and Diwar was insignificant.

Season wise the highest salinity was recorded during the pre-monsoon season in Tuvem and Diwar at 24 and 31 psu respectively. The seasonal variations followed a well defined cycle with a minimal salinity during the monsoon season, gradually increasing during the post-monsoon and attaining a maximum during the pre-monsoon season (Figure 8 b & 9 b). At the Tuvem it ranged from 1.5 (SD \pm 1.03; n=4) during the monsoon season to 22.8 (SD \pm 3.4; n=4) psu during the pre-monsoon season, while at the Diwar it ranged from 4.0 (SD \pm 3.1; n=4) during the monsoon season to 27.8 (SD \pm 2.4; n=4) during the pre-monsoon season. With the onset of monsoon, there was a prominent drop in the salinity at both the sites. In general, the variation in salinity at both the Tuvem and Diwar were comparable.

4.1.2. Dissolved oxygen

The Dissolved oxygen in the mangrove water of Tuvem and Diwar varied from 1.26 (May) to 4.79 ml L⁻¹ (July) and 1.29 (November) to 5.03 ml L⁻¹ (August) respectively (Figure 10 a & 11 a).

The seasonal mean DO levels at the Tuvem ranged from 1.9 during the post monsoon ($SD \pm 0.6$, $n=4$) to 3.8 ml. L^{-1} ($SD \pm 0.8$, $n=4$) during the monsoon season while at the Diwar it ranged from 1.9 ($SD \pm 0.6$, $n=4$) to 4.0 ml L^{-1} ($SD \pm 1.0$, $n=4$), respectively for the post monsoon and monsoon seasons (Figure 10 & 11 b). In general, it was observed that the DO levels at both the Tuvem and Diwar were higher during the monsoon months in contrast to the pre and post monsoon months. Throughout the period of sampling there was no significant variation in DO between the two sites. The lowest value of DO was observed at Tuvem during the pre-monsoon while at Diwar lowest DO values were observed during post monsoon season (Figure 10 b & 11 b).

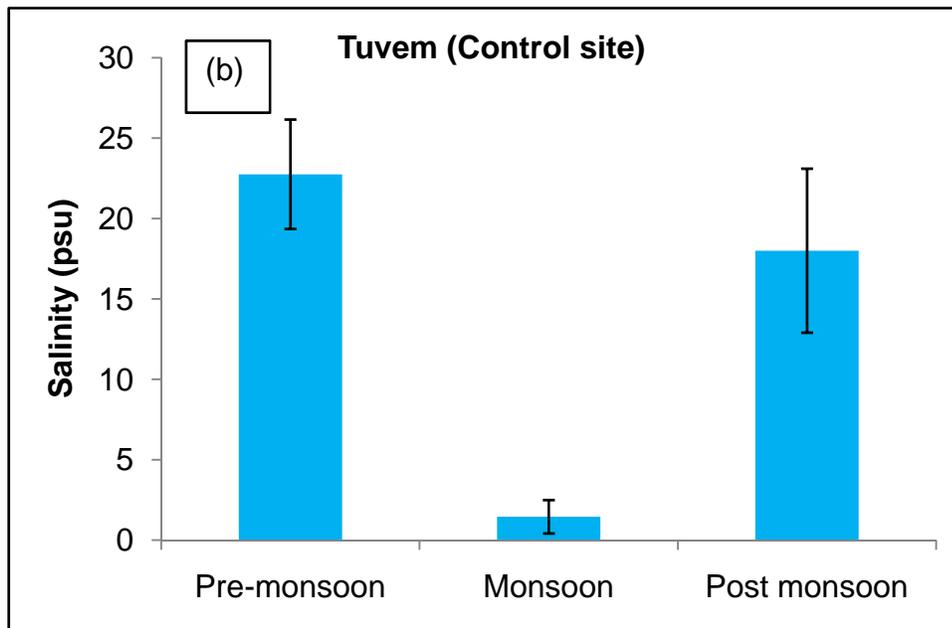
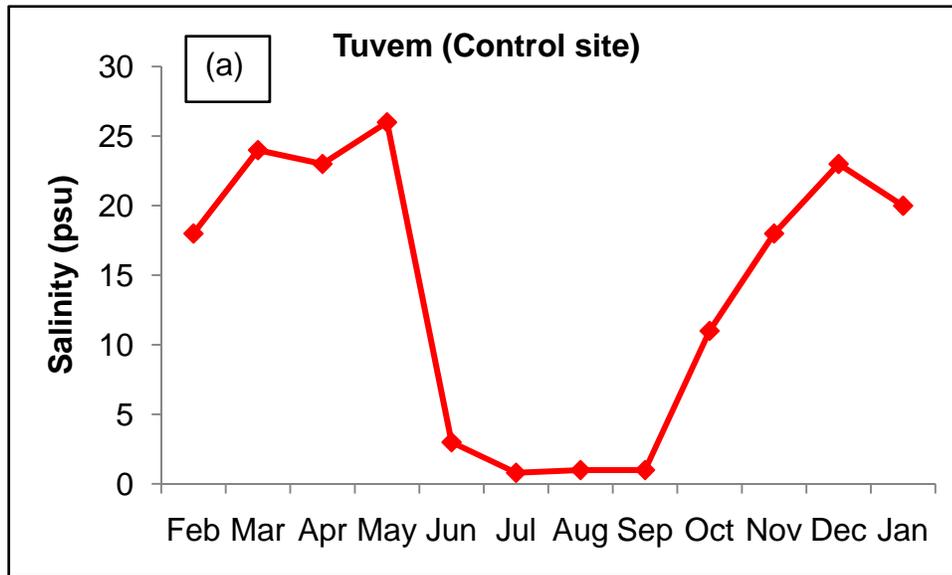


Figure: 8. Monthly (a) and seasonal (b) variation in salinity of mangrove water at Tuvem. Bars indicate \pm SD.

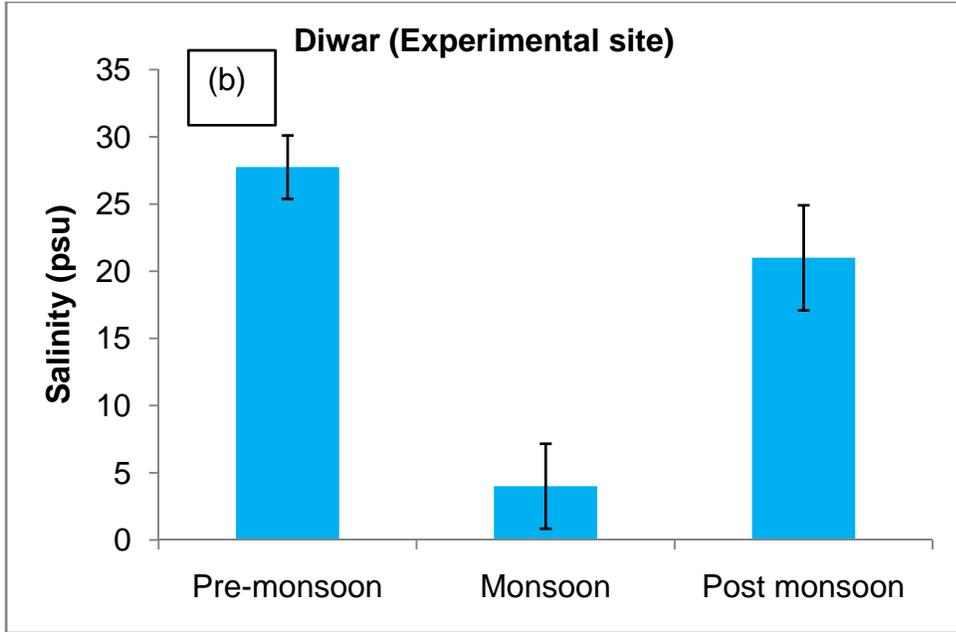
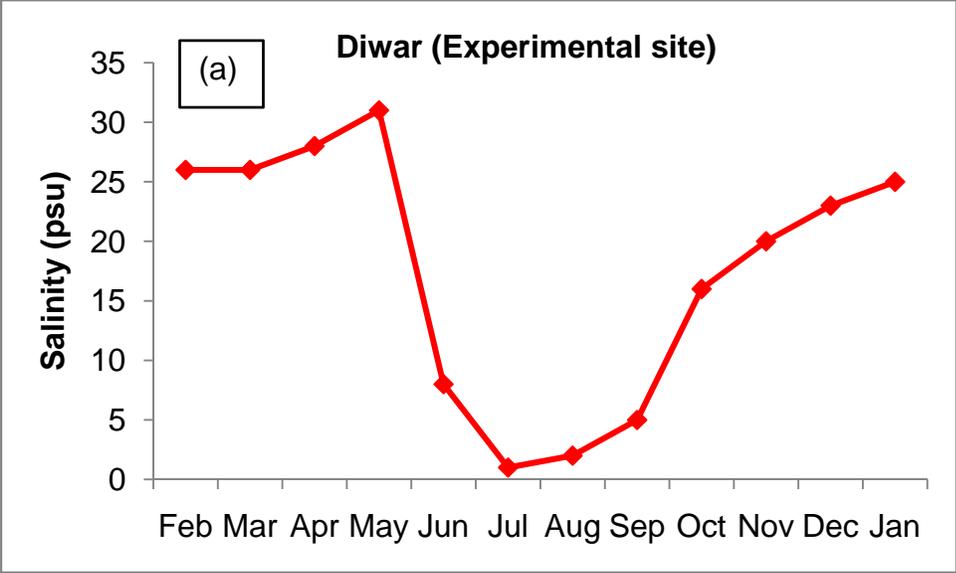


Figure: 9. Monthly (a) and seasonal (b) variation in salinity of mangrove waters at Diwar. Bars indicate \pm SD.

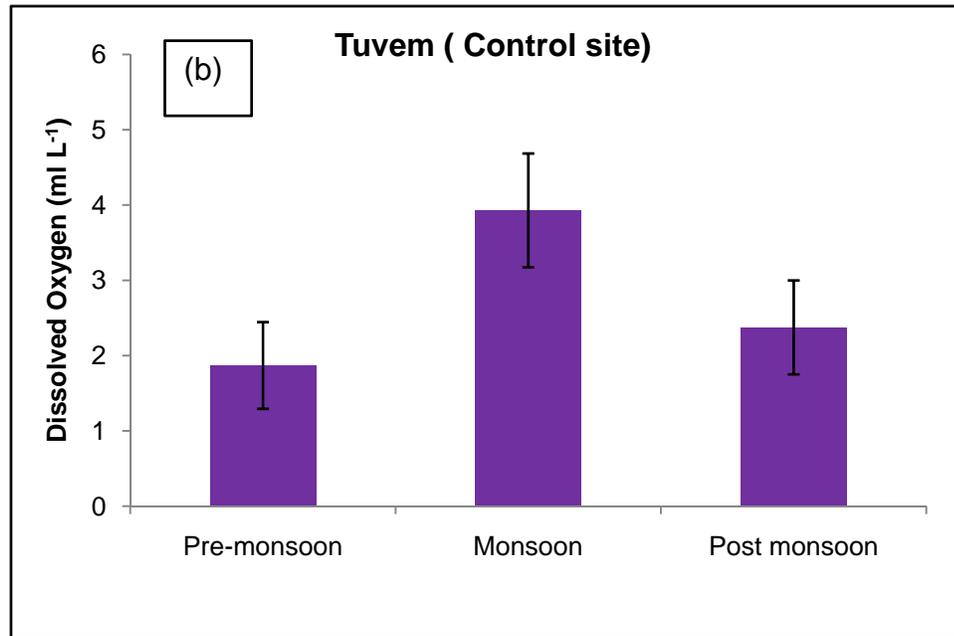
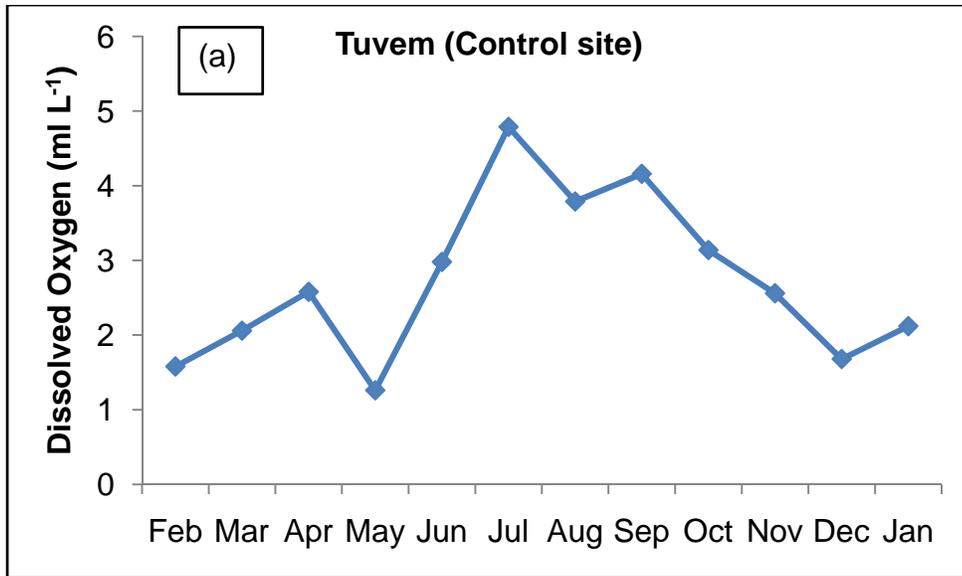


Figure: 10. Monthly (a) and seasonal (b) variation in dissolved oxygen of mangrove waters at Tuvem. Bars indicate \pm SD.

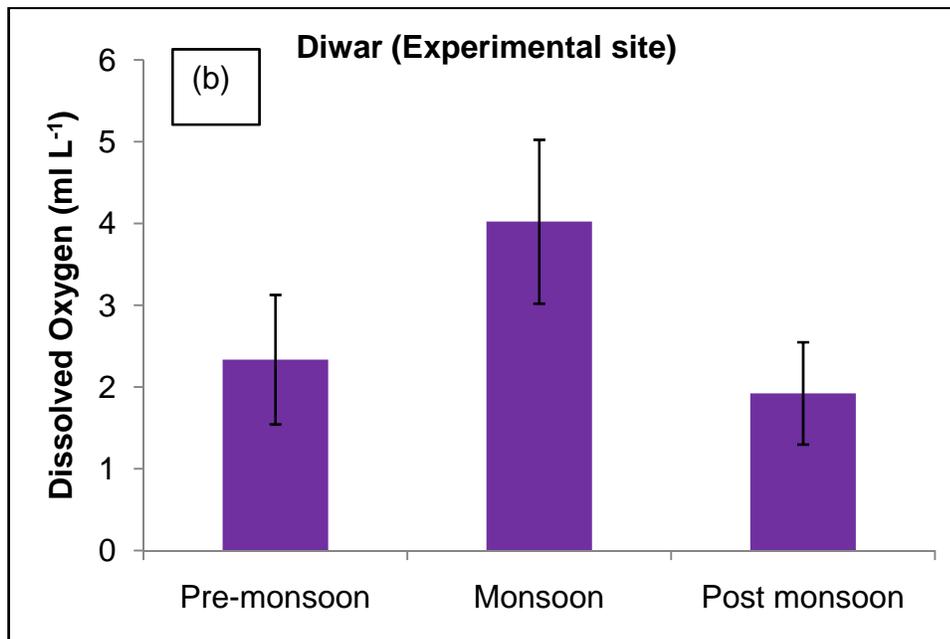
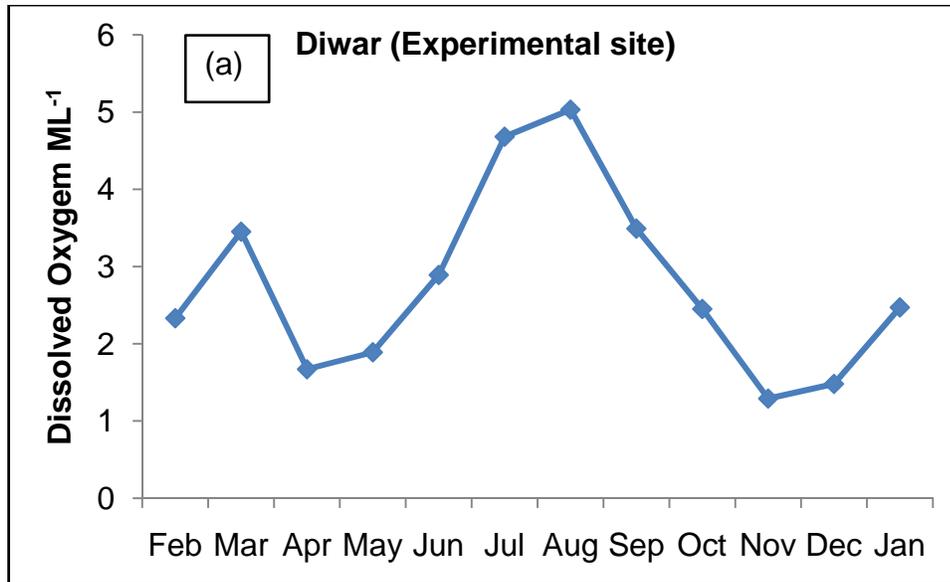


Figure: 11. Monthly (a) and seasonal (b) variation in dissolved oxygen of mangrove waters at Diwar. Bars indicate \pm SD.

4.2. Sediment geochemistry:

4.2.1. Temperature:

During the entire period of study (2007-2008), the down core variation in temperature at Tuvem (Figure 12) ranged from 25.1 to 30.3 °C (mean=27.0± 1.3 °C; n=60) while at the Diwar it varied from 24.1 to 31.4 °C (mean=26.8± 1.6°C; n=60). Analysis of variance (ANOVA) showed that there was a high significant monthly variation in sediment temperature profiles at both the Tuvem (df=11, p<0.001) and Diwar (df=11, p<0.001) (Table 5 & 6). However, no statistically significant variation was observed between the five depths (0-10 cm at 2 cm interval) in temperature, at both Tuvem and Diwar sites. Moreover, throughout the period of sampling there was no significant variation in temperature between the two sites for neither of the measured depth intervals. Seasonal and annual mean of temperature variation for different depth intervals for both Tuvem and Diwar are given in Table 2. In general it could be observed that at both Tuvem and Diwar, the pre-monsoon months were warmer than the monsoon and post-monsoon months. The post-monsoon months being the coolest.

4.2.2. pH and redox potential:

Monthly, down core variation in pH is illustrated in Figure 13. At Tuvem the down core variation in pH ranged from 6.7 to 7.1 (mean=6.92± 0.10; n=60), while at the Diwar it varied from 6.6 to 7.3 (mean=6.91± 0.14; n=60). ANOVA showed a highly significant variation between depth in sediment pH profiles at both Tuvem (df=4, p<0.001) and Diwar (df=4, p<0.001) sites (Table 5 & 6). There was no statistically

significant monthly variation at Tuvem while at Diwar, the monthly variation was significant (df=11, $p < 0.05$) (Table 6). Seasonal and annual mean of pH for different depth intervals at both Tuvem and Diwar site are given in Table 3.

Down core variation in Eh (Figure 13) at Tuvem ranged from -341.5 to 289.5 (mean= 38 ± 132.9 ; n=60), while at Diwar it varied from -140.4 to 301.7 (mean= 87.66 ± 115.23 ; n=60) throughout the study period. ANOVA showed that there was a significant monthly variation in sediment Eh profiles at Tuvem (df=11, $p < 0.05$) whereas the monthly variation at Diwar was insignificant (Table 5 & 6). Between the depths, a high significant variation in Eh at both Tuvem (df=4, $p < 0.001$) and Diwar (df=11, $p < 0.001$) was seen. Seasonal and annual means of Eh for different depth intervals at both Tuvem and Diwar sites are given in Table 4.

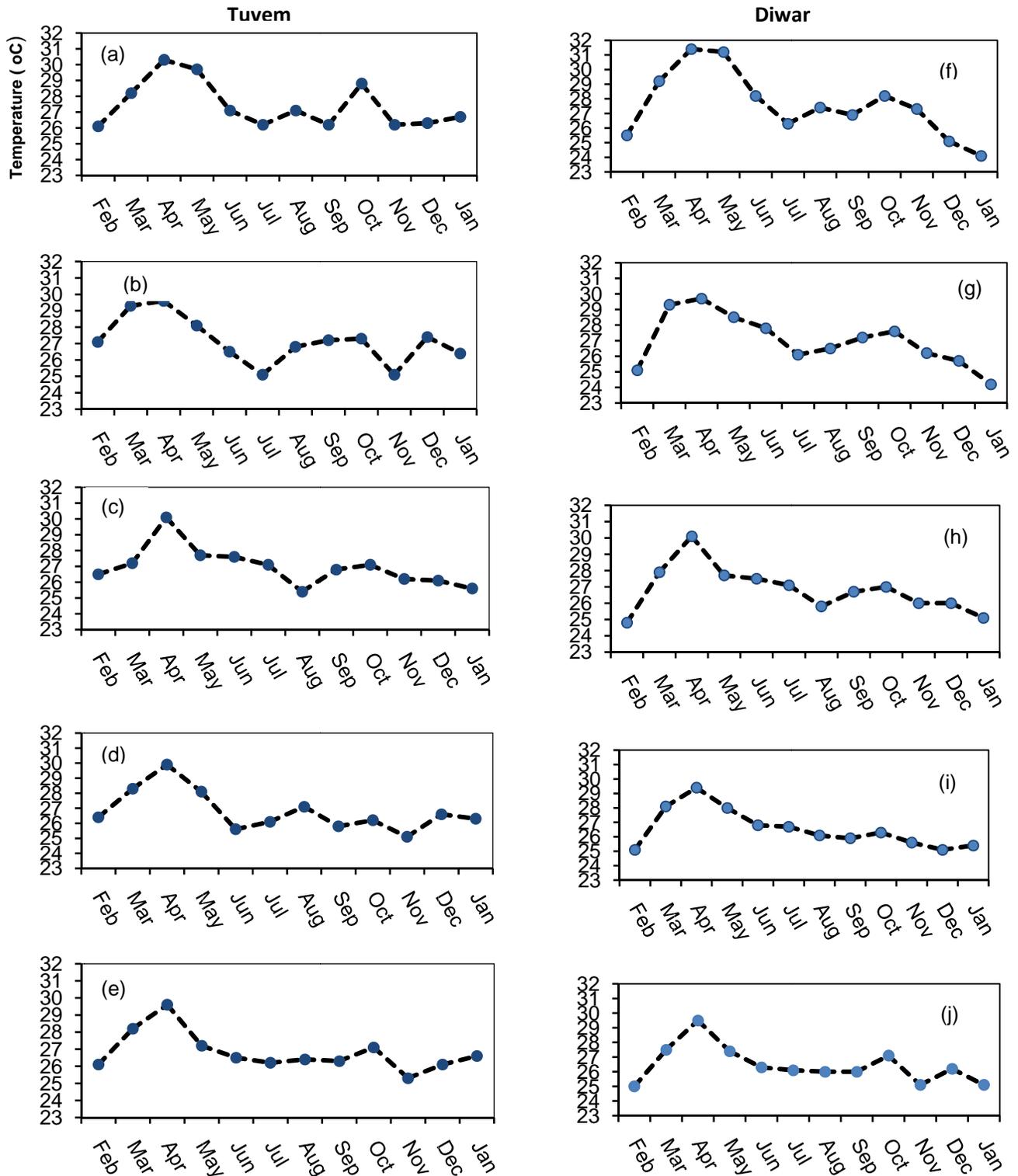


Figure: 12. Monthly down core variation of temperature at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

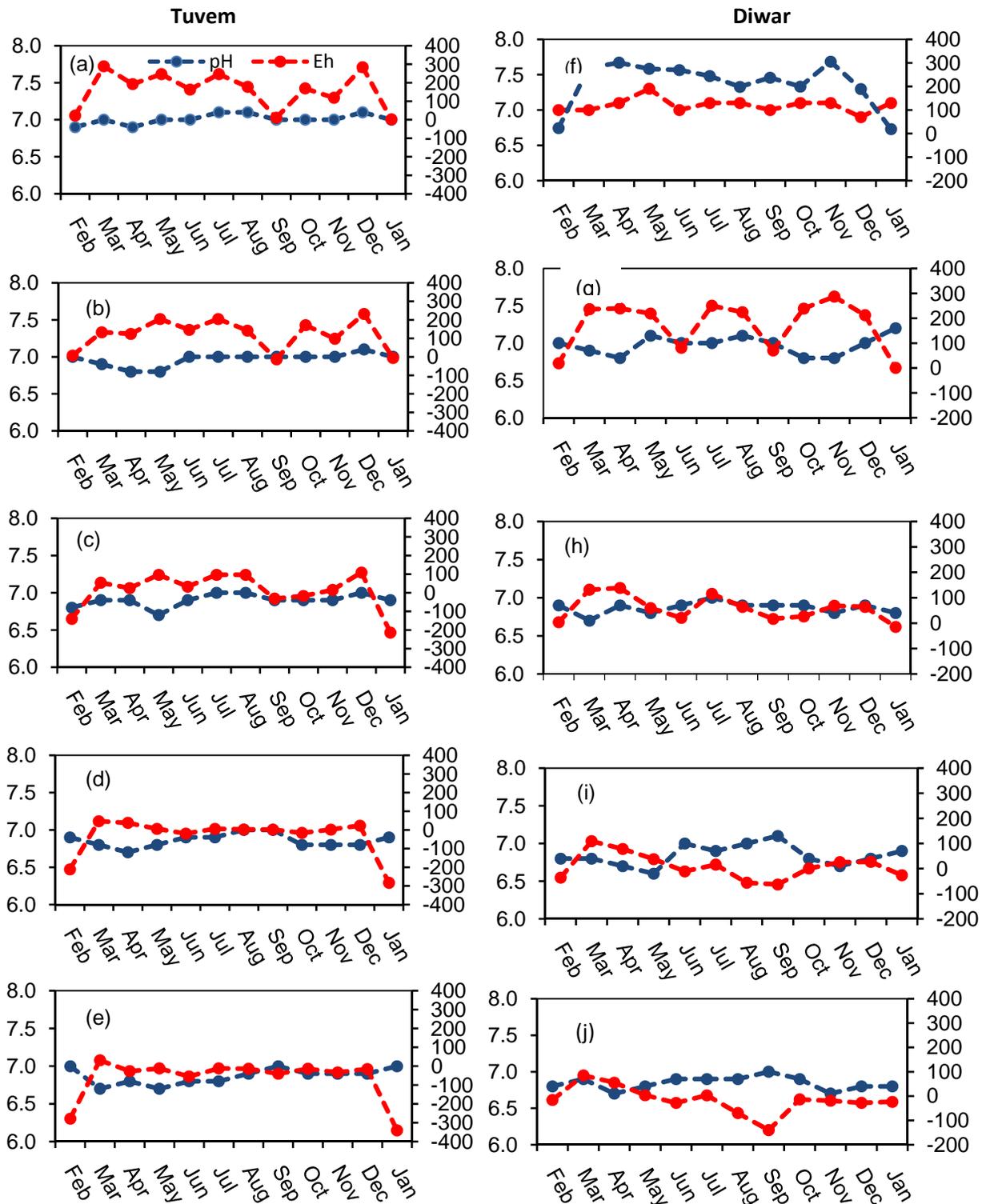


Figure: 13. Monthly down core variation of pH and Eh (mV) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0- 2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm. Primary y and secondary y axis refers to pH and Eh respectively.

Table: 2. Seasonal and annual mean \pm SD of temperature ($^{\circ}$ C) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	28.6 \pm 1.9	26.7 \pm 0.5	27.0 \pm 1.2	27.4 \pm 1.5
2 to 4	28.5 \pm 1.2	26.4 \pm 0.9	26.6 \pm 1.0	27.2 \pm 1.4
4 to 6	27.9 \pm 1.6	26.7 \pm 0.9	26.3 \pm 0.6	26.9 \pm 1.2
6 to 8	28.2 \pm 1.4	26.2 \pm 0.7	26.1 \pm 0.7	26.8 \pm 1.4
8 to 10	27.8 \pm 1.5	26.4 \pm 0.1	26.3 \pm 0.8	26.8 \pm 1.1
Diwar (Experimental site)				
0 to 2	29.3 \pm 2.7	27.2 \pm 0.8	26.1 \pm 1.9	27.6 \pm 2.3
2 to 4	28.2 \pm 2.1	26.9 \pm 0.8	25.9 \pm 1.4	27.0 \pm 1.7
4 to 6	27.6 \pm 2.2	26.8 \pm 0.7	26.0 \pm 0.8	26.8 \pm 1.4
6 to 8	27.7 \pm 1.8	26.4 \pm 0.4	25.6 \pm 0.5	26.5 \pm 1.3
8 to 10	27.4 \pm 1.8	26.1 \pm 0.1	25.9 \pm 1.0	26.4 \pm 1.3

Table: 3. Seasonal and annual mean \pm SD of pH at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	6.9 \pm 0.1	7.1 \pm 0.1	7.0 \pm 0.1	7.0 \pm 0.1
2 to 4	6.8 \pm 0.1	7.0 \pm 0.0	7.0 \pm 0.1	7.0 \pm 0.1
4 to 6	6.8 \pm 0.1	6.9 \pm 0.1	6.9 \pm 0.1	6.9 \pm 0.1
6 to 8	6.8 \pm 0.1	7.0 \pm 0.1	6.8 \pm 0.1	6.8 \pm 0.1
8 to 10	6.8 \pm 0.1	6.8 \pm 0.1	6.9 \pm 0.1	6.8 \pm 0.1
Diwar (Experimental site)				
0 to 2	7.1 \pm 0.1	7.0 \pm 0.1	7.0 \pm 0.1	7.1 \pm 0.1
2 to 4	6.9 \pm 0.1	7.0 \pm 0.1	6.9 \pm 0.1	6.9 \pm 0.1
4 to 6	6.8 \pm 0.1	6.9 \pm 0.1	6.8 \pm 0.1	6.8 \pm 0.1
6 to 8	6.7 \pm 0.1	7.0 \pm 0.1	6.8 \pm 0.1	6.8 \pm 0.1
8 to 10	6.8 \pm 0.1	6.9 \pm 0.1	6.8 \pm 0.1	6.8 \pm 0.1

Table: 4. Seasonal and annual mean±SD of Eh (mV) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	188.0±117.1	150.2±99.0	143.0±117.4	160.2±102.9
2 to 4	111.7±81.8	120.6±93.5	124.7±102.6	1120.1±84.2
4 to 6	9.6±103.4	48.9±61.3	-27.8±135.8	10.4±100.0
6 to 8	-30.3±121.9	-2.4±11.6	-69.4±144.1	-34.8±102.8
8 to 10	-71.8±140.1	-30.4±20.7	-101.0±160.3	-67.2±115.7
Diwar (Experimental site)				
0 to 2	220.3±131.3	238.1±29.4	178.7±118.5	212.1±97.2
2 to 4	178.1±106.7	156.4±93.9	184.1±126.6	173.4±100.2
4 to 6	83.1±63.8	54.3±46.0	36.3±38.6	58.6±50.1
6 to 8	47.2±62.5	-28.1±37.6	7.0±25.1	9.0±51.7
8 to 10	32.3±46.3	-59.0±61.9	-21.1±6.0	-16.8±56.2

Table: 5. Analysis of variance (ANOVA) of different parameters in sediments at Tuvem, and between Tuvem and Diwar.

Variables	Sediment	d.f.	F-value	F-critical	P-value*
Redox Potential	Between depths	4	11.25	2.53	9.65E-07
	Between seasons	2	0.53	3.15	0.58
	Between sites	1	4.69	3.92	0.03
pH	Between depths	4	6.50	2.52	0.0002
	Between seasons	2	8.87	3.15	0.0004
	Between sites	1	0.005	3.92	0.94
Moisture content	Between depths	4	1.45	2.54	0.23
	Between seasons	2	5.09	3.15	0.009
	Between sites	1	5.03	3.92	0.03
Density	Between depths	4	3.82	2.53	0.008
	Between seasons	2	1.59	3.15	0.211
	Between sites	1	3.06	3.92	0.08
Sand	Between depths	4	0.13	2.53	0.96
	Between seasons	2	60.75	3.15	7.47E-5
	Between sites	1	39.59	3.92	5.5 E-9
Silt	Between depths	4	1.73	2.53	0.16
	Between seasons	2	0.76	3.15	0.46
	Between sites	1	34.60	3.91	3.86 E-8
Clay	Between depths	4	0.22	2.54	0.93
	Between seasons	2	59.38	3.15	1.15 E-14
	Between sites	1	24.41	3.92	2.59 E-6
Salinity	Between depths	4	2.86	2.54	0.03
	Between seasons	2	31.25	3.16	6.87E-10
	Between sites	1	4.75	3.92	0.03
Sulfate	Between depths	4	1.97	2.53	0.11
	Between seasons	2	31.36	3.15	6.51E-10
	Between sites	1	9.15	3.92	0.003
Sulfide	Between depths	4	0.85	2.53	0.49
	Between seasons	2	3.00	3.15	0.05
	Between sites	1	316.33	3.92	3.49E-35

TOC	Between depths	4	2.53	1.72	0.16
	Between seasons	2	3.15	6.71	0.002
	Between sites	1	3.92	27.10	8.25E-7
LOM	Between depths	4	1.90	2.53	0.12
	Between seasons	2	1.65	3.15	0.20
	Between sites	1	58.46	3.92	6.12E-12
Iron	Between depths	4	0.03	2.53	0.99
	Between seasons	2	3.87	3.15	0.03
	Between sites	1	120.22	3.92	1.02E-19
AODC	Between depths	4	0.58	2.54	0.68
	Between seasons	2	21.35	3.88	0.001
	Between sites	1	2.12	3.92	0.15
Heterotrophic Counts	Between depths	4	0.30	2.54	0.87
	Between seasons	2	33.76	3.68	2.8E-6
	Between sites	1	0.99	3.92	0.31
SRB MPN acetate	Between depths	4	0.86	2.54	0.50
	Between seasons	2	2.36	3.7	0.13
	Between sites	1	51.7	3.92	6.4E-11
SRB MPN lactate	Between depths	4	0.99	2.53	0.41
	Between seasons	2	3.37	3.68	0.06
	Between Sites	1	12.53	3.92	0.0005
SRB Agar shake acetate	Between depths	4	0.46	2.54	0.76
	Between seasons	2	1.58	3.88	0.25
	Between sites	1	3.23	3.92	0.07
SRB Agar Shake lactate	Between depths	4	0.50	2.54	0.73
	Between seasons	2	1.03	3.88	0.38
	Between sites	1	14.24	3.92	0.0003
Sulfate reduction rates	Between depths	4	1.41	2.54	0.24
	Between seasons	2	5.30	3.16	0.008
	Between sites	1	23.06	3.9	4.65E-6

*Significant values ($p < 0.05$) are in bold

Table: 6. Analysis of variance (ANOVA) of different parameters measured in sediments at Diwar.

Variables	sediments	df	F-Value	F-critical	P Value*
Redox potential	Between depth	4	21.88	2.54	7.61E-11
	Between season	2	0.70	3.16	0.50
pH	Between depth	4	9.70	2.54	5.2E-6
	Between season	2	3.77	3.16	0.02
Moisture content	Between depth	4	0.09	2.54	0.98
	Between season	2	30.67	3.16	9.09E-10
Density	Between depth	4	3.37	2.54	0.015
	Between season	2	10.76	3.16	0.0001
Sand	Between depth	4	68.18	2.54	0.95
	Between season	2	48.76	3.16	4.52E-13
Silt	Between depth	4	0.17	2.54	0.95
	Between season	2	23.31	3.15	4.01E-08
Clay	Between depth	4	0.24	2.54	0.91
	Between season	2	38.79	3.16	2.32E-11
Salinity	Between depth	4	1.99	2.54	0.10
	Between season	2	34.16	3.16	1.77E-10
Sulfate	Between depth	4	0.57	2.54	0.69
	Between season	2	75.75	3.15	8.87E-17
Sulfide	Between depth	4	3.74	2.54	0.009
	Between season	2	21.06	3.16	1.42E-07
TOC	Between depth	4	0.09	2.54	0.98
	Between season	2	38.57	3.16	2.54E-11
LOM	Between depth	4	0.52	2.54	0.71
	Between season	2	5.06	3.16	0.009
Iron	Between depth	4	0.20	2.51	0.94
	Between season	2	160.56	3.88	2.18E-9

Continued table.....

AODC	Between depth	4	1.00	2.54	0.41
	Between season	2	14.94	3.89	0.0006
Heterotrophic- counts	Between depth	4	0.31	2.54	0.87
	Between season	2	16.87	3.88	0.0003
SRB MPN acetate	Between depth	4	0.77	2.54	0.62
	Between season	2	2.09	3.89	0.17
SRB MPN lactate	Between depth	4	0.04	2.54	0.99
	Between season	2	2.43	3.15	0.09
SRB Agar- shake acetate	Between depth	4	0.82	2.54	0.51
	Between season	2	3.65	0.06	3.89
SRB Agar shake lactate	Between depth	4	4.18	2.54	0.005
	Between season	2	0.57	3.89	0.58
Sulfate reduction rates	Between depth	4	0.45	2.54	0.77
	Between season	2	87.26	3.16	4.47E-18

*Significant values ($p < 0.05$) are in bold

4.2.3. Water content:

Monthly down core variation in water content is illustrated in Figure 14. At Tuvem the down core variation in water content ranged from 19.81 to 65.2 (%) (mean= 47.1 ± 11.0 ; n=60), while at Diwar it varied from 25.8 to 61.9 (mean= 42.6 ± 11.0 ; n=60). ANOVA showed no significant variation between the depths in sediment water content profiles at both Tuvem and Diwar. However there was a significant monthly variation in sediment moisture content profiles at both Tuvem (df=11, $p < 0.001$, n=12) and Diwar (df=11, $p < 0.001$, n=12) sites (Table 5 & 6). The significant variation was also observed between Tuvem and Diwar (df=1, $p < 0.05$, n=2) (Table 5). Seasonal and annual mean of sediment water content for different depth intervals at both Tuvem and Diwar are given in Table 7.

4.2.4. Density:

During the entire study period, at Tuvem the down core variation in sediment density (Figure 15) ranged from 1.01 to 1.35 gm cm⁻³ (mean= 1.20 ± 0.09 gm cm⁻³; n=60) while at Diwar it varied from 1.02 to 1.20 gm cm⁻³ (mean= 1.2 ± 0.1 gm cm⁻³; n=60). ANOVA showed that there was a significant monthly variation in sediment density profiles at both Tuvem (df=11, $p < 0.05$, n=12) and Diwar (df=11, $p < 0.05$, n=12) (Table 5 & 6). A statistically significant variation between the depths was observed in density at both Tuvem (df=4, $p < 0.05$, n=5) and Diwar (df=4, $p < 0.05$, n=5) sites (Table 5 & 6). Moreover, throughout the period of sampling there was no significant variation in density between Tuvem and Diwar sites. Seasonal and annual mean of density for different depth intervals at both Tuvem and Diwar site are given in Table 8. In general it was observed that at both Tuvem and Diwar, the seasonal sediment density was highest during the pre-monsoon.

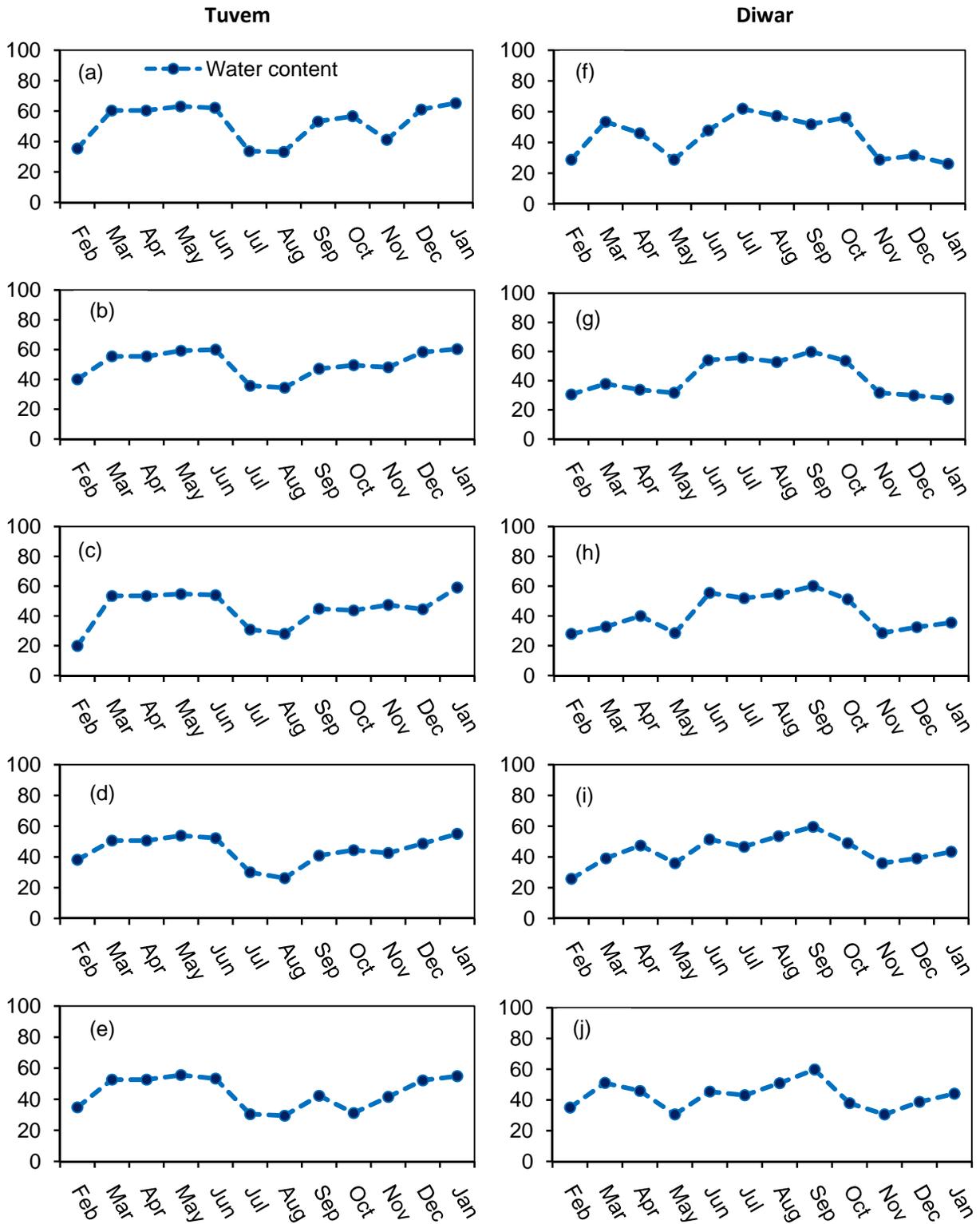


Figure: 14. Monthly down core variation of sediment water content (%) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar - (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

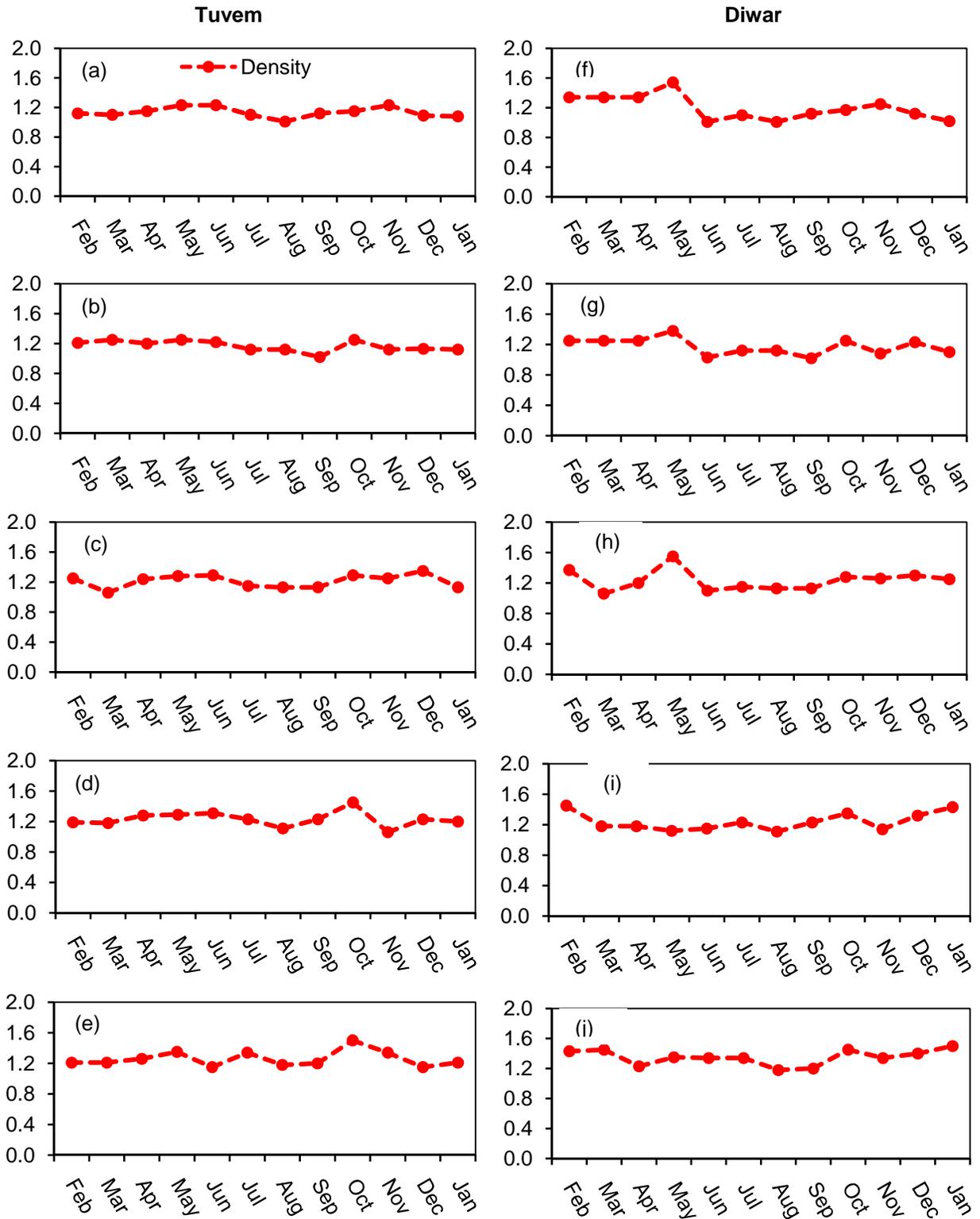


Figure: 15. Monthly down core variation of sediment sites density (gm cm⁻³) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar– (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

Table: 7. Seasonal and annual mean±SD of water content (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	54.8±13.0	45.5±14.5	55.9±10.5	52.1±12.5
2 to 4	52.6±8.52	44.3±11.9	54.1±6.2	50.3±9.4
4 to 6	45.4±17.1	39.4±12.2	48.7±7.1	44.5±12.2
6 to 8	48.3±7.0	37.5±11.7	47.7±5.5	44.5±9.3
8 to 10	49.0±9.5	38.9±11.3	44.9±10.8	44.2±10.5
Diwar (Experimental site)				
0 to 2	39.3±12.5	55.0±6.1	35.6±13.9	42.2±13.4
2 to 4	33.5±3.2	55.6±3.1	35.7±12.1	41.6±12.4
4 to 6	32.3±5.5	55.5±3.4	36.9±9.9	41.6±12.2
6 to 8	37.1±9.0	52.8±5.4	41.9±5.7	43.9±9.3
8 to 10	40.6±9.5	49.8±7.4	37.8±5.5	42.7±8.7

Table: 8. Seasonal and annual mean±SD of density (gm cm⁻³) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	1.2±0.1	1.1±0.1	1.1±0.1	1.1±0.1
2 to 4	1.2±0.02	1.1±0.1	1.2±0.1	1.2±0.1
4 to 6	1.2±0.1	1.2±0.1	1.3±0.1	1.2±0.1
6 to 8	1.2±0.1	1.2±0.1	1.2±0.2	1.2±0.1
8 to 10	1.3±0.1	1.2±0.1	1.3±0.2	1.3±0.1
Diwar (Experimental site)				
0 to 2	1.4±0.1	1.1±0.1	1.1±0.1	1.2±0.2
2 to 4	1.3±0.1	1.1±0.1	1.2±0.1	1.2±0.1
4 to 6	1.3±0.2	1.1±0.02	1.3±0.02	1.2±0.1
6 to 8	1.2±0.1	1.2±0.06	1.3±0.1	1.2±0.1
8 to 10	1.4±0.1	1.3±0.1	1.4±0.1	1.4±0.1

4.2.5. Iron content:

A wide difference in the annual variation in the sediment iron content (Figure 16) between Tuvem (3.6 to 8.6%; mean=6.3± 1.5%; n=60) and Diwar (3.3 to 32.3%; mean=17.8± 8.1%; n=60) was noted. ANOVA showed a significant monthly variation at Tuvem (df=11, p<0.001, n=12) and a insignificant down core variation (Table 5). However at Diwar, there was a significant monthly variation (df=11; p<0.001, n=12) and no significant down core variation (Table 6). Throughout the period of sampling, significant variation in the iron content between Tuvem and Diwar sediments in all the depth intervals viz. 0-2 (df=1; p<0.001, n=2), 2-4 (df=1; p<0.001, n=2), 4-6 (df=1; p<0.001, n=2), 6-8 (df=1; p<0.001, n=2) and 8-10 cm (df=1; p<0.001, n=2) was observed (Table 5). Seasonal and annual mean of iron content for different depth intervals at both the sites are given in Table 9. It could be observed that in general there is an enrichment of iron in the sediments of Diwar as compared to at Tuvem. Pre-monsoon season showed the highest enrichment at Diwar.

Other metals:

Seasonal variation in heavy metal viz. Mn, Co, Zn, Cu, Cr and Pb, at Tuvem and Diwar is depicted in Figure 17. Values are represented at a scale of Log₁₀. Seasonal fluctuation in concentration of metals was highly significant (p< 0.001; df=2) at both the sites. Mn concentrations at Tuvem were lower as compared to Diwar where maximum concentrations of 0.28% were recorded. While the concentration of Co, Cu, Cr and Pb were 32.45 ppm, 49.5 ppm, and 28.25 ppm respectively at Tuvem, and almost double of these values were recorded at Diwar.

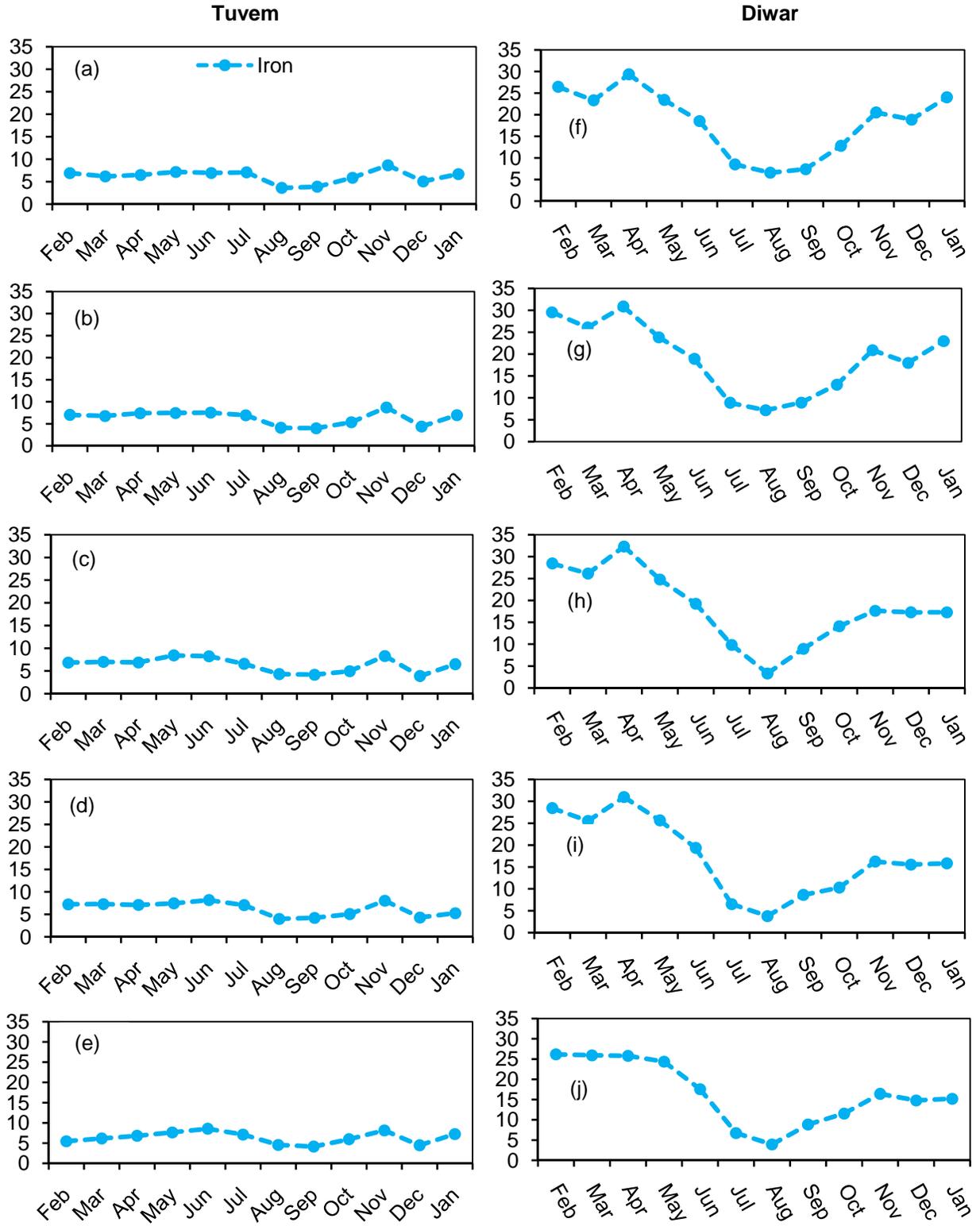


Figure: 16. Monthly down core variation of sediment Iron (%) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

Table: 9. Seasonal and annual mean \pm SD of iron (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	6.7 \pm 0.4	5.4 \pm 1.9	6.6 \pm 1.5	6.2 \pm 1.4
2 to 4	7.1 \pm 0.3	5.6 \pm 1.9	6.3 \pm 1.9	6.4 \pm 1.5
4 to 6	7.3 \pm 0.8	5.8 \pm 1.9	5.9 \pm 1.9	6.3 \pm 1.6
6 to 8	7.3 \pm 0.1	5.8 \pm 2.1	5.7 \pm 1.6	6.2 \pm 1.6
8 to 10	6.5 \pm 0.9	6.1 \pm 2.1	6.4 \pm 1.6	6.3 \pm 1.5
Diwar (Experimental site)				
0 to 2	25.6 \pm 2.9	10.2 \pm 5.6	19.0 \pm 4.7	18.3 \pm 7.7
2 to 4	27.6 \pm 3.2	11.0 \pm 5.3	18.7 \pm 4.3	19.1 \pm 8.1
4 to 6	27.9 \pm 3.3	10.4 \pm 6.6	16.6 \pm 1.7	18.3 \pm 8.6
6 to 8	27.7 \pm 2.6	9.6 \pm 6.80	14.5 \pm 2.8	17.2 \pm 8.9
8 to 10	25.6 \pm 0.8	9.3 \pm 5.90	14.5 \pm 2.1	16.4 \pm 7.8

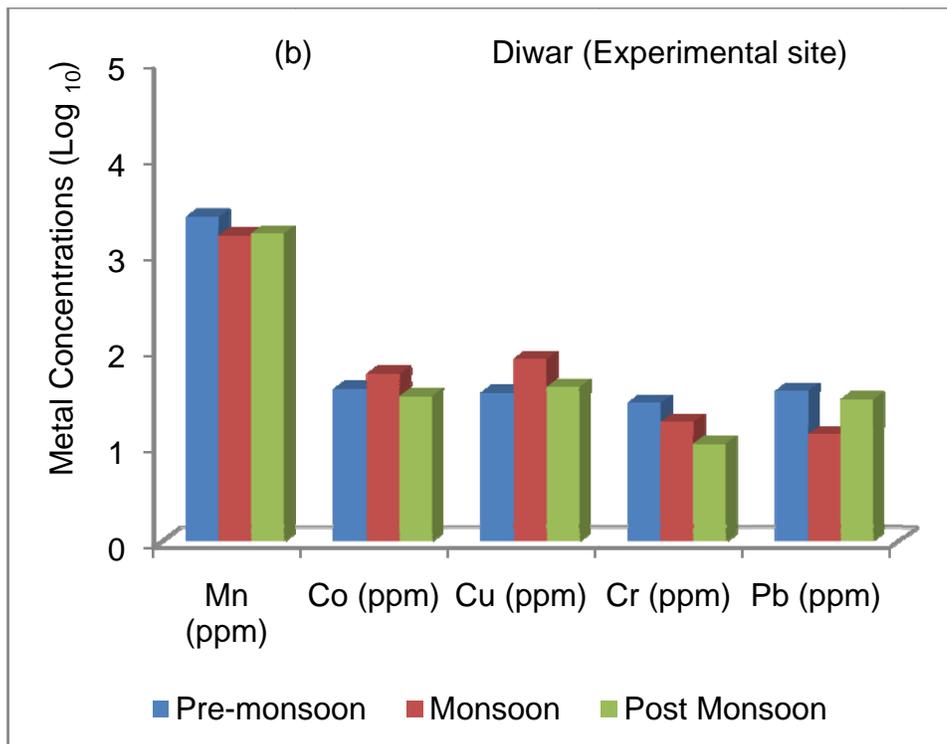
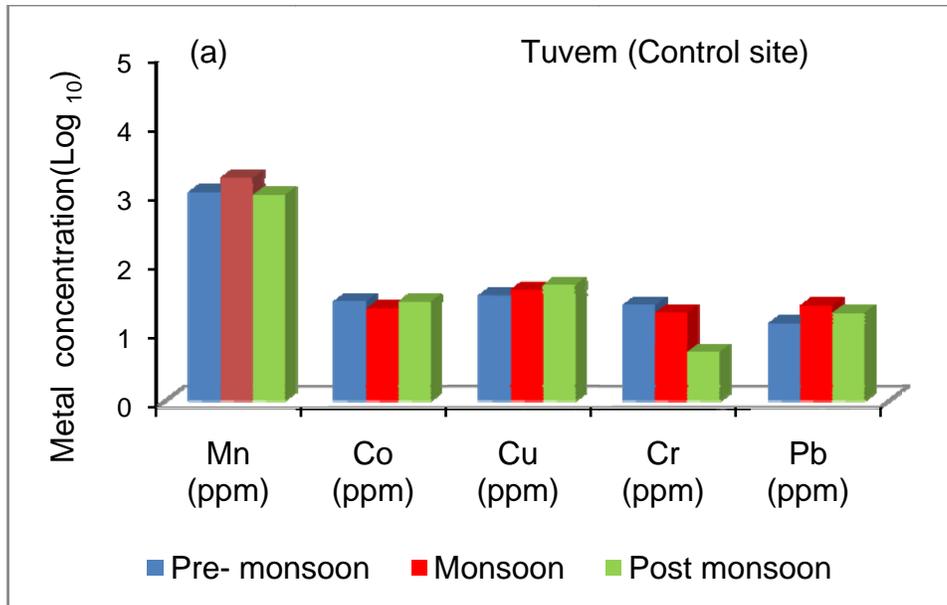


Figure: 17. Seasonal variation of sediments; Mn, Co, Cu, Cr and Pb at Tuvem (a) and Diwar (b).

4.2.6. Total organic carbon:

Total organic carbon (TOC) concentrations varied widely (Figure 18) at both Tuvem (1.09 to 5.7%; mean=3.2± 1.1%; n=60) and Diwar (0.5 to 4.2%; mean=2.1± 1.1; n=60). ANOVA showed a significant monthly variation at Tuvem (df=11; p<0.001, n=12) and Diwar (df=11, p<0.001, n=12) site, but down core variation was not significant at either of the sites (Table 5 & 6). Throughout the period of sampling, TOC differed significantly (df=1, p<0.001, n=1) between Tuvem and Diwar (Table 5). In general TOC was higher during pre-monsoon and post monsoon at Tuvem, while at Diwar TOC was higher during monsoon season. Seasonal and annual mean of TOC for different depth intervals at both Tuvem and Diwar site are given in Table 10.

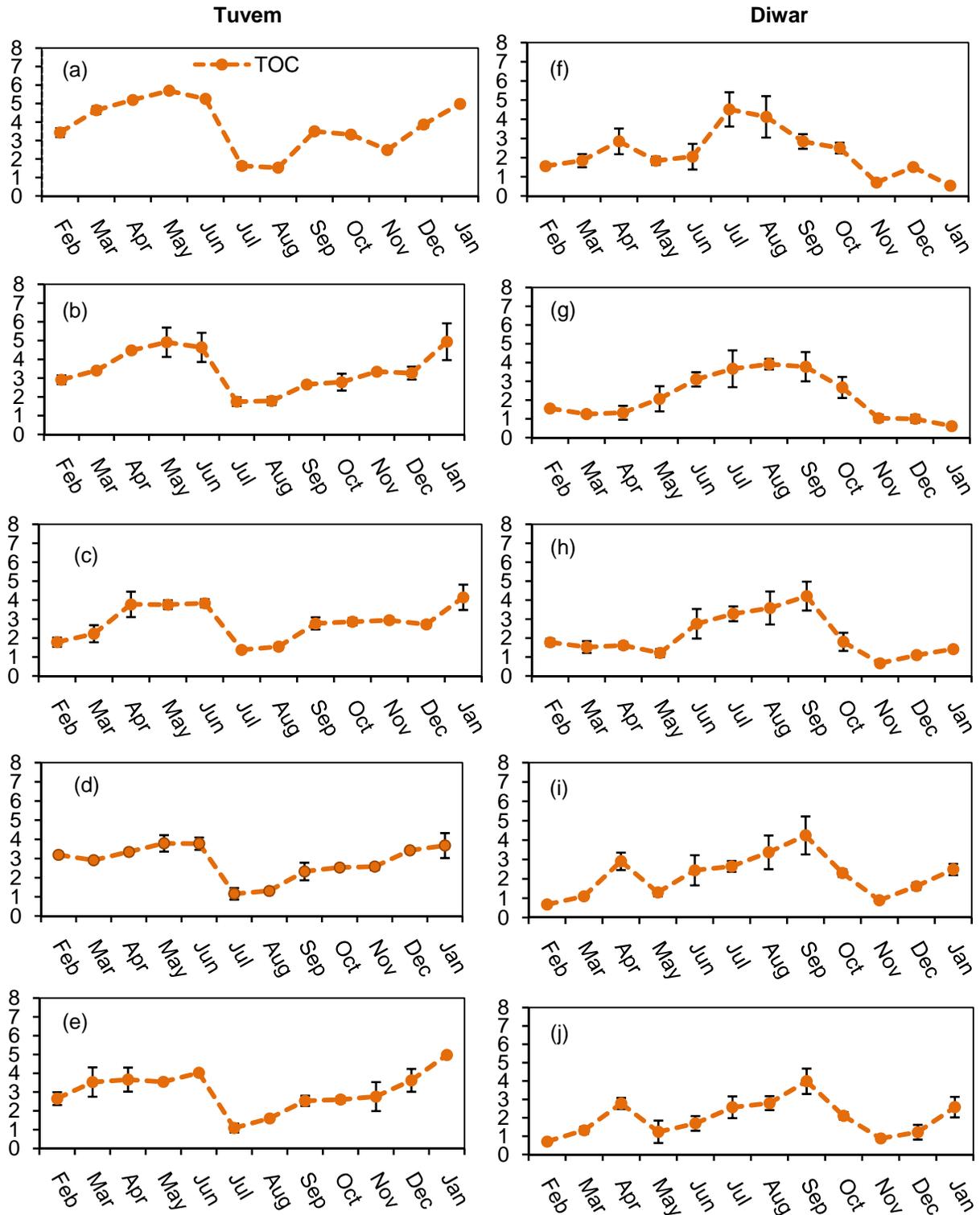


Figure: 18. Monthly down core variation of sediment TOC(%) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

Table: 10: Seasonal and annual mean±SD of total organic carbon content (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	4.7±1.1	3.1±1.8	3.7±1.0	3.8±1.4
2 to 4	3.9±1.1	2.7±1.4	3.6±1.1	3.4±1.1
4 to 6	2.9±1.0	2.4±1.2	3.2±0.7	2.8±0.9
6 to 8	3.3±0.4	2.1±1.2	3.1±0.6	2.8±0.9
8 to 10	3.4±0.5	2.3±1.3	3.5±1.1	3.1±1.1
Diwar (Experimental site)				
0 to 2	2.0±0.6	3.4±1.1	1.3±0.9	2.2±1.2
2 to 4	1.6±0.4	3.6±0.4	1.3±0.9	2.2±1.2
4 to 6	1.5±0.2	3.5±0.6	1.3±0.5	2.1±1.1
6 to 8	1.5±0.9	3.2±0.8	1.8±0.7	2.2±1.1
8 to 10	1.5±0.9	2.8±0.9	1.7±0.8	2.0±1.0

4.2.7. Labile Organic Matter:

4.2.7.1. Carbohydrate, Protein and Lipid

Monthly, down core variation in carbohydrate is illustrated in Figure 19. At Tuvem, the down core variation in carbohydrate ranged from 0.07 to 1.28% (mean=0.42± 0.3; n=60), while at Diwar it varied from 0.08 to 0.32% (mean=0.17± 0.06; n=60). ANOVA showed a significant monthly variation in sediment carbohydrate profiles at both Tuvem (df=11, p<0.001, n=12) and Diwar (df=4, p<0.001, n=12) sites (Table 5 & 6). There was no statistically significant down core variation at both Tuvem and Diwar sites (Table 5 & 6). A significant variation was also observed between both sites (df=1, p<0.001, n=2) (Table 5). Seasonal and annual mean of carbohydrates for different depth intervals at both Tuvem and Diwar are given in Table 11. In general, the carbohydrate content was higher at Tuvem throughout the study period.

During the entire study period, the down core variation at Tuvem with respect to protein concentrations (Figure 19) ranged from 0.1 to 0.85 % (mean=0.4± 0.2; n=60) while at Diwar site it varied from 0.09 to 0.4% (mean=0.2± 0.09%; n=60). ANOVA showed that there was a significant monthly variation in sediment protein profiles at both Tuvem (df=11, p<0.001, n=12) and Diwar sites (df=11, p<0.001, n=12) (Table 5 & 6). There was no statistically significant variation between the depths at both Tuvem and Diwar sites. However, throughout the period of sampling there was a high significant variation (df=1, p<0.001, n=2) in protein between Tuvem and Diwar sites (Table 5). Seasonal and annual mean of protein for different depth intervals at both Tuvem and Diwar sites are given in

Table 12. In general it could be observed that at Tuvem site protein was higher during pre-monsoon and higher at Diwar site during the monsoon season.

Down core variation in lipid (Figure 19) at Tuvem ranged from 0.046 to 0.23% (mean=0.13± 0.05; n=60), while at Diwar it varied from 0.08 to 0.23% (mean=0.14± 0.04; n=60). With ANOVA, there was neither a monthly variation nor a down core variation at both Tuvem as well as Diwar. Throughout the period of sampling there was no a significant variation in sediment lipids between Tuvem and Diwar. Seasonal and annual mean of lipids for different depth intervals at both Tuvem and Diwar site are given in Table 13.

4.2.8. Total Labile Organic Matter:

The total Labile organic matter (LOM) varied widely (Figure 20) at both Tuvem (0.34 to 2.3%; mean=0.96± 0.4%; n=60) and Diwar sites (0.3 to 0.8%; mean=2.1± 1.1; n=60). ANOVA showed a significant monthly variation at Tuvem (df=11; p<0 .001, n=12) and Diwar (df=11, p<0.001, n=12), but variation between the depth was not significant at either of the site (Table 5 & 6). Throughout the period of sampling, LOM differed significantly (df=1, p<0.001, n=1) between Tuvem and Diwar sites (Table 5). Seasonal and annual mean of LOM for different depth intervals at both Tuvem and Diwar site are given in Table 14.

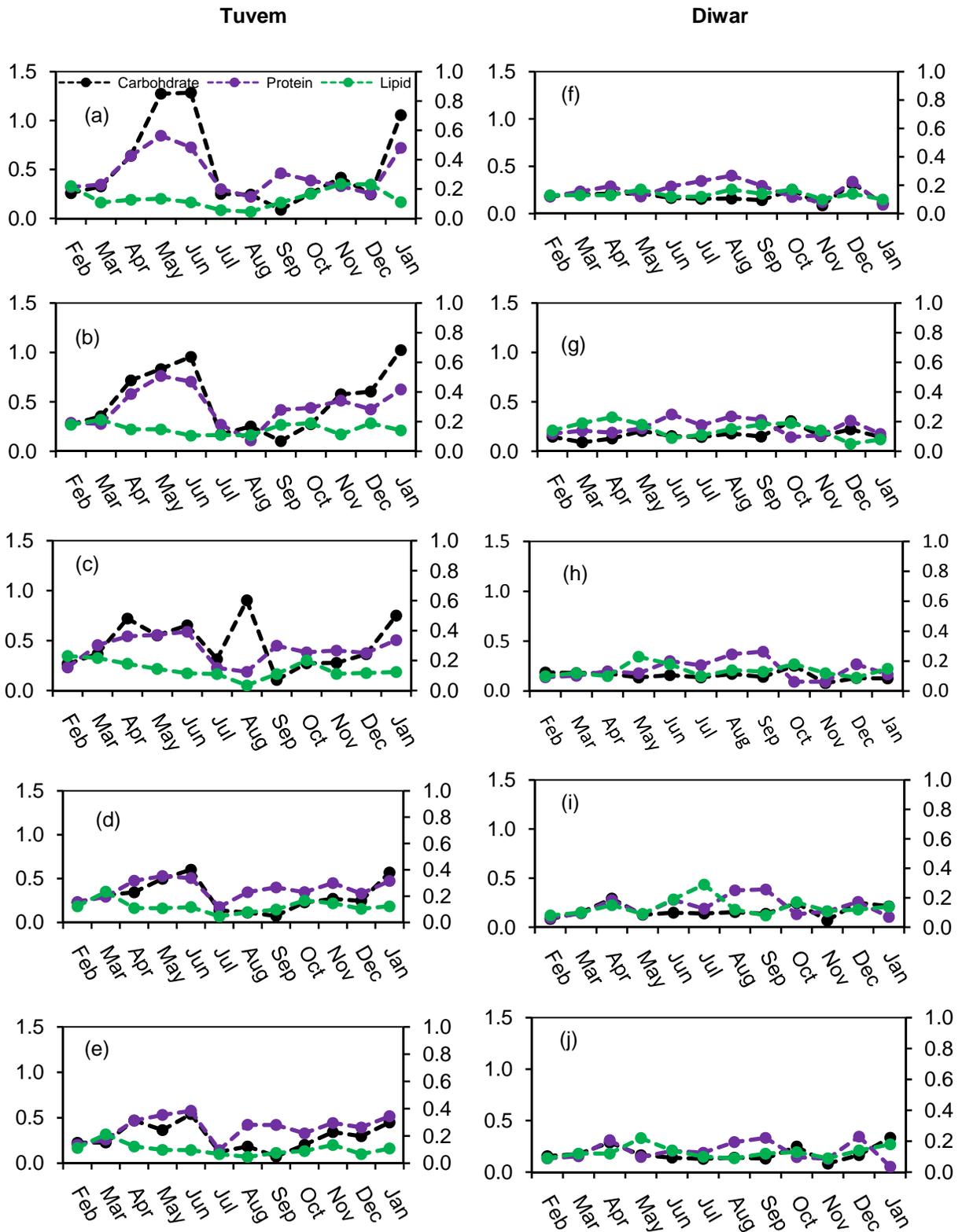


Figure: 19. Monthly down core variation of sediment: carbohydrate(%), protein(%) and lipid (%) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar– (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm. Primary y refers to carbohydrate and protein; and secondary y refers to lipid.

Table: 11. Seasonal and annual mean \pm SD of carbohydrate content (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	0.6 \pm 0.5	0.5 \pm 0.6	0.5 \pm 0.4	0.5 \pm 0.5
2 to 4	0.5 \pm 0.2	0.4 \pm 0.4	0.6 \pm 0.3	0.5 \pm 0.3
4 to 6	0.5 \pm 0.2	0.5 \pm 0.4	0.4 \pm 0.2	0.5 \pm 0.2
6 to 8	0.3 \pm 0.1	0.2 \pm 0.2	0.3 \pm 0.2	0.3 \pm 0.1
8 to 10	0.3 \pm 0.1	0.2 \pm 0.2	0.3 \pm 0.1	0.3 \pm 0.1
Diwar (Experimental site)				
0 to 2	0.2 \pm 0.01	0.2 \pm 0.01	0.2 \pm 0.10	0.2 \pm 0.06
2 to 4	0.1 \pm 0.05	0.2 \pm 0.02	0.2 \pm 0.07	0.2 \pm 0.06
4 to 6	0.2 \pm 0.02	0.2 \pm 0.02	0.1 \pm 0.07	0.2 \pm 0.04
6 to 8	0.2 \pm 0.09	0.1 \pm 0.01	0.2 \pm 0.08	0.2 \pm 0.07
8 to 10	0.2 \pm 0.06	0.1 \pm 0.01	0.2 \pm 0.10	0.2 \pm 0.07

Table: 12. Seasonal and annual mean \pm SD of protein content (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	0.53 \pm 0.5	0.4 \pm 0.6	0.4 \pm 0.4	0.5 \pm 0.2
2 to 4	0.5 \pm 0.3	0.4 \pm 0.4	0.5 \pm 0.3	0.5 \pm 0.2
4 to 6	0.4 \pm 0.2	0.4 \pm 0.4	0.4 \pm 0.2	0.4 \pm 0.1
6 to 8	0.4 \pm 0.1	0.4 \pm 0.2	0.4 \pm 0.2	0.4 \pm 0.1
8 to 10	0.4 \pm 0.1	0.4 \pm 0.2	0.4 \pm 0.1	0.4 \pm 0.1
Diwar (Experimental site)				
0 to 2	0.2 \pm 0.05	0.3 \pm 0.05	0.2 \pm 0.10	0.3 \pm 0.09
2 to 4	0.2 \pm 0.02	0.3 \pm 0.05	0.2 \pm 0.08	0.2 \pm 0.08
4 to 6	0.2 \pm 0.02	0.3 \pm 0.06	0.2 \pm 0.08	0.2 \pm 0.10
6 to 8	0.2 \pm 0.08	0.3 \pm 0.09	0.2 \pm 0.07	0.2 \pm 0.10
8 to 10	0.2 \pm 0.08	0.3 \pm 0.07	0.2 \pm 0.10	0.2 \pm 0.09

Table: 13. Seasonal and annual mean \pm SD of lipid content (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	0.2 \pm 0.05	0.08 \pm 0.03	0.2 \pm 0.06	0.14 \pm 0.06
2 to 4	0.2 \pm 0.03	0.1 \pm 0.03	0.2 \pm 0.04	0.15 \pm 0.04
4 to 6	0.2 \pm 0.04	0.1 \pm 0.04	0.1 \pm 0.04	0.14 \pm 0.06
6 to 8	0.1 \pm 0.06	0.08 \pm 0.03	0.1 \pm 0.03	0.12 \pm 0.05
8 to 10	0.1 \pm 0.05	0.07 \pm 0.02	0.1 \pm 0.03	0.10 \pm 0.04
Diwar (Experimental site)				
0 to 2	0.1 \pm 0.02	0.1 \pm 0.02	0.1 \pm 0.03	0.14 \pm 0.02
2 to 4	0.2 \pm 0.04	0.1 \pm 0.04	0.1 \pm 0.06	0.14 \pm 0.05
4 to 6	0.1 \pm 0.06	0.1 \pm 0.03	0.1 \pm 0.04	0.14 \pm 0.04
6 to 8	0.1 \pm 0.03	0.2 \pm 0.1	0.1 \pm 0.03	0.14 \pm 0.06
8 to 10	0.1 \pm 0.06	0.1 \pm 0.02	0.1 \pm 0.40	0.13 \pm 0.04

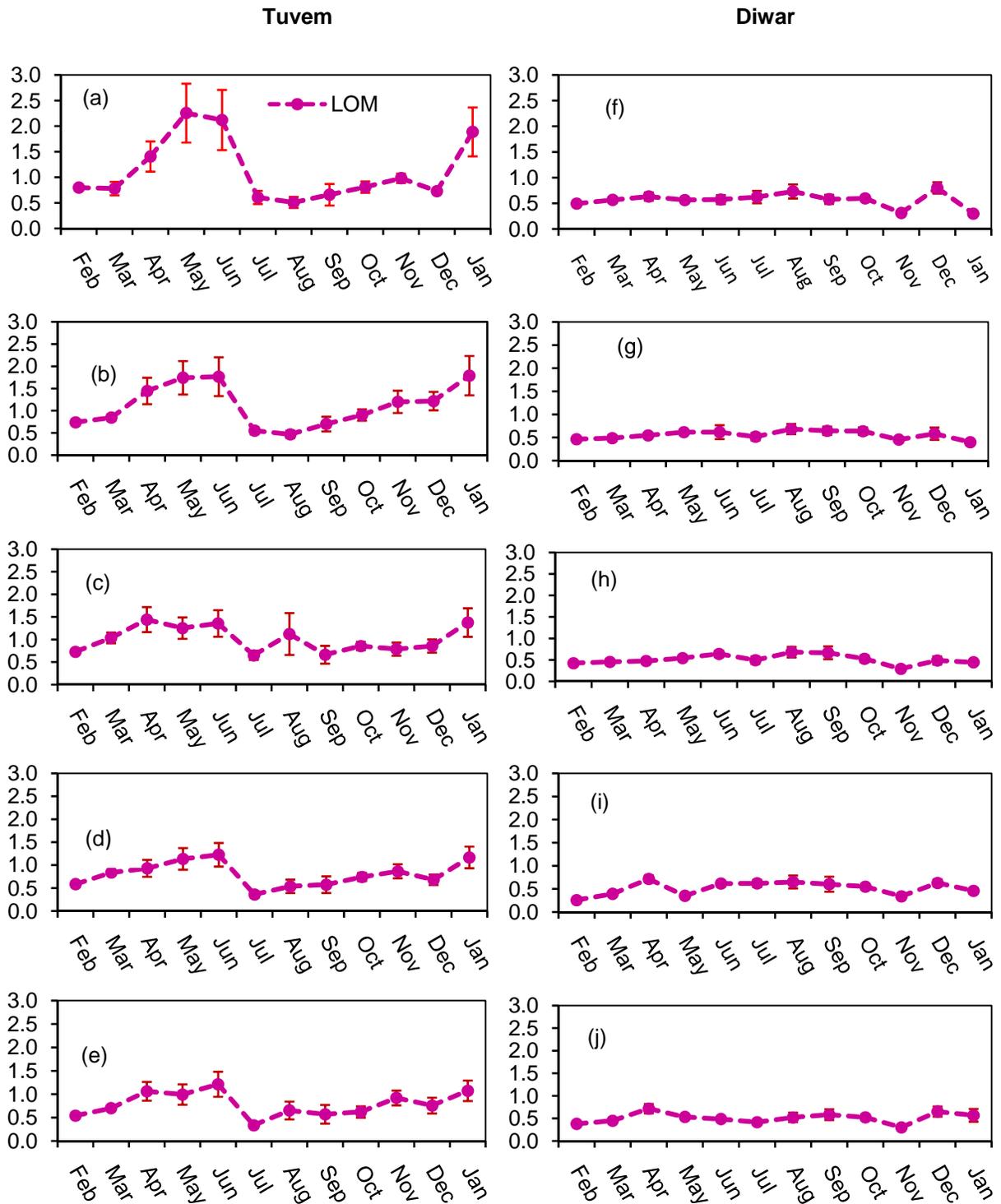


Figure: 20. Monthly down core variation of sediment labile organic matter (%) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar– (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm. Bars \pm represent SD of the mean (n=3).

Table: 14. Seasonal and annual mean \pm SD of labile organic matter content (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	1.3 \pm 0.7	1.0 \pm 0.8	1.1 \pm 0.5	1.1 \pm 0.6
2 to 4	1.2 \pm 0.5	0.9 \pm 0.6	1.3 \pm 0.4	1.1 \pm 0.5
4 to 6	1.1 \pm 0.3	1.0 \pm 0.3	1.0 \pm 0.3	1.0 \pm 0.2
6 to 8	0.9 \pm 0.2	0.7 \pm 0.4	0.9 \pm 0.2	0.8 \pm 0.3
8 to 10	0.8 \pm 0.2	0.7 \pm 0.4	0.8 \pm 0.1	0.8 \pm 0.3
Diwar (Experimental site)				
0 to 2	0.6 \pm 0.05	0.6 \pm 0.07	0.5 \pm 0.2	0.6 \pm 0.1
2 to 4	0.5 \pm 0.07	0.6 \pm 0.07	0.5 \pm 0.1	0.6 \pm 0.1
4 to 6	0.5 \pm 0.5	0.6 \pm 0.08	0.4 \pm 0.1	0.5 \pm 0.1
6 to 8	0.4 \pm 0.2	0.6 \pm 0.02	0.5 \pm 0.1	0.5 \pm 0.1
8 to 10	0.5 \pm 0.1	0.5 \pm 0.07	0.5 \pm 0.1	0.5 \pm 0.1

4.2.9. C/N ratio:

Seasonal, down core variation in C/N ratio is illustrated in Figure 21. At Tuvem, the down core variation in C/N ratio ranged from 8.2 to 16.4 (mean=11.3± 2.1; n=30), while at Diwar it varied from 1.1 to 15.2 (mean=8.3± 4.5; n=30). ANOVA showed a highly significant monthly variation in sediment C/N ratio profiles at both Tuvem (df=5, p<0.001, n=6) and Diwar (df=5, p<0.001, n=6) sites (Table 5 & 6). There was no statistically significant variation between the depths at both Tuvem and Diwar sites. A significant variation in C/N ratio was seen between Tuvem and Diwar (df=1, p<0.01, n=2) sites (Table 5). In general, the C/N ratio was higher at Diwar throughout the study period.

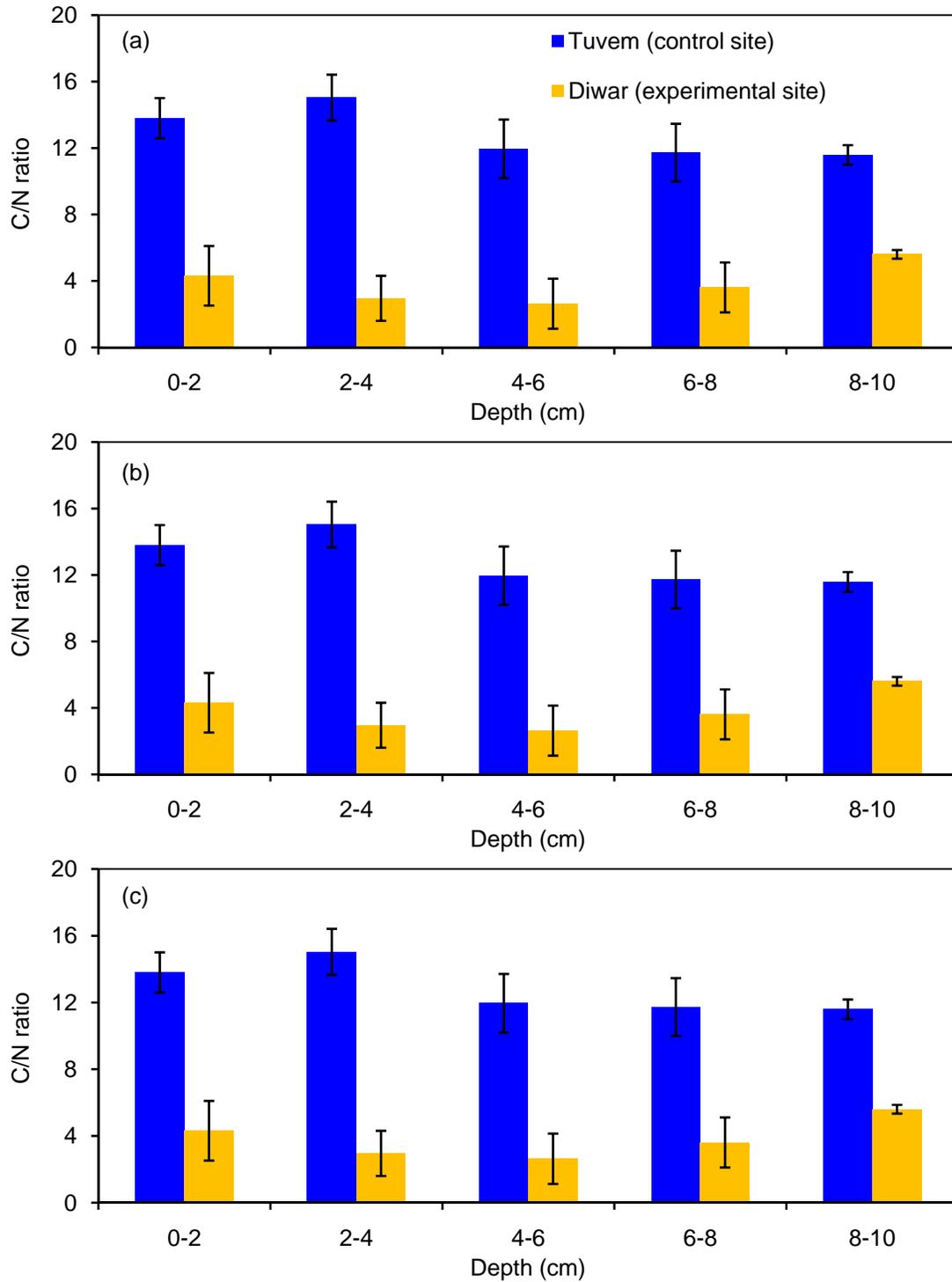


Figure: 21. Seasonal down core variation of C/N ratio at Tuvem and at Diwar – (a) Pre-monsoon, (b) Monsoon, (c) Post monsoon. Bars \pm represent SD of the mean (n=3).

4.2.10. Sediments granulometry (sand, silt & clay):

The monthly, down core variation of the sand fraction in the sediment is depicted in Figure 22. At Tuvem, the down core variation in sediment sand ranged from 2.1 to 48.5% (mean= 13.0 ± 13.5 ; n=60), while at Diwar it varied from 6.4 to 76.9% (mean= 31.9 ± 18.9 ; n=60). ANOVA showed a highly significant monthly variation in sediment sand profiles at both Tuvem (df=11, $p < 0.001$, n=12) and Diwar (df=4, $p < 0.001$, n=12) (Table 5 & 6). A significant variation in the sand fraction was seen on comparing Tuvem and Diwar (df=1, $p < 0.001$, n=2) (Table 5). Seasonal and annual mean of sand for different depth intervals at both Tuvem and Diwar are given in Table 15. In general sand content was higher at Diwar throughout the study period irrespective of the depth.

During the entire study period at Tuvem, the down core variation in silt the silt fraction (Figure 22) ranged from 15.7 to 44.1 % (mean= 24.6 ± 4.9 ; n=60) while at Diwar it varied from 6.07 to 30% (mean= $18.2 \pm 6.8\%$; n=60). ANOVA showed that there was a significant monthly variation in sediment silt profiles at the Diwar (df=11, $p < 0.001$, n=12) with no significant monthly variation at Tuvem (Table 5 & 6). However, throughout the period of sampling there was a high significant variation in silt fraction (df=1, $p < 0.001$, n=2) between Tuvem and Diwar sites Table 5. Seasonal and annual mean of silt (%) for different depth intervals at both Tuvem and Diwar site are given in Table 16. In general it could be observed that at Tuvem average silt was almost constant in all the three seasons while at Diwar silt (23 %) was higher during monsoon than non monsoon seasons.

Clay varied widely (Figure 22) at both Tuvem (33.7 to 78.6%; mean=62.5± 13.4; n=60) and Diwar (21.6 to 75.5%; mean=49.9± 14.4; n=60). ANOVA showed a very significant monthly variation at Tuvem (df=11; p<0 .001, n=12) and Diwar (df=11, p<0.001, n=12), but down core variation was not significant at both sites (Table 5 & 6). Throughout the period of sampling, sediment clay (%) differed significantly (df=1, p<0.001, n=1) between Tuvem and Diwar sites (Table 5). In general clay (%) was higher at Tuvem. Seasonal and annual mean of clay for different depth intervals at both Tuvem and Diwar sites are given in Table 17.

It could be observed that clay is the prominent contributor to the granulometry at Tuvem as well as Diwar sediment. At Tuvem and Diwar, the second prominent contributor was silt and sand respectively.

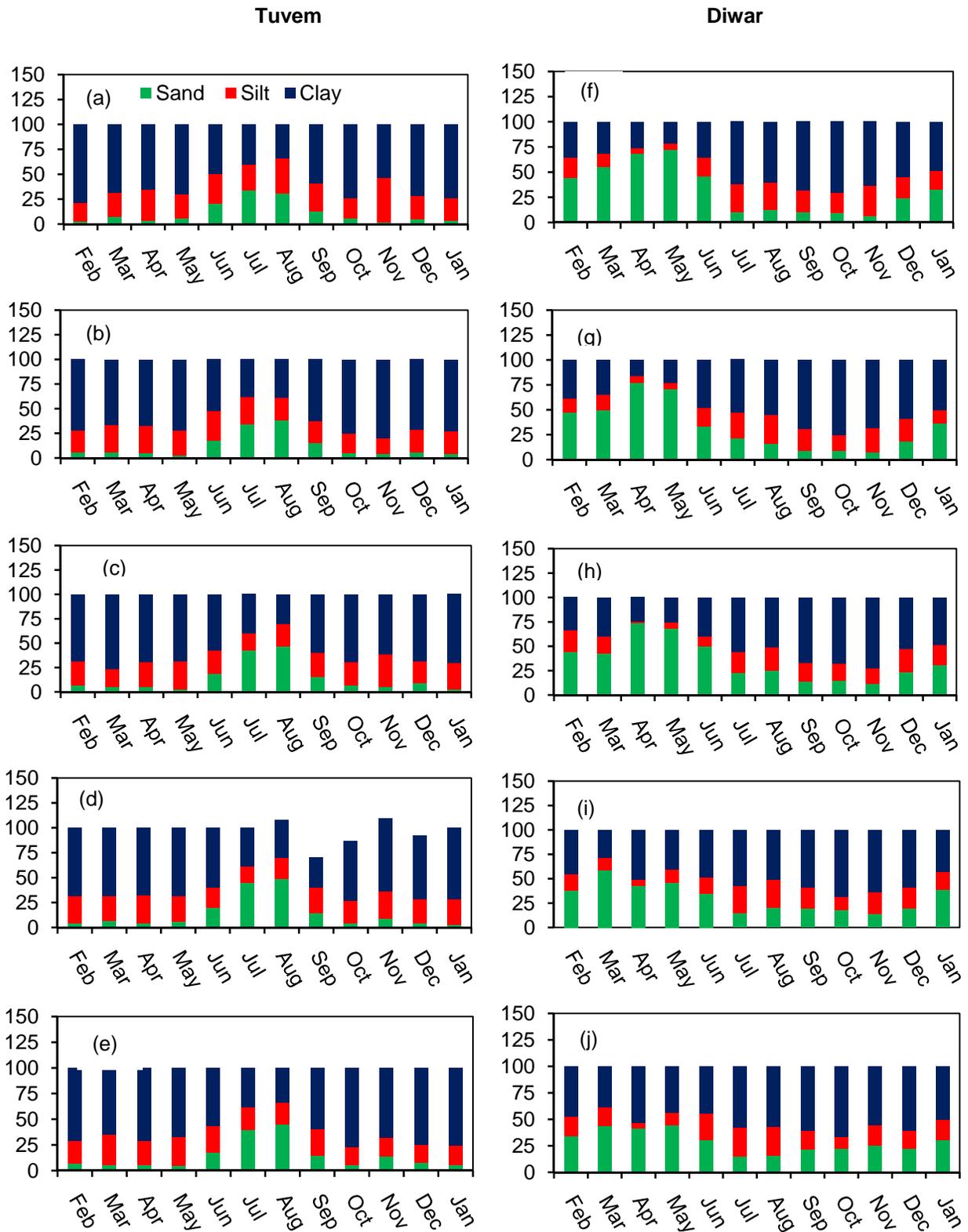


Figure: 22. Monthly down core variation of sediment: sand, silt & clay (%) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

Table: 15. Seasonal and annual mean \pm SD of sand (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	4.4 \pm 2.2	24.4 \pm 9.70	4.0 \pm 1.6	10.9 \pm 11.3
2 to 4	4.6 \pm 1.7	26.5 \pm 11.6	4.7 \pm 1.0	11.9 \pm 12.4
4 to 6	4.7 \pm 1.9	31.0 \pm 16.2	5.6 \pm 2.7	13.7 \pm 15.4
6 to 8	5.2 \pm 1.3	31.9 \pm 17.5	5.0 \pm 2.7	14.0 \pm 16.0
8 to 10	5.5 \pm 0.9	29.3 \pm 15.4	8.0 \pm 3.8	14.3 \pm 13.9
Diwar (Experimental site)				
0 to 2	59.8 \pm 12.8	19.8 \pm 17.3	18.4 \pm 12.4	32.7 \pm 23.9
2 to 4	60.0 \pm 15.1	19.6 \pm 10.2	17.4 \pm 13.4	32.7 \pm 24.0
4 to 6	57.3 \pm 16.1	28.0 \pm 15.5	20.0 \pm 15.5	35.0 \pm 20.9
6 to 8	46.0 \pm 8.9	22.4 \pm 8.50	22.4 \pm 10.9	30.3 \pm 14.5
8 to 10	41.0 \pm 4.6	21.0 \pm 7.10	25.2 \pm 3.80	28.8 \pm 10.2

Table: 16. Seasonal and annual mean \pm SD of silt (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	25.0 \pm 5.2	29.7 \pm 4.2	27.7 \pm 11.0	27.5 \pm 7.0
2 to 4	25.9 \pm 2.9	25.3 \pm 3.8	20.6 \pm 3.0	23.9 \pm 3.9
4 to 6	24.8 \pm 4.4	22.3 \pm 3.7	27.1 \pm 5.1	24.7 \pm 4.4
6 to 8	26.4 \pm 1.5	20.8 \pm 3.9	25.0 \pm 2.2	24.1 \pm 3.5
8 to 10	25.9 \pm 3.3	23.6 \pm 2.5	18.3 \pm .1	22.6 \pm 4.0
Diwar (Experimental site)				
0 to 2	11.6 \pm 6.9	23.7 \pm 4.5	22.2 \pm 5.3	19.3 \pm 7.6
2 to 4	11.2 \pm 5.0	24.0 \pm 4.6	19.2 \pm 5.6	18.1 \pm 7.2
4 to 6	11.9 \pm 9.7	19.0 \pm 6.0	19.8 \pm 3.5	16.9 \pm 7.3
6 to 8	12.7 \pm 4.4	23.8 \pm 5.5	19.4 \pm 3.8	18.6 \pm 6.3
8 to 10	13.4 \pm 6.0	24.6 \pm 4.3	16.5 \pm 3.9	18.2 \pm 6.6

Table: 17. Seasonal and annual mean \pm SD of clay (%) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	70.6 \pm 5.7	45.9 \pm 11.2	68.3 \pm 9.7	61.6 \pm 14.3
2 to 4	69.5 \pm 3.1	48.2 \pm 11.7	74.7 \pm 3.7	64.1 \pm 13.7
4 to 6	70.6 \pm 3.9	46.8 \pm 14.1	68.3 \pm 1.9	61.6 \pm 13.6
6 to 8	68.4 \pm 1.0	41.8 \pm 12.4	67.1 \pm 6.3	59.2 \pm 14.7
8 to 10	68.6 \pm 2.8	47.1 \pm 12.9	73.7 \pm 4.0	63.1 \pm 14.0
Diwar (Experimental site)				
0 to 2	28.6 \pm 6.1	56.5 \pm 14.4	59.4 \pm 9.7	48.2 \pm 17.4
2 to 4	27.9 \pm 10.5	56.6 \pm 9.0	63.4 \pm 10.8	49.3 \pm 18.5
4 to 6	31.0 \pm 7.0	53.3 \pm 11.2	60.2 \pm 11.4	48.1 \pm 15.9
6 to 8	41.3 \pm 9.7	53.8 \pm 5.2	58.2 \pm 11.1	51.1 \pm 11.1
8 to 10	45.8 \pm 6.2	54.8 \pm 7.2	58.3 \pm 7.0	53.0 \pm 8.3

4.3. Pore water:

4.3.1. Sulfate:

Pore water sulfate concentrations (Figure 23) varied widely at both Tuvem (3.1 to 14.4 mM, mean=7.4± 3.0 mM, n=60) and Diwar (2.7 to 17.3 mM, mean=9.6± 4.7 mM, n=60). ANOVA showed a significant monthly variation at both Tuvem (df=11, p<0.001, n=12) and Diwar sites (df=11, p<0.001, n=12), but no variation between the depths was seen at both Tuvem and Diwar (Table 5 & 6). A significant variation in sediment sulfate concentration was seen throughout the year in Tuvem and Diwar (df=1, p<0.05, n=2) (Table 5). Seasonal and annual mean of sulfate concentrations for different depth intervals at both Tuvem and Diwar site are given in Table 18. In general, there was a decrease in sulfate values at Tuvem and Diwar during the monsoon months as compared to pre and post-monsoon season concentrations.

4.3.2. Sulfide:

Sediment pore water sulfide concentrations (Figure 23) varied widely at both Tuvem (0.23 to 1.92 mM, mean=1.2± 0.5 mM, n=60) and Diwar (0.018 to 0.17 mM, mean=0.073± 0.04 mM, n=60). ANOVA did not show any significant monthly and down core variation in sulfide at Tuvem. However at Diwar, a significant monthly (df=11, p<0.001, n=11) and down core (df=5, p<0.05, n=4) variation was observed (Table 6). A significant variation was seen between the sediment sulfide content at Tuvem and Diwar (df=1, p<0.05, n=2) (Table 5). Seasonal and annual mean of sulfide concentrations for different depth intervals at both Tuvem and

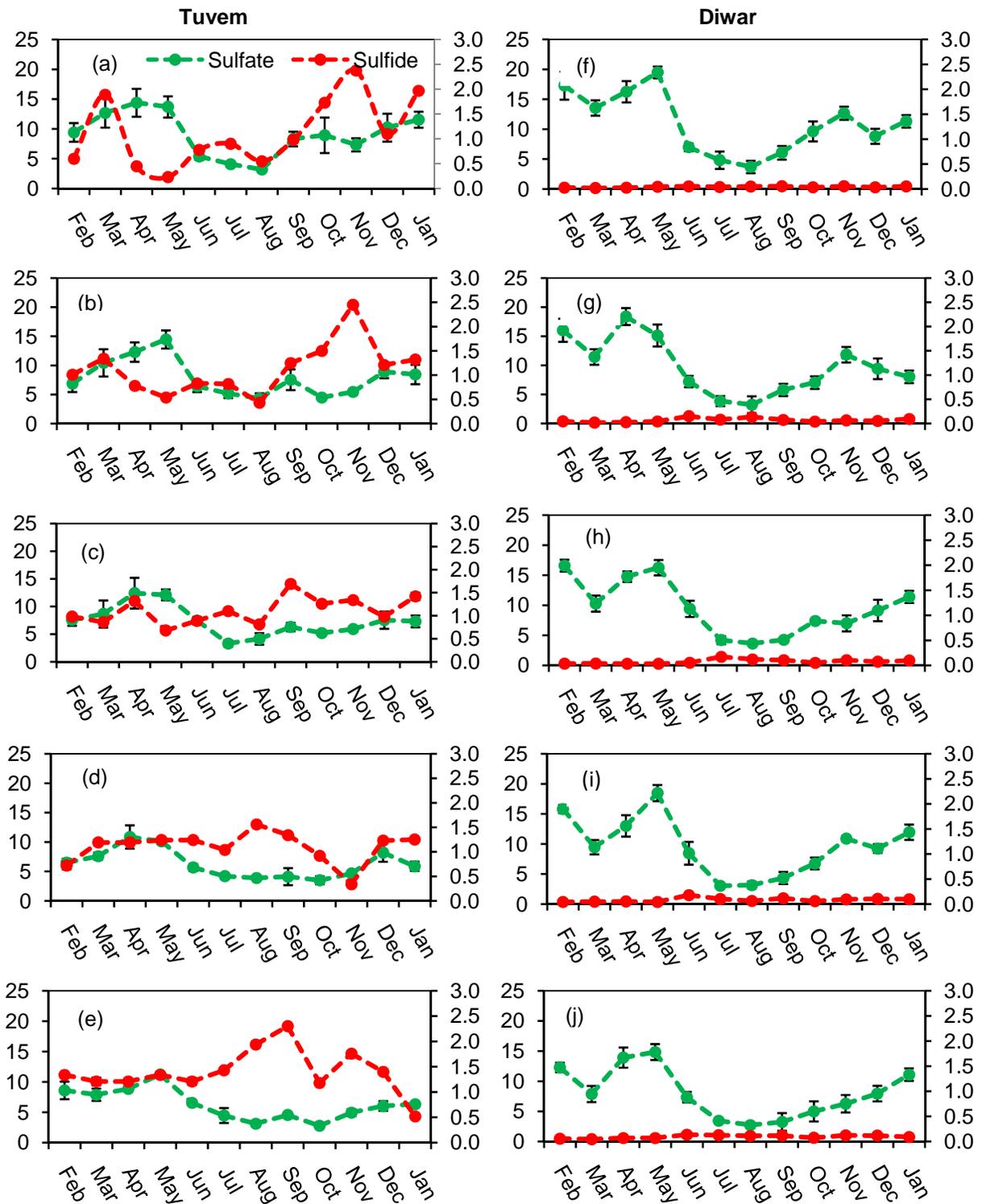


Figure: 23. Monthly down core variation sediment: sulfate (mM) and sulfide (mM) at Tuvem– (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0- 2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm. Primary y and secondary y axis in all cases refer to sulfate and sulfide respectively. Bars \pm represent SD of the mean (n=3).

Table: 18. Seasonal and annual mean±SD of sulfate concentration (mM) at different intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	12.6±2.2	5.26±2.4	9.5±1.8	9.1±3.7
2 to 4	11.0±3.2	5.9±1.4	6.8±2.2	7.9±3.2
4 to 6	10.2±2.4	5.3±1.9	6.5±1.1	7.3±2.8
6 to 8	8.7±2.1	4.5±0.8	5.6±2.0	6.3±2.5
8 to 10	9.15±1.5	4.7±1.4	5.0±1.6	6.3±2.5
Diwar (Experimental site)				
0 to 2	16.7±2.5	5.4±1.4	10.6±1.7	10.9±5.1
2 to 4	15.3±2.9	5.0±1.8	9.1±2.1	9.8±4.9
4 to 6	14.5±2.9	5.4±2.7	8.7±2.0	9.5±4.6
6 to 8	14.2±3.9	4.7±2.5	9.7±2.3	9.5±4.8
8 to 10	12.3±3.1	4.2±2.1	7.6±2.6	8.0±4.2

Table: 19. Seasonal and annual mean±SD of sulfide concentration (mM) at different intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	0.79±0.75	0.80±0.19	1.79±0.53	1.13±0.69
2 to 4	0.92±0.34	0.83±0.33	1.62±0.57	1.12±0.53
4 to 6	0.96±0.27	1.12±0.40	1.25±0.19	1.11±0.30
6 to 8	1.09±0.24	1.30±0.22	0.94±0.42	1.11±0.32
8 to 10	1.28±0.08	1.72±0.49	1.22±0.52	1.40±0.44
Diwar (Experimental site)				
0 to 2	0.03±0.01	0.05±0.01	0.05±0.01	0.04±0.01
2 to 4	0.03±0.01	0.11±0.04	0.06±0.02	0.07±0.04
4 to 6	0.04±0.00	0.11±0.05	0.08±0.02	0.08±0.04
6 to 8	0.05±0.00	0.12±0.05	0.09±0.02	0.08±0.04
8 to 10	0.06±0.01	1.23±0.02	0.10±0.02	0.10±0.03

Diwar site are given in Table 19. In general, the sulfide values were higher at Tuvem as compared to Diwar during all three seasons.

4.3.3. Salinity:

Annual pore water salinity (Figure 24) varied widely at both Tuvem (5.0 to 20.0 psu, mean= 9.7 ± 3.7 psu mM, n=60) and Diwar (3.0 to 22.0 mM, mean= 11.4 ± 4.7 psu, n=60). ANOVA showed a significant monthly variation of sediment salinity both at Tuvem (df=11, $p < 0.001$, n=12) and Diwar (df=11, $p < 0.001$, n=12) (Table 5 & 6). Down core variation was significant at Tuvem (df=4, $p < 0.05$, n=4) while at Diwar there was no significant variation between the depths. A significant variation was seen between Tuvem and Diwar (df=1, $p < 0.05$, n=2) sediments (Table 5). Seasonal and annual mean of salinity concentrations for different depth intervals at both Tuvem and Diwar sites are given in Table 20.

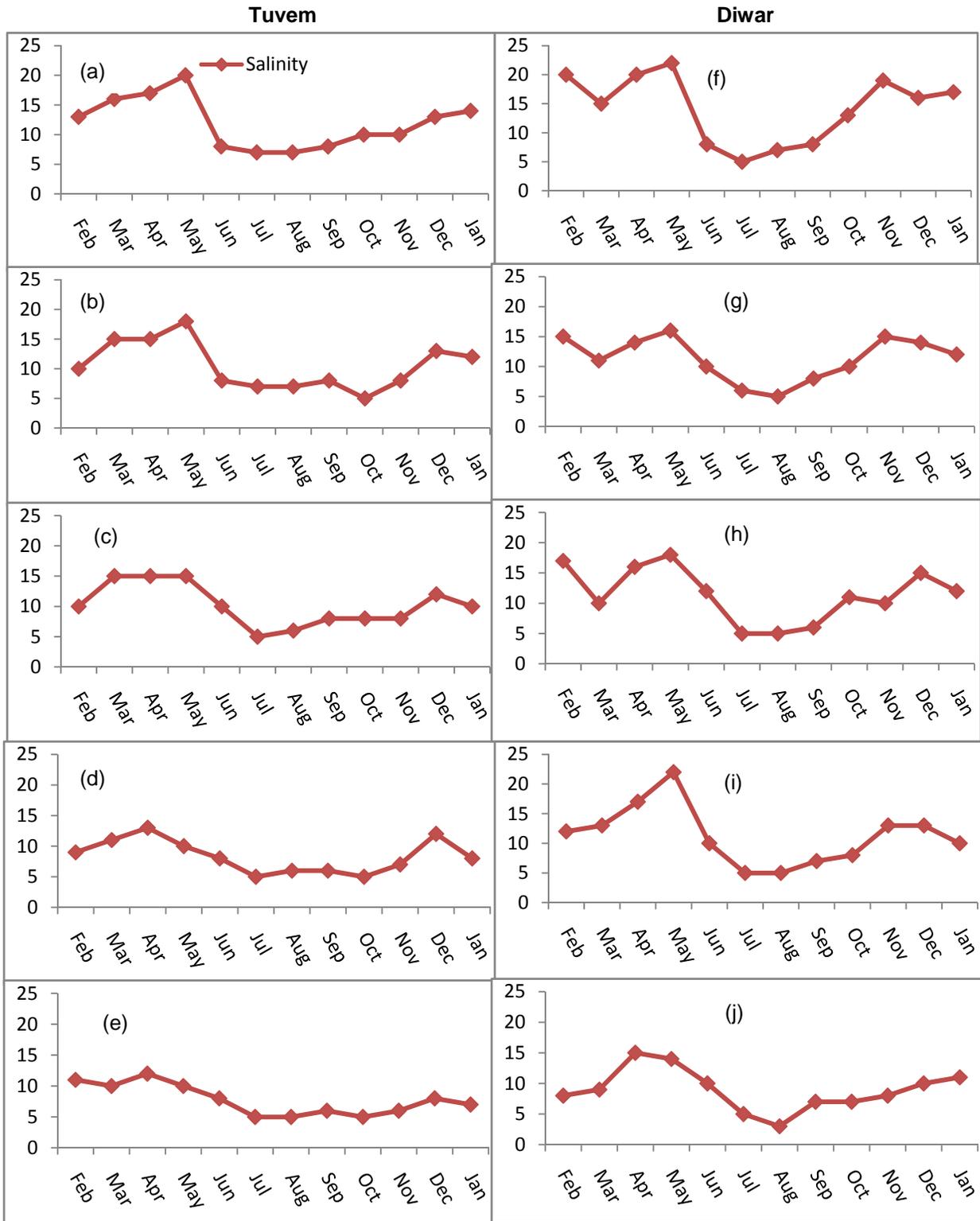


Figure: 24. Monthly down core variation of salinity (psu) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm. Bars± represent SD of the mean (n=3).

Table: 20. Seasonal and annual mean \pm SD of salinity at different intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	16.5 \pm 2.99	7.5 \pm 0.6	11.7 \pm 2.0	11.9 \pm 4.27
2 to 4	14.5 \pm 3.3	7.5 \pm 0.6	9.5 \pm 3.7	10.5 \pm 4.03
4 to 6	13.8 \pm 2.5	7.3 \pm 2.2	9.5 \pm 1.9	10.2 \pm 3.46
6 to 8	10.8 \pm 1.7	6.3 \pm 1.3	8.0 \pm 2.9	8.33 \pm 2.71
8 to 10	10.8 \pm 1.0	6.0 \pm 1.4	6.5 \pm 1.3	7.75 \pm 2.49
Diwar (Experimental site)				
0 to 2	19.3 \pm 3.1	7.0 \pm 1.4	16.3 \pm 2.5	14.2 \pm 5.9
2 to 4	14.0 \pm 2.2	7.3 \pm 2.2	12.8 \pm 2.2	11.3 \pm 3.7
4 to 6	15.3 \pm 3.6	7.0 \pm 3.4	12.0 \pm 2.2	11.4 \pm 4.5
6 to 8	16.0 \pm 4.5	6.8 \pm 2.4	11.0 \pm 2.5	11.3 \pm 4.9
8 to 10	11.5 \pm 3.5	6.3 \pm 3.0	9.0 \pm 1.80	8.9 \pm 3.40

4.4. Bacterial Abundance and Distribution:

4.4.1. Total cell counts:

Total cell counts are presented in the Figure 25. The total cell counts range from 0.47 to 3.23×10^{10} cells g^{-1} dry weight sediment at Tuvem with a mean of $1.53 \pm 0.68 \times 10^{10}$ cells g^{-1} dry weight sediment, $n=60$) and in Diwar from 0.75 to 3.4×10^{10} cells g^{-1} with a mean of $1.72 \pm 0.75 \times 10^{10}$ cells g^{-1} dry weight sediment, $n=60$. It was observed that both at the Tuvem and Diwar sites, the total cell counts were higher during the monsoon months compared to the non monsoon months and ranged from 10^{9-10} cells g^{-1} wet weight sediment. It was also seen that the average abundance, as recorded by total cell counts, was the lowest during the post - monsoon season at Tuvem ($1.21 \pm 0.19 \times 10^{10}$ cells g^{-1} dry weight sediment) and Diwar ($1.36 \pm 0.43 \times 10^{10}$ cells g^{-1} dry weight sediment). Seasonal and annual mean (\pm SD) of total cell counts is presented in Table 21. There was a significant monthly variability at both Tuvem ($df=11$, $p<0.001$, $n=12$) and Diwar ($df=11$, $p<0.001$, $n=12$) with no down core variation at either of the sites Table 5 & 6.

4.4.2. Total heterotrophic bacterial abundance and distribution:

Total heterotrophic bacterial counts (Figure 26) varied widely at both Tuvem (0.05 to 4.5×10^5 CFU g^{-1} wet weight sediment; (mean= $1.1 \pm 1.2 \times 10^5$ CFU g^{-1} wet weight sediment; $n=60$) and Diwar (0.11 to 3.3×10^5 CFU g^{-1} wet weight sediment; (mean= $9.2 \pm 8.2 \times 10^4$ CFU g^{-1} wet weight sediment; $n=60$). ANOVA showed a significant monthly variation ($df=12$, $p<0.001$, $n=12$) at the Tuvem, while there was no significant down core variation (Table 5 & 6). Similarly, at

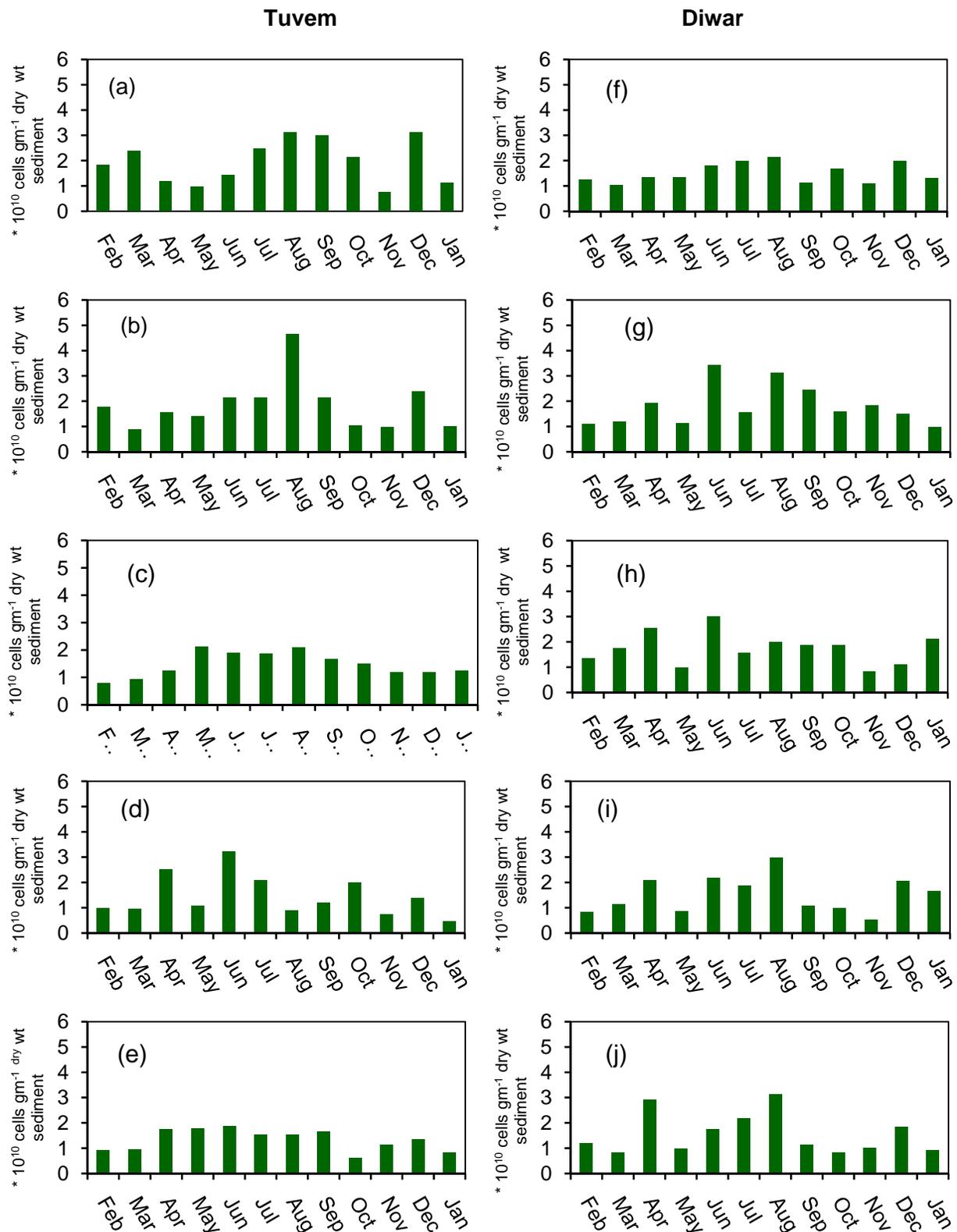


Figure 25: Monthly down core variation of total cell counts at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

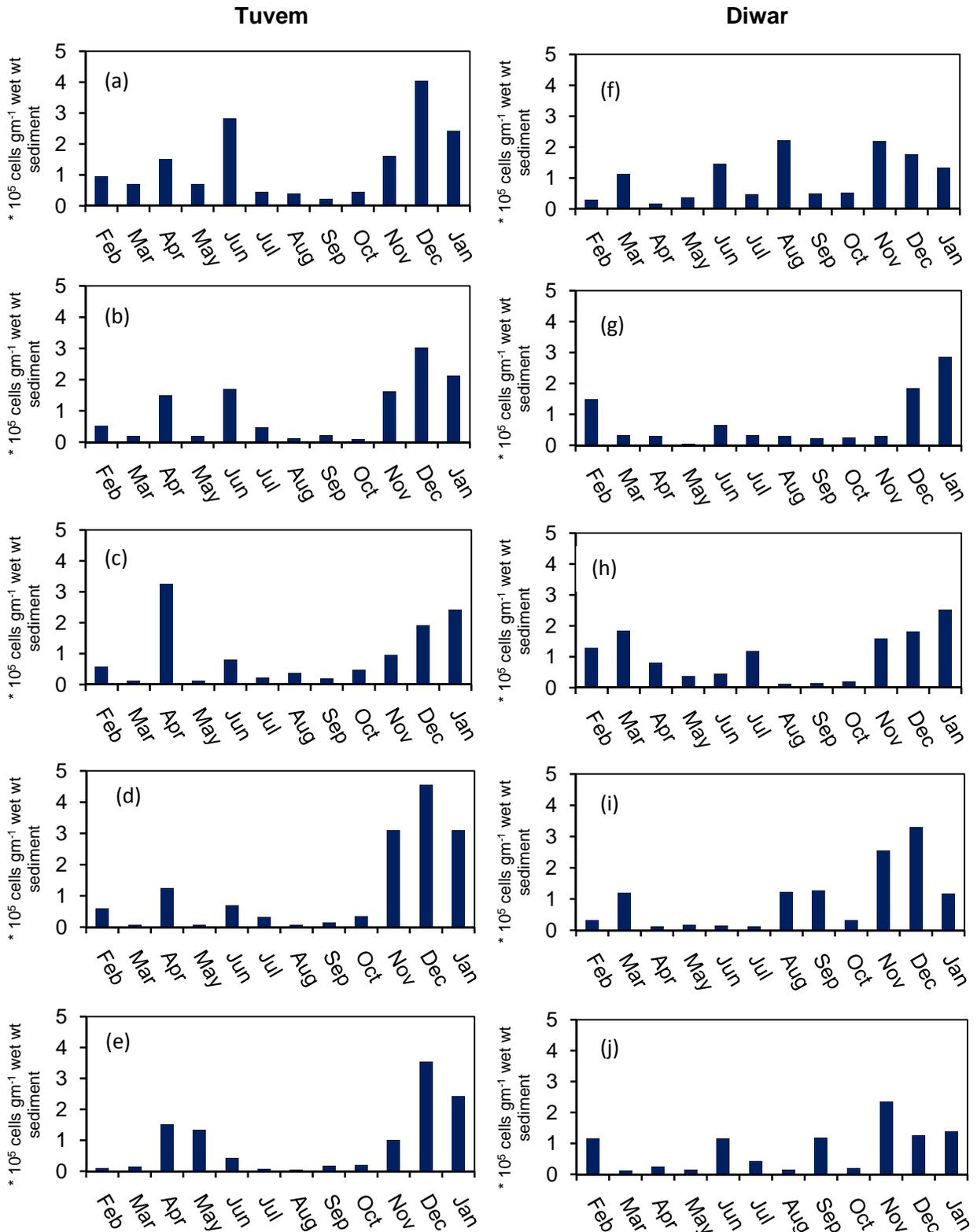


Figure 26: Monthly down core variation in total heterotrophic count at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

Table: 21. Seasonal and annual mean±SD of total cell counts ($\times 10^{10}$ cells g^{-1} dry weight sediment) at different depth intervals at the Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	1.7±0.7	2.1±0.6	1.5±0.6	1.7±0.6
2 to 4	1.4±0.8	1.9±1.1	1.1±0.7	1.5±0.8
4 to 6	1.2±0.6	2.0±0.8	1.3±0.7	1.5±0.7
6 to 8	1.4±0.5	1.6±0.2	1.0±0.3	1.3±0.4
8 to 10	1.5±1.0	2.1±0.8	1.2±0.5	1.6±0.8
Diwar (Experimental site)				
0 to 2	1.6±0.6	2.5±0.8	1.8±1.0	2.0±0.9
2 to 4	1.2±0.2	1.8±0.4	1.5±0.4	1.5±0.4
4 to 6	1.4±0.4	2.8±1.3	1.4±0.7	1.8±1.0
6 to 8	1.3±0.4	2.6±0.8	1.5±0.4	1.8±0.8
8 to 10	1.3±0.6	1.9±0.2	1.3±0.1	1.5±0.5

Table: 22. Seasonal and annual mean±SD of total heterotrophic count ($\times 10^5$ cells g^{-1} wet weight sediment) at different depth intervals at the Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	1.0±0.4	1.0±1.2	2.1±1.5	1.4±1.2
2 to 4	0.6±0.6	0.6±0.7	1.7±1.2	1.0±1.0
4 to 6	1.0±1.5	0.4±0.3	1.4±0.9	1.0±1.0
6 to 8	0.5±0.6	0.3±0.3	2.8±1.8	1.2±1.5
8 to 10	0.8±0.8	0.2±0.2	1.8±1.5	1.0±1.1
Diwar (Experimental site)				
0 to 2	0.5±0.4	1.2±0.8	1.4±0.7	1.0±0.7
2 to 4	0.5±0.6	0.4±0.2	1.3±1.3	0.7±0.9
4 to 6	1.0±0.6	0.5±0.5	1.5±1.0	1.0±0.8
6 to 8	0.4±0.5	0.7±0.6	1.8±1.3	1.0±1.0
8 to 10	0.4±0.5	0.7±0.5	1.3±0.9	0.8±0.7

Diwar, there was significant monthly variation ($df=11$ $p<0.001$, $n=12$) throughout the period of sampling and no significant variation was noticed in the total heterotrophic counts between Tuvem and Diwar irrespective of the depths. Seasonal and annual mean ($\pm SD$) of total heterotrophic bacterial counts are given in Table 22. It could be seen that in general, there was an increase in total heterotrophic bacterial counts at Tuvem during the post-monsoon months as compared to pre-monsoon and monsoon months. Moreover, the total heterotrophic bacterial counts at Diwar site were higher than Tuvem throughout the three seasons.

4.4.3. Abundance and distribution of sulfate reducing bacteria (SRB):

4.4.3.1. Agar-shake method:

4.4.3.1.1 Acetate:

Retrievable SRB counts (Figure 27) on acetate as a carbon source varied widely at both Tuvem (0.24 to 4.8×10^3 CFU g^{-1} wet weight sediment; mean= $1.9 \pm 1.4 \times 10^3$ wet weight sediment; $n=60$) and Diwar site (0.23 to 4.9×10^3 CFU g^{-1} wet weight sediment; mean= $1.5 \pm 1.0 \times 10^3$ wet weight sediment ($n=60$)). ANOVA did not show a significant monthly and down core variation at Tuvem and Diwar. At Tuvem a significant monthly ($df=11$, $p<0.001$, $n=12$) variation was observed with no variations between the depths (Table 5). Throughout the period of sampling, there was no significant variation in retrievable SRB counts on acetate as a carbon source between Tuvem and Diwar. Seasonal and annual mean of retrievable SRB counts for different depth intervals at both Tuvem and Diwar are given in Table 23. The general trend observed was the retrievable SRB counts at Tuvem were higher than Diwar site for all the three seasons.

4.4.3.1.2 Lactate:

Lactate was a more preferred substrate as compared to acetate. Retrievable SRB counts (Figure 28) on lactate as a carbon source varied widely at both Tuvem (0.37 to 4.9×10^3 CFU g^{-1} wet weight sediment; mean= $2.4 \pm 1.3 \times 10^3$ wet weight sediment; n=60) and Diwar (0.05 to 4.2×10^3 CFU g^{-1} wet weight sediment; mean= $1.5 \pm 1.1 \times 10^3$ wet weight sediment; n=60). ANOVA showed a significant monthly (df=11, $p < 0.05$, n=12) variation while down core variation was insignificant at Tuvem. At Diwar, there was no significant monthly variation while variation between the depths was significant (df=4, $p < 0.01$, n=5). Throughout the period of sampling there was a significant (df=1, $p < 0.001$, n=2) variation in retrievable SRB counts on lactate as a carbon source between both the sites (Table 5). Seasonal and annual mean of retrievable SRB counts for different depth intervals at both sites are given in Table 24. It was observed that in general the retrievable SRB counts at the Tuvem were higher than the Diwar site for all the three seasons.

4.4.3.2. Most Probable Number method (MPN):

4.4.3.2.1. Acetate:

MPN counts of SRB (Figure 29) on acetate as a carbon source varied widely at both Tuvem (0.04 to 4.5×10^5 cells g^{-1} wet weight sediment; mean= $1.2 \pm 1.2 \times 10^5$ cells g^{-1} wet weight sediment; n=60) and Diwar (0.15 to 3.5×10^5 cells g^{-1} wet weight sediment; mean= $1.0 \pm 1.0 \times 10^5$ cells g^{-1} wet weight sediment n=60). In the surficial sediments, acetate counts were highest (2×10^5 cells) in the month of september at Tuvem and in the month of march (9.5×10^5 cells) at Diwar. ANOVA showed no significant monthly nor down core variation at Tuvem as well as Diwar, throughout

the period of sampling there was a significant variation ($df=1$, $p<0.001$, $n=2$) in MPN counts between Tuvem and Diwar (Table 5). Seasonal and annual mean ($\pm SD$) of MPN counts for different depth intervals at both Tuvem and Diwar are shown in Table 25. The MPN counts were higher at Tuvem.

4.4.3.2.2. Lactate:

MPN counts of SRB (Figure 30) with lactate as a carbon source showed wide variation at both Tuvem (0.3 to 4.5×10^5 cells g^{-1} wet weight sediment; mean= $2.2 \pm 1.6 \times 10^5$ cells g^{-1} wet weight sediment; $n=60$) and Diwar (0.2 to 4.5×10^5 wet weight sediment; mean= $1.3 \pm 1.2 \times 10^5$ wet weight sediment; $n=60$). In the surficial sediments, lactate counts were highest (4.5×10^5 cells) in the month of June at Tuvem and in the month of October (9.5×10^5 cells) at Diwar. No significant monthly nor down core variation was observed in Tuvem sediments. However at Diwar there was a significant monthly variation ($df=11$, $p<0.01$, $n=11$), with no down core variation (Table 6). Throughout the period of sampling a significant variation ($df=1$, $p<0.001$, $n=2$) (Table 5) in MPN counts of SRB between Tuvem and Diwar were seen being higher at Tuvem during all the three seasons. Table 26.

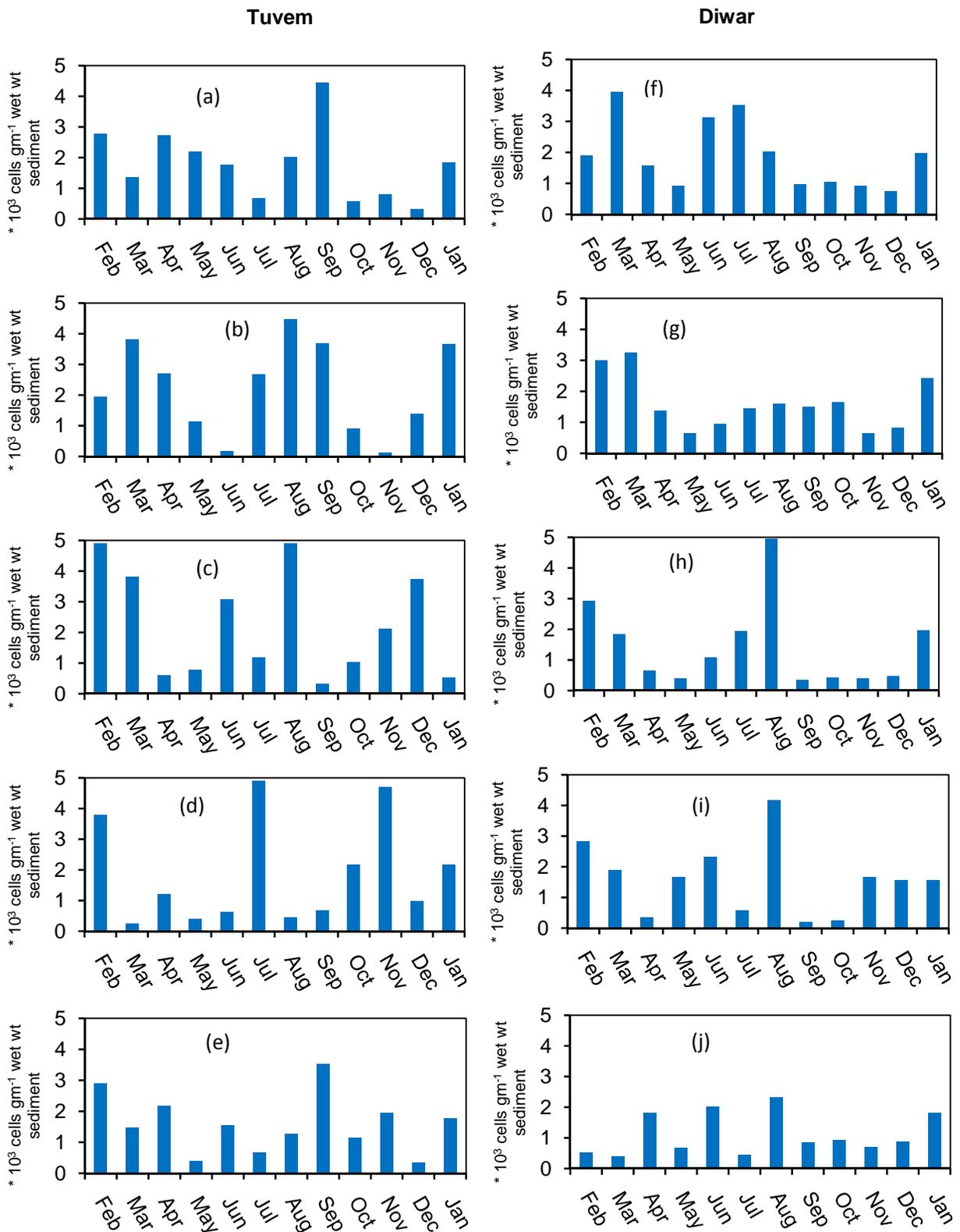


Figure: 27. Monthly down core variation SRB (agar shake acetate) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

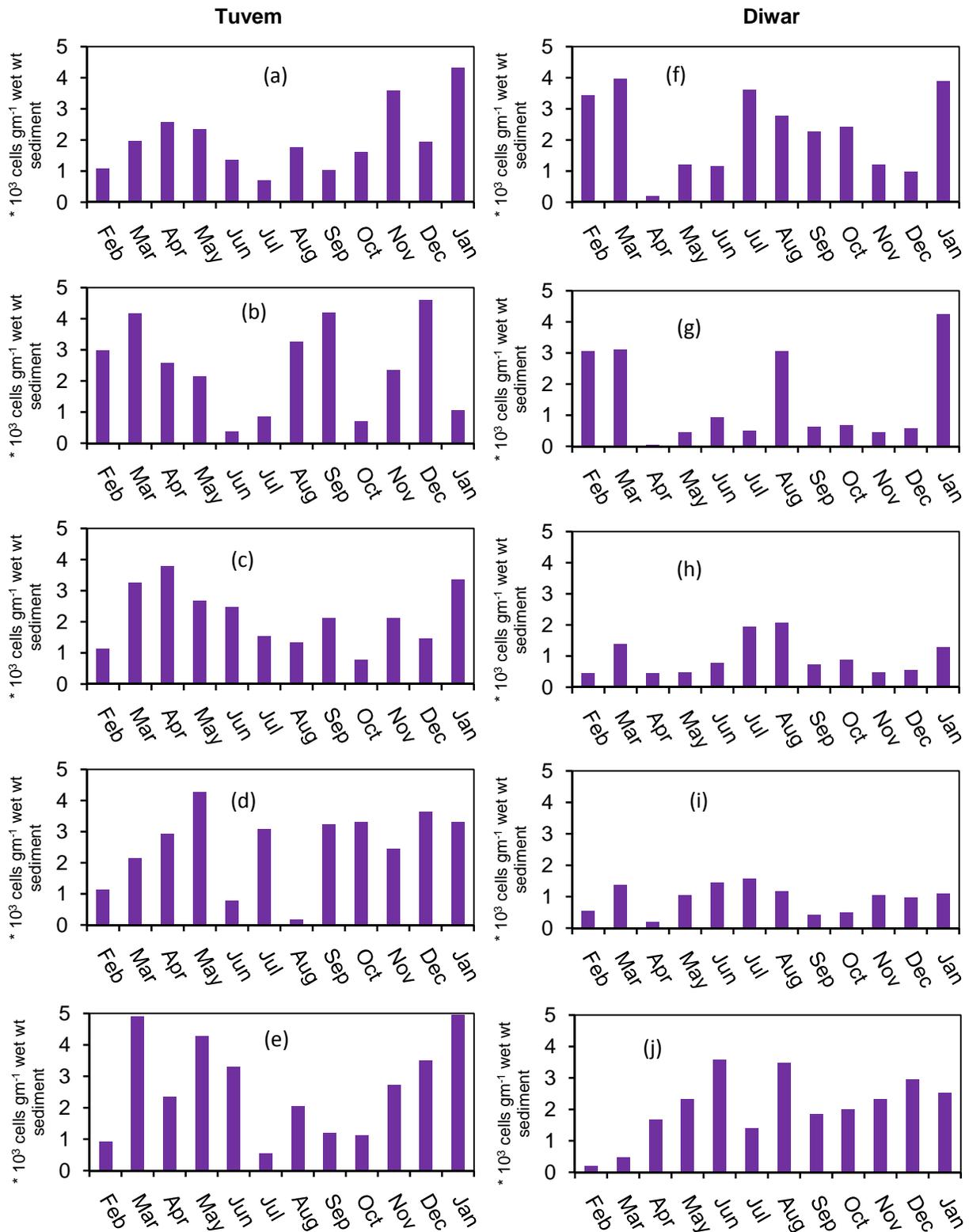


Figure: 28. Monthly down core variation SRB (agar shake lactate) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

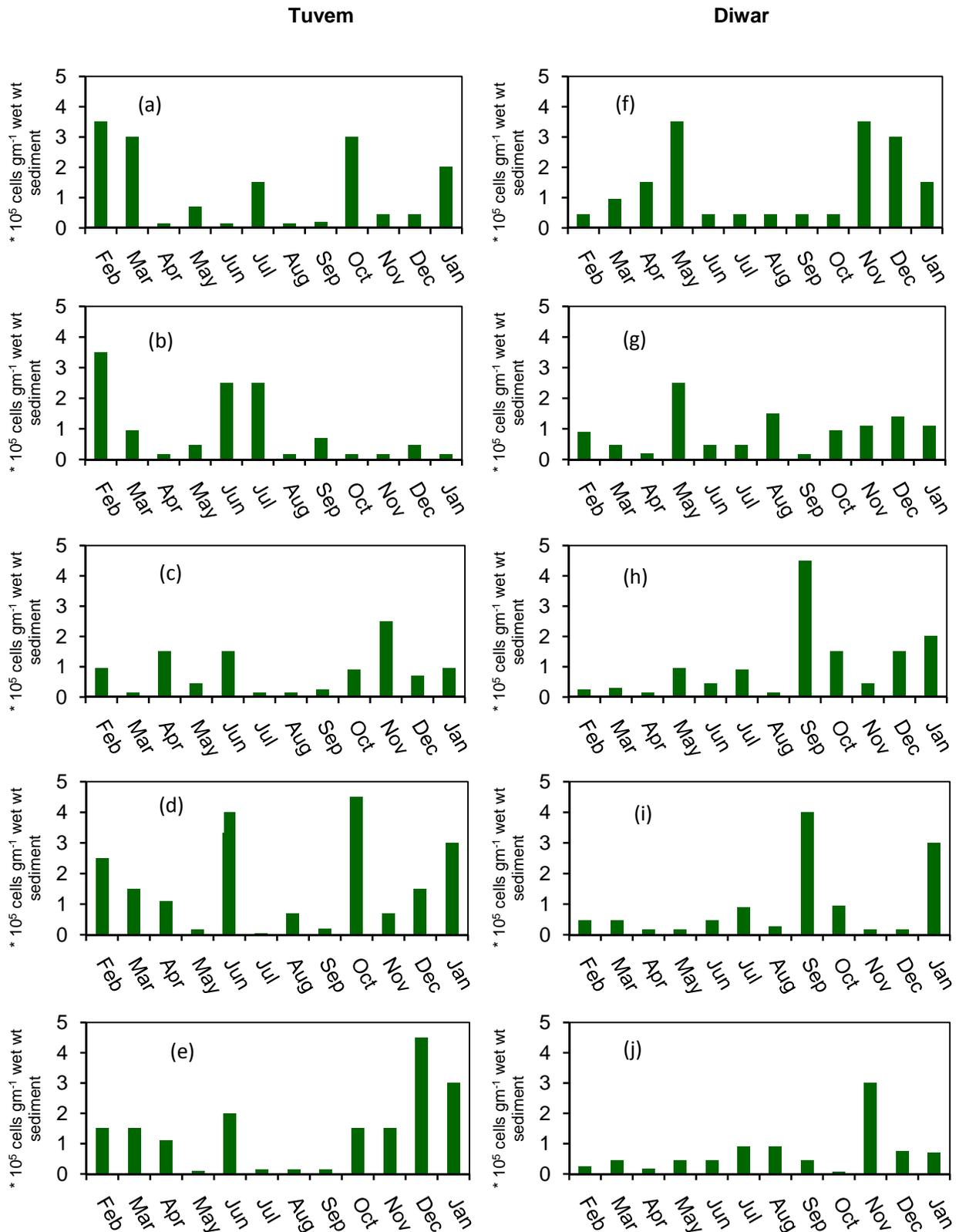


Figure: 29. Monthly down core variation SRB (MPN acetate) at Tuvem– (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

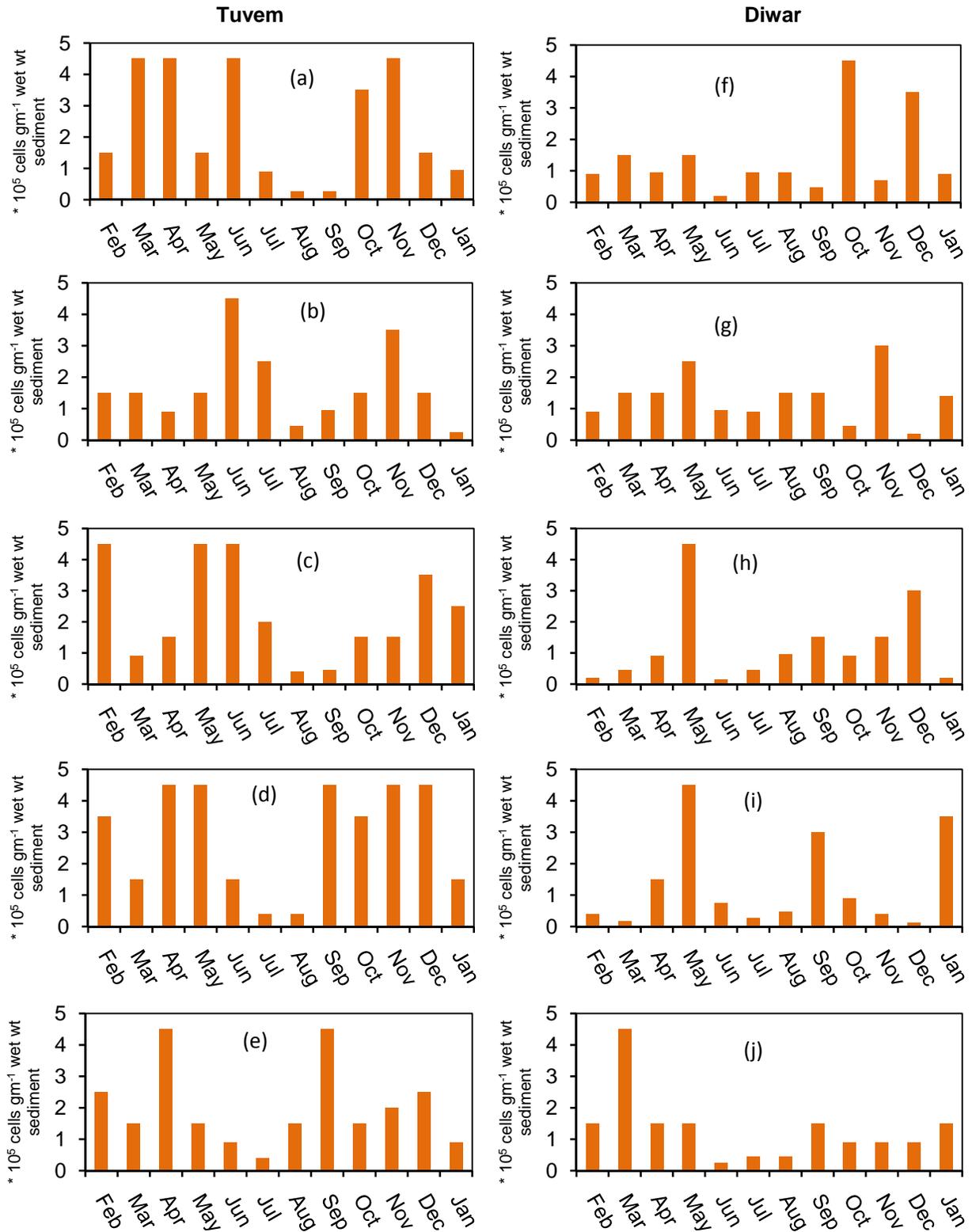


Figure: 30. Monthly down core variation SRB (MPN lactate) at Tuveem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

Table: 23. Seasonal and annual mean±SD of SRB agar shake acetate ($\times 10^3$ cells g^{-1} wet weight sediment) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	2.3±0.6	2.2±1.6	0.9±0.7	1.8±1.7
2 to 4	2.4±1.1	2.7±1.9	1.5±1.5	2.2±2.2
4 to 6	2.5±2.2	2.4±2.0	1.9±1.4	2.2±2.0
6 to 8	1.4±1.7	1.7±2.2	2.5±1.7	1.9±1.7
8 to 10	1.7±1.1	1.8±1.2	1.3±0.7	1.6±1.5
Diwar (Experimental site)				
0 to 2	2.1±1.3	2.4±1.2	1.2±0.5	1.9±1.1
2 to 4	2.1±1.3	1.4±0.3	1.4±0.8	1.6±0.8
4 to 6	1.5±1.1	2.1±2.0	0.8±0.8	1.5±1.3
6 to 8	1.7±1.0	1.8±1.8	1.3±0.7	1.6±1.1
8 to 10	0.9±0.6	1.4±0.9	1.1±0.5	1.1±0.7

Table: 24. Seasonal and annual mean±SD of SRB agar shake lactate ($\times 10^3$ cells g^{-1} wet weight sediment) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	2.0±0.7	2.1±0.5	1.7±0.9	2.0±1.1
2 to 4	3.0±0.9	2.3±1.6	1.5±1.0	2.4±1.5
4 to 6	2.7±1.1	3.0±0.6	2.6±1.0	2.2±1.0
6 to 8	2.6±1.3	2.5±1.5	2.8±1.5	2.6±1.3
8 to 10	3.1±1.8	3.7±1.1	2.6±1.6	2.6±1.6
Diwar (Experimental site)				
0 to 2	2.2±1.8	2.4±1.0	2.1±1.3	2.3±1.3
2 to 4	1.7±1.6	1.3±1.2	1.5±1.8	1.5±1.4
4 to 6	0.7±0.5	1.4±0.7	0.8±0.4	1.0±0.6
6 to 8	0.8±0.5	1.1±0.5	0.9±0.3	0.9±0.4
8 to 10	1.2±1.0	2.6±1.1	2.5±0.4	2.0±1.0

Table: 25. Seasonal and annual mean±SD of SRB, MPN acetate ($\times 10^5$ cells g^{-1} wet weight sediment) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon	Annual
0 to 2	1.8±1.7	0.5±0.7	1.5±1.3	1.3±1.3
2 to 4	1.3±1.5	1.5±1.2	0.2±0.2	1.0±1.2
4 to 6	0.8±0.6	0.5±0.7	1.3±0.8	0.8±0.7
6 to 8	1.3±1.0	1.2±1.9	2.4±1.7	1.7±1.5
8 to 10	1.0±0.7	0.6±1.0	2.6±1.4	1.4±1.3
Diwar (Experimental site)				
0 to 2	1.6±1.3	0.5±0.0	2.1±1.4	1.4±1.2
2 to 4	1.0±1.0	0.6±0.6	1.1±0.2	0.9±0.7
4 to 6	0.4±0.4	1.5±2.0	1.3±0.7	1.1±1.2
6 to 8	0.3±0.2	1.4±1.8	1.1±1.3	1.0±1.3
8 to 10	0.3±0.1	0.7±1.3	1.1±1.3	0.7±0.8

Table: 26. Seasonal and annual mean±SD of SRB, MPN lactate ($\times 10^5$ cells g^{-1} wet weight sediment) at different depth intervals at Tuvem and Diwar sites.

Tuvem (Control site)				
Depth Interval (cm)	Pre-monsoon	Monsoon	Post - monsoon	Annual
0 to 2	3.0±1.7	1.5±2.0	2.6±1.7	2.4±1.8
2 to 4	1.4±0.3	2.1±1.8	1.7±1.3	1.7±1.2
4 to 6	2.9±1.9	1.8±1.9	2.3±1.0	2.3±1.6
6 to 8	3.5±1.4	1.7±1.9	3.5±1.4	2.9±1.7
8 to 10	2.5±1.4	1.8±1.8	1.7±0.7	2.0±1.3
Diwar (Experimental site)				
0 to 2	1.2±0.3	0.6±0.4	2.4±1.9	1.4±1.3
2 to 4	1.6±0.7	1.2±0.3	1.3±1.3	1.4±0.8
4 to 6	1.5±2.0	0.8±0.6	1.4±1.2	1.2±1.3
6 to 8	1.6±2.0	1.1±1.3	1.2±1.5	1.3±1.5
8 to 10	2.3±1.5	0.7±0.6	1.0±0.3	1.3±1.0

4.5. Sulfate Reduction Rates:

4.5.1. S^{35} based estimation (tracer technique):

Sulfate reduction rates (SRR) in sediment cores (0-10 cm with 2 cm interval) (Figure 31) varied widely at both Tuvem (50.2 to 698.7 $nM\ cm^{-3}\ d^{-1}$; mean= $247.5 \pm 162.3\ nM\ cm^{-3}\ d^{-1}$; n=60) and Diwar (23.3 to 294.5 $nM\ cm^{-3}\ d^{-1}$; mean= $138.5 \pm 67.8\ nM\ cm^{-3}\ d^{-1}$; n=60). A significant monthly variation (df=12, $p < 0.001$, n=12), and no significant down core variation at Tuvem and Diwar was seen (Table 5 & 6). Throughout the period of sampling, a significant variation (df=1, $p < 0.001$, n=2) was noticed in SRR between Tuvem and Diwar sites (Table 5). It could be seen that in general SRR was higher (ca. 2 times) during non monsoon seasons as compared to the monsoon season at Tuvem site. While a reverse trend was observed at the Diwar site i.e SRR was higher during monsoon season as compared to pre-monsoon (ca. 3 times) and post monsoon (ca. 2 times).

Integrated sulfate reduction rates (0-2 to 10 cm) are shown in Figure 32 a & b. Integrated SRR showed a wide variation at both Tuvem (10.4 to 63.4 $mM\ M^{-2}\ d^{-1}$; mean= $35.2 \pm 19.6\ mM\ M^{-2}\ d^{-1}$; n=12) and Diwar site (6.28 to 63.37 $mM\ M^{-2}\ d^{-1}$; mean= $21.2 \pm 15.2\ mM\ M^{-2}\ d^{-1}$; n=12). A significant variation (df=1, $p < 0.05$, n=1) between Tuvem and Diwar site was seen (Table 5). At Tuvem integrated SRR was higher (ca. 2 times) during the non monsoon seasons as compared to monsoon season. While at the Diwar it was higher during the monsoon season as compared to pre- monsoon (ca.4 times) and post monsoon season (ca. 2 times) (Figure 33).

In general, the average SRR and integrated SRR were higher at Tuvem as compared to Diwar. The average SRR was (55%) higher while the integrated SRR was 60 % higher at Tuvem than Diwar sediments.

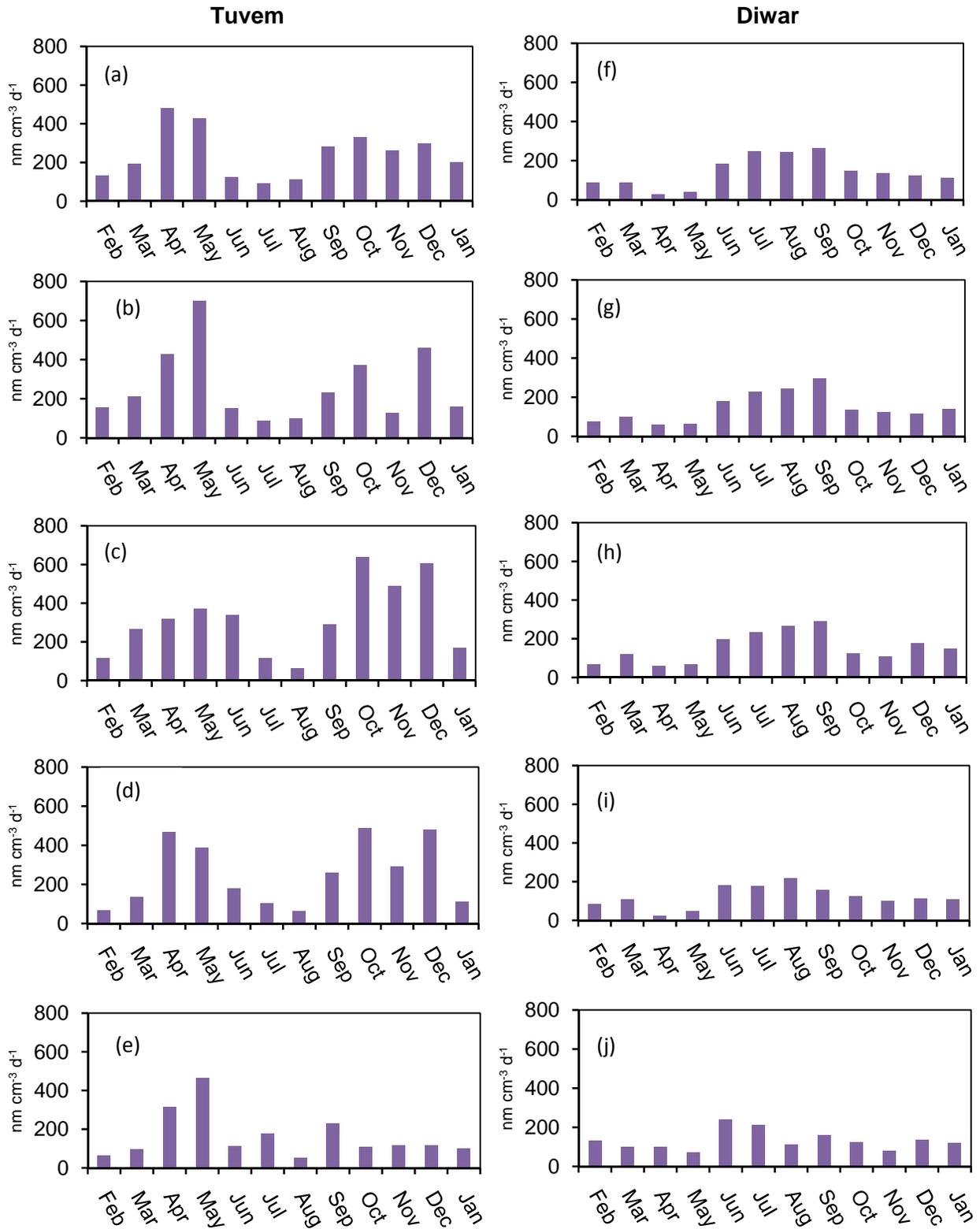


Figure: 31. Monthly down core variation in sulfate reducing activity ($\text{nm cm}^{-3} \text{d}^{-1}$) at Tuvem – (a) 0-2 cm, (b) 2-4 cm, (c) 4-6 cm, (d) 6-8 cm, (e) 8-10 cm and at Diwar – (f) 0-2 cm, (g) 2-4 cm, (h) 4-6 cm, (i) 6-8 cm, (j) 8-10 cm.

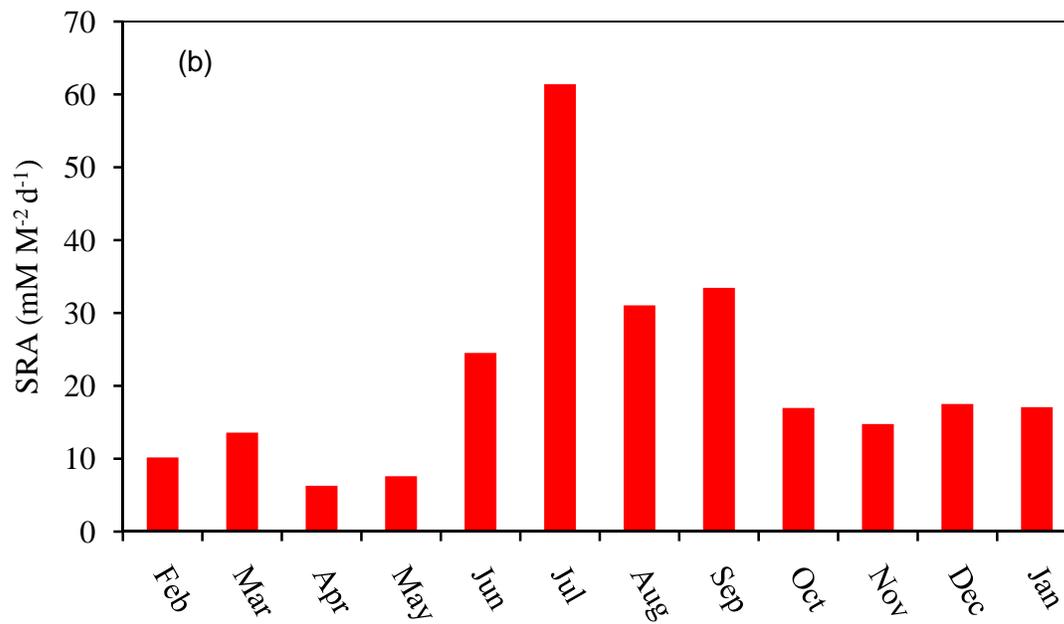
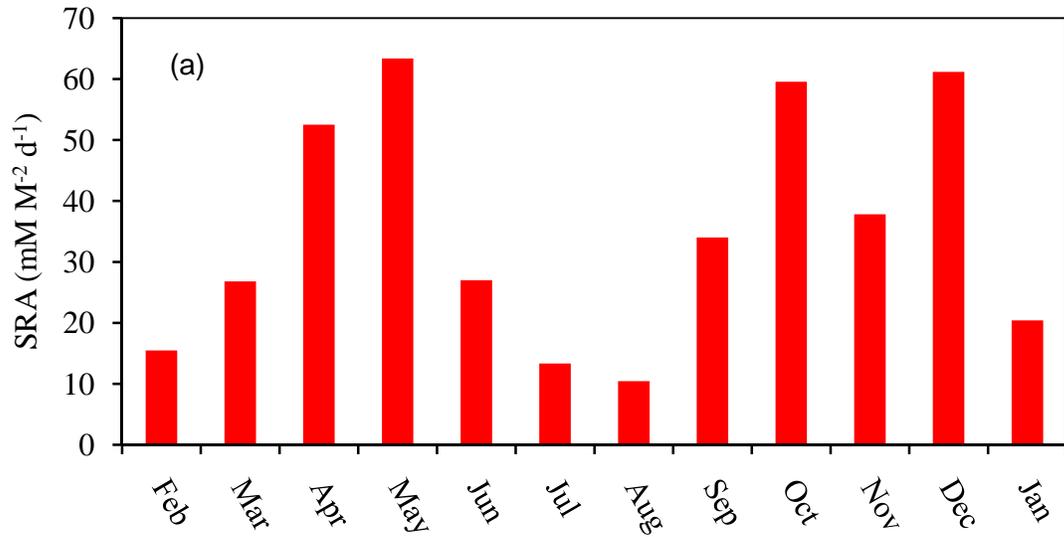


Figure: 32. Monthly down core variation in integrated sulfate reducing activity ($\text{mM m}^{-2} \text{d}^{-1}$) at Tuvem – (a) and at Diwar – (b).

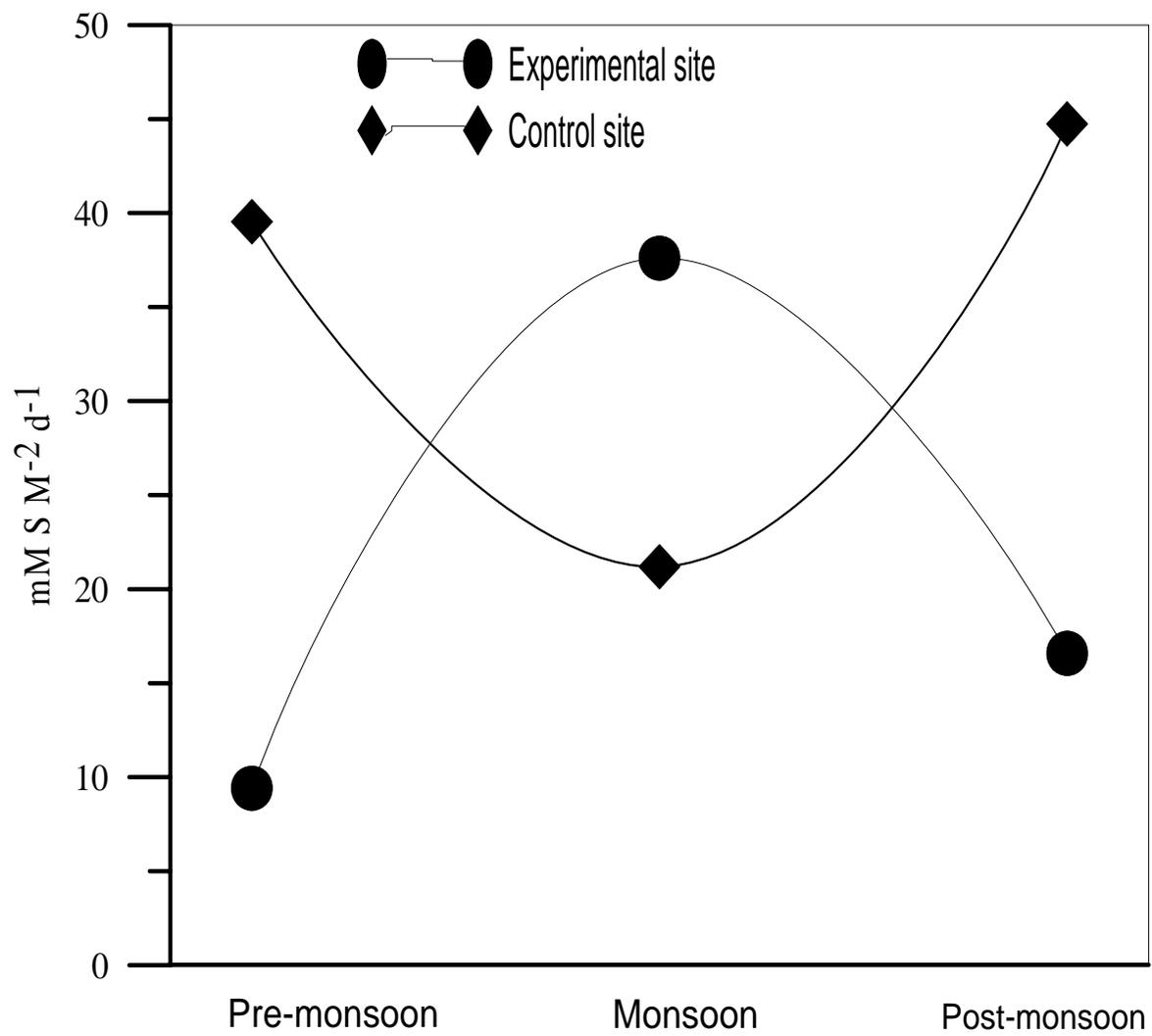


Figure: 33. Seasonal comparison of integrated (up to 10 cm) sulfate reduction rates at Tuveem (control) and Diwar (experimental) sites.

Table: 27. Correlation matrix between the physicochemical parameters at Tuvem. (n=60)

	SRA	MPN Acetate	MPN Lactate	Sulfate	Sulfide	Salinity	Eh	pH	TOC	LOM	Sand	Silt	Clay	Temp	Density	AODC	HC*	Iron
SRA	1.00																	
MPN Acetate	0.00	1.00																
MPN Lactate	0.19	0.24	1.00															
Sulfate	0.50	0.11	0.21	1.00														
Sulfide	-0.13	-0.11	0.10	-0.15	1.00													
Salinity	0.47	0.14	0.20	0.94	-0.22	1.00												
Eh	0.29	-0.24	0.03	0.25	-0.11	0.33	1.00											
pH	-0.3	-0.09	-0.19	-0.3	0.10	-0.16	0.29	1.00										
TOC	0.32	0.28	0.27	0.69	-0.14	0.64	0.06	-0.10	1.00									
LOM	0.26	0.08	0.16	0.52	-0.17	0.54	0.17	-0.02	0.80	1.00								
Sand	-0.5	-0.4	-0.4	-0.6	-0.05	-0.6	0.13	0.27	-0.6	-0.4	1.00							
Silt	0.23	-0.02	0.08	0.24	-0.06	0.23	0.18	-0.11	0.17	0.17	-0.21	1.00						
Clay	0.39	0.38	0.37	0.50	0.08	0.49	-0.2	-0.24	0.58	0.30	-0.9	-0.15	1.00					
Temp	-0.09	-0.01	0.12	-0.01	-0.06	0.00	-0.1	-0.02	-0.16	-0.17	0.06	-0.14	-0.01	1.00				
Density	0.33	0.12	0.27	-0.04	0.03	-0.10	-0.2	-0.5	0.03	0.02	-0.20	-0.05	0.22	0.07	1.00			
AODC	-0.07	-0.07	0.14	-0.01	-0.07	-0.06	0.25	0.12	0.05	0.22	0.40	0.03	-0.4	-0.06	-0.10	1.00		
HC	0.19	0.34	0.21	0.26	-0.07	0.28	0.01	0.12	0.50	0.44	-0.4	0.15	0.37	-0.01	-0.08	0.04	1.00	
Iron	-0.01	0.20	0.20	0.27	-0.05	0.24	-0.0	-0.4	0.32	0.40	-0.3	0.16	0.20	0.03	0.22	-0.11	0.19	1.00

Red color value=significant at the 0.01 level

Green color value=Correlation is significant at the 0.05 level

SRA=Sulfate Reducing Activity

HC=Heterotrophic Counts

TOC=Total Organic Carbon

LOM=Labile Organic Matter

AODC=Acridine Orange Direct Counts

Table: 28. Correlation matrix between the physicochemical parameters at Diwar. (n=60).

	AODC	Clay	Density	Eh	HC	iron	LOM	WC	pH	Salinity	Sand	Silt	SRA	MPN Acetate	MPN Lactate	Sulfide	Sulfate	Temp	TOC	
AODC	1.00																			
Clay	0.19	1.00																		
Density	-0.36	-0.29	1.00																	
Eh	0.01	-0.16	-0.21	1.00																
HC	-0.09	0.10	-0.11	-0.10	1.00															
iron	-0.50	-0.68	0.28	0.16	0.01	1.00														
LOM	0.54	0.10	-0.21	0.19	-0.3	-0.3	1.00													
WC	0.47	0.35	-0.38	0.01	-0.3	-0.6	0.58	1.00												
pH	0.22	-0.06	-0.15	0.34	0.03	-0.21	0.22	0.21	1.00											
Salinity	-0.34	-0.50	0.26	0.27	0.02	0.77	-0.19	-0.6	-0.03	1.00										
Sand	-0.26	-0.95	0.34	0.15	-0.18	0.75	-0.07	-0.4	-0.01	0.57	1.00									
Silt	0.34	0.54	-0.33	-0.07	0.28	-0.7	-0.03	0.26	0.16	-0.5	-0.8	1.00								
SRA	0.52	0.50	-0.51	-0.03	0.04	-0.8	0.32	0.64	0.28	-0.7	-0.6	0.64	1.00							
MPN Acetate	-0.11	0.04	0.16	0.14	0.12	-0.12	-0.03	-0.11	0.39	0.07	-0.08	0.15	0.00	1.00						
MPN Lactate	-0.05	-0.03	0.16	0.10	-0.3	0.12	0.08	0.00	0.08	0.21	0.11	-0.24	-0.19	0.19	1.00					
Sulfide	0.25	0.38	-0.13	-0.5	0.09	-0.5	0.04	0.29	0.01	-0.6	-0.4	0.38	0.48	0.10	-0.15	1.00				
Sulfate	-0.43	-0.66	0.41	0.19	-0.07	0.87	-0.3	-0.6	-0.15	0.89	0.7	-0.6	-0.77	-0.04	0.20	-0.58	1.00			
Temp	-0.01	-0.08	0.10	-0.05	-0.04	0.14	0.02	-0.07	0.03	-0.01	0.07	-0.03	-0.12	0.09	0.02	-0.14	0.05	1.00		
TOC	0.52	0.27	-0.35	0.02	-0.3	-0.6	0.67	0.87	0.33	-0.6	-0.3	0.26	0.61	-0.03	0.04	0.30	-0.56	0.11	1.00	

Red Color value=Correlation is significant at the 0.01 level
 Green color value=Correlation is significant at the 0.05 level
 AODC=Acridine Orange Direct Count
 HC=Heterotrophic counts
 LOM=Labile Organic Matter
 WC=Water Content
 SRA=Sulfate Reducing Activity
 TOC=Total Organic Carbon

4.6. Interrelationship of microbiological and physico-chemical parameters

Tuvem (Control site): Irrespective of month and depth, SRR positively correlated with sulfate ($r=0.50$, $p<0.001$), salinity ($r=0.47$, $p<0.001$), Eh ($r=0.29$, $p<0.05$), TOC ($r=0.32$, $p<0.01$), LOM ($r=0.26$, $p<0.05$) and density of the sediments ($r=0.38$, $p<0.01$) while negatively correlated with sand ($r=-0.50$, $p<0.001$) and pH ($r=0.30$, $p<0.05$) (Table 27).

The positive correlation between sulfate and SRR is contributed towards the positive influence of sulfate on SRR during the pre-monsoon ($r=0.80$, $p<0.001$, $n=20$) and monsoon seasons ($r=0.60$, $p<0.001$, $n=20$). While during post monsoon, sulfate ($r=0.05$, $p>0.05$, $n=20$) does not regulate the SRR. During the pre-monsoon and monsoon seasons SRR is positively correlated with TOC ($r=0.64$, $p<0.001$, $n=20$; $r=0.40$, $p<0.01$, $n=20$, respectively) and LOM also has a positive influence on the SRR during the pre-monsoon ($r=0.77$, $p<0.001$, $n=20$) and monsoon ($r=0.40$, $p<0.01$) seasons. During the post monsoon, TOC ($r=-0.45$, $p<0.01$, $n=20$) and LOM ($r=-0.35$, $p<0.01$, $n=20$) has negative correlation with the SRR in sediments (Table 27).

Total organic carbon in sediments was positively correlated with LOM, ($r=0.80$, $p<0.001$, $n=60$), clay contents of the sediments ($r=0.58$, $p<0.001$, $n=60$), and iron ($r=0.32$, $p<0.05$, $n=60$) has a strong negative correlation ($r=-0.60$, $p<0.001$, $n=60$) with sand (Table 27).

Sulfate reducing bacteria (SRB) was positively correlated with TOC ($r=0.28$, $p<0.05$, $n=60$) and clay ($r=0.38$, $p<0.01$, $n=60$). Acridine orange direct count has a positive ($r=0.4$, $p<0.01$, $n=60$) relationship with sand and is

negative with clay ($r=-0.40$, $p<0.01$, $n=60$). Heterotrophic bacterial count on nutrient agar plates positively correlated with TOC ($r=0.50$, $p<0.001$, $n=60$), LOM ($r=0.44$, $p<0.001$, $n=60$), clay ($r=0.32$, $p<0.01$, $n=60$), clay ($r=0.37$, $p<0.01$, $n=60$) and sulfate ($r=0.26$, $p<0.05$, $n=60$). Pearson's correlation coefficient (r) for each parameter is given in Table 27 and significant values are highlighted in the Table with red ($p<0.01$) and green ($p<0.05$) (Table 27).

Diwar (Experimental site): In general throughout the study period sulfate reduction rates were controlled by iron ($r=-0.76$, $p<0.001$), clay ($r=0.50$, $p<0.001$), LOM ($r=0.32$, $p<0.01$), silt ($r=0.64$, $p<0.001$), sand ($r=-0.61$, $p<0.001$) and TOC ($r=0.61$, $p<0.0001$). Iron has the maximum negative influence on SRR and it is responsible for 58% of the variability in the SRR in sediments (Table 28).

During the pre-monsoon season SRR was negatively controlled by iron ($r=-0.33$, $p<0.01$, $n=20$) and negatively correlated with LOM ($r=-0.43$, $p<0.01$, $n=20$), salinity ($r=-0.77$, $p<0.001$, $n=20$) and sulfate ($r=-0.63$, $p<0.001$, $n=20$). However silt had a positive influence ($r=0.58$, $p<0.001$, $n=20$) on SRR (Table 28).

Throughout the study period TOC was positively correlated with water content ($r=0.87$, $p<0.001$), clay ($r=0.27$, $p<0.05$) and, LOM also increased with increase ($r=0.67$, $p<0.001$) in TOC. Total organic carbon was negatively correlated to iron ($r=-0.64$, $p<0.001$), sand ($r=-0.30$, $p<0.05$) and sediment density ($r=-0.35$, $p<0.01$) (Table 28).

Sulfate reducing bacterial counts did not correlate with any individual physico-chemical parameters. AODC was positively correlated with TOC ($r=0.52$, $p<0.001$), LOM ($r=0.54$, $p<0.001$) and water content ($r=0.47$, $p<0.001$). AODC was negatively correlated with iron ($r= -0.50$, $p<0.001$), sand ($r= -0.26$, $p<0.05$) and sulfate($r= -0.43$, $p<0.01$). Pearson's correlation coefficient (r) for each parameter is given in Table 28 and significant values are highlighted in the Table with red ($p<0.01$) and green ($p<0.05$) (Table 28).

In general it was observed that at Tuvem and Diwar, TOC, LOM, and clay positively influenced the SRR while sand has a negative control over the SRR at both Tuvem and Diwar. Sulfate and iron were the main governing factor at Tuvem and Diwar respectively.

(A) Tuvem

Variable	PC1	PC2	PC3	PC4	PC5
SRA	0.251	-0.045	-0.363	-0.261	-0.218
MPN Acetate	0.160	0.217	0.324	0.249	0.051
MPN Lactate	0.188	0.137	-0.037	0.263	-0.577
Sulfate	0.362	-0.180	-0.126	-0.161	0.050
Sulfide	-0.063	0.165	0.141	-0.137	-0.404
Salinity	0.353	-0.216	-0.077	-0.197	0.057
Eh	0.038	-0.457	-0.198	-0.138	-0.270
pH	-0.143	-0.304	0.472	-0.170	-0.205
TOC	0.372	-0.128	0.167	0.118	0.058
LOM	0.295	-0.255	0.082	0.271	0.096
Sand	-0.375	-0.211	-0.106	0.215	0.038
Silt	0.112	-0.194	-0.236	0.052	0.155
Clay	0.338	0.283	0.193	-0.236	-0.094
Temperature	-0.034	0.119	-0.048	0.034	-0.208
Density	0.083	0.359	-0.389	0.214	-0.192
AODC	-0.061	-0.348	-0.047	0.477	-0.333
HC	0.231	-0.081	0.385	0.175	-0.075
Iron	0.191	0.096	-0.122	0.414	0.299

(B) Diwar

Variable	PC1	PC2	PC3	PC4	PC5
SRA	0.341	-0.089	0.041	-0.106	0.075
MPN Acetate	0.010	0.002	0.555	0.430	0.129
MPN lactate	-0.079	-0.209	0.057	0.496	-0.421
Sulfate	-0.365	-0.032	0.029	-0.053	-0.060
Sulfide	0.236	0.202	-0.118	0.205	0.266
Salinity	-0.325	-0.058	0.168	-0.091	-0.156
Eh	-0.064	-0.372	0.379	-0.267	-0.271
pH	0.074	-0.306	0.485	0.061	0.285
LOM	0.135	-0.483	-0.141	0.046	0.049
Sand	-0.320	-0.206	-0.124	-0.044	0.344
Silt	0.288	0.182	0.255	-0.089	-0.012
Clay	0.286	0.184	0.043	0.100	-0.448
Temperature	-0.042	-0.022	0.031	0.196	0.364
Density	-0.191	0.148	-0.074	0.486	0.139
AODC	0.217	-0.292	-0.089	-0.108	0.195
HC	0.025	0.349	0.336	-0.316	0.189
Water content	0.270	-0.314	-0.189	0.038	0.007
Iron	-0.360	0.014	-0.024	-0.140	0.017

Significant loading factors are in bold

AODC=Acridine orange direct counts, HC=Heterotrophic counts, LOM=Labile organic matter, SRA=Sulfate reducing activity

Table: 29. Coefficients in the linear combinations of variables making up PC's – (A) Tuvem (control site) and – (B) Diwar (experimental site).

At Tuvem, principal component analysis revealed that component one accounted for 28.7% of variation and it mainly consisted of SRA, sulfate, salinity, TOC, LOM, clay and sand. With the second component, the cumulative variation was 41.8% where the key players were pH, Eh and density. Except sand, all significant parameters had a positive load factor. (Table 29 a).

At Diwar, the first component accounted for 36.4% variation which included SRA, clay, silt, and water content with positive load factor and iron, salinity sand and sulfate having a negative load factor. With the second component, the cumulative variation was 49% where the key players were pH, Eh, density and labile organic matter. (Table 29 b). Throughout the study, principal components with more than two Eigen values were selected.

4.7. Factors influencing sulfate reduction rates:

4.7.1. Physico-chemical characteristics of sediments used for slurry experiments:

The pH of the sediment collected for slurry experiments was 7.1. The average redox potential value being 23.6 mV. The pore water salinity, sulfate and sulfide values were 7.1 psu, 4.10 mM, and 0.90 mm respectively. The water content in the sediments was 34%. The total organic carbon analyzed was 1.63% whereas the labile organic matter content was 0.61% of the dry weight of sediments. Granulometry appeared almost homogenous with respect to the distribution of sand, silt and clay. The values were 33.7%, 25.6% and 40.6% for sand clay and silt respectively. The iron and manganese content in the sediments was 3.66% and 0.17% respectively.

4.7.2. Sediment Amendments:

Change in sulfate reduction rates with and without amendment of different sources is portrayed in Figure (34 A, B, C & D). Without amendments, SRR were $168 \text{ nM gm}^{-1} \text{ day}^{-1}$. Average sulfate reduction rates decreased with iron additions in slurry experiments, compared to the controls. An increase (14%) in sulfate reduction rates were observed with an ammendment of 50 ppm iron. Further increase in concentration of iron, lead to a gradual decrease in SRR with a 25% decrease with 100ppm of iron and finally a 93% decrease with 1000ppm of iron concentrations (Figure 34 A). SRR at 1000 ppm drastically decreased to $38.6 \text{ nM gm}^{-1} \text{ day}^{-1}$. Manganese ammendements did not show

any significant change in sulfate reduction rates (Figure 34 B). Amendment with labile carbon with lactate and acetate together did not influence the sulfate reduction rates considerably. An average of 8.33% increase in SRR was observed after amendment with one mM of labile organic carbon (Figure 34 C). Beyond one mM LOC, there was no significant difference in SRR. With sulfate amendments, a 10.11% increase in SRR was observed after five mM increase in sulfate. (Figure 34 D). No significant change in SRR was observed with higher amendment of sulfate in the sediments.

It was thus seen that iron addition, had the maximum influence on the SRR in the sediments at 1000 ppm of iron as compared to 1000 ppm of manganese.

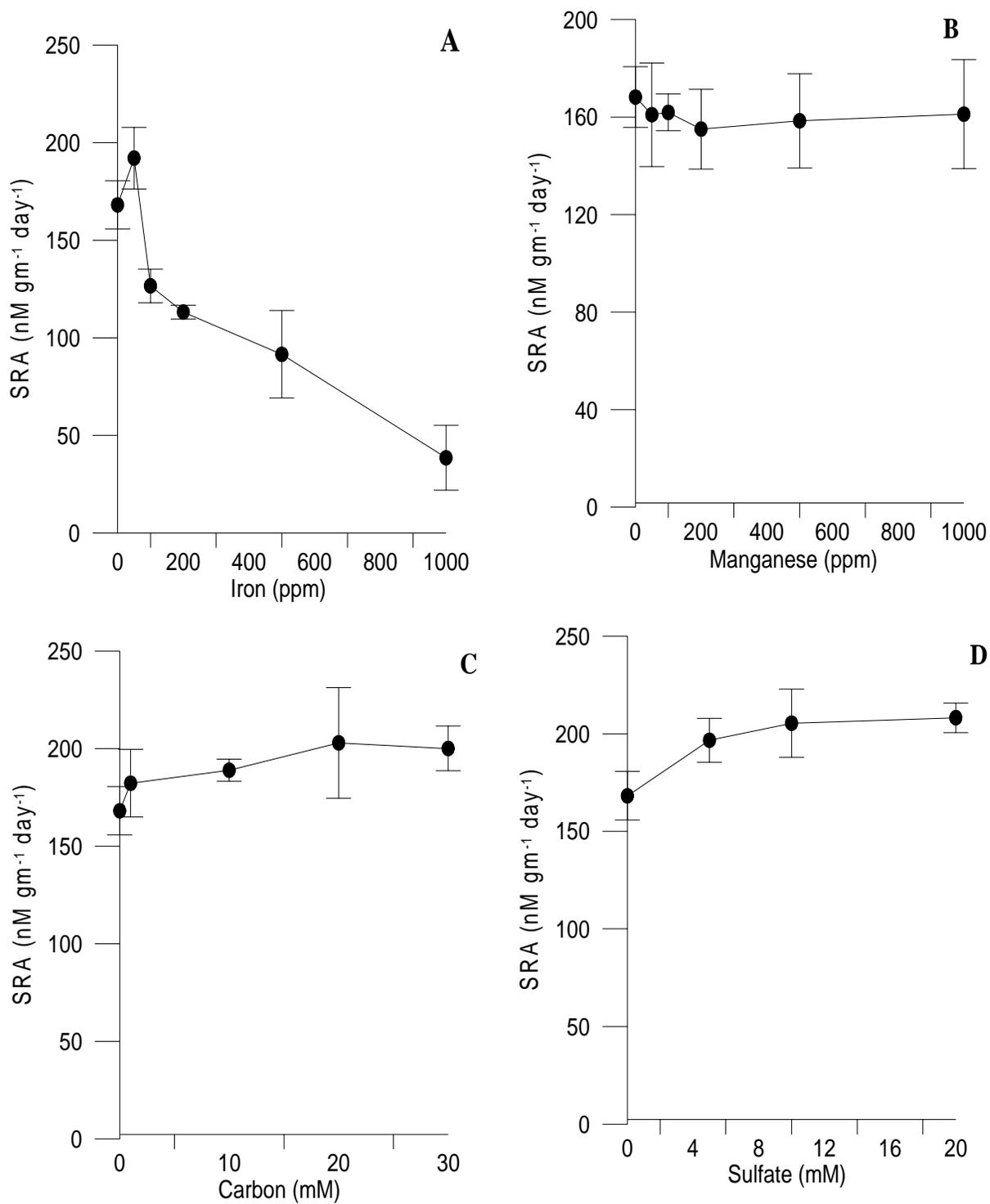


Figure: 34. Change in sulfate reduction rates with ammendment of (A) Iron, (B) mangaeense, (C) carbon, (D) sulfate in Tuvem sediments.

4.8. Sediment Sulfate budget at Chapora and Mandovi estuary:

From the results of the present study, the areal SRR for the total, mangrove areas at the Chapora and the Mandovi estuary were calculated seasonally. The area occupied by mangroves at Chapora and Mandovi estuaries is 218.5 and 700 hectares respectively (Mohmad H.H.N, 2008). The rates were calculated monthly and recorded as the average of four months for one season. The areal sulfate reduction rates at Chapora estuary ranged from 10.43 (monsoon) to 63.68 (post monsoon) mM m⁻² d⁻¹. The amount of sulfur reduced as sulfate was ca. two times higher during the non-monsoon seasons as compared to the monsoon season. The amount of sulfate reduced in the total area ranged from 266 to 1624 tones S-SO₄²⁻ yr⁻¹ and the mean values for the pre-monsoon, monsoon and post monsoon were 1009±566, 541±285 and 1142±495 tones S-SO₄²⁻ yr⁻¹ respectively. The areal sulfate reduction rates at the Mandovi estuary varied from 6.28 to 61.29 mM S-SO₄²⁻ m⁻² day⁻¹. The amount of sulfur reduced in the total mangrove area as sulfate, varied from 541 to 5023 tones yr⁻¹. The annual average for the S-SO₄²⁻ reduction at Diwar was 1733 tones yr⁻¹. (Table 30).

4.9. Carbon mineralization via sulfate reduction:

An average 2 moles of organic carbon are oxidized for every 1 moles of sulfate reduced (Jorgensen 1977). Redfield's stoichiometry for organic matter oxidation by sulfate reduction C_{org}: SO₄²⁻ = 2.1 (Volkov et al, 1998). Thus based on this relationship carbon mineralization has been calculated. At the Chapora estuary, carbon mineralization rates via sulfate reduction varied from

91.39 to 535.98 gm C m⁻² d⁻¹. The carbon mineralization for the total area (218.5 hectare) ranged from 255 to 1216 tones C yr⁻¹. At the Mandovi estuary the carbon mineralization via the process undertaken (i.e sulfate reduction) varied from 55.04 to 537.78 gm C m⁻² d⁻¹. The carbon mineralization in 700 hectares was 385 to 3763 tones C yr⁻¹. The annual average carbon mineralized via sulfate reduction at Chapora and Mandovi estuary was 673 and 1299 tones C yr⁻¹ respectively. The seasonal and total carbon mineralized at the Chapora and Mandovi estuary is shown in Table 31.

The mangrove area covered at Chapora estuary is 0.31 times the area at the Mandovi estuary. The carbon mineralized via sulfate reduction is 0.51 times higher than the carbon mineralized at the Mandovi estuary.

Table: 30. Seasonal comparison of areal sulfate reduction at whole study area -Chapora and Mandovi estuary.

Chapora Estuary			Total area	
Seasons	SRR(10cm) mmolS. m ⁻² day ⁻¹	Area (hec.)	Sulfate reduced (kmol.day ⁻¹)*10 ⁷	Sulfate reduced (tones. yr ⁻¹)
Pre-monsoon	39.54±22.19	218.5	86.4±48.5	1009±566
Monsoon	21.20±11.18	218.5	46.3±24.4	541±285
Post monsoon	44.74±19.41	218.5	97.8±42.4	1142±495
Annual av.	35.15±17.60		76.8±42.7	897±499
Mandovi Estuary				
Pre-monsoon	9.41±3.22	700	65.9±22.6	770±263
Monsoon	37.60±16.30	700	26.3±114	3074±1332
Post monsoon	16.58±1.24	700	116±8.6	1355±101
Annual av.	21.19±6.92		148±107	1733±1245

Table: 31. Seasonal comparison of carbon mineralization via sulfate reduction at Chapora and Mandovi estuary.

Chapora Estuary				Total area
Seasons	mM C mineralized m⁻². d⁻¹	gmC mineralized m⁻². yr⁻¹	Area (Hectare)	C mineralized tones. yr⁻¹
Pre-monsoon	79.08±44.39	346.36±194.41	218.5	757±425
Monsoon	42.39±22.38	185.65±97.99	218.5	406±214
Post monsoon	89.49±38.82	391.95±170.04	218.5	856±372
Average	70.32±39.12			673±374
Mandovi Estuary				
Pre-monsoon	18.82±6.44	82.45±28.22	700	577±198
Monsoon	75.19±32.60	329.36±142.81	700	2305±999
Post monsoon	33.15±2.48	145.24±10.85	700	1016±76
Average	42.39±30.45	185.68±133.38		1299±934

4.10. K value (The degree of recalcitrance):

Westrich and Berner, (1984) describe the importance of the amount of organic matter (G) and, the reactivity of decomposable organic matter. They explained that in a simple first order G-model, the material denoted as CH_2O is assumed to be decomposed at an overall rate directly proportional to its own concentration. Thus G is indicative of the reactivity of the available organic carbon. This can be expressed as $dG/dt = -K(G)$. K is the first order decay constant for decomposition via sulfate reduction and t is the time. The annual down core variation in k values at Tuvem (Table 32) ranged from 0.00046 to 0.014 month^{-1} . While at Diwar, it varied from 0.00034 to 0.0069 month^{-1} . ANOVA showed a significant monthly variation in k value profiles at Tuvem and Diwar ($df=11$, $p<0.001$, $n=12$). There was no statistically significant down core variation at both Tuvem and Diwar. However, a significant monthly variation ($df=1$, $p<0.001$, $n=2$) in k values between Tuvem and Diwar were noted. Seasonal mean k values for different depth intervals at both sites are given in Table 32. The constant k was calculated monthly and the values of four months were pooled to represent one season. When the values were depth integrated (10 cm), the k values varied at Tuvem from 0.002 to 0.053 month^{-1} and at Diwar from 0.0021 to 0.021 month^{-1} (Figure 35). The variability between the two sites was not as significant ($df=1$, $p<0.05$, $n=2$) as the non integrated k values. ($df=2$, $p<0.0001$, $n=2$). However k calculated on an annual basis was 0.25 yr^{-1} at Tuvem site and 0.097 yr^{-1} at Diwar site. The degree of recalcitrance expressed as k was thus higher at Tuvem compared to Diwar.

Table: 32. Seasonal, mean±SD of k value (month⁻¹) at different depth intervals at Tuvem and Diwar.

CONTROL SITE (Tuvem)			
Depth Interval (cm)	Pre-monsoon	Monsoon	Post-monsoon
0 to 2	0.0092±0.0064	0.0028±0.0023	0.0055±0.001
2 to 4	0.01±0.008513	0.0023±0.0017	0.0054±0.003
4 to 6	0.0052±0.0034	0.0036±0.0037	0.0089±0.004
6 to 8	0.0057±0.0046	0.0023±0.0020	0.0065±0.004
8 to 10	0.0053±0.0047	0.0020±0.0013	0.0024±0.0004
EXPERIMENTAL SITE (Diwar)			
0 to 2	0.00074±0.0003	0.004±0.0021	0.001±0.0008
2 to 4	0.0007±0.0002	0.005±0.0012	0.001±0.0008
4 to 6	0.0008±0.0002	0.005±0.0021	0.001±0.0004
6 to 8	0.0005±0.0002	0.003±0.0008	0.001±0.0007
8 to 10	0.0018±0.0005	0.003±0.0009	0.001±0.0008

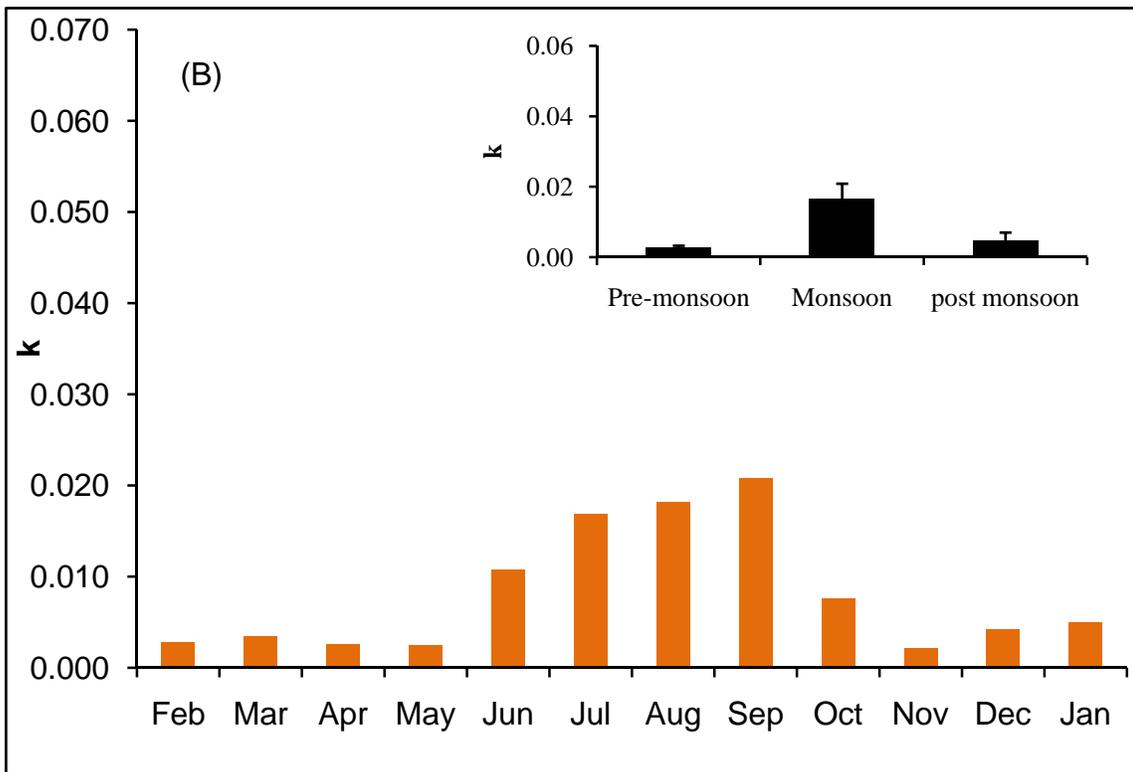
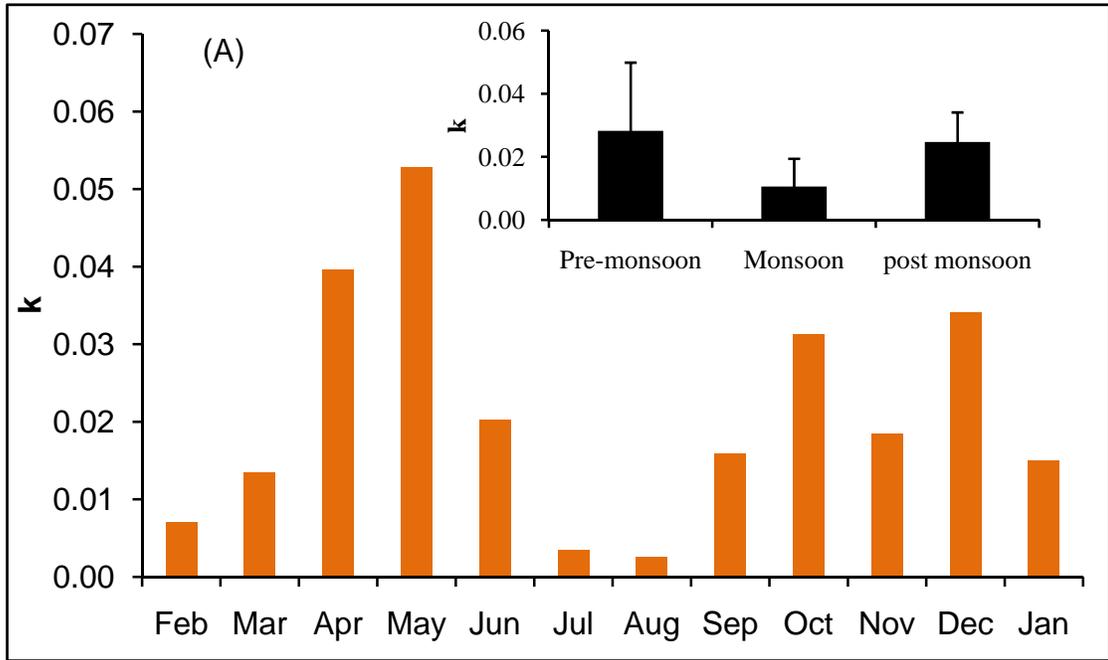


Figure: 35. Monthly variation in integrated k values (month⁻¹): (A) Tuvem and (B) Diwar. Inset shows the seasonal values.

4.11. Identification of sulfate reducing bacteria:

Pure colonies (88) from agar shake tubes were sub-cultured and checked for their purity before they were inoculated in liquid media. Their affiliations have been established using the standard methods.

Twelve different genera were retrieved from the mangrove sediments which were identified by classical taxonomy, morphology, sporulation and biochemical tests. The dominant genus was *Desulfovibrio* sp. During the study period the SRB from the mangrove sediment belong to the following genera (in order of frequency of recovery) by classical taxonomy:

1. *Desulfococcus multivorans* (16)
2. *Desulfovibrio desulfuricans* (11)
3. *Desulfobacter postgatei* (10)
4. *Desulfovibrio baculatus* (9)
5. *Desulfotomaculum orientis* (8)
6. *Desulfovibrio vulgaris* (8)
7. *Desulfomicrobium* sp. (6)
8. *Desulfosarcina* sp. (6)
9. *Desulfobulbus propionicus* (5)
10. *Desulfotomaculum acetooxidans* (4)
11. *Desulfomonas pigra* (4)
12. *Desulfonema limicola* (1).

Out of 88 isolates 24 (27%) were representative of 0-2 cm depth. Out of 24 isolates the most dominant group was *Desulfococcus multivorans* (5%) followed by *Desulfovibrio baculatus*. All the genera reported have been isolated from 0-2 cm, which shows the maximum diversity of sulfate reducers in the surficial layer. Out of 24 SRB, 21 were isolated on lactate and three on acetate and 50% were able to utilize both lactate as well as acetate. 83% of the isolates could use ethanol as a substrate. The isolates showed a wide range of salinity tolerance. 58% of the isolates were able to tolerate upto 2% amendment of NaCl in media of 15 psu

salinity. Out of 24 isolates, 37.5% were benzoate degraders. Of the 24 isolates obtained, 15 showed peaks characteristics of desulfovirdin (Table 33).

Among the 88 isolates, only 6% were representatives of 2-4 cm depth. The most dominant group was *Desulfovibrio* sp. This depth showed the minimum diversity which may be misinterpreted as the number of representative SRB were low as compared to the other depths (Table 33).

At 4-6 cm depth, 19 representatives were identified with classical taxonomy. Eleven species of SRB were isolated from 4-6 cm depth.

Among the 88 isolates, 9 % were representative of 6-8 cm depth. The most dominating group was *D. baculatus*. Eight species of SRB was isolated from 6-8 cm depth.

Among the 88 isolates 17 % were representative of 8-10 cm depth. Out of 19 isolates *Desulfotomaculam orentis* and *Desulfovibrio baculatus* was the dominant. Ten species of the SRB were present at 8-10 cm depth.

Table: 33. Cytological, pigment, physiological characteristics and biochemical tests of SRB isolates and their affinity to existing genera

Characteristics	SKKL1	SKKL2	SKKL3	SKKL4	SKKL5	SKKL6	SKKL7	SKKL8	SKKL9	SKKL10
Location	D, 0-2	D, 6-8	T, 4-6	D, 6-8	T, 2-4	D, 8-10	T, 0-2	D, 0-2	T, 0-2	D, 4-6
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Rod	Cocci	rod	spiral	Vibrio	Ovoid	spiral	Vibrio	rod	rod
Gram Character	+	-	+	-	-	-	-	-	-	-
Motility	+	-	+	+	+	-	+	+	-	+
Spore	-	-	+	-	-	-	-	-	-	-
Desulfoviridin	-	DSV	-	-	DSV	-	-	DSV	DSV	DSV
Electron donor used										
Acetate	-	+	-	+	-	+	+	+	+	-
Lactate	+	+	+	+	+	+	+	+	+	+
Formate	-	+	-	+	-	-	+	+	-	-
Ethanol	-	+	-	+	+	-	+	+	+	-
Pyruvate	+	+	+	+	-	-	+	-	-	-
Fumarate	-	+	-	+	-	-	+	+	-	-
Malate	-	+	-	+	-	-	+	+	-	+
Benzoate	-	-	-	-	-	-	-	-	-	-
Glucose	-	-	-	+	+	+	+	+	+	-
Propionate	-	-	-	+	+	-	+	-	-	-
Butyrate	-	-	-	+	+	-	+	-	-	-
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	-	+
Sulfite	+	+	+	+	+	+	+	+	-	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	+	-	-	-	-
SOD	-	-	-	-	-	-	-	-	-	-
NADH oxidase	-	-	-	-	-	-	-	-	+	-
Cytochrome	b	A	b	c	c	a	c	c	a	c
NaCl (opt%)	0	1	1	1	0	1	0	1	2	0
NaCl (range)	0-2	0-4	0-3	0-4	0-2	0-4	0-2	0-4	0-4	0-3
Identity	<i>Desulfotomaculum orentis</i>	<i>Desulfococcus Multivorians</i>	<i>Desulfotomaculum orentis</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfovibrio vulgaris</i>	<i>Desulfosarcina sp</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfomonas pigra</i>	<i>Desulfomicrobium sp</i>

Characteristics	SKKL11	SKKL12	SKKL13	SKKL14	SKKL15	SKKL16	SKKL17	SKKL18	SKKL19	SKKL20
Location	T,2-4	D, 8-10	D, 0-2	D, 4-6	T, 8-10	T, 6-8	D, 8-10	D, 0-2	T, 0-2	D, 4-6
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	spiral	Vibrio	Spherical	Vibrio	Small rods	spiral	spiral	tiny rods	Small rod	rod
Gram Character	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Spore	-	-	-	-	-	-	-	-	-	-
Deulfo viridin	-	DSV	DSV	DSV	-	-	-	DSV	DSV	DSV
Electron donor used										
Acetate	+	-	+	-	-	+	+	+	+	-
Lactate	+	+	+	+	+	+	+	+	+	+
Formate	+	+	+	-	-	+	+	+	+	-
Ethanol	+	+	+	+	-	+	+	+	+	-
Pyruvate	+	-	+	-	-	+	+	+	+	-
Fumarate	+	+	+	-	+	+	+	+	+	-
Malate	+	+	+	-	-	+	+	+	+	+
Benzoate	-	-	+	-	-	-	-	+	+	-
Glucose	+	+	+	+	-	+	+	+	+	-
Propionate	+	+	+	+	-	+	+	+	+	-
Butyrate	+	+	+	+	-	+	+	+	+	-
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	+	+
Sulfite	+	+	-	+	+	+	+	+	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	-	+	-	-	-	-	+	+	-
Cytochrome	c	c	c	c	c	c	c	a	a	c
NaCl (opt%)	1	0	2	1	0	1	0	0	1	1
NaCl (range)	0-4	0-2	0-4	0-4	0-4	0-4	0-3	0-2	0-4	0-3
Identity	<i>Desulfovibrio baculatus</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfovibrio vulgaris</i>	<i>Desulfomicrobium sp</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfococcus multivorans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfomicrobium sp</i>

Characteristics	SKKL21	SKKL22	SKKL23	SKKL24	SKKL25	SKKL26	SKKL27	SKKL28	SKKL29	SKKL30
Location	D, 8-10	T, 6-8	T, 4-6	D, 0-2	D, 4-6	D, 4-6	T, 8-10	T0-2	T, 0-2	D, 6-8
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Big rods	Big rods	Lemon shape	Spherical	oval	oval	Single ovoid	rods	Lemon shape	oval
Gram Character	-	-	-	-	-	-	-	+	-	-
Motility	+	+	-	+	+	+	+	+	+	+
Spore	+	+	-	-	-	-	-	+	-	-
Desuloviridin	-	-	DSV	DSV	-	DSV	-	DSR	-	-
Electron donor used										
Acetate	-	-	+	+	-	-	+	+	-	-
Lactate	+	+	+	+	+	+	-	+	+	+
Formate	-	-	+	+	-	-	-	-	-	-
Ethanol	-	-	+	+	+	+	-	-	+	+
Pyruvate	+	+	+	+	-	-	-	-	-	-
Fumarate	-	-	+	+	+	-	+	-	-	+
Malate	-	-	+	+	-	-	+	-	-	-
Benzoate	-	-	+	+	+	-	+	+	-	+
Glucose	+	-	+	+	-	+	-	-	-	-
Propionate	-	-	+	+	-	+	-	-	+	-
Butyrate	-	-	+	+	-	+	-	+	-	-
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	+	-
Sulfite	+	+	+	+	-	+	+	-	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	-	+	-	-	-	-	+	+	-
Cytochrome	b	B	b	c	c	c	c	b	a	c
NaCl (opt%)	0	1	1	1	1	0	0	1	0	0
NaCl (range)	0-3	0-4	0-3	0-4	0-3	0-4	0-3	0-4	0-2	0-3
Identity	<i>Desulfotomaculum orientis</i>	<i>Desulfotomaculum Orientis</i>	<i>Desulfococcus multivorans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfobacterium sp</i>	<i>Desulfovibrio vulgaris</i>	<i>Desulfosarcina variabilis</i>	<i>Desulfotomaculum acetooxidans</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfobacteri postgatei</i>

Characteristics	SKKL31	SKKL32	SKKL33	SKKL34	SKKL35	SKKL36	SKKL37	SKKL38	SKKL39	SKKL40
Location	T, 0-2	D, 0-2	D, 4-6	D, 8-10	T, 4-6	T, 6-8	D, 0-2	D, 8-10	T, 8-10	T, 8-0
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Rod shape	Rod shape	Rod shape	Rod shape	Ovoid	spiral	spiral	tiny rods	Small rod	rod
Gram Character	-	-	-	-	-	-	-	-	-	-
Motility	-	+	+	+	+	+	+	+	+	+
Spore	-	-	-	-	-	-	-	-	-	-
Desulfovirdin	DSV	-	-	-	-	-	-	DSV	DSV	DSV
Electron donor used										
Acetate	+	-	-	-	+	+	+	+	+	-
Lactate	+	+	+	+	+	+	+	+	+	+
Formate	-	-	-	-	-	+	+	+	+	-
Ethanol	+	+	+	+	-	+	+	+	+	-
Pyruvate	-	-	-	-	-	+	+	+	+	-
Fumarate	-	-	-	-	+	+	+	+	+	-
Malate	-	-	-	-	-	+	+	+	+	+
Benzoate	-	-	-	-	+	-	-	+	+	-
Glucose	+	-	-	-	+	+	+	+	+	-
Propionate	-	+	+	+	-	+	+	+	+	-
Butyrate	-	-	-	-	-	+	+	+	+	-
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	-	+	+	+	+	+	+	+	+	+
Sulfite	-	+	+	+	+	+	+	+	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	+	+	-	-	-	-	+	+	-
Cytochrome	c	a	a	a	c	c	c	a	a	c
NaCl (opt%)	0	1	0	0	0	1	0	0	1	1
NaCl (range)	0-3	0-4	0-4	0-3	0-1	0-4	0-3	0-4	0-4	0-2
Identity	<i>Desulfomanas pigra</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfosarcina variabilis</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfococcus multivorans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfomicrobium Sp.</i>

Characteristics	SKKL41	SKKL42	SKKL43	SKKL44	SKKL45	SKKL46	SKKL47	SKKL48	SKKL49	SKKL50
Location	D, 0-2	D, 4-6	T, 4-6	T, 6-8	T, 6-8	T, 8-10	D, 0-2	T, 0-2	D, 8-10	T, 0-2
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Vibrio	Vibrio	Rod	Rod	rod	tiny rods	spiral	spiral	Small rod	Small rod
Gram Character	-	-	-	-	-	-	-	+	-	-
Motility	+	+	+	+	+	+	+	Gliding	+	+
Spore	-	-	-	+	-	-	-	-	-	-
Desulfoviridin	DSV	DSV	DSV	-	DSV	DSV	-	DSV	DSV	DSV
Electron donor used										
Acetate	-	-	+	-	-	+	+	+	+	+
Lactate	+	+	+	+	+	+	+	+	+	+
Formate	+	+	+	-	-	+	+	-	+	+
Ethanol	+	+	+	-	-	+	+	-	+	+
Pyruvate	-	-	+	+	-	+	+	-	+	+
Fumarate	+	+	+	-	-	+	+	+	+	+
Malate	+	+	+	-	+	+	+		+	+
Benzoate	-	-	-	-	-	+	-	+	+	+
Glucose	+	+	+	-	-	+	+	-	+	+
Propionate	+	+	+	-	-	+	+	-	+	+
Butyrate	+	+	+	-	-	+	+	-	+	+
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	+	+
Sulfite	+	+	+	+	+	+	+	+	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	+	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	-	-	-	-	+	-	-	+	-
Cytochrome	c	C	a	b	c	a	c	c	c	c
NaCl (opt %)	1	0	1	0	1	1	0	1	NO Opt	1
NaCl (range)	0-4	0-3	0-4	0.3	0-4	0-3	0-4	0-4	0-4	0-4
Identity	<i>Desulfovibrio desulfuricans</i>	<i>Desulfovibrio Desulfuricans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfotomaculum orientis</i>	<i>Desulfomicrobium Sp.</i>	<i>Desulfococcus multivorans</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfonema limicola</i>	<i>Desulfococcus multivorans</i>	<i>Desulfococcus multivorans</i>

Characteristics	SKKL51	SKKL52	SKKL53	SKKL54	SKKL55	SKKL56	SKKL57	SKKL58	SKKL59	SKKL60
Location	T, 8-10	D, 8-10	D, 2-4	D, 6-8	T, 0-2	D, 0-2	T, 4-6	T, 4-6	T, 2-4	D2-4
Isolated	Lactate	Lactate	Acetate	Acetate	Acetate	Acetate	Lactate	Acetate	Acetate	Acetate
Cell morphology	rod	Vibrio	spiral	vibrio	rod	rods	Vibrio	rod	ovoid	Small rod
Gram Character	+	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+
Spore	+	-	-	-	-	-	-	-	-	-
Desulfovirdin	-	DSV	DSV	DSV	DSV	-	-	-	-	-
Electron donor used										
Acetate	+	+	+	+	-	+	-	+	+	+
Lactate	+	+	+	+	+	+	+	+	+	+
Formate	-	-	-	-	-	+	-	+	-	-
Ethanol	-	+	+	+	-	-	+	-	-	-
Pyruvate	-	-	-	-	-	-	-	-	-	-
Fumarate	-	+	+	+	-	-	-	-	+	-
Malate	-	-	-	-	+	-	-	-	+	+
Benzoate	-	-	-	-	-	-	-	-	+	-
Glucose	-	+	+	+	-	+	+	+	-	+
Propionate	-	-	-	-	-	-	+	-	-	-
Butyrate	+	-	-	-	-	-	+	-	-	-
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	+	+
Sulfite	-	+	+	+	+	-	+	+	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	+	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	-	-	-	-	+	-	-	-	-
Cytochrome	b	C	c	c	c	c	c	c	c	c
NaCl (opt %)	1	0	1	0	0	2	0	1	0	0
NaCl (range)	0-3	0-4	0-4	0-4	0-3	0-4	0-2	0-4	0-3	0-4
Identity	<i>Desulfotomaculum acetooxidance</i>	<i>Desulfovibrio Desulfuricans</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfomicrobium Sp.</i>	<i>Desulfobacter postgatei</i>	<i>Desulfovibrio vulgaris</i>	<i>Desulfobacter postgatei</i>	<i>Desulfosarcina Sp.</i>	<i>Desulfococcus multivorans</i>

Characteristics	SKKL61	SKKL62	SKKL63	SKKL64	SKKL65	SKKL66	SKKL67	SKKL68	SKKL69	SKKL70
Location	D, 0-2	T, 6-8	D, 4-6	T, 6-8	D, 0-2	D, 2-4	T, 8-10	D, 4-6	T, 0-2	D,8-10
Isolated	Acetate	Acetate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Filament	rods	Vibrio	rod	oval	ovoid	vibrio	rods	spherical	oval
Gram Character	+	-	-	-	-	-	-	-	-	-
Motility	+	+	+	-	+	+	+	+	-	+
Spore	-	-	-	-	-	-	-	-	-	-
Desulfovirdin	DSV	-	DSV	-	-	-	DSV	DSV	DSV	-
Electron donor used										
Acetate	+	+	-	+	-	+	+	+	+	-
Lactate	+	+	+	+	+	-	+	+	+	+
Formate	-	-	-	-	-	-	+	+	+	-
Ethanol	+	+	+	+	+	-	+	+	+	+
Pyruvate	-	-	-	-	-	-	-	-	+	-
Fumarate	-	-	-	-	-	+	+	+	+	+
Malate	-	-	-	-	-	+	+	+	+	-
Benzoate	-	-	-	+	+	+	-	-	+	+
Glucose	+	-	+	-	-	-	+	+	-	-
Propionate	-	-	+	-	-	-	-	-	+	-
Butyrate	-	-	+	-	-	-	-	-	-	-
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	+	-
Sulfite	-	+	+	-	-	+	+	+	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	-	+	-	-	-	-	-	+	-
Cytochrome	c	c	c	c	c	c	c	c	b	c
NaCl (opt %)	1	1	0	1	1	0	1	1	1	0
NaCl (range)	0-3	0-4	0-3	0-3	0-4	0-3	0-4	0-4	0-4	0-3
Identity	<i>Desulfomaonas pigra</i>	<i>Desulfobacter</i> Sp.	<i>Desulfovibrio vulgaris</i>	<i>Desulfobacter</i> Sp.	<i>Desulfobacterium</i> Sp.	<i>Desulfosarcina variabilis</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfobacteri postgatei</i>

Characteristics	SKKL71	SKKL72	SKKL73	SKKL74	SKKL75	SKKL76	SKKL77	SKKL78	SKKL79	SKKL80
Location	T, 8-10	D, 0-2	D, 4-6	D, 6-8	T, 0-2	T, 0-2	D, 8-10	T, 4-6	D, 4-6	D, 4-6
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Big rods	Big rods	Lemon shape	Spherical	oval	oval	Single ovoid	rods	Lemon shape	oval
Gram Character	-	-	-	-	-	-	-	+	-	-
Motility	+	+	-	+	+	+	+	+	+	+
Spore	-	+	-	-	-	-	-	+	-	-
Desulfovirodin	-	-	DSV	DSV	-	-	-	-	-	DSV
Electron donor used										
Acetate	-	-	+	+	-	-	+	+	-	-
Lactate	+	+	+	+	+	+	-	+	+	+
Formate	-	-	+	+	-	-	-	-	-	-
Ethanol	-	-	+	+	+	+	-	-	+	+
Pyruvate	+	+	+	+	-	-	-	-	-	-
Fumarate	-	-	+	+	+	+	+	-	-	-
Malate	-	-	+	+	-	-	+	-	-	-
Benzoate	-	-	+	+	+	+	+	+	-	-
Glucose	-	-	+	+	-	-	-	-	-	+
Propionate	-	-	+	+	-	-	-	-	+	+
Butyrate	-	-	+	+	-	-	-	+	-	+
Electron acceptor used										
Sulfate	+	+	+	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+	+	+	+
Sulfite	+	+	+	+	-	-	+	-	+	+
Biochemical tests										
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-
SOD	-	-	-	-	+	-	-	-	-	-
NADH oxidase	-	-	+	-	-	-	-	+	+	-
Cytochrome	b	c	b	c	c	c	c	b	a	c
NaCl (Opt %)	0	1	1	1	1	0	1	1	0	0
NaCl (range)	0-1	0-3	0-3	0-4	0-4	0-4	0-4	0-3	0-4	0-4
Identity	<i>Desulfotomaculum orientis</i>	<i>Desulfotomaculum orientis</i>	<i>Desulfococcus multivorans</i>	<i>Desulfococcus multivorans</i>	<i>Desulfobacterium sp</i>	<i>Desulfobacter postgatei</i>	<i>Desulfosarcina variabilis</i>	<i>Desulfotomaculum acetooxidans</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfovibrio Vulgaris</i>

Characteristics	SKKL81	SKKL82	SKKL83	SKKL84	SKKL85	SKKL86	SKKL87	SKKL88
Location	D, 0-2	T, 2-4	D, 4-6	D, 6-8	T, 0-2	T, 4-6	D, 8-10	T, 4-6
Isolated	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate	Lactate
Cell morphology	Rod shape	vibrio	Rod shape	Rod shape	Vibrio	spiral	spiral	Big rods
Gram Character	-	-	-	-	-	-	-	-
Motility	-	+	+	+	+	+	+	+
Spores	-	-	-	-	-	-	-	+
Desulfovirdin	DSV	DSV	-	-	DSV	-	-	-
Electron donor used								
Acetate	+	+	-	-	-	+	+	-
Lactate	+	+	+	+	+	+	+	+
Formate	-	+	-	-	-	+	+	-
Ethanol	+	+	+	+	+	+	+	-
Pyruvate	-	-	-	-	-	+	+	+
Fumarate	-	-	-	-	-	+	+	-
Malate	-	+	-	-	-	+	+	-
Benzoate	-	-	-	-	-	-	-	-
Glucose	+	-	-	-	+	+	+	+
Propionate	-	-	+	+	+	+	+	-
Butyrate	-	-	-	-	+	+	+	-
Electron acceptor used								
Sulfate	+	+	+	+	+	+	+	+
Thiosulfate	-	+	+	+	+	+	+	+
Sulfite	-	+	+	+	+	+	+	+
Biochemical tests								
Oxidase	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-
SOD	-	-	-	-	+	-	-	-
NADH oxidase	-	-	+	-	-	-	-	-
Cytochrome	c	c	a	a	c	c	c	b
NaCl (opt %)	0	1	1	1	1	0	1	1
NaCl% (range)	0-3	0-2	0-4	0-3	0-4	0-4	0-4	0-3
Identity	<i>Desulfomanas pigra</i>	<i>Desulfovibrio desulfuricans</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfobulbus propionicus</i>	<i>Desulfovibrio vulgaris</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfovibrio baculatus</i>	<i>Desulfotomaculum orientis</i>

4.12. Bioremediation Mediated by Sulfate Reducing Bacteria:

4.12.1. Bioadsorption studies:

The cobalt concentration slightly to about 485 ppm in 30 days amounting to about 3% cobalt removal by bioadsorption (Figure 39). Thus bioadsorption seemed to play only a minor role in the overall cobalt removal.

4.12.2. Bioremediation using active cells of SKKL8:

The kinetics of bioremediation of cobalt during the growth of SKKL8 was studied as function of time. The pH of the experimental medium with 500 ppm cobalt increased from 6.2 to 6.6 after 7 days of growth while it further increased from 6.6 to 8.0 by the end of 30 days (Figure 36). In the absence of cobalt' (positive control) pH increased from 6.7 to 8.3 by the end of 30 days. While in media without SKKL8 with cobalt (negative control), the pH remained constant throughout the study. In the presence of 500 ppm cobalt, Eh decreased from 52 mV to -82.5 mV at the end of 30 days, while for the positive control Eh decreased from 100 mV to -210 mV after 30 days of incubation (Figure 37). In case of negative control no significant change was observed in Eh. In the presence of 500 ppm cobalt, sulfate concentrations reduced from 18 mM to 10 mM while in case of positive control there was a reduction upto 8.0 mM (Figure 38). No change in sulfate concentration was noted in the negative control. A fall in level of cobalt from 500 ppm to 86 ppm by the end of 30 days, indicated removal of cobalt mediated by SKKL8. However in the control without SKKL8 the fall was limited to 465 ppm (Figure 39). A lag phase of 7 days was observed when the culture was

incubated in the presence of cobalt. While in the control without Co it was only 3 days (Figure 40).

4.12.3. Bioremediation using hydrogen sulfide stripped of bacteria and Co at 5000 ppm:

An actively growing culture of SKKL8 was subjected to treatment by purging the inert nitrogen gas. This resulted in the expulsion of bacterially produced hydrogen sulfide gas from the media which was channelized into a sterile cobalt chloride solution (5000 ppm). Cobalt in the supernatant was reduced to non detectable levels by the end of 12 hrs. This was accompanied by the precipitation of cobalt.

4.12.4. Characterization of precipitate:

The chemical composition of the biogenic and negative control precipitate varied widely (Figure 41). The concentrations were interpreted using SEM-EDS values. In the biogenic precipitate, cobalt contributed upto 11.63% and sulfur upto 8.33%. In case of negative control, the precipitated cobalt was 0.47% and sulfur was 1.53%.

4.12.5. Characterization of SKKL8:

SKKL8 was Gram negative and comma shaped. The strain used lactate, acetate, formate, malate, ethanol and glucose as electron donors however no growth was observed with benzoate. In addition to sulfate, SKKL8 culture could use sulfite and thiosulfate as electron acceptors. The culture tolerated upto 3.5% NaCl.

Phylogenetic analysis of the 1333 base pairs sequenced established the identity of the strain to belong to genus *Desulfovibrio* of the delta subclass of Proteobacteria.

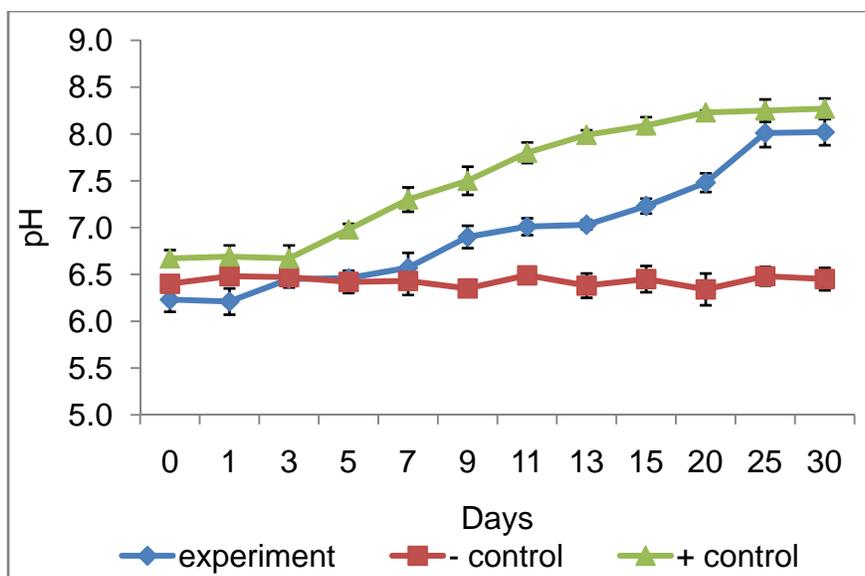


Figure: 36. Change in pH of the medium during the growth of SKKL8 in the presence (experiment) and absence (+ control) of cobalt. Negative control was without SKKL8 and with added cobalt. The bars indicate \pm SD (n=3).

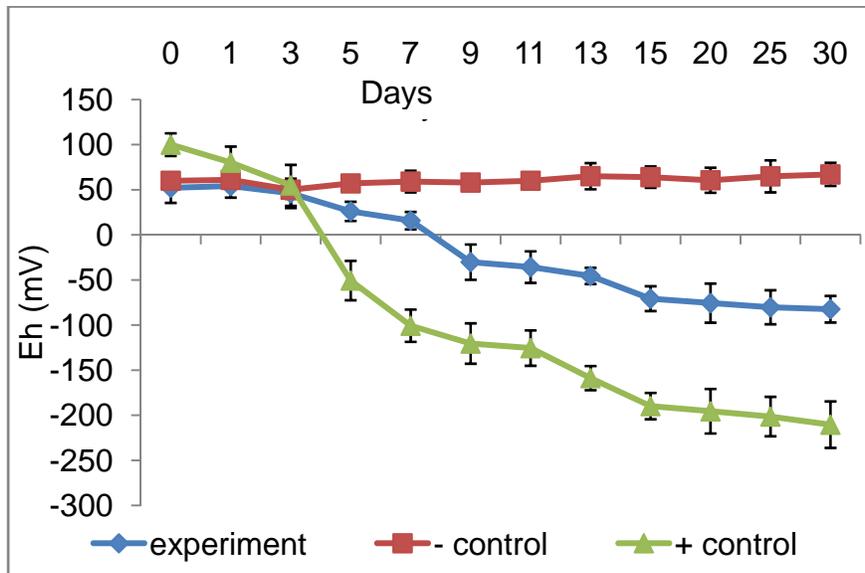


Figure: 37. Change in Eh of the medium during the growth of SKKL8 in the presence (experiment) and absence (+ control) of cobalt. Negative control was without SKKL8 and with added cobalt. The bars indicate \pm SD (n=3).

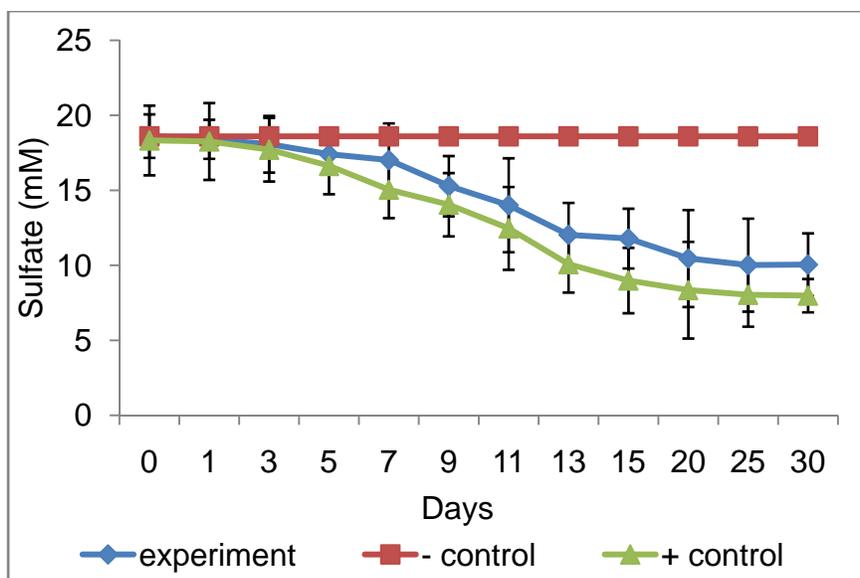


Figure: 38. Change in sulfate concentrations of the medium during the growth of SKKL8 in the presence (experiment) and absence (+ control) of cobalt. Negative control was without SKKL8 and with added cobalt. The bars indicate \pm SD (n=3).

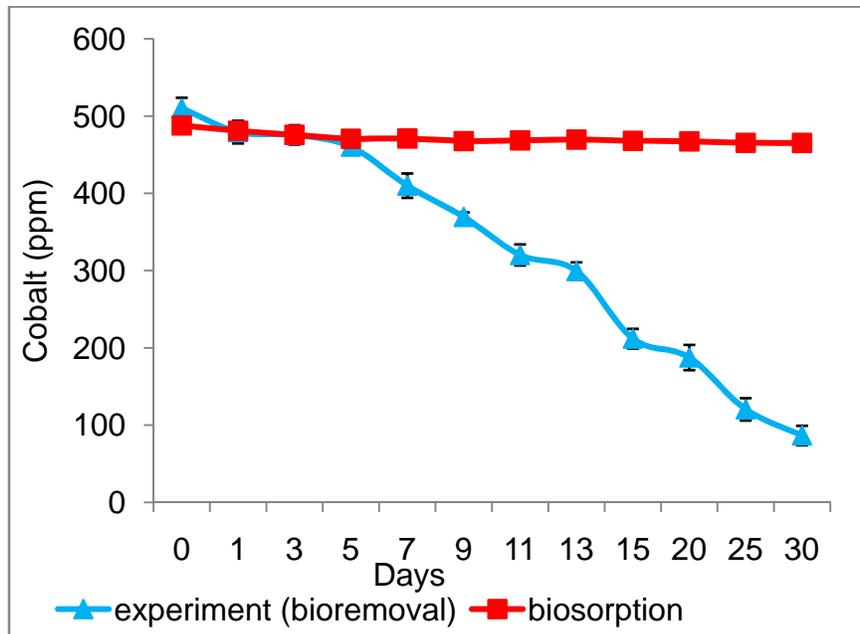


Figure: 39. Residual concentration of cobalt in the incubations under aerobic (biosorption) and anaerobic condition (bioremoval) with the culture-SKKL8. The bars indicate the \pm standard deviation.

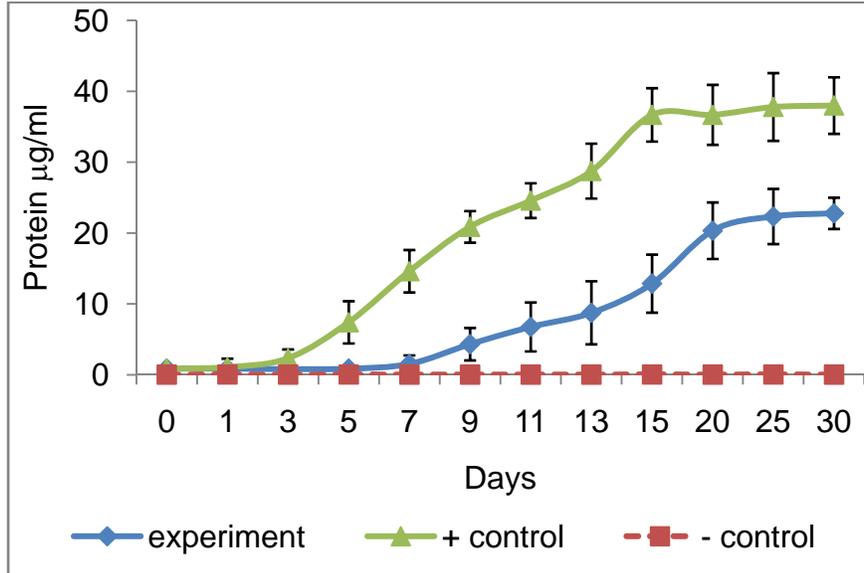
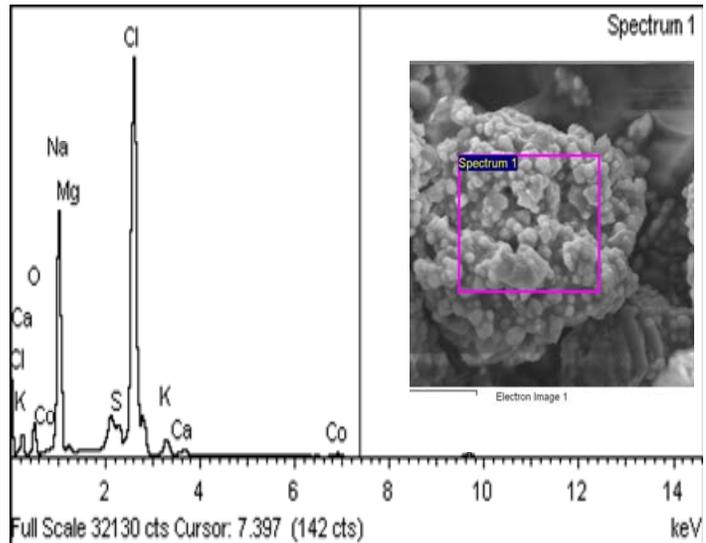


Figure: 40. Change in protein concentration of the medium during the growth of SKKL8 in the presence (experiment) and absence (+ control) of cobalt. Negative control was without SKKL8 and with added cobalt. The bars indicate \pm SD (n=3).

Control

Element	Weight%	Atomic%
O	23.36	35.96
Na	28.91	30.97
Mg	0.76	0.77
S	1.99	1.53
Cl	41.26	28.66
K	2.00	1.26
Ca	0.61	0.37
Co	1.12	0.47
Totals	100.00	



Experimental

Element	Weight%	Atomic%
O	47.04	70.29
Na	1.67	1.73
Mg	0.56	0.55
Si	0.68	0.58
S	11.17	8.33
Cl	10.22	6.89
Co	28.66	11.63
Totals	100.00	

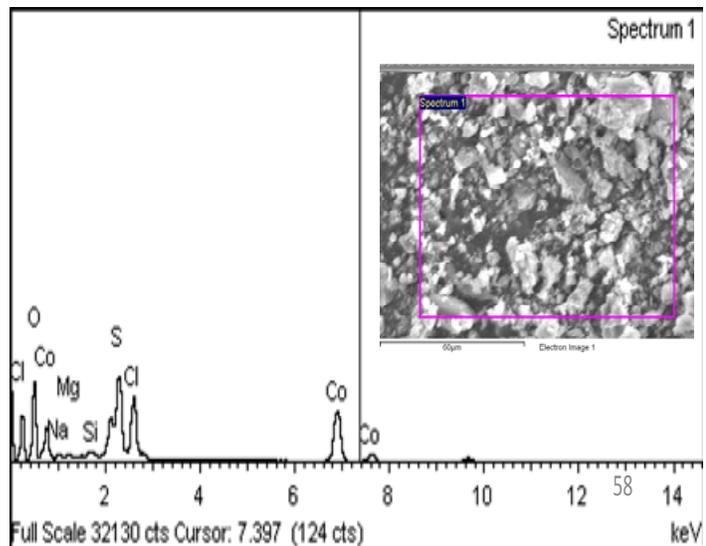


Figure: 41. SEM- photomicrograph and Energy Dispersive X-ray analysis of cobalt precipitated produced by SKKL8 and under the control condition.

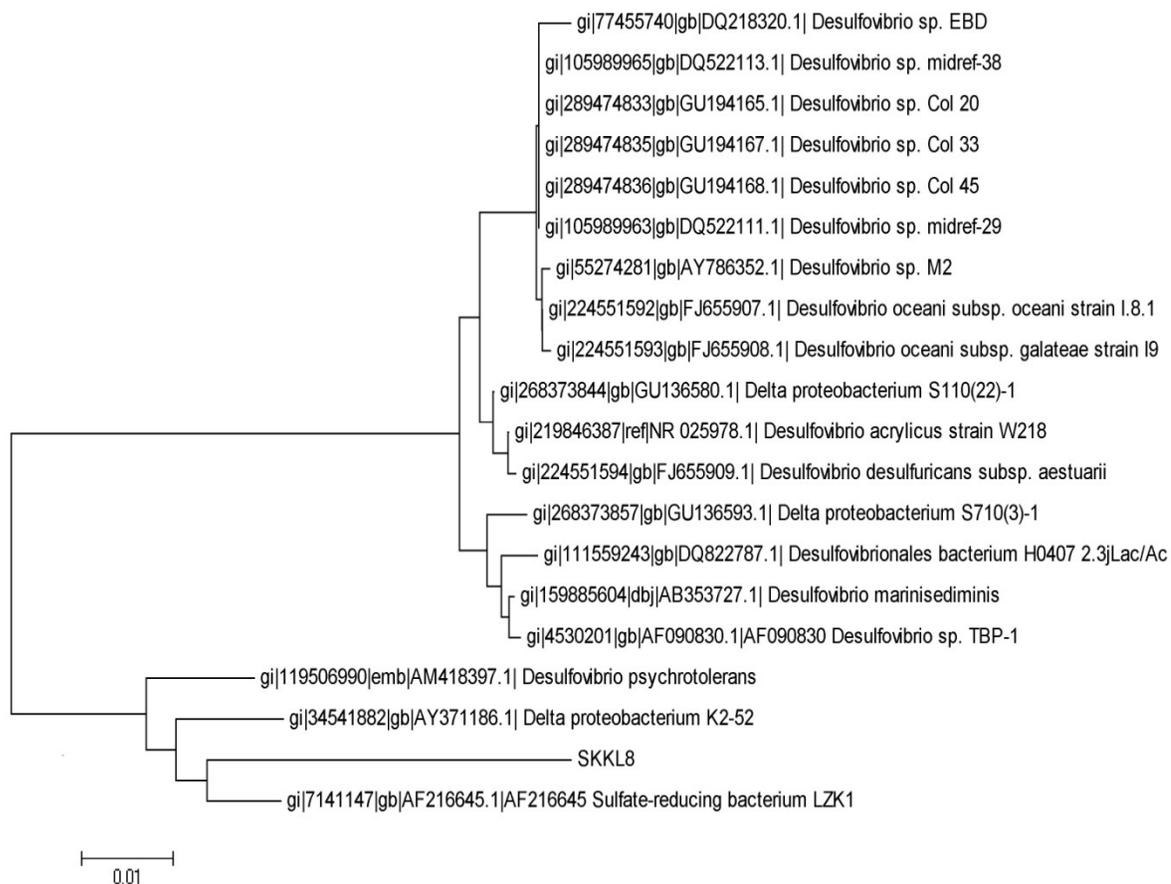


Figure: 42. Phylogenetic tree showing the affiliation of the 16S rRNA gene sequence of strain SKKL8 within the delta Proteobacteria. Gene bank accession numbers are shown in parentheses. The tree was generated by the neighbor-joining method.

Chapter 5

Discussion

Microbial activity controls the mineralization of organic matter in intertidal soils and sediments, thereby influencing pore water nutrient availability and the speciation of redox sensitive ions (Paerl and Pinckney, 1996). Organic matter oxidation is coupled to the reduction of an oxidant by terminal metabolism and production of a variety of metabolites, including sulfide, nitrogen and nitrous oxide gases, reduced iron and manganese, and methane which are indicative of sulfate reduction, denitrification, metal reduction, and methanogenesis respectively. This process recycles complex organic matter back to inorganic forms such as bicarbonates, ammonium and phosphate, which are critical for primary production in oligotrophic mode (Paerl and Pinckney, 1996). Mangrove soils are usually nutrient deficient (Boto and Wellington, 1984; Alongi and Sasekumar, 1992; Alongi, 1996) though rich in organic matter, suggesting highly throughput recycling of the inorganic nutrients (Holguin et al. 2001). Sulfate reduction is an important process for re-mineralization of organic matter but this process is influenced by spatial and temporal variability (Kristensen et al. 1988). Overall sulfate reduction accounts for 10 to 90% (50% on an average) of C_{org} oxidation in the coastal sediments (Thandrup et al. 1996). The high productivity of mangroves is thus sustained by internal nutrient recycling, which could be coupled to organic matter mineralization. Mangrove soils can be both hypersaline and at the same time biochemically reducing (Kathiresan and Bingham, 2001). Increased rainfall during the wet season can affect pore water salinity, redox potential, pH and other soil biogeochemical processes (Alongi et al. 1999; 2004). Earlier studies of benthic metabolism and nutrient transformations in mangrove soils, fringing oceans or

rivers have a marked relationship between organic matter availability, elemental cycling and mangrove density (Nedwell et al. 1994; Sherman et al. 1998). This study focuses on the sulfate reduction pathway, understanding the factors regulating and modulating this process in the mangrove sediments. The carbon and sulfate budget in Mandovi and Chapora estuary, with respect to sulfate reduction has also been discussed.

5.1. Sulfate reduction rates: Process and Control:

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 impact. A number of environmental factors are known to influence SRR including bacterial abundance, availability of the sulfate, availability and lability of organic carbon, temperature, salinity and pH (Conell and Patrick, 1968; Roychoudhury et al. 1998; Brandt et al. 2001; Kostka et al. 2002; Roychoudhury, 2004; Meir et al. 2005; Pallud and Van Cappellen, 2006; Roychoudhury and McCormic, 2006). Some of the other factors include the availability and location of nutrient sources (Fredrickson et al. 1991; Martino et al. 1998; Detmers et al. 2001), availability of water (Jones et al. 1989), redox conditions (Hamilton, 1998; Okabe et al. 1999) and relative ease of transport through the surrounding medium (Nacro et al. 1996). Since the rates and factors controlling the sulfate reduction process varied spatially and temporally, the present study was undertaken to quantify the sulfate reduction rates and the factors controlling the process.

In the present study, the SRR measured in the mangroves of Tuvem (control site) and Diwar (experimental site) together range from a minimum of 6.28 to a maximum of 63.4 mM m⁻² d⁻¹ which were comparable to SRR reported by various researchers (Table 34) like Alongi et al. (2004) in the Malaysian mangroves, and Kerkar and Loka Bharathi, (2007) in salt pans of Goa at Ribandar which range from 0.002–65.75 mM m⁻² d⁻¹. However our rates are higher than those reported by Kristensen et al. (1992) in the mangrove sediments of Pukhet Thailand and lower than the SRR reported from salt marshes in Germany (Al-Raei et al. 2009) and by Schubert et al. (2000) in Chilean coast sediments. The SRR in the present study are also lower than those reported by Alongi et al. (2005) which range from 19-281 mM m⁻² d⁻¹ in mangrove sediments of China (Table 34).

Sulfate reducing bacteria 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 decreases with depth, pH does not have a fixed pattern.

Table: 34. Sulfate reduction rates in present study compared with published rates.

Location		Sulfate reduction rates		References	
Mangrove, Pakistan		2.5-16.1	mM m ⁻² d ⁻¹	Kristensen et al. (1992)	
Sediment Off Chile, Germany		40-430	nM cm ⁻³ d ⁻¹	Schubert et al. (2000)	
Mangrove, Pukhet, Thailand		5-140	nM cm ⁻³ d ⁻¹	Kristensen et al. (2000)	
Mangrove, Southern Thailand		0.6-16.9	mM m ⁻² d ⁻¹	Alongi et al. (2001)	
Mangrove, Vietnam		0.2-13	mM m ⁻² d ⁻¹	Alongi et al. (2001)	
Mangrove, Malaysia		19-53	mM m ⁻² d ⁻¹	Alongi et al. (2004)	
Mangrove, China		19-281	mM m ⁻² d ⁻¹	Alongi et al. (2005)	
Ribander salt pan, Goa- India		0.002-66	mM m ⁻² d ⁻¹	Kerkar et al. (2007)	
Salt marshes, Germany		0.9-106	mM m ⁻² d ⁻¹	AL-Raie et al. (2009)	
Intertidal mud flat, Korea		226-1516	nM cm ⁻³ d ⁻¹	Hyun et al. (2009)	
Present study					
Seasons		SRR		Integrated SRR	
Diwar	Pre-monsoon	23.32-131.94	nM cm ⁻³ d ⁻¹	6.2-13.6	mM m ⁻² d ⁻¹
	Monsoon	112.6-294.49	nM cm ⁻³ d ⁻¹	24.5-61.4	mM m ⁻² d ⁻¹
	Post monsoon	79.22-147.31	nM cm ⁻³ d ⁻¹	14.8-33.4	mM m ⁻² d ⁻¹
Tuvem	Pre-monsoon	62.55-698.66	nM cm ⁻³ d ⁻¹	15.5- 63.4	mM m ⁻² d ⁻¹
	Monsoon	50.21-338.14	nM cm ⁻³ d ⁻¹	10.43-34.0	mM m ⁻² d ⁻¹
	Post monsoon	99.88-635.86	nM cm ⁻³ d ⁻¹	20.41-61.2	mM m ⁻² d ⁻¹

5.1.1. Effect of Temperature:

Experiments with sediments and microorganisms show temperature to be an important environmental variable, since it controls the composition of the community and activity of individual organisms. Sulfate reducing bacteria feature a temperature dependent metabolic rates, which is generally comparable with the effect of temperature on heterotrophic bacteria (Jorgensen, 1977a & b). Al-Raei et al. (2009) showed the impact of temperature on the sulfate reduction in sediments. Mainly temperature played a significant role in temperate regions since seasonal variability of temperature is higher. In the present study, we discuss a tropical mangrove ecosystem, where - down core sediment temperatures during the entire year of sampling, at Tuvem (Figure 12) ranged from 25.1 to 30.3°C (mean= 27.0 ± 1.3°C; n= 60) and at Diwar from 24.1 to 31.4 °C (mean= 26.8±1.6°C; n=60). Since the temperatures do not show a high seasonal variation, it did not play a major role in these tropical mangroves. Thus temperature did not show any significant relationship with the SRR in both Tuvem and Diwar site.

5.1.2. pH:

SRB have a preference to environment of pH 6 to 8 (Widdel, 1988; Hao et al. 1996). However Foti et al. (2007) reported the sulfate reducing activity at pH 11 in hyper-saline Soda Lake. Observation of SRB in acidic environments may be explained by the existence of micron-niches where SRB thrive in pockets with a higher pH (Hao et al. 1996). Thus, organisms with a low metabolic energy yield like SRB (Hamilton, 1998) might be especially susceptible to low pH. But in the last decades, considerable evidence has emerged that sulfate reduction at pH

value below 5 is prevalent. Acidophilic organisms maintain an elevated pH in the cytosol, which requires energy (Lowe et al. 1993). Furthermore metabolic products of SRB viz. H₂S and organic acids are known to be potentially toxic at low pH. In the present study at Tuvem, the down core variation in pH ranged from 6.70 to 7.10, while at Diwar it varied from 6.60 to 7.30. A highly significant down core variation in sediment pH profiles at both Tuvem (df = 4, p<0.001) and Diwar (df = 4, p <0.001) was observed. Ferreira et al. (2007) has reported a similar pH range in the mangrove swamp of Brazil. Generally in mangrove ecosystems, there is an active decomposition of tree litter. Further, hydrolysis of tannins which release various acids (Liao, 1990) and/or the oxidation of sulfide pyrite which release the dissolved ferrous iron (Stumm and Morgan, 1996) are known to be responsible for a shift towards more acidic conditions. As noted by the Middelburg et al. (1996) for sediments in the mangrove forest in Kenya, mangrove vegetation can affect the acid base and redox condition in several ways: (1) by the translocation of oxygen to their roots and release to sediments; (2) via the uptake of NH₄ by the trees leading to H⁺ release; and /or (3) via CO₂ respired by the roots, resulting in lower pH. In the present study, the pH variation was minimal and mostly within a neutral range which could be due to a balancing activity between TDLO (*Thiobacillus denitrificans* like organism) and SRB.

5.1.3. Water content:

Another important factor that affects the SRR is the water content of the sediments as it helps in transportation of nutrients. A positive correlation (p<0.05, n=60) between SRR and water content at both the sites was observed, revealing

the importance of water in solubilizing degraded nutrients in a dissolved form to the sediment bacteria. Such influence has also been observed by Jones et al. (1989), Musslewhite et al. (2007) in deep terrestrial subsurface sediments. In mangrove forests in Pakistan, Kristensen et al. (1992) reported the total microbial activity to reduce during the desiccation.

5.1.4. Eh:

The importance of redox potential (Eh) for the biological and chemical processes in marine sediments has been discussed by Fenchel, (1969); Whitefield (1969) and others. Bacterial sulfate reduction often takes place in negative redox potentials as described in many studies (Postgate, 1959). We have observed that the down core variation in Eh at Tuvem to range from -341.5 to 289.5 while at Diwar it varied from -140.4 to 301.7. Ferreira (2007) has also reported the oxic, suboxic and anoxic conditions in the mangrove sediments where the redox potential vary from 366 mV at top layer and 4 mV at 30 cm depth. Even in the surface sediments when the redox potential was +200 mV to 300 mV, sulfate reduction was reported which may be due to the presence of reducing micro-niches where SRR occurs, despite the oxidizing conditions in the surrounding (Jorgensen, 1977a). Since many biological and chemical processes depend on the combined effect of oxidizing and reducing environments, this micro-niche is probably of general importance for the metabolism of sediments.

5.1.5. Iron:

Iron plays both a direct and indirect role in modulation of SRR. Our results showed that during the pre-monsoon season at experimental site of Diwar, the average concentration of iron was 2.67 times higher (26.94%) than the monsoon season (10.08%). The average pre-monsoon SRR ($75.46 \text{ nM cm}^{-3} \text{ d}^{-1}$) was 0.35 times the monsoon rates ($216.14 \text{ nM cm}^{-3} \text{ d}^{-1}$). 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 elevated iron concentrations inhibited the SRR in the mangrove sediments at Diwar, while at Tuvem iron did not limit the SRR. Few studies have been carried out to study 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. (Thamdrup, 2000). Impact of Fe^{3+} addition in biofilms in sewers was put forth by Zhang et al. (2009), showing inhibitory activity (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). Alternatively iron could directly inhibit SRR (Gonzalez- Silva et al. 2009).

Probable mechanism for the inhibition of SRR in mangrove sediments:

- ❖ Lovley and Klug, (1983) and Van Bodegom et al. (2004) suggested that the inhibition of sulfate reduction and methane production by Fe^{3+} in sediments was caused by the competition of Fe^{3+} -reducing bacteria with sulfate-reducing bacteria and methanogens for common electron donors, primarily acetate and hydrogen (products of bacterial fermentation). The concentrations of hydrogen and acetate are generally maintained at low levels in-situ, by the action of Fe^{3+} -reducing organisms, thus making hydrogen and acetate unavailable for uptake for SRB and methanogens for metabolism (Lovley and Phillips, 1986b; 1987).
- ❖ Utgikar et al. (2002) hypothesized that metal sulfide deposits on the surface of SRB or methanogenic cells could cause the inhibition in activity of these bacteria. Ferrous sulfide precipitation in the vicinity of bacterial cells may reduce access of reactants (sulfate and VFAs) to the necessary enzymes. Thus, the metabolic rate of bacteria gets reduced (Utgikar et al. 2002). Fe^{3+} , like many heavy metals, could also deactivate enzymes of microorganisms by reacting with their functional groups, denature proteins of microorganisms and compete with essential cations utilized by microorganisms, which cause adverse effects on the activities of microorganisms (Mazidji et al. 1992; Atlas and Bartha, 1998). This deposit of metal sulfides may also potentially alter the final products of

sulfate production. Tuvem being a relatively pristine environment has lower concentrations of iron deposits in its sediments. However in Diwar the sediments have higher iron concentrations as a result of mining activities in the vicinity. The high concentration above 3.67 % (36700 ppm)– iron has been seen to be inhibiting SRR in these sediments. Further a continuous deposition of elevated iron concentrations may have a toxic effect on the SRB and thus influence the SRR and in turn the carbon mineralization rates in these mangrove ecosystems. However there is a need of fundamental research at the cellular and enzymatic levels to further verify the above hypothesis. Carbon mineralization in these systems could be linked to different redox cycles.

5.1.6. Organic carbon:

Mangroves are known to be highly productive ecosystems (global litter fall of 100 Tg C y^{-1} ; Jennerjahn and Ittekkot, 2002). Recent estimates show that as much as 11% of the total organic carbon inputs across the coastal zone (i.e. through riverine transport) are of mangrove origin. Carbon fixed by mangroves could be significant in the carbon budget of the coastal zone (Jennerjahn and Ittekkot, 2002). A strong interaction exists between the intertidal zone and the adjacent aquatic environment. Both import (terrestrial material, phytoplankton, sea grasses, etc.) and export (mangrove-derived organic matter) are expected to have major consequences for the carbon dynamics in both compartments (Hemminga et al. 1994; Bouillon et al. 2003; Marchand et al. 2003). The imported carbon sources have an important trophic role in sustaining macro-invertebrate

communities of the estuarine zones (Bouillon et al. 2002). Mineralization however, could represent a major fate for exported organic matter (Bouillon et al. 2002; Borges et al. 2003). Similarly, estimates of total organic carbon available (Alongi, 1988; Alongi et al. 1993; Middelburg et al. 1996; Alongi et al. 2000) indicate that intense mineralization takes place in intertidal mangrove ecosystems. A continuous range of mangrove ecosystem types exist. They vary from 'retention' systems where sediments are rich in organic carbon almost entirely of local origin, to 'flow-through' systems with mineral sediments (i.e. relatively low in organic carbon) where the organic matter can be dominated by imported sources (Bouillon et al. 2003). The SRB play an important geochemical role in organic matter decomposition. This process involves the SRB and their enzymes (which effect fermentation) (Westrich and Berner, 1984). SRB, themselves are not able to consume complex organic matter and can thrive only in cooperation with saprophyte micro-flora, which degrade these complex organic substrates (Matveev et al.1990). Microbial enzymes hydrolyze the original detritus and form low molecular components (acetate, hydrogen etc.) which are thereafter consumed by SRB. In this study, organic carbon revealed lowest values of 2.50 (± 0.30) and 1.48 (± 0.23) %, at Tuvem and Diwar sites respectively during the monsoon season and post monsoon season respectively. This could again be attributed to the higher flow rates and lower residence times associated with the South West monsoons (Krishnan, 2009). Highest accumulation of TOC in sediments at Tuvem, was in the post-monsoon season ($3.49 \pm 0.27\%$) while at Diwar it was seen in the monsoon season ($3.28 \pm 0.30\%$). High values of TOC at Diwar could be attributed to the

influx of terrestrial organic carbon due to the South West monsoons. This is supported by the high C/N values during the monsoon at Diwar (15.02). The trend for TOC concentration in sediments was post monsoon > pre-monsoon > monsoon and monsoon > post-monsoon > pre-monsoon at Tuvem and Diwar respectively. Though there was no monthly variability at Tuvem and Diwar, there was considerable inter seasonal variability at both the sites (Tuvem, $p < 0.01$, $df = 2$; Diwar, $p < 0.1$, $df = 2$).

At Tuvem and Diwar a positive relationship between SRR and TOC exists ($r = 0.319$, $p < 0.05$, $n = 60$ and $r = 0.615$, $p < 0.001$, $n = 60$ respectively), suggesting TOC to be a limiting factor for SRR. Surprisingly at Diwar, during pre-monsoon, a negative correlation between SRR and TOC ($r = -0.545$, $p < 0.01$, $n = 20$) was evident. This relationship is probably indirectly due to high iron concentrations which are known to inhibit SRR. The non significant depth-wise variability in total organic matter at both the sites indicates a vertical translocation of metabolizable organic substrate within the sediments (Howarth and Teal, 1979), either due to the subsurface root growth or due to downwards transport of newly deposited organic matter from the surface by bioturbation. In sub-tidal coastal sediments, where most of the freshly deposited organic matter remains at or near the surface, a sharp peak of sulfate reduction close to the sediment surface is normally followed by a rapidly decreasing rate with depth. More than 80% of the total activity is usually concentrated in the upper 10 cm of the sediment (Berner and Westrich, 1985; Swider and Makin, 1989).

5.1.7. C/ N ratio & labile organic matter:

Heterotrophic bacteria generally need organic substrates with a C/N ratio of 10 or less for maintenance and growth (Fenchel and Blackburn, 1979). To characterize the source of the organic matter in the mangrove sediments, C/N values were measured. Generally low C/N values of 5-7 are characteristic for marine organic material (Redfield et al. 1963). Values higher than 20 indicate a terrestrial source of organic matter (Scheffer and Schachtschabel, 1984). During degradation of marine organic matter, protein content is rapidly utilized by the mangrove flora and fauna and therefore the C/N ratio, increases up to ten (Emery and Uchupy, 1984). On the other hand, it has been shown that adsorption of ammonium on clay mineral can significantly lower the C/N values (Muller, 1977). Our sediment samples were treated with HCl to minimize this error. Besides C/N ratio correlate with our labile organic matter concentrations, calculated as the sum of protein, carbohydrate and lipids concentrations in sediments. At Diwar, a high average C/N ratio signature (13.85) of sediments signifies a terrestrial source of organic matter during the monsoon season, which is considered to be less labile. However high SRR ($216.14 \text{ nM cm}^{-3} \text{ d}^{-1}$) during this season, suggest that lability of organic matter plays relatively little role in dictating the SRR in Diwar sediments. What about LOM at this region? 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.

Low SRR in spite of high lability of substrate could be due to the inhibitory effect of excess iron in Diwar sediments.

To examine the labile components of living and detritus organic material total protein, lipid and carbohydrate contents were estimated. The pooled values of all the components represented the total labile organic matter. At both Tuvem and Diwar, the percentage of labile organic matter were correlated to the total organic carbon content, ($r=0.80$, $p<0.001$, $n=60$) and ($r=0.67$, $p<0.001$, $n=60$) respectively. Our finding suggests that 16.8 to 72.8 % of the total organic carbon is present as readily utilizable (labile organic matter) form at Tuvem. A subsurface maximum (4-6 cm) for labile organic matter was also observed at Tuvem for all the three seasons. At Diwar the LOC values ranged from 13.7 to 65.3% of the total organic carbon. Schubert et al. (2000) has shown the labile organic matter in marine sediments to be 70%. The low value of labile organic matter during the monsoon season is consistent with the high C/N ratio. Since the organic matter brought to the estuary is of the terrestrial origin, the total organic carbon during the monsoon season is mainly recalcitrant in nature. As expected labile organic matter has a significant correlation with sulfate reduction rates at both the sites. The relations were $r=0.26$, $p<0.05$, $n=60$ and $r=0.32$, $p<0.05$, $n=60$ at Tuvem and Diwar sites respectively (Table 34). The organic content could be governed by granulometry of the sediment.

5.1.8. Granulometry (sand, silt and clay):

The physical and geochemical properties of the subsurface medium in which SRB live contribute greatly to the distribution, number and metabolic activity

of these bacteria. Numerous studies have found significant shifts in bacterial populations and the metabolisms of the populations when the geochemical properties of the surrounding sediments change. Chappel and Lovely, (1990) found higher rates of metabolism in a sediment, likewise, Fredrickson et al. (1991) reported that after a shift in aquifer sediments type from a relative porous sandstone to a less porous material, there was a decrease in the ability of the bacterial populations to utilize different substrate as well as an overall decrease in bacterial numbers. Musslewhite, (2007) gives a concluded remark that sulfate reducing activity was not statistically correlated with sediments type (sand, silt and clay).

In the present study, a significant monthly variation in sediment sand profiles at both Tuvem ($df = 11, p < 0.001, n=12$) and Diwar ($df = 4, p < 0.001, n=12$) sites was noticed. Also a significant variation in the sand fraction was noted between Tuvem and Diwar ($df=1, p<0.001, n=2$). As the speed of water fluxes within the mangrove forests continuously fluctuate, the grain characteristics of the soil may also vary significantly within the same region. Because of the physiographic position of the soil, the sediment supply depends on both tidal action and fluvial discharge, which create a more energetic sedimentary environment capable of transporting and accumulating sand. Upkong, (1997) suggested that the intensity of the sedimentation and transport processes in mangrove forest may vary depending on the physiographic position and will also lead to variations in grain size distribution. Upkong, (1997) attributes the occurrence of sandy soils to the predominance of terrestrial over tidal sediment. In the present study at both

Tuven as well as Diwar, the sediment SRR had a negative correlation with the sand fraction ($r = -0.47$, $p < 0.001$, $n = 60$ and $r = -0.6$, $p < 0.001$, $n = 60$ respectively). Al- Raei et al. (2009) also observed the low sulfate reduction rates in sandy areas as compared to the mud and mixed flats. Kerkar and LokaBharathi, (2007) demonstrated high rate of sulfate reduction rates in clay and silt dominated salt pans. In the present study, since the sand has a negative correlation with TOC ($r = -0.64$, $p < 0.001$, $n = 60$ and $r = -0.30$, $p < 0.01$, $n = 60$) at both Tuven and Diwar respectively, low substrate availability could be the reason for low sulfate reduction in sandy sediments. At both the sites, clay has a positive correlation with the sediment SRR ($r = 0.39$, $p < 0.01$, $n = 60$) and ($r = 0.50$, $p < 0.0001$, $n = 60$) for Tuven and Diwar respectively. Clay also showed a positive correlation with TOC at a significant level ($p < 0.01$) at both the sites. The scarcity of the substrate availability also has an impact on sediment SRR in the clay fraction. Chatterjee et al. (2007) also showed a strong positive correlation between clay fraction and total organic carbon. The correlation is attributed to the affinity of these small particles to entrap organic carbon (Sarkar et al. 2004). Besides the influence of organic carbon, SRR could also be governed the sulfate concentrations.

5.1.9. Sulfate:

Sulfate concentration in marine environment is ca. 28 mM (De Wit, 1992) which is greater than other electron acceptors, i.e. O_2 , NO_3^{2-} combined (King, 2001). Mangrove soil can be both hyper-saline as well as with low salinity. During the monsoon season, influx of fresh water causes decrease in the pore water sulfate concentration. SRB use sulfate as the terminal electron acceptor for

growth. Reduced sulfur compounds can be used either by colorless sulfur bacteria or the colored photosynthetic bacteria finally resulting in the production of sulfate as a metabolite. In the present study the sulfate concentration in mangrove sediments varied from 2.73 to 18.47 mM. Sulfate concentrations do not show any depth wise variability at both the sites. This is probably due to the benthic macrofauna pumping respiratory water down into the burrows and thereby renewing the pore-water sulfate. 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 observed that it has negative influence on the sediment SRR at Diwar. About 63% of the variation in SRR is apparently negatively influenced by sulfate concentration ($r = -0.796$, $p < 0.001$, $n = 60$). However, this influence may not directly be due to sulfate, but due to the high iron content ($r = 0.867$, $p < 0.001$, $n = 60$). This correlation may be incidental or due to higher iron solubility under reduced salinity i.e. lower sulfate concentration. Alternatively when the salinity is low, SRR would be negatively related to sulfate especially when rates of sulfide oxidation are low. This co-variation is more conspicuous 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 sediment SRR at Tuvem suggesting that sulfate is the limiting factor at this station ($r = 0.498$, $p < 0.001$, $n = 60$). Sulfate is known to limit SRR in limnetic environments. 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 ($>15\text{mM}$) in coastal aquifers, enhanced the SRR.

They showed the K_s (half saturation constant for sulfate) value ranged from 3.5 to 7.5 mM while the other authors have showed the value of K_s to range from 240 μM to 1.63 mM in the marine environments (Boudreau and Westrich, 1984; Roychoudhury et al. 1998; Roychoudhury et al. 2003b). Trimmer et al. (1997) showed that sulfate reduction rates were in the same range to that found in the fresh water part of an estuary as brackish/marine part of the same estuary. High sulfate reduction rates in freshwater environments indicate that resident SRB were able to cope up with low sulfate concentrations (Ingvorsen and Jorgensen, 1984). The by product of SRR, sulfide can also influence the abundance and activity of SRB.

5.1.10. Sulfide:

Only about 10% of the sulfide produced during sulfate reduction is considered to be permanently buried in marine sediments and the rest is re-oxidized (Thoden- Andersen and Jorgensen, 1989). Free sulfide may be toxic to all the bacteria because it reacts with metal ions and functional groups of electron carriers, amino acids and metabolic coenzymes (Hao et al. 1996). Compared to other bacteria, SRB produce H_2S as a major product of their energy metabolism. Studies show H_2S to be the most toxic form of sulfide to the cell (Moosa and Harrison, 2006) which can be explained by the higher membrane permeability of the uncharged gas. Koschorreck, (2008) observed that typically 50% inhibition of sulfate reduction occurs at H_2S concentrations between 2 to 15 mM. In the present study pore water sulfide concentrations varied widely at both Tuvem (0.23 to 1.92 mM) and Diwar sites (0.018 to 0.17 mM). The higher concentration of

sulfide in the sediment pore water of Tuvem as compared to Diwar is suggestive 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). At Diwar, generally low H₂S levels measured, may be due to its precipitation with dissolved iron. Concurrent sulfide oxidation by the activity of sulfide oxidizers and limited oxidation of sulfide during centrifugation. Al-Raei et al. (2009) has shown that high concentrations of sulfide in the intertidal sediments ranged from 4 mM to 12 mM while Ferreira, (2007) has recorded the sulfide concentrations to vary from 5 µM to 38 µM in the upper 10 cm of sediments. Low values of sulfide were attributed to the high reactive Fe²⁺ concentrations. Sulfide production may not only be due to the general abundance of SRB but also due to the active fraction in a SRB community.

5.1.11. Abundance of SRB and SRR:

The estimation of sulfate reducers was carried out by both MPN serial dilutions and agar shake method on different sources of carbon i.e. lactate and acetate. The abundance of sulfate reducing bacteria in the mangrove sediments, varied dynamically throughout the year. Sediment SRB counts were in the order of 10³⁻⁶ g⁻¹ by the MPN serial dilution method. These bacterial numbers are comparable to the earlier studies on mangrove and estuarine systems around Goa estimated by either agar shake or MPN serial dilution method (Saxena et al. 1988; LokaBharathi et al. 1991; Kerkar and Bharathi, 2010). However agar shake method reveals abundance in the order of 10¹⁻³ g⁻¹. In spite of the limitation of the

agar shake method, it was adapted in the study as this approach helps to isolate representative cultures. The medium was amended with low concentrations of 1mM substrate to improve the retrievability. Also the natural water from the creeks in mangrove areas was used to prepare the media. In the marine and estuarine sediments, Parkes et al. (1993) also showed the same order of retrievable SRB on agar shake method. Interestingly the bacterial density was also comparable to that in marine coastal waters in sediments of the temperate regions (Brandt et al. 2001; Kondo et al. 2004). Hao et al. (2011) showed SRB counts in the range of 10^6 cfu/gm dry weight of the sediments in the mangrove ecosystem in China. However Kondo et al. (2004) demonstrated that in the marine sediments retrievable counts were 1000 times and MPN serial dilution counts at least 10 times lesser than the SRB counts in terms as *dsr* gene copy number. Kondo et al. (2004) showed the SRB counts in the order of 10^8 in marine and estuarine sediments by competitive PCR technique. However this technique presumed that all the cells to have only a single copy of the *dsr* gene and that copy number should indicate the cell numbers of SRB in sediments. However, *dsr* copy number may vary with different species *Desulfobacter vibrioformis*, *Desulfobulbus rhabdoformis* (Larsen et al. 2000), *Desulfovibrio vulgaris* (Karkhoff-Schweizer et al. 1995) *Desulfitobacterium hafniense* and *Archaeoglobus fulgidus* (Dahl et al. 1993) have a single copy of *dsr* but more than one copy of *dsr* have been detected in some *Desulfovibrio* species therefore competitive PCR may overestimate the number of SRB.

In the present study the proportion of cultivable SRB by MPN serial dilution method, relative to the total bacterial counts ranges from 0.00005 to 0.0047%. Our reports are consistent with Hao et al. (2011) results, which showed the same order of proportion of SRB in comparison to total bacteria counts, while the earlier reports depict the high proportions in other marine sediments. Previous estimates of the sulfate reducer abundance, is mostly in the range of 2 to 15% in near surface marine sediments (Raven-schlag et al. 2000; Kondo et al. 2004; Lehours et al. 2005). The above mentioned authors have used the uncultivable approaches to enumerate the SRB in sediments i.e. real time PCR, competitive PCR etc. The lower proportion of SRB in the present study either could be attributed to the regional difference or the technique used for the enumeration of SRB.

At both Tuvem and Diwar lactate degraders were homogeneously distributed up-to 10 cm of depth while acetate degraders had inconsistent patterns of abundance. At Tuvem acetate degraders were more abundant at 0-2 cm and at 6-10 cm of depth while at Diwar acetate degraders were high at 0-2 cm. A gradual decrease was observed with increase in depth. In general the lactate degraders were dominating groups in these sediments. Similar observations were reported by Taylor and Parkes, (1985) and Sham et al. (1999) in other marine sediments. The difference with the depth in the distribution of different substrate utilizing bacteria was probably due to the difference in unbound and freely available fatty acids. The difference in this pattern between the two sites may be attributed to the

variation in different mangrove plants, since the root exudates play a significant role in fatty acid composition and the diversity of bacteria in that specific location.

In the present study the SRR and density of the SRB do not relate significantly during the entire study period. A negative correlation between SRR and MPN counts on acetate at Tuvem during the pre-monsoon ($r = -0.44$, $p < 0.01$, $n = 20$) and post monsoon season ($r = -0.25$, $p < 0.05$, $n = 20$) has been observed. There are reports which show that acetate metabolism by SRB is inhibited at high salinities and thus resulting in the accumulation of acetate (Boone et al. 1993; Brandt and Ingvorsen, 1997; Kerkar and Bharathi, 2010). This observation is in the line with the results of recent experimental and modeling studies, which imply a lack of correspondence between the vertical microbial community structure and the vertical distribution of metabolic activities in sediments exhibiting high rates of microbial respiration (Koretsky et al. 2005; Thullner et al. 2005).

Previous work has shown that less than one order of magnitude change in SRB numbers may cause difference of several folds in measured metabolic activity (Ulrich, 1999). Another possibility may be that the medium used along with the carbon source in MPN method may have restricted the growth of some fastidious SRB which has resulted in a non significant relation between the two studied parameters.

5.2. Principal component analysis:

In order to appreciate the effect of environmental parameters on SRR, PCA analysis was carried out. PC1 at Tuvem and Diwar included essentially similar parameters except iron which was the dominating factor at Diwar. Hence, it is

suggested that that this parameter had a strong role in bringing about the variation between the two systems. At Diwar, five components accounted for 72.3% variation while at Tuvem, five components accounted for only 65%, suggesting that Tuvem is a comparatively stabler ecosystem as the variation is controlled by more factors. Diwar is a simpler system, perhaps less stable as the variation is controlled by fewer factors. At Diwar, clustering of SRR and Fe in the same component but with positive and negative loading respectively suggest the negative influence of iron on SRR. At Tuvem, SRR and Fe was not in the same component. These results suggest that iron could play a negative role on SRR when iron concentrations were high in Diwar. When iron concentrations were low in Tuvem, it did not affect SRR significantly. The above results have also been complemented by our laboratory experiments where iron has shown a retarding effect on SRR. The correlation matrix and principal component analysis showed comparable results. At Tuvem TOC, LOM, clay and sulfate has the positive loading factor, while sand has the negative loading factor at both the sites, which suggests the negative impact of sand on the SRR in these mangrove sediments. The negative loading of sulfate, at Diwar site may be fortuitous due to the iron that co-varies with the sulfate which in turn varies with salinity.

Thus different environmental parameters have varying degree of influence on the number and activity of SRB. In order to complement field observations laboratory experiments were also carried out in mesocosms.

5.3. Mesocosm experiments:

Experiments with sediment slurries elucidate some of the aspects of iron, manganese, sulfate and TOC regulation 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 compare trend at the two sites. The background concentration of total iron in sediments used for slurry experiments was 3.66%. Supplementing with 50 ppm (0.005%) of Fe^{3+} had a stimulatory effect (14%) on the SRR, however increasing Fe^{3+} to 100 ppm (0.01 %) and above, reduced the SRR. Further increase in iron concentrations up to 1000 ppm (0.1 %) brought about a decrease in the SRR up to 93%). These results show that Fe^{3+} had a strong inhibitory effect on the SRR at concentrations above 100 ppm (0.01%) in mangrove sediments.

In coastal sediments, microbial Mn reduction is generally concluded to be of little significance in carbon oxidation, due to a relatively low abundance and shallow vertical penetration of Mn oxides. Besides, studies of microbial Mn reduction are often hampered by the spatial resolution of available techniques (Thamdrup, 2000). Because the sedimentation of Mn and Fe oxides in most sediment is much lower than the benthic carbon oxidation rates, an efficient recycling of reduced Mn and Fe must take place when levels of Mn and Fe reduction are significant in carbon oxidation (Canfield et al. 1993). Since the

manganese concentrations were high up-to 1700 ppm and 2407 ppm at Tuvem and Diwar respectively, slurry experiments were conducted at the different concentrations of Mn as ($\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$) salt amendment. This was done to expound the effect of Mn amendment on sulfate reduction in the sediments. The laboratory results showed that sulfate reduction is not inhibited nor altered by the amendment of manganese up to 1000 ppm. This could be due to the fact that manganese reduction may not be a part of the carbon mineralization process in the sediments. There is thus no competition for a common substrate between sulfate reducers and Mn reducers. Other possibility is that the manganese reduction may be at its peak values showing that the substrate limitation (Mn) was not a factor controlling the manganese reduction in these sediments. It may be possible that the manganese reducers may not adept at reducing the Mn at a short period of time in slurry experiments.

In the present study, the SRR in sediments has a positive correlation ($r=0.50$, $p<0.0001$, $n=60$) with pore water sulfate at Tuvem. In the slurry experiments, the background values of sulfate were 4.1 mM. With such high background concentrations, further amendment with 5.0 mM sulfate, increased the SRR by 10 % after which a further increase did not bring about a significant change in sulfate reduction. In an earlier study, Roychoudhury et al. (2006) had shown that in a coastal aquifer, amendments of sulfate with a background value of 15 mM resulted in an increase in SRR. This could be due to the unavailability of the sulfate present in the sediments to the SRB. Another possibility could be that

some factor may be inhibiting the permeability of cells to the substrate through the membrane.

An Increase of 8 % in SRR with labile organic matter in the form of lactate and acetate amended sediments was observed when compared to the SRR of unamended sediments. During the monsoon, the C/N ratio at Tuvem was very high. This shows the signature of terrestrially derived organic matter sourced through South West monsoons. Since this organic matter is generally more recalcitrant, the sulfate reduction may not be so efficient. Addition of labile organic carbon resulted in an increase in SRR. Earlier studies also have shown the increasing effect on SRR with amendment of labile organic carbon (lactate) sediments (Schubert et al. 2000; Kerkar and Bharathi, 2007).

5.4. k value (Degree of recalcitrance):

Sulfate reducing activity, in marine ecosystems, especially the coastal regions contributes to as much as 50% organic carbon turnover (Jorgensen, 1982). Sulfate respiration accounts for 70-90% of the total respiration in salt marsh sediments, where total respiration rates are 2.5 to 5.5 g C cm⁻² d⁻¹. Besides sulfate, organic carbon levels also influence SRA which is an important reaction for the production of reactive by products and the regeneration of the nutrients. Westrich and Berner, (1984) stressed the importance of the amount of organic matter (G) and, the reactivity of decomposable organic matter. They explained that in a simple first order G-model, the material denoted as CH₂O is assumed to be decomposed at an overall rate directly proportional to its own concentration. Thus G is indicative of the reactivity of the available organic carbon.

$$dG/dt=-k(G).$$

Where k is the recalcitrance of the substrate depicted as reaction rate coefficient and calculated from SRA and TOC. We have applied a single reactivity constant meant for anaerobic systems where k is the first order decay constant via sulfate reduction. As SRB are more adept at utilizing secondary metabolites present in anaerobic systems, we have used only a single k meant for the recalcitrant or less labile substrate. The k value at Tuvem varied from 0.002 to 0.01 month⁻¹. While at Diwar k varied from 0.00046 to 0.0049 month⁻¹ which are the minimum and maximum values calculated throughout the year. These values are low, reflecting the high decomposability of organic matter in these mangrove ecosystems. Organic matter of marine origin is known to be more easily degradable than terrestrial origin. At Tuvem the k value during the pre-monsoon and post monsoon were 0.028 and 0.025 month⁻¹ respectively while during the monsoon there was an increase in k value (0.011 month⁻¹). The difference in k value may be attributed to the influx of terrestrial origin organic matter with South West monsoon. These results are consistent with the high C/N ratio during the monsoon season as compared to the non monsoon seasons. Absence of significant depth wise variability in k values, evinced a homogenic response in the sediments. At Diwar, the same pattern of the k value was observed. The k values were 0.0028 month⁻¹, 0.017 month⁻¹ and 0.0047 month⁻¹ during the pre-monsoon, monsoon and post monsoon seasons. These results were consistent with the low C/N ratio i.e the influence of marine origin of organic matter during the non monsoon seasons as compared to the monsoon seasons. However, if we compare the two sites, the average k values at Tuvem were one order higher than Diwar. Westrich and

Berner, (1984) proposed that the pool of decomposable sedimentary organic matter is actually composed of various groups of compounds that have different reactivities with regards to the decomposition. Based on this concept, seasonal and spatial differences in k values can be explained by the series of changes, the organic matter undergoes during different seasons and at different sites.

The k values calculated on the annual basis were 0.245 month⁻¹ and 0.097 month⁻¹ at Tuvem and Diwar. The lower values at Diwar suggestive of higher lability of the substrate perhaps due to anthropogenic forcing. The present k values could not be compared with the k values of any other tropical mangrove ecosystem due to the unavailability of research on the degree of recalcitrance of organic matter in mangroves. However comparison of C/N ratio between Diwar and Tuvem reflect the degree of recalcitrance, with an average C/N ratio (8.3) at Diwar being lower than at Tuvem (11.3). However these values have been compared to other marine and estuarine k values from different sources of organic matter. Our values are much lower than the k values of 14 reported by Grill and Richards, (1964) (as cited by Marten et al. 1978) and the range 11-26 given by Otuski and Hanya, (1972) (as cited by Skopintsev, 1976) and those reported by Westrich and Berner, (1984), which were around 24 yr⁻¹. However the k values of the Schubert et al. (2000) ranged from 0.001 to 0.01 month⁻¹ in 0-20 cm sediment core, off the central Chilean coast. Kerkar and Bharathi, (2010) have reported the k value to range from 0.0021 to 0.086 month⁻¹ and 3.2 yr⁻¹ in the Ribandar salt pan of Goa. Schubert et al. (2000) suggest that the source and type of organic matter play a major role in the distribution of SRR. They also opine that the organic

matter being degraded may be simply diluted by non-reactive components including terrestrially derived organic matter. Thus the average LOM/TOC ratio suggest that the labile substrate at Tuvem (0.25) is more diluted than at Diwar (0.32). Consequently the dynamics of the availability of labile organic matter and its interaction with other environmental parameters could affect SRR.

5.5. Carbon mineralization via sulfate reduction:

The water-logged mangrove sediments are usually anoxic, even those which close to the sediment surface (~2 mm). Roots of trees and in-faunal burrows may translocate oxygen deep into the sediment. Except for a network of narrow oxic rhizospheres and burrow walls, the bulk sediment generally remains largely anoxic (Kristensen and Alongi, 2006). The mineralization of organic carbon in mangrove sediments is therefore primarily mediated by various microbial fermentation and respiration processes. Aerobic microorganisms have the enzymatic capacity for complete oxidation of organic carbon to CO₂, while more important anaerobic degradation processes occur stepwise involving several competitive types of bacteria. Thus, large organic molecules are first split into small moieties by fermenting bacteria. These small molecules are then oxidized completely to CO₂ by anaerobic respiring bacteria using electron acceptors in the following sequence according to the energy yield: Mn₄⁺, NO₃⁻, Fe₃⁺ and SO₄²⁻ (Canfield et al. 2006). In the present study we have made attempts to try and quantify the carbon mineralization rates via the sulfate reduction process in the mangrove sediments.

From the results of the present study, the areal SRR for the whole study area could be estimated on a seasonal basis. The annual amount of sulfate reduced in the top integrated 10 cm surface sediments was then calculated.

The sulfate reduced for the total mangrove area of the Chapora estuary (218.5 hectare) was calculated to be 1009, 541 and 1142 tons of sulfate reduced per year in the top 10 cm of a mangrove area, during the pre-monsoon, monsoon and post monsoon respectively. Hence areal benthic organic matter mineralization by microbial sulfate reduction is two times higher during the non monsoon seasons as compared to the monsoon season. The results correspond to an estimated average benthic organic carbon mineralization via sulfate reduction of $307.99 \text{ g C m}^{-2} \text{ yr}^{-1}$ ($672.97 \text{ tons y}^{-1}$). When using total area why are you reducing to per m^{-2} . At Diwar 770, 3074 and 1355 tons per year of sulfate reduced during the pre-monsoon, monsoon and post monsoon seasons respectively in the top integrated 10 cm sediments of an area of 700 hectare. The results correspond to an average benthic organic carbon mineralization of $185.68 \text{ g C m}^{-2} \text{ yr}^{-1}$ ($1299.74 \text{ tons y}^{-1}$). These values are higher than that values reported by AL-Raei et al. (2009) in temperate intertidal sediments. Alongi et al. (2004) has reported carbon mineralization via sulfate reduction to range from $166.11 \text{ gm C m}^{-2} \text{ yr}^{-1}$ to $466.28 \text{ g C m}^{-2} \text{ yr}^{-1}$ in the mangrove sediments of peninsular Malaysia. Kerkar and Bharathi, (2010) estimated these rates to range from $543 \text{ g C m}^{-2} \text{ yr}^{-1}$ during monsoon to $4188 \text{ gm C m}^{-2} \text{ yr}^{-1}$ during pre-monsoon in the salt pans of Ribandar, Goa and attributed the high values to the high degradation coefficient of SRB. In the present study the difference in the value of carbon mineralization via the sulfate

reduction between the two sites could be attributed to the high iron content since iron is a more preferred electron acceptor than the sulfate in the sediments (Thandrup, 2000). The carbon mineralization by iron reduction may be the dominant process at Diwar while sulfate reduction may be the dominant process at Tuvem. Thus iron may have a direct or indirect inhibition impact on the sulfate reduction process at Diwar. Further emphasis on varied aspects of this research is required to estimate the carbon mineralization via other process i.e oxic respiration, denitrification, metal reduction, and the contribution of each process for the carbon mineralization in these mangrove sediments on monthly and seasonal basis. There is a need for more detailed measurements of these processes in diverse mangrove systems. Besides the need for more quantitative process studies, complementary approaches to determine the contribution of mangroves and other carbon sources to various fluxes and process rates are required to better constrain the major sinks of mangrove carbon.

5.6. Identity of sulfate reducing bacteria in mangrove swamps:

Using biochemical characterization it was possible to tentatively identify, 32% of 88 isolates as *Desulfovibrio* sp. These isolates have been grouped under 8 genera and 12 species i.e. *Desulfococcus multivorians*, *Desulfovibrio desulfuricans*, *Desulfobacter postgatei*, *Desulfovibrio baculatus*, *Desulfotomaculum orentis*, *Desulfovibrio vulgaris*, *Desulfomicrobium* sp, *Desulfosarcina*, sp, *Desulfobulbus propionicus*, *Desulfotomaculum acetooxidans* sp, *Desulfomonas piagra* and *Desulfonema limicola*. Such increased variety has been noted earlier in mangrove ecosystems (LokaBharathi et al. 1991). Lactate was the most

preferred substrate. Out of 88 isolates 86 can use the lactate as electron donor. Out of 88 isolates 51 can use the acetate as a substrate. The abundance of lactate utilization over acetate utilization has been reported by LokaBharathi et al. (1988) in mangrove sediments. Some of the SRB isolates were able to utilize substrate i.e. formate (34), ethanol (61), pyruvate (33), fumarate (42), malate (42), benzoate (28), glucose (50), propionate (41) and butyrate (36). Thus these SRB are nutritionally versatile and therefore ecologically competitive. SRB have been reported to degrade other compounds like benzene and monoaromatic hydrocarbon such as toluene and xylene (Edwards and Galic, 1992; Flybridge Lovely, 1995). The genera like *Desulfosarcina* and *Desulfococcus* are also isolated in the present study, which is known to be metabolically versatile (Harms et al. 1999). The presence of spore forming *Desulfotomaculum acetoxidans* could indicate the likely deposits of manure in these mangrove swamps, since *D. acetoxidans* is known to inhabit gastrointestinal tracts of animals (Laanbroek and Pfennig, 1981). The *Desulfococcus multivorians* isolated in the present study can utilize the aromatic compound like benzoate. Peters et al. (2004) also have reported a *D. multivorians* strain which could utilize the benzoate and its utilization by strain was attributed to Fe-S enzyme benzoyl CoA reductase. In present study the most abundant sp was *Desulfovibrio*. The *D. vibrio* commonly found in aquatic environments with high level of organic carbon, as well as in the water logged soil (Madigan & Martinko, 2006). In the Other studies of Taketani et al. (2010) in mangrove sediment of Brazil have also emphasized the occurrence of this genus. In the present study 32% of the isolates decomposed benzoate. The occurrence of

p-hydroxy benzoic acids and other phenolic acids in these mangrove sediments was reported by Karnath et al. (1975). Around 50% isolates can use propionate as a substrate. Propionate is a common end product of many fermentations. Further, more propionate is produced from long chain fatty acids with odd numbers of carbon atoms by a syntrophic culture of a hydrogen producing acetogenic bacterium and a hydrogen consuming organism (McInerney et al. 1979). The presence of formate, pyruvate, malate and other substrate utilizers in these mangrove sediments suggested, lactate and possibly acetate may not be the only primary substrate for sulfate reduction in anaerobic sediments, which is also reported by LokaBharathi et al. (1988). LokaBharathi et al. (1991) have also highlighted the presence of *D. desulfuricans*, *D. salexigens*, *Desulofatoma maculum orientis*, *D. acetoxidans*, *Desulfosarcina variabilis* and *Desulfococcus multivorians* in mangrove sediments.

The SRB isolates from the present study were able to tolerate a wide range of salt concentrations. It is known that SRB can tolerate a wide range of salt concentrations (LokaBharathi et al. 1991). Moreover, these mangrove swamps along Mandovi and Chapora estuary are continuously exposed to wide diurnal seasonal and annual fluctuations in salinity which makes these habitats euryhaline in nature.

The diversity of SRB in marine sediments has been investigated by clone libraries of the 16S rRNA gene (Devereux and Mundfrom, 1994; Bowman and McCuaig, 2003; Purdy et al. 2003a). A more powerful approach for the detection of SRB is the use of functional genes which encode enzymes that play an

important part in the sulfate-reduction pathway, such as *dsrAB*, which encodes the dissimilatory sulphite reductase (Wagner et al. 1998), or *aprBA*, which encodes the dissimilatory adenosine-5'-phosphosulfate reductase (Meyer et al. 2007). Cloning or denaturing gradient gel electrophoresis of PCR amplified 16S rRNA (Dhillon et al. 2003; Dar et al 2005), *dsr* (Minz et al. 1999; Geets et al. 2006; Dar et al. 2007) or *aprA* (Meyer et al. 2007) gene fragments have been used to determine the diversity of SRB in many different habitats. Recently, a DNA microarray, the SRP-PhyloChip (Loy et al. 2002), has been used to detect SRB in natural samples, such as acidic fen soils (Loy et al. 2004). However, these methods have the disadvantage as they provide little or no information on the number of SRB cell counts that are present. Using different probes, 2% to 21% of the prokaryotic DNA in marine sediments has been shown to represent total SRB numbers in these ecosystems (Rooney-Varga et al. 1997; Sahm et al.1999a; Ravensschlag et al. 2000). This group includes metabolically versatile organisms, from the genera *Desulfosarcina* and *Desulfococcus*, and species with 16S rRNA sequences that are up-to 18% divergent (Aeckaersberg et al.1991; Widdel and Bak, 1992; Harms et al. 1999).

Quantitative real-time PCR is a highly sensitive technique that can be used to quantify the number of SRB, and has been used, to determine the number of SRB in rice field soils (Stubner, 2002; 2004), soda lakes (Foti et al. 2007) and industrial waste water (Ben-Dov et al. 2007). Moreover, this technique can also be used to study the expression of functional genes, such as *dsrAB* (Neretin et al. 2003). Another technique that can be used to quantify the number of SRB is

fluorescence *in situ* hybridization (FISH), which also allows their spatial distribution to be visualized (Dar et al. 2007; Lockner et al. 2007). Different probes have been developed to target the rRNA of different taxonomic groups of SRB (Stahl et al. 2007). Mussmann et al. (2005) used a combination of FISH with catalysed reporter deposition (CARD–FISH) to study the vertical distribution of SRB in intertidal mud-flat samples. They found that up to 11% of all cells were SRB and that organisms related to the genera *Desulfosarcina* and *Desulfobulbaceae* dominated the surface layer of the sediment. Comparative sequencing and hybridization of ribosomal RNA techniques would perhaps indicate more diversity in these mangrove swamps.

5.7. Cobalt precipitation using SRB:

SRB are important bioremediators of heavy metals and precipitate them as metal sulfides. Selection of the most efficient SRB was carried out on the basis of the precipitation of cobalt chloride as cobalt sulfide with minimal loss of cell viability. In the present study the cobalt concentrations in mangrove sediments has been estimated to range from 18 to 64 ppm which are much above the permissible levels (10 ppm). Cobalt concentrations in the mangrove sediments may be higher due to the high concentration of iron and manganese. It is known that cobalt combines with the hydroxide of iron and manganese as well as silty minerals (Barańkiewicz D, Siepa J, 1999). Alagarasamy, (2006) has reported the cobalt concentration in Mandovi estuary sediments ranged from 2.5 to 45.3 ppm.

Only few studies have dealt with the role of SRB as a bioremediating agent for cobalt (Tebo et al. 1984; Luoma, 1990; White et al. 1998; Krumholz, 2003).

While the studies on other metals viz. chromium (Chardin et al. 2002), Hg (Haristha et al. 2002), As, Cd, Cr, Cu and Zinc (Viggi, 2010), Uranium (Boonchayaanant, 2010) Cd and Zn (Goncalves et al. 2007) have been carried out globally in different laboratories. In the present study, Co has been considered as a model for metal precipitation by sulfate-reducing bacteria under the laboratory conditions (Krumholz, 2003)

Though Co has important biological functions, it is required only at low concentrations and can become toxic at micro and millimolar concentrations (Ehlich, 1997; Nies, 1992). Metals become toxic to the organism when their concentration is higher than the demand for metabolism, resulting in inhibition of metabolic pathways usually by strongly binding to enzymes, forming unwanted radicals or less stable reaction products (Raab & Feldmann, 2003). The toxicity of Co appears to be due to unregulated cell growth triggered by internal sequestration of the metal, resulting in enlarged cells and their subsequent rupture (Runa et al. 2009).

In the present study an increase in pH was coupled to the release of biogenically produced hydrogen sulfide, which results in the precipitation of metal as cobalt sulfide. The variation in the redox potential as a function of time was observed during the growth of SKKL8. The initial Eh measured in the experiment was around 100 mV. Further the addition of resazurine facilitated visual assessment of change in Eh. At the beginning of the experiment blue color was observed In tubes with SRB minus Co, blue color disappeared within 5 days However in tubes with cobalt and SRB, the decoloration was within 7 days of

incubation with 500 ppm cobalt. A similar observation was reported by Radhika, et al. (2006) in case of zinc precipitation using *Desulfotomaculam nigrificans* and Saini et al. (2001) in an experiment with *Desulfovibrio desulfuricans* in presence of lead.

The growth of SKKL8 in the presence of Co (500 ppm) was monitored throughout the study period. Cobalt concentration showed a reduction from ~500 ppm to 86 ppm after an incubation period of 30 days.

The effect of cobalt (II) on the growth of SKKL8 is depicted in Figure 40. The lag phase in the control was observed for 3 days. However addition of cobalt (500 ppm) delayed the lag phase of SKKL8 to 7 days. Even though the growth recovered after 7 days, it never reached the control without cobalt. The effect of cobalt on the growth of SRB has not been reported so far and hence attempted in the present study. Moreover the concentration factor (up to 6) calculated for cobalt in Diwar mangrove sediments was highest among the analyzed metals. However reports on the effect of cobalt on other microorganisms are used as a comparison. Ainsworth et al. (1980), has reported an increase of lag time, with the increase of Co (II) concentration in the growth medium of *Klebsiella pneumoniae*. Cobalt has been identified as a trace element by a number of researchers (Wood et al. 1975; Jefferson et al. 2001) but only a few microorganisms were able to grow at relatively high Co (II) concentrations. Schmidt and Schlegel (1989) have isolated, bacterial strains able to grow at CoCl_2 concentration of up to 20 mM (= 1178.6 mg L) from metal processing wastewater treatment plants. Otth et al. (2005) have reported that *Aeromonas butzleri* was able to grow at extreme Co (II) concentration of 80 mM (= 4714.4 mg L⁻¹).

In the present study, abiotic precipitation or biosorption of the added cobalt concentrations, reduced to about 485 ppm from an initial concentration of 500 ppm after a 30 day incubation revealing this process playing a minor role in cobalt precipitation.

Using the set-up described in section (3.8.3.3) by purging the bacterially produced hydrogen sulfide gas through the cobalt chloride solution, the precipitation of the desired metal was achieved. It is worthy of mention that concentrations of cobalt (5000 ppm) could be successfully precipitated as sulfide in the period of 24 hrs utilizing the sulfide stripped from a fresh, fully grown culture. These findings hold promise for further scale up and application to typical waste water systems such as industrial effluents and aquifers. Bioremediations by sulfate reduction has a significant advantage over other chemical processes as this metal can be recovered as a non toxic eco-friendly product. In addition the conventional treatment by chemicals could yield non desirable end products. The distinct advantages of biogenic sulfide precipitation vis a vis precipitation as a hydroxide or by other chemical processes, particularly in the remediation of waste water containing low concentration of deleterious metal ions cannot be overemphasized.

The precipitate produced during the growth of SKKL8 in the presence of the cobalt chloride (500 ppm) and precipitate produced by media interaction with cobalt chloride (negative control) was characterized with respect to its chemical composition. The ratio of the cobalt to sulfur precipitated is higher in case of the biogenic precipitate as compared to the chemically precipitated cobalt.

Sulfate reducing bacteria and their activity in the mangrove swamps of Goa.

The highly productive mangrove environments are an ecological asset and economic resource. Being ecologically sensitive, any loss of these buffer zones could have severe impacts on several biogeochemical cycles. The biogeochemical cycle of sulfur being very complex could be one of the most affected.

Sulfate reduction occupies a central position within the global sulfur cycle. It is the predominant process converting sulfate to its reduced form- hydrogen sulfide. The condition in sediments due to its low oxygen content, errand the process upto a great extent. Despite the importance of sulfate reduction, only a few studies have explored the factors regulating this process in mangroves and no single set of factors has emerged consistently as the regulator of sulfate reduction rates. Hence, the aim of the present study is to understand the principle factors influencing sulfate reduction rates in mangrove ecosystems. In order to address this aim, the study had the following objectives.

- To quantify the abundance of sulfate reducing bacteria.
- To identify the sulfate reducing bacteria
- To quantify the sulfate reducing activity in sediments and understand the influence of environmental factors
- Bioremediation of heavy metal using SRB to immobilize Co.

To fulfill the above objectives, both field observations and laboratory experiments were carried out. The observations covered a one year period at Diwar influenced

by ferromanganese ore mining (experimental site) and Tuvem (control site), relatively pristine and free from mining influence. The experimental site at Diwar facilitated the study on the effect of metals on sulfate reduction.

Sediment cores were analyzed at 2 cm intervals up to 10 cm. Ambient physico-chemical parameters like salinity, dissolved oxygen, sediment temperature, pH and Eh were measured along with pore water sulfate and sulfide. Bulk sediment properties like total organic carbon, labile organic matter, iron, manganese and other heavy metals were measured using standard techniques. Sulfate reduction rates were measured by ^{35}S radioisotope technique. Total and viable bacteria, general heterotrophs and sulfate reducers were enumerated in all the sections of the core. Representative cultures of sulfate reducing bacteria were checked for their metal tolerance and subsequently for bioremediation of cobalt. Some of the isolates from the mangrove sediments were identified by both biochemical and molecular techniques.

Experiments were conducted to quantify the effect of various abiotic factors on sulfate reduction by amendments with iron, manganese, sulfate and dissolved organic carbon.

Salient results:

1. At Tuvem and Diwar sites, total bacterial counts were higher during the monsoon months compared to the non monsoon months and ranged from 10^9 to 10^{10} cells g^{-1} dry weight sediment.
2. Total heterotrophic bacterial counts at Tuvem ranged from 0.05 to 4.5×10^5 CFU g^{-1} dry weight sediment. At Diwar it was from 0.11 to 3.3×10^5 CFU g^{-1} dry

weight sediment. The metabolites of these bacteria are important substrates for SRB.

3. The abundance of SRB in the sediments was in the order of 10^{3-6} g^{-1} by the MPN method. The proportion of cultivable SRB relative to the total bacterial counts ranged from 0.00005 to 0.0047%.
4. At both Tuvem and Diwar, lactate degraders were homogeneously distributed upto 10 cm of depth while acetate degraders had inconsistent patterns of distribution.
5. Total genera retrieved from the mangrove sediment was twelve as identified by classical taxonomy. The prominent genus was *Desulfovibrio sp.* (20) The SRB isolates showed affinity to the following genera :

Desulfococcus multivorans (16), *Desulfovibrio desulfuricans* (11) ,
Desulfobacter postgatei (10), *Desulfovibrio baculatus* (9) ,
Desulfotomaculum orientis (8) and *Desulfovibrio vulgaris* (8)
Desulfomicrobium sp. (6) and *Desulfosarcina sp.* (6) *Desulfobulbus propionicus* (5) *Desulfotomaculum acetooxidans* (4) and *Desulfomonas pigra* (4) and *Desulfonema limicola* (1).

6. These SRB had the ability to grow on a wide range of substrates i.e. acetate, benzoate, butyrate, fumarate, formate, lactate, malate, propionate, with preference for lactate. Metabolic diversity of this group suggests a more general participation in the flow of carbon and electrons in anoxic mangrove sediments of Diwar and Tuvem.

7. Average sulfate reduction rates (SRR) at Tuvem were $247.50 \pm 162.28 \text{ nM cm}^{-3} \text{ d}^{-1}$, and at Diwar were $138.46 \pm 67.79 \text{ nM cm}^{-3} \text{ d}^{-1}$. SRR peaked at Tuvem during the post monsoon season while at Diwar rates peaked during monsoon season. The average SRR was 55% higher at Tuvem.
8. Integrated SRR (0-2 to 10 cm) at Tuvem varied from 10.4 to $63.7 \text{ mM M}^{-2} \text{ d}^{-1}$ while at Diwar it ranges from 6.28 to $61.3 \text{ mM M}^{-2} \text{ d}^{-1}$.
9. Estimation of the amount of sulfate reduced via sulfate reduction in the mangroves of Chapora estuary (location Tuvem) which covers an area of 218.5 hectares was 897 tons y^{-1} . The corresponding value for Mandovi estuary (location Diwar) with an area of 700 hectares was 1733 tons y^{-1} .
10. The annual average carbon mineralized via sulfate reduction in mangrove sediments at Chapora and Mandovi estuary was 673 and 1299 tons y^{-1} respectively.
11. Though the mangrove area at Chapora covers only 0.31 times the area of that at Mandovi, the carbon mineralized via sulfate reduction was 0.51 times the Mandovi, suggesting that the carbon flux could be much higher when iron ambient concentrations are low.
12. Besides iron, the recalcitrance of the substrates also affects SRR. The average reaction rate coefficient k which is indicative of recalcitrance of substrate was 0.245 and 0.097 month^{-1} at Tuvem and Diwar respectively suggesting a higher lability of substrate at Diwar. This lability of organic matter was 2.5 times higher at Diwar than at Tuvem.

13. The average iron concentration was nearly 18% at Diwar and only 6% at Tuvem. Consequently at Diwar SRR, was governed by iron concentrations at an r-value of -0.76 ($p < 0.001$, $n = 60$), suggesting that ca. 58% of the variation in SRR was influenced by variation in ambient iron concentrations. Thus our study showed that ambient iron concentrations influence SRR negatively and counters the positive influence of organic carbon. Consequently, the influence can cascade to other biogeochemical processes in the mangrove swamps especially mineralization of organic matter to carbon dioxide by sulfate respiration.
14. Only in the control site SRR related to TOC ($r = 0.32$) and clay ($r = 0.39$). These parameters in turn related to SRB ($r = 0.27$ and $r = 0.38$ respectively) suggesting that TOC governs the distribution of SRB and dictates their activity. In Diwar such control of TOC on number and activity was not evident as TOC could be non limiting in the experimental site.
15. Principal component analysis showed that at Diwar, five components accounted for 72 % variation while at Tuvem, five components accounted for only 65%, suggesting that Tuvem is a comparatively stable ecosystem as the variation is controlled by more factors. Diwar is a simpler system, perhaps less stable as the variation is controlled by fewer dominant factors notably iron.
16. Though iron was a controlling factor for SRR at Diwar, there could have been other metals like Mn and Co influencing the process. One of the Isolate SKKL8, with the high potential of precipitating metals was used for testing

bioremediating potential with cobalt, as this metal has been observed to range from 18 to 64 ppm in mangrove sediments.

17. Subsequently SKKL8 was identified as *Desulfovibrio vulgaris* by 16S rDNA technique. It showed 98% similarity with type strain in NCBI data base.
18. While 5% of the cobalt was bioadsorbed by dead cells, 80% was bioremediated by active cells. Hydrogen sulfide stripped from exponentially grown cells could precipitate all the 5000 ppm in 24 hrs. These results suggest that bioremediation using the sulfide stripped from bacteria is more efficient than bioadsorption by dead cells or bioremediation by active cells.
19. These findings hold promise for further scale up and application to typical waste water systems such as industrial effluents and aquifers.

Conclusion: 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 show that iron inhibited SRR but the concentration of iron in Diwar sediments was more inhibitory than stimulatory to this process. However, at Tuvem, the factors controlling SRR were sulfate concentrations and TOC. Even though iron was the key controlling factor for SRR at Diwar, the sulfate concentration, moisture content and total organic carbon, may have synergistically played a role in controlling the sulfate reduction rates at both the sites. The upstream ferromanganese transport in the Mandovi is probably the main contributing source of excess of iron to this estuary and thus possibly affecting the biogeochemical processes of carbon mineralization in these

mangrove swamps. These findings could have implications on the flux of greenhouse gases such as CO₂ from the mangrove sediments. More importantly, SRB are potential and perennial source of bioremediating agents under anaerobic conditions which are cost effective.

Future prospects: The stimulatory or inhibitory effects of other metals on SRB and SRA would throw more light on the details of changes in the community and the activity. These studies would help us to appreciate the total CO₂ flux from these sediments at a higher resolution. It would also provide a set of SRB able to bioremediate other metals as well.

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Annexure I

Manuscript Published:

- ✓ **Kuldeep Attri**, Savita Kerkar. (2011). “Seasonal assessment of Heavy metals pollution in tropical mangrove sediments Goa, India” *Journal of Ecobiotechnology*. 3(8), 9-15.
- ✓ **Kuldeep Attri**, Savita Kerakar, P.A. Loka Bharathi. (2011) “Sulfate reducing activity in tropical mangrove sediments and the factors controlling the process” *Estuarine, coastal and shelf science*, 95, 156-164.

Abstracts Published at Conferences:

- ✓ Savita Kerkar, **Kuldeep Attri** (2012). The effect of a temperature rise on the sulfate reducing rates in cold marine sediments of Kongsfjord (Svalbard, Arctic Ocean). *Science & geopolitics of Arctic and Antarctic*, March 9-11, New Delhi.
- ✓ Krishan, K.P, **Kuldeep Attri**, Lokabharathi P.A, (2006). “Metabolic plasticity: some unusual combination of inorganic carbon and nitrogen metabolism in marine benthic bacteria”. *Proceedings of 7th Asia Pacific Marine Biotechnology Conference* (Kochi).
- ✓ Savita Kerkar, **Kuldeep Attri** (2009). “Bioremediation of heavy metals using sulfate reducing bacteria”. *Proceedings of National Seminar on Sustainable Waste Management: Problems and Solutions* (Manglore).
- ✓ **Kuldeep Attri**, Savita Kerkar. (2009). Sulfate reducing activity in tropical mangrove sediments and the factors controlling the process. *Proceedings of Association of Microbiologist of India* (Pune).

To be communicated:

- **Kuldeep Attri, Savita Kerkar & Loka Bharathi.** “Immobilization of cobalt using the Sulfate reducing bacteria isolated from mangrove sediments. (*Geomicrobiology J*).
- **Kuldeep Attri, Savita Kerkar & Loka Bharathi.** “Implication of sulfate reduction in mangrove swamps of Goa” (*Estuary and Coast*).
- **Kuldeep Attri, Savita Kerkar & Loka Bharathi** “Ambient heavy metal concentrations inhibit SRA: Implications in mangrove swamps. (*Environmental Pollution*).

Appendix

1. Dissolved oxygen:

Winkler A: 60 g of potassium (KI) and 30 g potassium hydroxide was dissolved in two separate beakers and volume was made up to 100 ml with distilled water (d/w).

Winkler B: 40 g of manganese chloride ($\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$) was dissolved in d/w and volume was made up to 100 ml in d/w.

2. Redox potential measurements:

Solution A:

Potassium ferrocyanide 4.22 g reagent grade $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$: 0.1M

Potassium ferricyanide 1.65 g reagent grade $\text{K}_3\text{Fe}(\text{CN})_6$: 0.05M

Place in volumetric flask. Add about 50 ml d/w and swirl to dissolve solids. Dilute to volume with d/w.

Solution B:

Potassium ferrocyanide 0.01 M 0.42 g

Potassium ferricyanide 0.05 M 1.65 g

Potassium fluoride: $\text{KF} \cdot 2\text{H}_2\text{O}$ 0.36 M 3.39 g

Place in a volumetric flask. Add about 50 ml d/w and swirl to dissolve the solids. Dilute the volume with d/w.

Transfer solution to beaker. Place the electrode in the solution and wait until reading stabilizes. The potential should be about 192 mV (Solution A). For solution B: 256mV. Difference: B-A= 66mV.

3. Protein:

Reagents:

1) Reagent A: 2% Na_2CO_3 in 0.1 N NaOH
2 g of Na_2CO_3 in 100 ml of 0.1 N NaOH

2) Reagent B: 0.5% CuSO_4 in 1% sodium potassium tartarate solution
0.5 g CuSO_4 in 50 ml d/w
1 g Na-K tartarate in 50 ml d/w
Mix both together

- 3) Reagent C: Mix 50 ml of Reagent A and 1 ml of Reagent B (freshly prepared)
- 4) Folin's reagent
Mix 5 ml of Folin ciocalteau phenol reagent and 5 ml d/w.
- 5) 0.1 N NaOH:
Weigh 0.4 g of NaOH pellets in 100 ml d/w.
- 6) 1 N NaOH
Weigh 40 g of NaOH in 1000 ml d/w

4. Carbohydrate:

Reagents:

- 1) 5% phenol reagent:
Weigh 5 g phenol in 100 ml d/w.
- 2) 5% TCA (Trichloroacetic acid):
Weigh 5 g TCA in 100 ml d/w.
- 3) Sulphuric acid reagent:
Dissolve 2.5 g of hydrazine sulfate in 500 ml reagent grade concentrated sulphuric acid (sp. gr. 1.84)

5. Lipids:

Reagents:

- 1) 0.5% dichromate in conc. H_2SO_4 (to be prepared in volumetric flask):
Dissolve 0.75 g $\text{K}_2\text{Cr}_2\text{O}_4$ (AR) in 10 ml H_2O with heating. Cool the solution. Make the solution to 500 ml with conc. H_2SO_4 (sp. gr. 1.82) [Add acid to H_2O]
- 2) Organic solvent:
Mix 200 ml of CHCl_3 (chloroform AR), 400 ml (methanol) [CH_3OH], 160 ML d/w. [ratio 5:10:4]
- 3) Chloroform: Analytical grade chloroform CHCl_3

6. Sulfate estimation (turbidometry):

Acidify the sample to pH 1 with 4N HCl. Let it boil for 10 minutes. Place the beaker in a water bath at 90 °C (10-12 hours), cool, adjust the pH to 7 with concentrated NaOH and then 0.5 N NaOH. Make the volume to 150 ml. The sample is ready for measurement. In a beaker with magnetic piece add 25 ml of sample and 1.25 ml conditioning solution. Agitate continuously and add 2 ml barium chloride. Agitate continuously during the addition keeping speed constant (1 minute). Incubate and read at end of 10 minutes (wave length 365 nm, glass cuvette)

Conditioner:

Dissolved 75 g NaCl in 300 ml d/w to which is added 100 ml of 95 % ethanol or isopropyl alcohol. To this add 30 ml concentrated HCl and 50 ml of glycerine. Filter the solution if turbid.

Barium Chloride solution ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ Loba Chemie, mol Wt. 244.28): 30%

Standard: 40 mg $(\text{NH}_4)_2\text{SO}_4$ in 1 liter DW. For estimation 10 ml of ammonium sulfate + 15 ml d/w (OD should be approximately 0.215- 0.220). Standard should be repeated every time.

7. Sulfide estimation:

1 ml of the sample is fixed in 10 ml of 2% Zinc acetate. To this add 5 ml DMPD, swirl once and quickly add 0.25 ml FAS. Shake and let stand for 10 minutes. Fill up volumetric flask to 50 ml (can be stored at this point for 2 days without loss of color intensity) make up the volume with d/w to 50 ml. Measure OD at 670 nm in a glass cuvette).

DMPD:

2 g dimethyl para-phenylene-diamine sulfate in 1 liter volumetric flask. Add 200 ml DW and then slowly add 200 ml concentrated H_2SO_4 (sp. wt. 83), place in ice box after cooling to room temperature fill p to 1 liter with d/w.

FAS:

50 g iron III ammonium sulfate, $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in a 500 ml volumetric flask add 10 ml concentrated H_2SO_4 and fill up to 500 ml with d/w.

Zinc acetate:

2% w/v zinc acetate in d/w add 1 drop of acetic acid per litre.

8. Chromous chloride preparation:

Immerse 100-200 g of zinc metal (granular 20-30 mesh size) in a solution of mercuric chloride (0.1 m in 1.0 m HCl) and mix gently. After metal acquires a bright metallic sheen, decant and save the mercuric chloride. Wash the zinc with tap water, transferring the first three rinses to containers for mercuric waste. Minimize the exposure of Zn to the atmosphere during these washes.

Prepare 2-3 litres of chromic chloride in a large Erlenmeyer flask, transfer the zinc to the second flask and add the chromic chloride (1 M in 1 M HCl). Stir until the solution turns deep blue from deep green. Do not seal the flask as it evolves hydrogen.

Decant the chromous chloride and store in sealed bottle. The solution remain stable for several weeks or until it appears blue-green.

9. Nutrient Agar (Himedia, Bombay)

Peptone	5.0 g
Beef extract	1.5 g
Yeast extract	1.5 g
Agar	15 g
Sea water (over laying)	1000 ml
pH	7.4

10. Shake agar method:

SRB were enumerated on modified Hatchikian's medium (Hatchikian 1972; Loka Bharathi and Chandramohan, 1985), SRB were quantified using the agar shake technique (Pfenning et al. 1981). Here 14 ml screw-capped culture tubes containing 12 ml medium and inoculum were gently tilted to allow mixing and then allowed to set. A sterile mixture of paraffin wax and oil (2:1 v/v) was then poured on top to maintain anaerobiosis. SRB were enumerated after 10-15 of incubation at room temperature. The numbers are expressed as average of triplicate tubes.

Hatchikian's Media:

NH ₄ Cl	2.0 g
K ₂ HPO ₄	0.2 g

Yeast extract	1.0 g
Trace elements (SL4)	5 ml
Agar (sloppy media)	0.8%

Trace elements solution: 500 ml

ZnSO ₄ .7H ₂ O	10 mg
MnCl ₂ .4H ₂ O	3 mg
H ₃ BO ₃	30 mg
CoCl ₂ .6H ₂ O	20 mg
CuCl ₂ .2H ₂ O	1 mg
NiCl ₂ .6H ₂ O	2mg
Na ₂ MoO ₄ .2H ₂ O	3 mg
CaCl ₂ .2H ₂ O	20 mg
Autoclave 15 minutes at 120 °C	

Liquid media additions:

FeSO₄.7H₂O: 20 mg/ 50 ml distilled water acidified with 1 drop of H₂SO₄ (1 ml to be added to 200 ml media)

Na₂S.9H₂O: 50% stock made in 10 ml test tube (0.5 ml to be added to 200 ml of media)

Solid media additions:

FeSO ₄	10% solution	1 ml/ 200 ml
Sodium thioglycolate	0.6 g/10 ml	2 ml/ 200 ml
0.5 N NaOH		0.8ml/200 ml

Sealer:

Wax-paraffin	100 ml melted
Paraffin oil	200 ml
(Mix distribute autoclave)	

11. Calculation for carbon mineralized via sulfate reduction

Calculation

C org : SO_4^{2-} = 2.1 (Volkov et al, 1998).

$$\text{SRR (mM m}^{-2}\text{d}^{-1}) \times 2 = \text{C mM m}^{-2}\text{d}^{-1}$$

$$(\text{C mM m}^{-2}\text{d}^{-1} \times 12)/1000 = \text{g C m}^{-2}\text{d}^{-1}$$

$$\text{g C m}^{-2}\text{d}^{-1} \times \text{Area (Km}^2) = \text{Tons C d}^{-1}$$

$$\text{Tons C d}^{-1} \times 365 = \text{Tons C y}^{-1}$$

SRR = Integrated sulfate reduction rates in upper 10 cm Sediment

mM = milli moles

C = carbon mineralized via sulfate reduction

G = gram

m^{-2} = meter square

d^{-1} = per day

y^{-1} = per year

eg. $\text{SRR} = 39.54 \text{ mM m}^{-2} \text{ d}^{-1}$

$$\text{Area} = 2.18 \text{ Km}^2$$

$$\text{Carbon mineralized} = 2 \times 39.54 = 79.08 \text{ C mM m}^{-2} \text{ d}^{-1}$$

$$79.08 \text{ C mM m}^{-2} \text{ d}^{-1} = 0.95 \text{ g C m}^{-2} \text{ d}^{-1}$$

$$0.95 \text{ g C m}^{-2} \text{ d}^{-1} \times 2.18 \text{ Km}^2 = 2.07 \text{ Tons C d}^{-1}$$

$$= 755 \text{ Tons C y}^{-1}$$

Annexure II

Seasonal Assessment of Heavy Metal Pollution in Tropical Mangrove Sediments (Goa, India)

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Article Info

Article History

Received : 29-05-2011
Revised : 25-07-2011
Accepted : 25-07-2011

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Abstract

Mangrove swamps along the Mandovi estuary, Goa are exposed to an influx of metal effluents from the ferromanganese mining activities. The present study was carried out to assess the seasonal concentrations of metals in the sediments of Divar, an anthropogenically-influenced mangrove swamp in the Mandovi estuary, and compared to Tuvem along the Chapora River, a relatively pristine mangrove site. In both the sites, the average heavy metal concentration in sediments decreased in the order: Fe > Mn > Zn > Cu > Co > Pb > Cr and showed a marked seasonal variability ($p < 0.001$; $df=2$). However, the Pollution Load Index (PLI) for Divar sediments was far greater (1.65-2.19) than that of Tuvem (0.91-1.3) reflecting the intensity of anthropogenic inputs into the ecosystem. Further, Muller geochemical index values for Divar sediments indicated that during pre and post-monsoon season, the sediments were moderately contaminated with Fe whereas at Tuvem, the sediments were below contamination levels. The comparison with Screening Quick Reference Table (SQuiRT) also revealed the poor sediment quality for Divar. The transport of ferromanganese ore along the Mandovi River could be a major source of the entry of heavy metals in this riverine system. The Effect Range- Low (ER-L) values for these elements exceeded the reference values suggesting a potential eco-toxicological risk to the benthic organisms and a possible transfer to higher trophic levels.

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Key Words: Metals, Pollution, Mangroves, Sediments, Mining

Introduction

With increase in urbanization and industrialization, many coastal regions have been subjected to considerable environmental stress [1, 2]. Nearshore marine ecosystems are especially prone to anthropogenic inputs due to their proximity to the coast. Sediments are important carriers of trace metals in the hydrological cycle and because metals are partitioned with the surrounding waters, they reflect the quality of an aquatic system. In tropical zones, the most dominant intertidal areas comprise of the mangrove fringes [3]. Out of the ~20 million hectares, Asia harbors the largest area of mangrove vegetation which includes 3% of the global mangrove forest in India [4, 5]. Mangroves are often exposed to heavy metal pollution due to a variety of factors ranging from processing and post development of metal ores, shipping, sewage and storm-water discharge [6]. Marine suspended matter with an elevated metal load, gets actively trapped within mangrove sediments [7].

Goa is richly endowed with industrial minerals like iron ore, manganese ore, bauxite, lime stone and dolomite etc. The commonly used practice of 'open cast' mining creates up-to three tons of waste for each ton of ore produced. This waste pollutes rivers and lakes, many of which run red with ore. The estuarine channel of the Mandovi river is crucial for the economy of the state since it is used to transport large quantities of ore to the Marmagao harbour. Lush mangrove vegetation fringes this estuarine system. The mining activity upstream in the watershed may influence the biological and geochemical conditions of these water bodies. Due to the bio-

accumulation potential and metal toxicity the persistence and cycling of heavy metals is of a serious concern in mangrove environments [7, 8, 9, 10]. Currently, studies assessing the potential problems related to heavy metal accumulation from the Indian mangroves regions are limited [11, 12, 13, 14]. Although the impacts of iron-ore processing on the surface sediments of the Mandovi estuary have been documented [15], their influence on the surrounding mangrove ecosystem is sparsely addressed. In the present study, we compare the seasonal variation in the concentration of heavy metals viz., Fe, Co, Cu, Cr, Mn, Pb and Zn in surficial sediments of two mangrove ecosystems of Goa viz. the relatively pristine site "Tuvem" and the anthropogenically-influenced site of "Divar". We have also evaluated the intensity of heavy metal pollution in the sediments through various indices, and we hypothesize that the mining activity adjoining the Mandovi estuary is the main source and cause of heavy metal pollution in these mangrove swamps. The measurement of the seasonal variation in trace metal concentrations and distribution in the sediments would give us a better understanding of the inputs of the accumulated metals in the mangrove ecosystem and thus the quality of local coastal environment.

Materials and Methods

Study area and sampling

The study included two mangrove forests located at Tuvem and Divar along the Chapora and Mandovi rivers in Goa respectively, located on the west coast of India (Fig. 1).

The site at Tuvem (15°39'94" N and 73°47'65" E) is set amidst coconut (*Cocos nucifera* L.), cashew (*Anacardium occidentale* L.), and banana (*Musa* L.) plantations and is comparatively less influenced by anthropogenic activities. The Divar mangrove ecosystem (15°30'35" N and 73°52'63" E) is separated from the mainland by the river Mandovi. *Rhizophora* sp., *Sonneratia* sp., *Avicennia* sp. and *Excoecaria* sp. are some of the dominant mangrove genera found along the Mandovi and Chapora estuaries. Iron ore beneficiation plants situated on the riverbanks carries out treatment and up-gradation of low grade ore. These plants use river water to wash the iron ore and in-turn discharge metal effluents directly into the aquatic system. Sediment samples (n=5) were collected during the low tide using PVC hand-held corers in the month of April (pre-monsoon), July (monsoon) and December (post-monsoon) of 2008. Upon collection, the cores were sealed at both the ends with sterile core caps and transported to the lab in an ice box for further analysis.

Physico-chemical and particle size analysis

Hydrogen ion concentration (pH) of the surface sediment was measured instantly upon sample arrival at the laboratory using a portable pH meter (Thermo Orion model 420A) following the manufacturer's instructions. Total organic carbon (TOC) was estimated using titrimetric wet oxidation method as described by Allen et al., [16]. The sediments used to estimate organic carbon and nitrogen content were dried at 60(±2)°C for 48 h. The samples were de-carbonated with HCl fumes and analyzed using an Elemental Analyzer (Thermo Finningan, Flash EA1112) with L-Cystine as standard. The precision of analysis was checked against NIST 1941b.

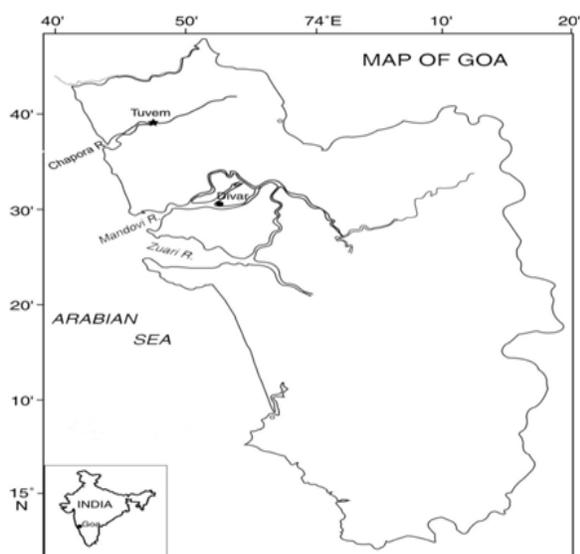


Fig. 1. Location of sampling sites along the Chapora and Mandovi rivers

Sub-samples for metal analysis were dried at 60(±2) °C for 48 h and disaggregated in an agate mortar before chemical treatment for Fe, Cu, Co, Cr, Mn, Pb and Zn following sediment digestion methods as described by Balaram et al., [17]. Briefly, a known quantity (0.2 g) of sediment was digested

in a Teflon vessel with a solution (10 ml) of concentrated HF, HNO₃, and HClO₄ (Merck,) in the ratio 7:3:1. The sediment was then dried on a hot plate in a fume hood chamber at 70°C for 4-6 h. The procedure was repeated with 5 ml of acid mixture. Further 2 ml of concentrated HCl was added followed by 10 ml of HNO₃. The residue was warmed and transferred to a clean, dry standard flask to make a final volume of 50 ml with double distilled water. The concentration of the metals were analyzed with an atomic absorption spectrophotometer (AAS; GBC 932 AA model) equipped with deuterium background corrections. Blank corrections were applied wherever necessary and the accuracy was tested using standard reference material MAG-1 (United Geological Survey) and GR-1 (Green River sediment). The particle size analysis was carried out by the wet sieving method for sand and the pipette method for silt and clay as reported by Day [18] and Carver [19].

Sediment quality assessment

To estimate the possible environmental consequences of metal pollution, our results were compared with Sediments Quality Values (SQV) using National Oceanic and Atmospheric Administration (NOAA) Screening Quick Reference Tables (SQiRTs) [20]. Concentration factor and Pollution Load Index (PLI) was used as described by [21].

The geoaccumulation index (I_{geo}) of Muller, [22] was used to determine the intensity of metal pollution. The index can be expressed as:

$$I_{geo} = \log_2 (C_n/1.5B_n)$$

Where, C_n = measured concentration of metal 'n' in the sediment

B_n = the background values for the metal 'n'.

The Factor 1.5 is a value intended to offset potential oscillations in background data resulting from lithological variations.

Statistical analyses

Significant variability in metal content was analyzed using two-factor analysis of variance (ANOVA) without replication in Analysis tool pack (*Microsoft Excel*). Pearson's correlation coefficients were used to assess inter-relationship between the abiotic parameters.

Results and Discussion

Sediment characteristics

The seasonal variation in environmental parameters at both the sampling locations has been presented in Table 1. The pH at both the sampling locations ranged between 6.82±0.08 to 6.98±0.13. Mangrove ecosystems have an active and continuous degradation of tree litter subsequently the hydrolysis of tannins release various acids [23] and/or the oxidation of sulfide pyrite which release the dissolved ferrous iron [24] is known to be responsible for a shift towards more acidic conditions. The present study reveals a high organic carbon content in the mangrove sediments. Thus, its degradation resulting in low pH is expected [23]. Grain size analysis in the mangrove sediments was clayey in nature during pre- and post-monsoon. However, there was a shift to sand dominated sediments during the monsoons which (up to 57.3±3.2% at Divar) could be attributed to the pre-dominance of terrestrial over tidal sediments [25].

Seasonal variation in heavy metal at Tuvem and Divar is depicted in Table 2. Seasonal fluctuation in concentration of

metals was highly significant ($p < 0.001$; $df=2$) at both the sites. During the pre-monsoon, the maximum concentration of Fe at Tuvem was 7.1% while at Divar it was almost 5 times higher at 32.3%. The concentration of Fe observed at Divar was higher than the values reported by Nair et al., [26] in the Ashtamudi estuary (0.11 to 0.39%) and Thomas et al., [11], along the Kerala coast (Table 3). A study by Alagarsamy [15] has shown that the concentration of Fe in the Mandovi estuary varies from 2.2 to 49.7%. An estuarine station in proximity to our study area (Divar) has reported maximum values of Fe to be approximately 12%. These high values of Fe in the mangrove sediment of Divar could be attributed to the precipitation of the respective metal sulfide compounds in anaerobic sediments [27]. These sulfides form a major sink for the heavy metals. Similarly, Mn concentrations at Divar were also higher as compared to Tuvem at maximum concentrations of 0.28%. These high concentrations of Mn and Fe at Divar could be explained by the the strong association of the geochemical matrix between the two elements. This association is not unusual and has been previously recognized by several authors [28, 29].

Concentration of Pb varied from 11.4 to 28.1 $\mu\text{g g}^{-1}$ at Tuvem and 7.9 to 50.5 $\mu\text{g g}^{-1}$ at Divar. As observed in Table 2, the present values were comparatively high at Divar during pre-monsoon and post-monsoon season which might be ascribed to river-borne sources [30], ore mining [15] and

agriculture practices in the basin [31]. Lead(Pb) concentration in clean coastal sediments is around 25 $\mu\text{g g}^{-1}$ or less [32] and average Pb values in Indian River sediments is about 14 $\mu\text{g g}^{-1}$ [33]. However the present values are below the USEPA (1996) prescribed maximum values of 90 $\mu\text{g g}^{-1}$ for non-polluted sediments [34]. The values observed in the present study, are also lower in comparison to the values reported in Kerala mangroves [11] and Tamil Nadu mangrove sediments [12] (Table 3). While the concentration of Cu, Co, Cr and Zn were 49.5 $\mu\text{g g}^{-1}$, 32.45 $\mu\text{g g}^{-1}$, 28.25 $\mu\text{g g}^{-1}$ and 64.25 $\mu\text{g g}^{-1}$ respectively at Tuvem, and almost double these values were recorded at Divar. Our values are lower than those reported from Maharashtra, where lethal concentration (LC50) have been assessed by Chourpagar and Kulkarni [35] for *Barytelphusa cunicularis*. Intensive anthropogenic activities such as mining in the upstream of Mandovi estuary, ferry services, sewage drainages from the mainland and other commercial activities are likely to be potential sources for the enrichment of these metals at Divar. Increased levels of metals like copper are known to accompany sewage sludge [36]. The enrichment of copper and cobalt in the mangrove sediments of Divar and Tuvem may be due to association with land-derived input of organic matter. During the monsoon season at Divar, most of the metal concentrations were lower, as compared to the non monsoon seasons, due to the restriction of ore transportation in the monsoon season.

Table 1: Seasonal variation in physico-chemical parameters at Tuvem and Divar sediments

	Tuvem			Divar		
	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
pH	6.82±0.08	6.96±0.11	6.98±0.13	6.84±0.17	6.98±0.08	6.88±0.08
TOC	4.09±0.74	1.40±0.29	3.38±0.43	2.29±0.76	3.33±0.80	1.29±0.27
C:N	9.22±0.4	11.07±2.24	11.81±1.03	2.7±1.9	14.6±0.4	6.01±1.2
Sand	4.41±0.98	39.18±5.12	6.36±1.80	31.7±14.1	57.3±3.2	21.5±2.8
Silt	27.5±3.01	21.5±5.10	22.2±2.52	5.41±2.32	26.05±2.72	21.5±2.8
Clay	68.1±2.18	39.3±1.01	71.5±2.22	62.8±14.6	16.8±5.1	57±3.37

Total organic carbon (TOC), and grain size content have been expressed as %.

Table 2: Seasonal variation in concentrations of heavy metals (\pm SD) at Tuvem and Divar

	Tuvem			Divar		
	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
Fe	6.93±0.34	6.93±0.22	4.41±0.42	29.85±2.51	8.10±1.43	16.90±1.69
Mn	0.11±0.007	0.18±0.003	0.097±0.01	0.25±0.03	0.15±0.04	0.16±0.009
Cu	32.75±3.84	39.54±4.65	46.35±2.23	34.95±3.93	78.30±10.15	40.15±5.98
Zn	43.65±5.35	49.44±10.38	36.93±6.21	91.25±3.61	101.2±17.12	114.65±8.65
Cr	24.43±14.10	18.85±4.21	5.1±2.10	27.65±6.0	17.55±2.30	10.35±2.75
Co	27.86±3.47	21.90±2.60	27.57±3.88	39.31±5.01	56.44±5.41	33.12±2.35
Pb	13.18±3.61	24.03±5.38	18.74±4.37	36.59±11.50	12.87±3.71	29.65±4.84

Except for Fe & Mn, values for total metal content have been expressed as $\mu\text{g g}^{-1}$ (n=5 at each sampling).

Table 3: A comparison of heavy metals ($\mu\text{g g}^{-1}$) reported from mangrove ecosystems in India

Location	Fe (%)	Mn (%)	Cu	Co	Cr	Pb	Zn	Reference
Kerala	4.7-12.1	0.032-0.11	652-845	159-261		1800-1950	1550-2372	[11]
Tamil Nadu	0.45-0.47	0.04-0.33	34-58	21-44	1.45-2.7	16-95		[12]
Bay of Bengal	0.18-2.69	0.02-0.06	7-44	6-14	24-111	9-28	44-163	[37]
Tuvem,Goa	3.9-7.4	0.09-0.17	27.3-49.5	18.4-33.1	3-28.3	11.4-28.1	29.5-64.3	Present study
Divar,Goa	6.5-32.3	0.15-0.28	31.8-94.3	31-63.7	7.8-36.8	7.9-50.5	79.5-123.3	Present study

Fe and Mn have been expressed as %.

Table 4: Screening quick reference table (SQiRT) for metals in marine sediments (Buchman, 1999).

Elements	Background	Threshold effect level (TEL)	Effect range low (ERL)	Probable effect level (PEL)	Effect range medium (ERM)	Apparent effect threshold (AET)
Fe (%)		-	-	-	-	22 (Neanthes)
Mn		-	-	-	-	0.026 (Neanthes)
Cu	10.0–25.0	18.7	34	108	270	390 (Microtox and oyster larvey)
Zn	7.0–38.0	124	150	271	410	410 (Infaunal community)
Cr	7.0–13.0	52.3	81	160	370	62 (Neanthes)
Co		-	-	-	-	10 (Neanthes)
Pb	4.0–17.0	30.2	46.7	112	218	400

Except for Fe and Mn which have been expressed as %, concentration of other metals is in $\mu\text{g g}^{-1}$; Threshold effect level (TEL) = Maximum concentration at which no toxic effects are observed; Effects range low (ERL) = 10th percentile values in effects or toxicity may begin to be observed in sensitive species; Probable effects level (PEL) = Lower limit of concentrations at which toxic effects are observed; Effects range median (ERM) = 50th percentile value in effects; Apparent effects threshold (AET) = Concentration above which adverse biological impacts are observed

Comparison with SQiRT

According to NOAA SQiRT (Table 4), Mn and Co concentration were above the AET while Fe was below the AET at Tuvem for all three seasons. High Mn, Co and Fe indicate their possible toxicity which may impart an adverse effect on the biota [20]. Zinc and Cr were below TEL for all three season (Table 2) at this location. At Divar, though Cu was below ERL, the toxic effects due to high Fe, Mn and Co concentration were evident as they exceeded the AET throughout the study period.

Concentration factor (CF) and Pollution Load Index (PLI)

At Tuvem, the concentration factor for elements such as Fe, Mn, Co and Cu were high (Table 5). The observed high values can be ascribed to the influence of external source

(agricultural, sewage runoff, intense fishing or recreational boating activities). Based on an annual average, the CF values were found to fall in the following sequence:

$$\text{Co} > \text{Mn} > \text{Cu} > \text{Fe} > \text{Pb} > \text{Zn} > \text{Cr}$$

At Divar, except for Cr, CF values for most of elements analyzed were high (>1) for all the three seasons. However in Tuvem, Co showed higher CF values based on an annual average:

$$\text{Co} > \text{Fe} > \text{Mn} > \text{Cu} > \text{Zn} > \text{Pb} > \text{Cr}$$

According to the PLI, lower values imply no appreciable input from anthropogenic sources [38]. The present study showed that the values of PLI were 0.91 to 1.29 at Tuvem and 1.65 to 2.19 at Divar (Fig. 2) indicating that the anthropogenic input at Divar is far greater as compared to Tuvem.

Table 5: Seasonal variation in concentration factor of Fe, Mn, Co, Zn, Cu, Cr and Pb at Tuvem and Divar

	Fe	Mn	Co	Zn	Cu	Cr	Pb
Tuvem (Chapora river)							
Pre-monsoon	1.39	1.77	2.79	0.61	1.31	0.70	0.66
Monsoon	1.39	2.85	2.19	0.70	1.58	0.54	1.20
Post-monsoon	0.88	1.62	2.76	0.52	1.85	0.15	0.94
Annual average	1.22	2.08	2.58	0.61	1.58	0.46	0.93
Divar (Mandovi estuary)							
Pre-monsoon	5.97	4.01	3.93	1.29	1.40	0.79	1.83
Monsoon	1.62	2.56	5.64	1.43	3.13	0.50	0.64
Post-monsoon	3.38	2.69	3.30	1.61	1.61	0.30	1.48
Annual average	3.66	3.09	4.29	1.44	2.05	0.53	1.32

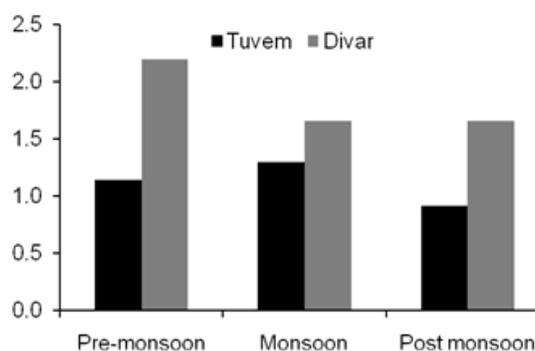


Fig. 2. Seasonal variation in Pollution Load Index at Tuvem and Divar.

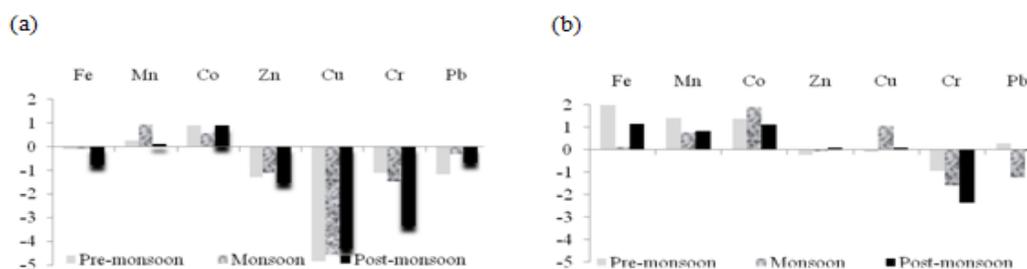


Fig. 3 . Geo-accumulation index for metals at Tuvem (a) and Divar (b).

Geoaccumulation index

The geoaccumulation index (I_{geo}) has been widely used as a measure of pollution in fresh water [39, 40] and marine sediments [32, 41]. Geo-accumulation index of different metals in Tuvem and Divar sediments is shown in Figs. 3a & b respectively. Crustal average values were taken as the baseline [42]. According to the I_{geo} classification, it could be inferred that at Tuvem, Co and Mn were among “uncontaminated to moderately contaminated” category, while other metals fell in the uncontaminated group for all the seasons.

At Divar, the sediments were moderately contaminated by Fe, Mn and Co during pre-monsoon while Zn, Cu, Cr and Pb were below contamination levels. During monsoon, moderate contamination from Co and Cu was observed. Whereas in the post-monsoon, moderate contamination of Co was persistent along with Fe while, all the other metals showed no contamination to the benthic environment. As the concentration of metals increased, the eco-toxicological risk also increased for the benthic organism and there is a possible transfer to higher trophic levels [20]. Vidya and Chandrasekaran [43] have shown that heavy metal concentration increase through various levels of food chain.

Inter-elemental and physico-chemical parameter relationship

In order to study the inter-elemental associations, the correlation coefficient of the elements were analyzed for Tuvem

and Divar. The analysis was carried out to understand the behavior of the metals during the transport to the mangrove ecosystem and to find out the source of origin of the metals. A positive correlation ($r=0.56$, $p<0.01$, $n=15$) was observed to exist between Fe and Mn at Tuvem (Table 6a), probably due to the strong association within the geochemical matrix of the two elements. The weak association of Mn with metals like Co, Zn and Cu suggest that Mn oxide may only be a minor host phase [15] for these elements in mangrove sediments of Divar. The variation in Fe was responsible for about 68% variation in the concentration of Mn ($r=0.82$, $p<0.001$, $n=15$; Table 6b). Fe-Mn mining upstream the Mandovi estuary, could be attributed to be a major source for the abundance of these elements in the Divar mangrove swamps. Fe also exhibited a positive correlation with Pb ($r=0.77$, $p<0.001$) and Cr ($r=0.53$, $p<0.001$) suggesting the adsorption of these elements by amorphous Fe-oxyhydroxide. Here, a significant correlation of C: N ratio with Cu ($r=0.93$, $p<0.001$), Co ($r=0.76$, $p<0.001$) and TOC ($r=0.60$, $p<0.01$) was also observed (Table 6b). In general, low C: N values of 5-7 are characteristic for marine organic matter [44] and values higher than 20 indicate a terrestrial source [45]. Except during the pre-monsoon season at Divar, C: N values recorded in the present study indicate considerable terrestrial influence at both the locations affirming the possible land-derived origin of these elements in the estuarine complex.

Table 6(a): Correlation matrix (r) for elements, organic carbon, sand, clay, silt, pH and C/N ratio at Tuvem (n=15). Significant r values have been highlighted in bold.

	Fe	Mn	Co	Zn	Cu	Cr	Pb	TOC	Sand	Clay	Silt	pH	C/N
Fe	1												
Mn	0.561	1											
Co	-0.246	-0.683	1										
Zn	0.521	0.541	-0.407	1									
Cu	-0.693	-0.080	-0.196	-0.008	1								
Cr	0.674	0.200	-0.048	0.070	-0.774	1							
Pb	0.091	0.559	-0.386	0.162	0.276	-0.052	1						
TOC	-0.226	-0.826	0.557	-0.321	-0.194	-0.150	-0.598	1					
Sand	0.429	0.946	-0.623	0.387	-0.013	0.169	0.600	-0.918	1				
Clay	-0.554	-0.988	0.672	-0.567	0.066	-0.190	-0.568	0.856	-0.967	1			
Silt	0.258	-0.224	0.074	0.459	-0.174	0.006	-0.342	0.568	-0.502	0.265	1		
pH	-0.343	0.164	-0.119	0.279	0.521	-0.458	0.227	-0.214	0.215	-0.182	-0.197	1	
C/N	-0.268	0.326	-0.288	0.461	0.711	-0.501	0.588	-0.387	0.289	-0.326	0.014	0.710	1

Table 6(b): Correlation matrix (r) for elements, organic carbon, sand, clay, silt, pH and C/N ratio at Divar (n=15). Significant r values have been highlighted in bold.

	Fe	Mn	Co	Zn	Cu	Cr	Pb	TOC	Sand	Clay	Silt	pH	C/N
Fe													
Mn	0.824												
Co	-0.540	-0.251											
Zn	-0.375	-0.393	-0.247										
Cu	-0.799	-0.435	0.844	0.035									
Cr	0.539	0.563	0.110	-0.614	-0.161								
Pb	0.779	0.545	-0.720	-0.169	-0.831	0.207							
TOC	-0.342	0.043	0.777	-0.298	0.785	0.361	-0.602						
Sand	-0.556	-0.290	0.869	-0.175	0.834	0.205	-0.816	0.856					
Clay	0.812	0.564	-0.841	-0.053	-0.921	0.109	0.892	-0.729	-0.925				
Silt	-0.938	-0.828	0.438	0.438	0.690	-0.639	-0.651	0.186	0.391	-0.712			
pH	-0.377	-0.017	0.355	0.029	0.524	-0.322	-0.116	0.385	0.199	-0.319	0.406		
C/N	-0.920	-0.677	0.768	0.153	0.936	-0.279	-0.843	0.605	0.783	-0.930	0.806	0.428	1

Conclusion

The impact and consequences of mining activities has caused extensive damage to the marine environment in Goa. The geo-accumulation index, contamination factor and PLI used to examine the intensity of metal pollution in the two tropical mangrove sediments in Goa, revealed high pollution in the anthropogenically-influenced site Divar as compared to the relatively pristine site of Tuvem. Mining activities in upstream locations of the adjoining Mandovi estuary, transportation of ferromanganese ore through the estuarine channel and sewage discharge are mostly accountable for the high pollution levels observed at Divar. Government and non-government organization including the local scientific communities need to be vigilant and initiate appropriate environmental pollution monitoring schemes to keep a check on the contamination of these indispensable mangrove ecosystems. These measures would help in minimizing heavy metal toxicity in these coastal estuarine ecosystems, especially the delicate and sensitive mangrove fringes.

Acknowledgements

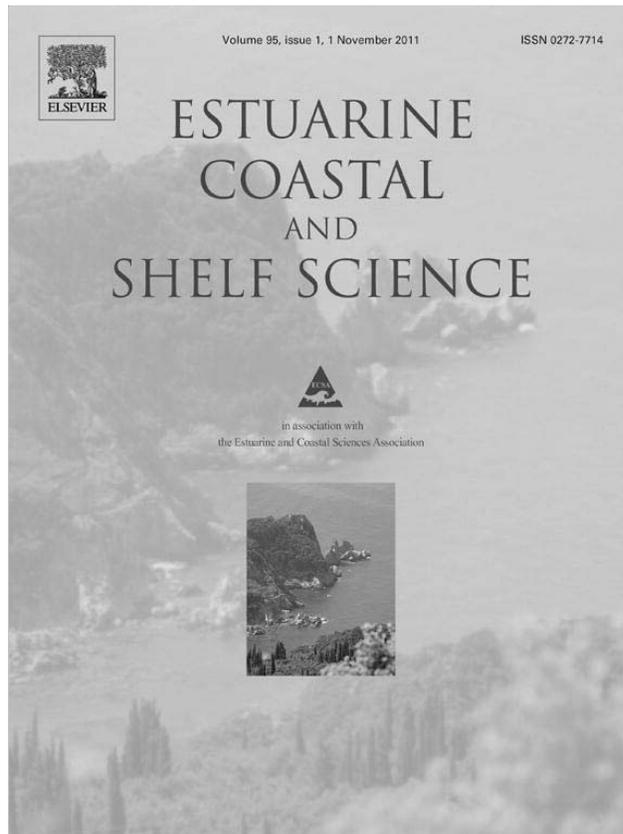
The authors express their gratitude to HRDG, CSIR, New Delhi for funding and the HOD, Department of Biotechnology, Goa University for the facilities. KA is greatly indebted to the Ministry of Earth Sciences, New Delhi for award of Junior Research Fellowship.

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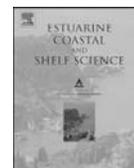
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Ambient iron concentration regulates the sulfate reducing activity in the mangrove swamps of Diwar, Goa, India

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ARTICLE INFO

Article history:

Received 25 November 2010

Accepted 21 August 2011

Available online 29 August 2011

Keywords:

iron
sulfate
organic matter
sulfate reduction
mangroves

ABSTRACT

In order to test the hypothesis that the ambient iron concentrations could regulate sulfate reducing activity (SRA) in mangrove areas, 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 coefficients between SRR and environmental parameters showed that 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 an r -value of -0.761 ($p < 0.001$, $n = 60$), suggesting that ca.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 supported our field observations. At an experimental manipulation of 50 ppm Fe^{3+} there was an increase in SRR but at 100 ppm an inhibitory effect was observed. At 1000 ppm Fe^{3+} there was a decrease in the SRR up to 93% of the 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.

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1. Introduction

Mangroves are one of the most productive ecosystems and occupy a large 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 20–40% 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, 1993). However, Kristensen et al. (1991)

have shown that sulfate reduction may account for up to 100% of total sediment metabolism (measured as CO_2 efflux) in mangrove sediments.

Several 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 Fe^{3+} has been reported by Lovley and Phillips (1987) and Van Bodegom et al. (2004). Recently, Krishnan and Loka Bharathi (2009) have shown that iron even modulates 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

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in terms of their biogeochemistry (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 geo-microbial processes in the mangrove sediments (Kristensen et al., 1992).

SRR has been occasionally quantified in tropical mangroves (Kristensen et al., 1988, 1992, 2000; Alongi et al., 2000, 2001, 2004) but seasonal monitoring is rarely reported. Although some cyclic studies have been outlined in temperate intertidal surface sediment (Al-Raei et al., 2009) and salt pans (Kerkar and Loka Bharathi, 2007), a 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) also state 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 the Tuvem and Diwar mangroves. The sampling site at Tuvem ($15^{\circ} 38.28'N$ and $73^{\circ} 47.71'E$) is on the Chapora estuary and that at Diwar ($15^{\circ} 30.42' N$, $73^{\circ} 52.28'E$) on the Mandovi estuary. The Diwar mangroves are separated from the mainland by the river Mandovi. The Mandovi river is heavily used for transportation of iron ore from mines located upstream. In addition, the iron ore extraction plants situated on the riverbank discharge effluents directly into the estuary. In contrast, Tuvem is free of such activity. Neither site is center of human habitation and agricultural practices (Fig. 1). Similar mangroves such as *Rhizophora* sp., *Sonneratia* sp., *Avicennia* sp. and *Excoecaria* sp. vegetate both the sites. Although the drainage is more at Diwar at 3580 million m^3 than at Tuvem at 588 million m^3 , the basin area is greater at Diwar at 1580 sq. km than at Tuvem at 255 sq. km, the ratio of basin area to drainage is almost equal at both the sites ~ 2.3 . (www.goaenvs.nic.in (ENVIS GOA)).

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 triplicate during the low tide using PVC hand-held corers of 15 cm length and 3.68 cm 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 h 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

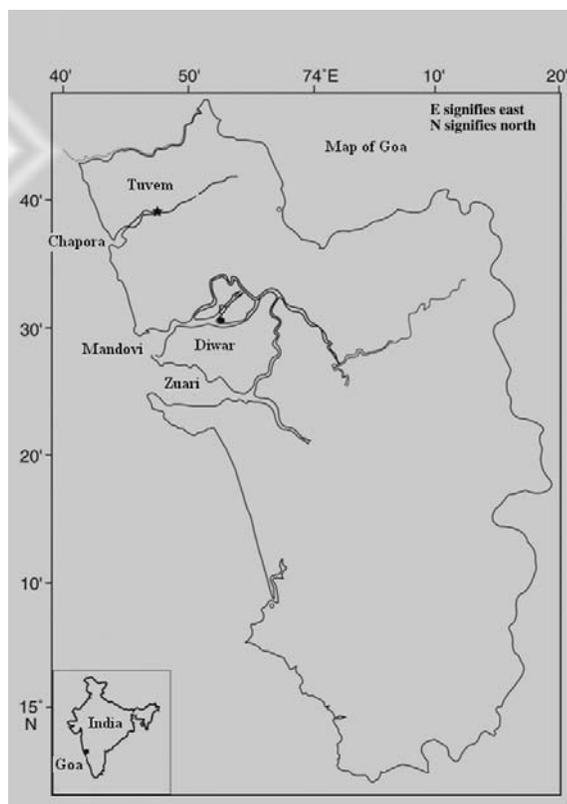


Fig. 1. Map with sampling locations.

manufacturer (Thermo Orion model 420A, USA). Another set was subsequently sectioned at 2 cm intervals for all analyses. 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): 0.5 gm of dried ($60^{\circ}C$) sediment in a 500 ml flask, 10 ml of 1N $K_2Cr_2O_7$ and 20 ml of acid mixture (2.5 gm of Ag_2SO_4 in Conc. H_2SO_4) were added and shaken for 1 min and allowed to stand for 30 min. About 200 ml distilled water, 10 ml of 85% H_3PO_4 , 0.2 gm of NAF and 0.5 ml diphenylamine indicator were added and titrated with Mohr's salt until the end point with glucose as standard.

The moisture content was estimated as loss in weight of wet sediment after drying at $80^{\circ}C$ for 24 h. The dried sediment was used to determine the C:N ratio with a CHN analyzer (Thermo Finningan, Flash EA1112). Subsamples for metal analysis were dried at $60^{\circ}C(\pm 2)$ for 48 h 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 which, for each sample, a known quantity (0.2 g) of sediment was digested in a Teflon vessel with 10 ml of concentrated HF, HNO_3 , and $HClO_4$ in a ratio of 7:3:1. The digested sediment was then dried and the procedure was repeated with 5 ml 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).

2.3. Pore water analysis: sulfide, sulfate and salinity

Sediment from the cores were extruded at 2 cm intervals and centrifuged at 5000 rpm for 10 min at 4 °C for collecting the 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 (0.5 ml) were fixed in a vial containing concentrated HCl (0.01 ml) and the parameter was determined turbidometrically by BaSO₄ precipitation (Clesceri 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 (2 ml) 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, 74 KBq from BARC, Mumbai) was injected into the section to distribute the label evenly and the activity was arrested at the end of 8 h by adding 5 ml (5% wt/vol) zinc acetate and frozen at –20 °C till further analysis. Radiotracer assay in the sediment was carried out using a single step chromium reduction assay (King, 2001). Radioactivity was measured using a liquid scintillation counter (Perkin Elmer Wallace 1409 DSA) and sulfate reduction rates (SRR) were calculated using the equation:

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

Where SRR = Sulfate reduction rates, H₂³⁵S = radioactivity of reduced sulfur in DPM, ³⁵SO₄²⁻ = radioactivity of sulfate at the beginning of incubation, ³²SO₄²⁻ = pore water sulfate concentration in μM SO₄²⁻ cm⁻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 2 ml of this aliquots sediment were put into 5 ml vials in triplicate and then amended with 2 ml 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 h and supplemented with 10 μl of radioactive sodium sulfate (³⁵SO₄²⁻, specific activity, 74 KBq) and incubated at room temperature for 8 h. 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 a 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. Maximum values of 219.6 ± 131 and 187.7 ± 117 mV were observed during the pre-monsoon season at Diwar and Tuvem respectively (Table 1). The upper sediment layer (0–2, 2–4 cm) was relatively oxic (Eh >145 mV) at both the sites. Minimum (–100 ± 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.

At Diwar, the sediment water content varied from 32 to 42% during the non-monsoon seasons and 50–54% during the monsoon season. However, at Tuvem the opposite was observed. The water content was high during the 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(±2.99) to 19.25(±2.99) and at Tuvem from 6.00(±1.41) to 16.50(±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

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.

7.38 mM with a maximum value 14.41 mM during pre-monsoon (Fig. 2). At both sites, seasonal variability in sulfate concentrations was 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, the 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).

3.3. Total organic carbon (TOC) of sediments

The lowest values of 2.5% (± 1.25) and 1.48% (± 0.73) were recorded during the monsoon season at Tuvem and post-monsoon season at Diwar respectively. The highest accumulation of TOC was observed at Tuvem during the pre-monsoon season at 3.64% (± 0.97) and in the monsoon season at Diwar with values of 3.28% (± 0.79) (Fig. 3). At both the sites, the 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 period, 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.

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 was significant (ANOVA, $F = 120.22, p < 0.001, df = 1$).

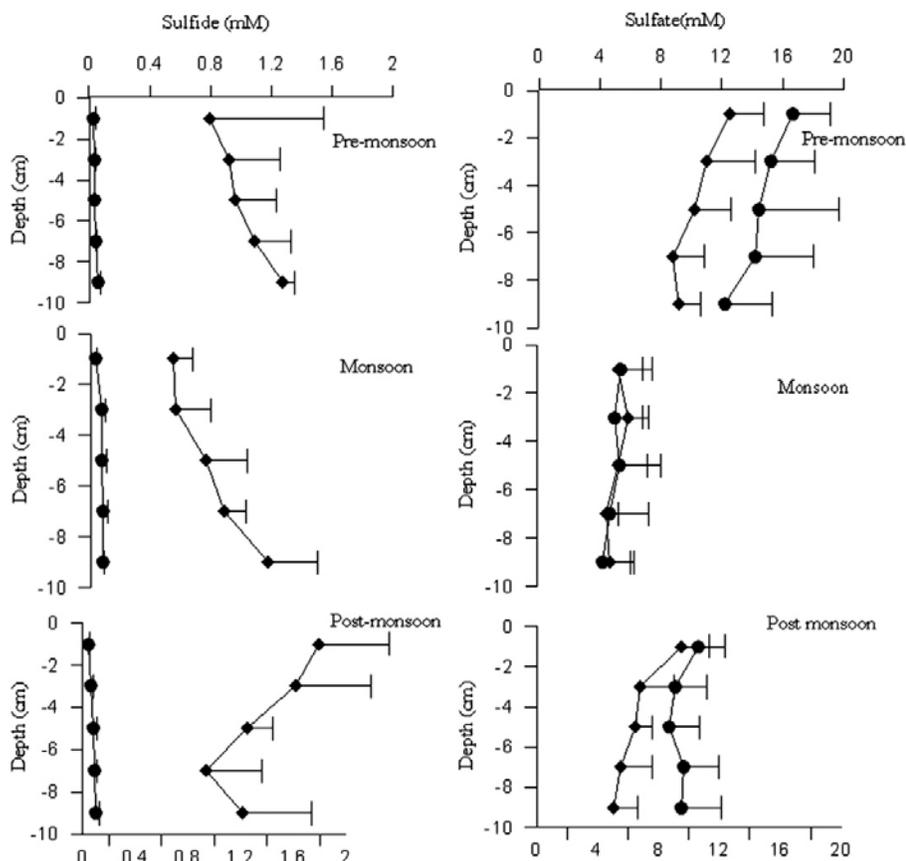


Fig. 2. Seasonal down-core variation in S^{2-} and SO_4^{2-} concentration. Diwar (○); Tuvem (□).

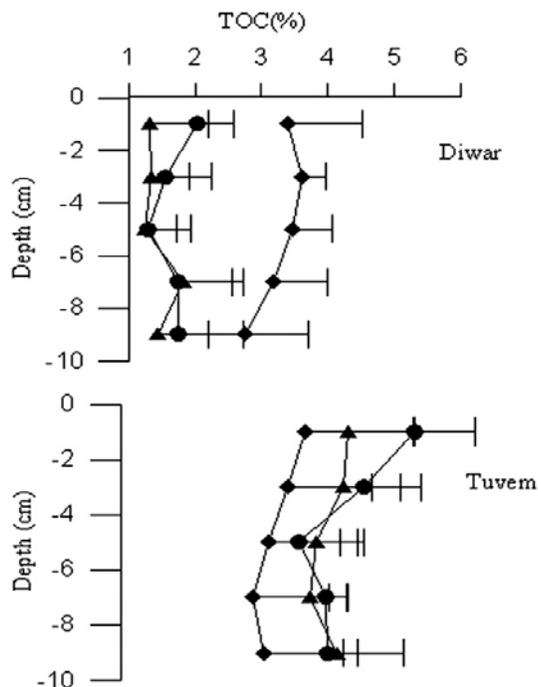


Fig. 3. Seasonal down-core variation in TOC (%). Pre-monsoon (\square); Monsoon (\square); Post-monsoon (\circ).

3.6. Sulfate reduction rate (SRR)

The 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, $F = 87.26$, $p < 0.001$, $df = 1$) and the 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 (ΣSRR , 0–10 cm) (Fig. 5) showed that at Diwar, the monsoon rates were ca.3 times higher than the pre-

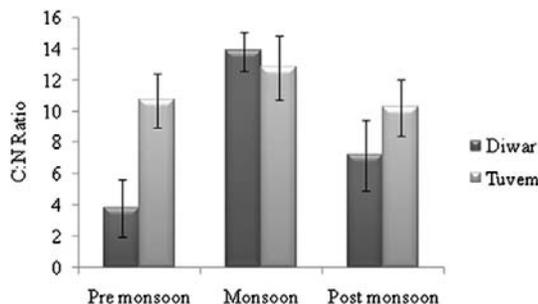


Fig. 4. Seasonal variation in C: N ratio at Diwar and Tuvem: Bars indicate the standard deviation.

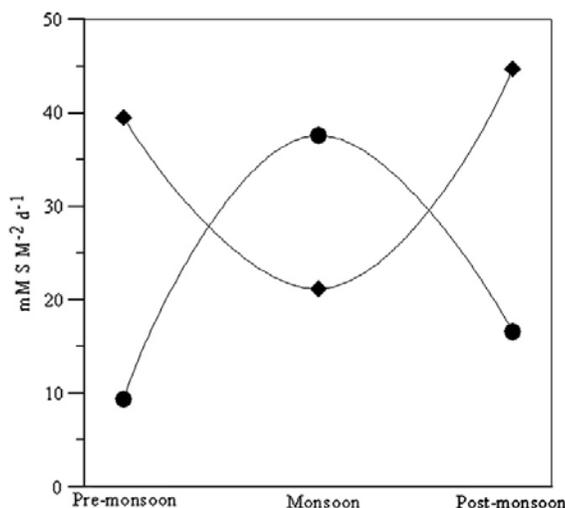


Fig. 5. Integrated sulfate reduction (ΣSRR) in upper 10 cm sediment core. Diwar (\square); Tuvem (\square).

monsoon period and double that at 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.

3.7. Laboratory experiments

3.7.1. 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%. A further increase in concentration led 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).

3.7.2. Interaction between microbial and environmental parameters

All variables have normal distributions 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 mangroves of Diwar and Tuvem in Goa range from 6.28 to $63.4 \text{ mM m}^{-2} \text{d}^{-1}$ which are comparable to SRR reported by Alongi et al. (2004) in the Malaysian mangroves and the range of

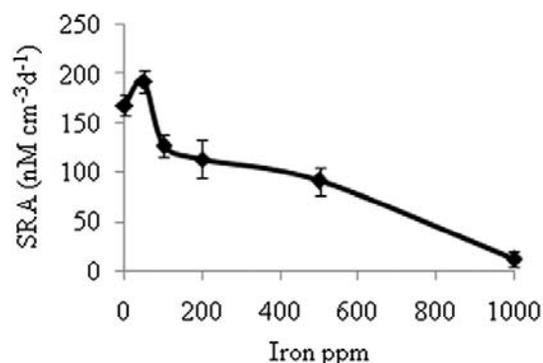


Fig. 6. Effect of increase in iron concentration on SRR in slurry experiments. Bar indicates the standard deviation.

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 (Schubert et al., 2000; Al-Raei et al., 2009). 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).

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. The water content of the sediments is also important as it helps transport 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 an 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. Although sulfate occurs at a concentration of 28 mM in the sea water and is not known to limit SRR in marine and estuarine sediments, it is apparent 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 co-variation with iron ($r = 0.867$, $p < 0.001$, $n = 60$)

(Fig. 7a) which 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). The 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 and McCormick (2006) also showed that an increase in sulfate concentration (>15 mM) in the coastal aquifer enhanced the SRR.

The higher concentration of sulfide in the pore water of Tuvem than Diwar indicates 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. By comparing our values with those measured by microelectrodes in salt marshes of Portugal by Sundby et al. (2005), their values ranged from non-detectable to 3 μm whereas 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 was 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. It is of note that at Diwar, a negative correlation between SRR and TOC ($r = -0.545$, $p < 0.01$, $n = 20$) was evident during the 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 the 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) whereas values >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, the high SRR of 216.14 nM cm⁻³ d⁻¹ during this

Table 2
Comparison of present sulfate reduction rates (SRR) to other coastal and estuarine ecosystems.

Locations	Sulfate reduction rates	Reference
Mangrove, Pakistan	2.5–16.1 mM m ⁻² d ⁻¹	Kristensen et al., 1992
Sediment Off Chile, Germany	40–430 nM cm ⁻³ d ⁻¹	Schubert et al., 2000
Mangrove, Pukhet, Thailand	5–140 nM cm ⁻³ d ⁻¹	Kristensen et al., 2000
Mangrove, Southern Thailand	0.6–16.9 mM m ⁻² d ⁻¹	Alongi et al., 2001
Mangrove, Vietnam	0.2–13 mM m ⁻² d ⁻¹	Alongi et al., 2001
Mangrove, Malaysia	19–53 mM m ⁻² d ⁻¹	Alongi et al., 2004
Mangrove, China	19–281 mM m ⁻² d ⁻¹	Alongi et al., 2005
Riband salt pan, Goa - India	0.002–66 mM m ⁻² d ⁻¹	Kerkar and Loka Bharathi, 2007
Salt marshes, Germany	0.9–106 mM m ⁻² d ⁻¹	Al-Raei et al., 2009
Intertidal mud flat, Korea	226–1516 nM cm ⁻³ d ⁻¹	Hyun et al., 2009
Present study	Sulfate reduction rates (nM cm ⁻³ d ⁻¹)	Integrated values (mM m ⁻² d ⁻¹)
Diwar		
Pre-monsoon	23.32–131.94	6.2–13.6
Monsoon	112.6–294.49	24.5–61.4
Post monsoon	79.22–147.31	14.8–33.4
Tuvem		
Pre-monsoon	62.55–698.66	15.5–63.4
Monsoon	50.21–338.14	10.43–34.0
Post monsoon	99.88–635.86	20.41–61.2

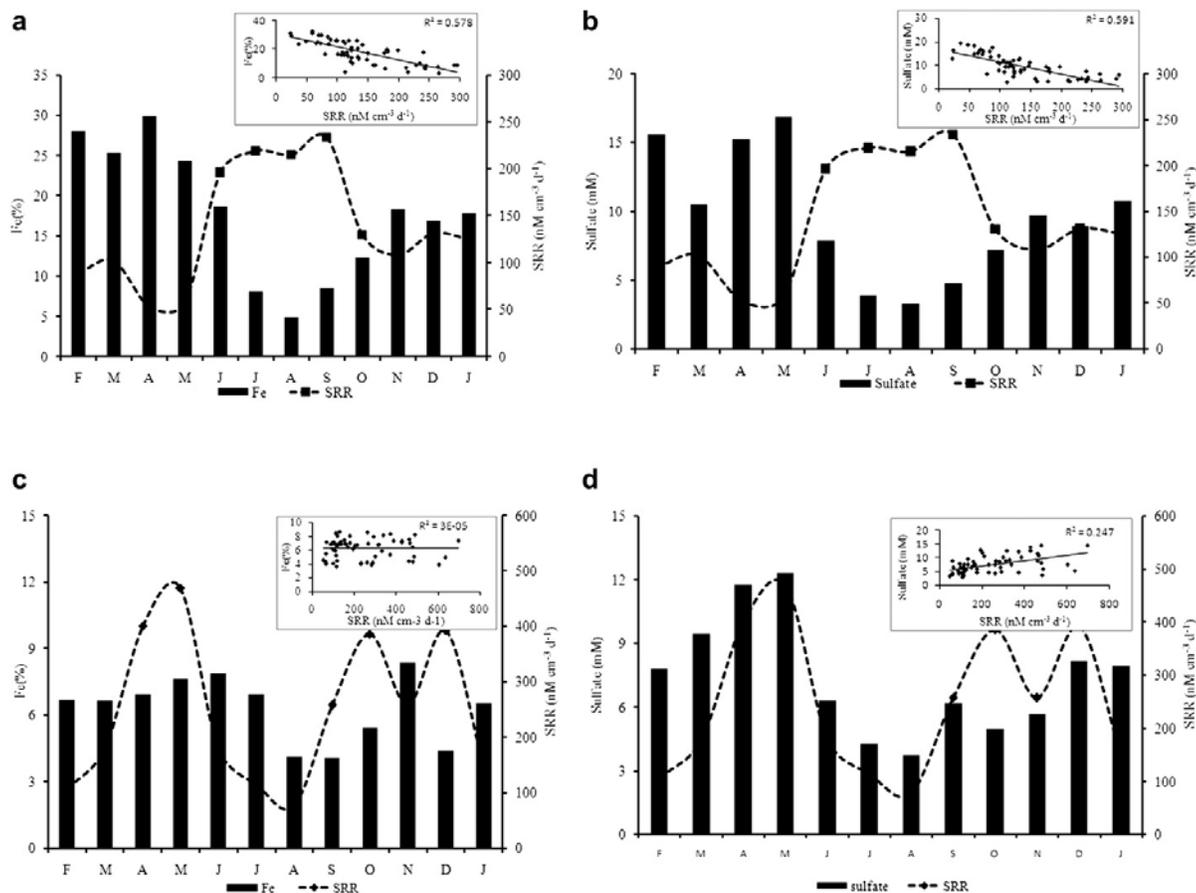


Fig. 7. SRR v/s Fe and SO_4^{2-} at Diwar (a & b) and Tuvem (c & d). [Monthly average values were represented since significant down core variability was not significant]. Inset show the extent of relatedness between the parameter.

season suggest that lability of organic matter plays a relatively small 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. The lower SRR despite the 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 10.08% during the 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 investigated 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 have 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 seagrass meadows demonstrated that iron addition suppresses the SRR at organic rich sites (Holmer et al., 2005, 2006). The suppressed sulfate reduction was due to a shift in bacterial metabolism to microbial iron reduction. The impact of Fe^{3+} addition in biofilms in sewers was put forth by Zhang et al. (2009), showing inhibitory activity upto 39–60% 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. The inhibitory effect of metallic ions on pure strains and mixed culture of SRB was also suggested 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).

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 the change in solid to solution ratio affecting substrate transport. A 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 this with 50 ppm of Fe^{+3} had a stimulatory effect (14%) on the SRR although increasing Fe^{+3} –100 ppm and above, reduced SRR. A 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 produces bisulfide (HS^-) that reacts with the dissolved metal and forms 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 such as 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 and Bartha, 1998).

Although the foregoing studies clearly indicate 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

We 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 show that iron inhibited SRR but the 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. Although 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 findings could have implications on the flux of greenhouse gases such as CO_2 from the mangrove sediments.

Acknowledgments

The authors are thankful to the HOD, Department of Biotechnology, Director NIO and HRDG, CSIR, New Delhi. C:N analyses were carried out at NIOT, Chennai. Attri K. is greatly indebted to the MoES New Delhi for the JRF award.

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