

Role of sulfur-oxidizing bacteria on the ecology in tropical mangrove sediments

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Abstract:

Mangroves are unique ecological niches for microorganisms which play major roles in biogeochemical cycles and in nutrient recycling. Sulfur compounds are primarily utilized by sulfur-oxidizing bacteria (SOB) and sulfate-reducing bacteria (SRB) which play an important role in mangrove metabolism. Besides, SOB oxidizes the toxic sulfide produced by SRB to inorganic sulfate utilized by mangroves and other microorganisms. Since the understanding of SOB role in mangrove ecosystem is scarce, the following questions are postulated. 1) Does the seasonal pattern influence abundance of SOB and sulfur-oxidation activity (SOA) in the mangrove sediments? 2) Which environmental variables support SOB and SOA? For this, sediments were sampled monthly for 13 months at Chorao, Goa, (southwest coast of India) for analysis of physicochemical, bacterial and sulfur related parameters. The average SOB population during pre-monsoon, monsoon and post-monsoon season was $1.99 \times 10^5 \text{g}^{-1}$, $2.0 \times 10^5 \text{g}^{-1}$ and $2.51 \times 10^5 \text{g}^{-1}$ respectively. The average SOA during pre-monsoon was 1.66mMd^{-1} , 0.92mMd^{-1} in monsoon and 2.09mMd^{-1} during post-monsoon season. Though the SOB did not have significant seasonal influence, SOA varied with season significantly. During the monsoon season SOB ($r^2=0.550$, $p<0.001$) and SOA ($r^2=0.467$, $p<0.001$) were influenced by total inorganic carbon. However, during the post-monsoon season SOA contributed to decrease in sulfide concentrations ($r^2=0.408$, $p<0.01$). Maximum carbon-fixation through SOA was in post-monsoon and least in pre-monsoon. Thus, SOB and SOA contribute by mineralization and detoxification to maintain homeostasis in the mangrove ecosystem.

Keywords: Mangrove ecosystem, environmental variables, carbon dioxide fixation, detoxification, sulfur-oxidizing bacteria, sulfur-oxidation activity,

Introduction

Mangroves dominate 75% of the tropical coastlines and are mainly found in the intertidal zones of estuaries between latitudes 25°N-25°S. Mangroves are the most productive ecosystems in the world and are of ecological and economic importance to the society (Alongi, 2002). Mangroves contribute towards soil formation and helps to stabilize coastlines, act as filters for upland runoff, and serve as habitat for many marine organisms and wildlife such as birds and reptiles and also produce large amounts of detritus that may contribute to productivity in offshore waters. One such ecosystem is along the Mandovi estuary, on the west coast of India, where the mangroves around the Chorao Island harbours a variety of mangrove species and also serves as a wildlife sanctuary (Sappal et al., 2014).

Mangrove ecosystems known to harbour a diverse microbial community that contributes to the bio-geochemical cycle and nutrient transformation by various mechanisms. These microbes participate in the degradation of complex molecules, ammonification, nitrification, denitrification, phosphate solubilization, mineralization of sulfur compounds as well as in the carbon flux and methane utilization (Abraham et al., 2004; Sherman et al., 1998; Holguin et al., 2001; Nedwell et al., 1994; Taketani et al., 2010). Microorganisms vary significantly in terms of the source, chemical form, and amount of essential elements they need (Charlson et al., 1992).

Among the bio-geochemical cycles, the sulfur cycle participates in various forms with different physicochemical properties and it is one of the major contributors for organic matter mineralization (Grinenko and Ivanov, 1983; Schroth et al., 2007). The key reaction in sulfur cycle is hydrogen sulfide that is released during sulfate reduction during the degradation of organic matter (Canfield et al., 1993). On the other hand hydrogen sulfide is re-oxidized and sulfate/sulfuric acid is produced during biological sulfur-oxidation by sulfur-oxidizing bacteria (Ivanov, 1983). Sulfide at a certain level can be detrimental to fishes (Tobler et al., 2006). Moreover, sulfide inhibits the respiration, oxygen release, nutrient uptake by plant roots and primary root growth (Bagarinao, 1992; Zhang et al., 2017). Sulfur-oxidizing bacteria play an important role in the detoxification of sulfide in water and sediment, coupling with nitrate reduction, and carbon dioxide fixation (Grote et al., 2012). Besides, they also have symbiotic relations with benthic organisms like those from the bivalve family *Lucinacea* (Liang et al., 2007). Moreover, microbial abundance and their activities in an ecosystem are influenced by different physical and chemical variables (Oller Costa et al., 2012) and different seasons (Liu et al., 2009). In view of the importance of SOB in maintaining ecosystem homeostasis, the present study aims at understanding the influence of environmental variables and the seasons on the abundance of SOB and sulfur-oxidation activity.

Materials and Methods:

Field studies

The investigation was carried out in tropical mangrove sediments in the Mandovi estuary along the west coast of India.

Site description

The sampling site was situated at 15°30'53"N, 73°51'27"E in the Mandovi estuary, Goa (Fig.1). The Mandovi-Zuari estuarine complex has approximately 12,000 ha of which the mangroves occupy 2000 ha in which 700 ha of mangrove region fringe the Mandovi estuary. Chorao Island (178 ha) is one of the best mangrove forests, comprising about 12 mangrove species and 8 associated species (Department of forest, Government of Goa). It also serves as a bird sanctuary and is rich in flora and fauna.

Sample collection and process

Sampling period

Monthly sampling was carried out at low tide from January 2013 to January 2014 in the Chorao mangroves swamps. The sampling covered three seasons, (n=20), i.e. Pre-monsoon (Feb-May; monsoon (June-September) and post-monsoon season (October-January).

Sample collection

Sediment cores up to 30 cms length were obtained, by using poly propylene core (~ 7 cm in diameter) and capped immediately at both ends. Physico-chemical parameters like temperature, pH, Eh, were recorded immediately by using IQ Scientific Waterproof ISFET pH/Ion Meter Kit (Cole-Parmer-99361-12) and sediment samples were transported to the laboratory for further analyses under cold conditions. Water from the sampling site was collected in sterile containers and used for media preparation for microbiological analyses and other laboratory experiments.

Analysis of physico-chemical parameters

A 21 cm long core was retrieved from the mangrove site of Mandovi estuary. On reaching the laboratory, the surface of the core was sectioned at 1 cm interval, while the next 4 sections were sub sampled at 5 cm interval. Each core section was further sectioned for analysis of labile organic matter, total inorganic carbon (TIC), grain size analysis, porosity, total sulfide, thiosulfate and sulfate concentration, and water sample for dissolved oxygen (DO).

Temperature, pH, Eh

The sediment core pipe used had holes of ~8 mm in diameter at 5 cm interval, in order to insert the probe. The outside of core was wrapped with tapes, which were removed during measurement of temperature, pH and Eh. Hydrogen ion concentration (pH) in the surficial sediments was measured using an Orion 4-Star Plus bench top pH/ISE meter (Thermo Fisher Scientific Inc., Waltham, MA). Sediment oxidation-reduction potential (Eh) was measured using an Orion platinum redox in combination with an Ag/AgCl₂ reference electrode (Thermo Fisher Scientific Inc.).

Total salinity

Salinity of overlying water was measured by using a hand held refractometer (model: S/Mill-E, ATAGO, Japan). The salinometer was calibrated according to the manufacturer's instructions by using distilled water. The pore water was collected by centrifugation procedure described by Edmunds and Bath, (1976). The samples were centrifuged at 1000 rpm for 15-30 minutes and the supernatant was used for analysis.

Total organic carbon and total organic matter

Total organic carbon was determined by wet oxidation method (El-Wakeel and Riley 1957) with a precision of 0.01%. Dried sediment was ground using a mortar and pestle, and sieved through a 200 µm mesh. About 0.3 grams sample from each sediment sections were taken and transferred to a conical flask. Organic matter present in sediment samples were oxidized by adding 25 ml of acid dichromate and the tubes were incubated at 60°C in a water bath for 1 hour. Then, 100 ml of distilled water was added and subsequently titrated against ferrous ammonium sulfate using diphenylamine as an indicator. A colour change from dark blue to green indicated the end point. A blank was run without sediment. The standard curve was plotted using glucose at varying concentrations.

Total organic matter (TOM) was calculated from the values of TOC, using a conversion factor of 1.724 following El Wakeel and Riley (1957).

Total inorganic carbon

The sediments were dried under 60°C and ground using mortar and pestle, and the samples were analyzed using UIC Coulometrics Inc. CM 5012 CO₂ coulometer coupled to a CM 5130 acidification module (Huffman, 1977; Engleman et al., 1985). For blank 10 ml of 2 % HCl was used, and for standard 12 mg of calcium carbonate (Merck) was used, 13 to 18 mg sample was used to detect TIC, and expressed as ppm or % of C.

Labile organic matter and labile organic carbon

The sum of total proteins, carbohydrates and lipids was expressed as labile organic matter (LOM). Carbohydrates (CHO), proteins (PRT) and lipids (LIP) were converted into carbon equivalents using conversion factors of 0.40, 0.49, and 0.70 for CHO, PRT, and LIP, respectively (Fichez, 1991; Fabiano and Danovaro, 1994). It's sum being the labile organic carbon (LOC). The nature and origin of the organic matter was estimated by the protein/carbohydrate ratio (Cauwet, 1978; Fichez, 1991).

Estimation of sulfide and sulfate

The cores were sectioned at particular intervals as mentioned before and sediments from different sections sampled, were centrifuged at 5000 rpm for 10 minutes at 4°C for collecting pore water. The supernatant was carefully drawn with a 5 ml syringe for the analysis of 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, 0.1g of sample was fixed in a vial containing concentrated HCl (0.01ml) and the parameter was determined turbidometrically by BaSO₄ precipitation (Clesceri et al., 1998).

Estimation of thiosulfate

Total thiosulfate was analyzed by method of Rodina (1972) Sediment samples (1.0g) from each section were pre-fixed with lead acetate, to remove organic matter which gives false positive result. The sediments were diluted with 10 ml of sterile sea water. Five ml of sample was taken and diluted with 95 ml distilled water to which 25 ml of diluted sulphuric acid and 12.5 ml of 0.0125N iodine was added and titrated immediately with standardized 0.0125 N thiosulfate until most of the iodine was consumed. A few drops of freshly prepared starch solution was added and titrated until the blue color just disappeared. Blanks were maintained and estimated as mentioned above. Thiosulfate (S₂O₃²⁻) was expressed as mM l⁻¹.

Sediment characteristics:

Porosity

Porosity, which is the measure of total pore spaces present within the sediment, is represented by the fraction of volume of void space over the total volume of the material. It is defined by the ratio: $\phi = V_v / V_T$, where, V_v is the volume of void space and V_T is the total bulk volume of the solution. In order to find out the porosity, the sediment samples were oven dried at 60°C. The dry bulk density and porosity of sediments was calculated from natural moisture content and grain specific gravity values using equations of soil mechanics (Budhu, 1999). The bulk density, ρ , was calculated by using specific gravity and void ratio

$$\rho = \frac{(G+e) \rho_w}{(1+e)}$$

where, G=specific gravity, e=void ratio and ρ_w =density of the water

Grain size analysis

Grain size analysis was done according to Gardner (1986). Sediment samples 5-10g from each section were desalinated by washing repeatedly with deionized water, followed by sieving the sample through a 300 μm sieve to separate the sand from the mud fraction and dried at 45 °C and treated overnight with 30% H_2O_2 for removal of organic matter. The samples were then soaked overnight in a 0.1% sodium hexametaphosphate (NaHMP) solution to aid sample dispersion. Samples were then analyzed through Malvern laser particle size analyzer (Mastersizer 2000).

Biochemical parameters

Estimation of carbohydrates

The estimation of carbohydrate was done by phenol sulfuric acid method (Kochert, 1978) using glucose as a standard. From each core 0.1 gm of sediment was taken in 15 ml centrifuge tube, to which 2 ml of 5 % TCA was added and placed in boiling water bath for 3 hrs at 80- 90°C. The tubes were cooled and centrifuged at 5000 rpm for 5 mins, from which 0.5 ml of supernatant was withdrawn and used for carbohydrates estimation. Later 0.5 ml of distilled water was added to the centrifuge tube followed by 1.0 ml of phenol reagent and 5 ml of concentrated sulfuric acid, mixed well, incubated for 30 min and the OD was measured at 490 nm.

Estimation of Protein

Estimation of protein was done by Lowry's method (1951). Dried sediment sample (0.1 g) was taken from each section, 2 ml of 1 N NaOH was added and mixed well, and kept at 100°C for 5 mins. Then the samples were centrifuged for 5 min at 4°C, and 0.5 ml of supernatant was taken in fresh vial and 0.5 ml distilled water and 5 ml of reagent C (50 ml of 2% of sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 gm in 100 ml distilled water+ 10 ml of 1.56% copper sulfate solution mixed with 10 ml of 2.37% sodium potassium tartarate solution) (1:1 ratio) was added. The samples were incubated in the dark condition for 10 mins, after which 0.5 ml of freshly prepared Folin-Ciocalteu reagent solution (1:1 dilution) was added, vortexed well and incubated for 20 min in the dark, followed by recording the OD at 750nm.

Estimation of Lipids

Estimation of lipids concentration in mangrove sediments was carried out by Bligh and Dyer (1959) as well as Marsh and Weinstein (1966). In 15 ml centrifuge tube 0.1g of dried sediment was taken, to which 1 ml of distilled water was added, vortexed for 1 min, followed by addition of 1.25 ml of chloroform and 2.5 ml of methyl alcohol and incubated overnight at 4°C. The samples were then centrifuged at 8000 rpm for 5 min, after which the supernatant was withdrawn using pipette and collected in separate vials, to which 1.25 ml of chloroform and 1.25 ml of distilled water was added. The tubes were shaken vigorously for 1 min and allowed to form a water - methanol - chloroform emulsion, and centrifuged at 8000 rpm for 5 min to collect the supernatant of hydro alcoholic fraction. To this supernatant 2 ml of dichromate solution was added, incubated in boiling water bath for 45 min, with intermittent shaking two to three times. The samples were diluted with 4.5 ml distilled water, and the OD was taken at 350/ 440 nm.

Determination of Dissolved oxygen

Dissolved oxygen (DO) concentration in the water was determined by using the traditional Winkler's titration (1888) method as described by Grasshoff et al. (1983). Water samples were collected at the site in acid-washed (10% HCl) 300 ml glass stopper bottles. The samples were immediately fixed using 2 ml of Winkler's A and B reagents. The DO bottles were gently shaken to mix the reagents and the precipitate formed was allowed to settle. In the laboratory, the precipitate was acidified by adding 2ml of sulfuric acid (50% v/v) and titrated against 0.01N sodium thiosulfate using starch as an indicator. End point was noted by observing color change from pale yellow to colorless. A blank was maintained using milli Q water. Potassium iodide (0.01N) was used as a standard. The dissolved oxygen concentration of water was expressed as the number of milliliters of dioxygen gas (O₂) per litre of water (ml l⁻¹).

Bacteriological parameters

Total bacterial counts

Sediment (0.1g) was taken in triplicates from different sections of cores and transferred to 10 ml of filter sterilized seawater. Tween 80 (50µl) was added and the mixture was sonicated at 15 mHz for 15 secs. The method of Hobbie et al. (1977) was used for the enumeration of total bacterial counts (TC) by epifluorescence microscopy. An aliquot of 5 ml sample was fixed using 250 µl of buffered formalin (2% final concentration) and filtered over a 0.2 µm black nuclepore polycarbonate filter paper (*Millipore*) and stained with acridine orange (final concentration 0.01% w/v). The sample was then incubated for 10 minutes and then filtered. Bacterial cells retained on the filter paper were

counted using *Nikon 50i* epifluorescence microscope equipped with a 100X oil immersion objective. Cells were counted from 10 microscopic fields and the total counts (TC) were expressed as number of cells g⁻¹ (dry sediment).

Total viable counts

Total viable counts (TVC) were enumerated according to Kogure et al. (1984). To 5 ml of sample and 50 µl of yeast extract (1%) was added, followed by addition of 50 µl of antibiotic cocktail mixture {nalidixic acid (0.002%, wt/vol) piromidic acid (0.001%, wt/vol), pipemidic acid (0.001%, wt/vol)} and incubated for 6 hrs. The samples were then fixed with 250 µl of buffered formalin (2% final concentration), stained with acridine orange (final concentration 0.01% w/v) for 10 minutes and then filtered over a 0.22 µm black nuclepore polycarbonate filter paper (*Millipore*). Bacterial cells retained on the filter paper were counted using *Nikon 50i* epi-fluorescence microscope equipped with a 100X oil immersion objective. Cells were counted from 10 microscopic fields and the TVC were expressed as number of cells g⁻¹ (dry sediment).

Determination of sulfur-oxidizing bacterial abundance

Sulfur-oxidizing bacterial population was enumerated using modified Leiske's medium (Rodina, 1972). The samples in triplicates from each section were diluted in 10 ml of autoclaved and filtered (0.22µM) saline water (0.85%), mixed gently and 100 µl of sample was spread plated on modified Leiske's medium, and the cells were expressed as CFU x 10⁵ l⁻¹.

Determination of sulfur-oxidation activity

Thiosulfate concentration was estimated as a measure of sulfur-oxidizing activity using modified Leiske's medium (LokaBharathi et al., 1988). Blank readings were subtracted from the experimental reading to obtain the final thiosulfate concentration which is expressed as S₂O₃²⁻ µM l⁻¹. Sediment samples (1.0g) from each section were pre-fixed with lead acetate, to remove organic matter which gives false positive result. The sediments were diluted with 10 ml of sterile sea water. Five ml of sample was taken and diluted with 95 ml distilled water to which 25 ml of diluted acid and 12.5 ml of 0.0125 N iodine was added and titrated immediately with standardized 0.0125 N thiosulfate until most of the iodine was consumed. A few drops of freshly prepared starch solution were added and titrated until the blue color just disappeared. Distilled water was used as blank.

Statistical analysis

Spearman's correlation analysis was performed on the normalized data to elucidate the relationship between environmental parameters and the bacterial abundance and their activity. The

variables were $\log(x + 1)$ transformed to normalize the data before analysis. Analysis of variance (ANOVA) was performed to analyze differences if any in sulfur bacterial abundance and activity during the three seasons. Principal Component Analysis (PCA) was performed using PRIMER version 6 (Clarke and Warwick, 1994). Only those variables with a load of > 0.2 were considered.

Results

The results are analyzed to understand the response of sulfur-oxidizing bacterial abundance and their activity to different physico-chemical parameters during the pre- monsoon, monsoon and post- monsoon seasons.

Physicochemical parameters:

Temperature did not show significant variation during the three seasons. The average temperature during pre-monsoon was 27.19°C, monsoon was 26.89°C and post-monsoon season was 27.01°C. pH values showed increasing trends with 6.36 during pre-monsoon, 6.86 during the monsoon and 6.92 in the post-monsoon season. Oxidation reduction potential (Eh) showed a lower value in the post-monsoon (4.24) as compared to that of the pre-monsoon (38.34) season. DO concentrations were 1.2 times lower in pre-monsoon (3.86 mg ml⁻¹) than that of the monsoon (4.67 mg/ml) season and dropped to 3.53 mg ml⁻¹ during the post-monsoon season (Table.1).

The total carbohydrate concentration did not show seasonal variation and ranged between 2.02 to 2.43 mg g⁻¹. During pre-monsoon, the protein concentration was 0.77 mg g⁻¹ which decreased during monsoon (0.58 mg g⁻¹) and increased ~ 4 times during post-monsoon (2.26 mg g⁻¹). During pre-monsoon, the lipid concentration (3.04 mg g⁻¹) was 2.2 times higher than that in the monsoon season (1.36 mg g⁻¹). In the pre-monsoon season the LOM (6.23mg g⁻¹), LOC (2.56 μg C g⁻¹) was comparatively more, than the values recorded in the monsoon and post-monsoon season (Table 1).

High concentration of TOC, TIC and TOM was observed during post-monsoon (7.20%, 0.08% and 12.38 %) as compared to pre-monsoon season. Similarly, sulfide concentration in the mangrove sediment was high during post-monsoon (0.98 mM) season as compared to other seasons (pre-monsoon: 0.76 mM, monsoon: 0.69 mM). In contrast to this parameter, thiosulfate and sulfate concentration was low during post- monsoon (1.28 mM, 10.67 mM) while it was high (1.32 mM, 15.85 mM respectively) during pre-monsoon season (Table. 1).

Bacterial parameters:

During the three seasons the TC of bacteria were 10⁸ g⁻¹, while TVC of bacteria were one to two orders lower between 10⁶ to 10⁷ g⁻¹. SOB numbers were 10⁵ g⁻¹ during all seasons and were in

the trend of post-monsoon > pre-monsoon > monsoon season (Table 1, Fig.2). However, season did not significantly influence SOB abundance.

Bacterial activity:

The sulfur-oxidizing activity (SOA) was generally present throughout all the seasons in the range of 0.92 mM g⁻¹ d⁻¹ to 2.09 mM g⁻¹ d⁻¹. The average SOA during pre-monsoon was 1.66 mM, while in the monsoon season the activity decreased to 0.92 mM, and during post-monsoon it increased by 2.3 times (Fig.2). This was corroborated by a significant difference in SOA between pre-monsoon and monsoon (p<0.001) and monsoon and post-monsoon season (p<0.001) Table 2. The same was true for the cell specific oxidation rate. The cell specific sulfur-oxidation rate was 0.97 and 0.9 nM cell⁻¹d⁻¹ during pre-monsoon and post-monsoon season respectively, while in the monsoon season it was ~2 times less than the other seasons (Table 1).

Statistical analysis:

During pre-monsoon, SOB negatively correlated with depth (n=20, r²=-0.183, p<0.05), and was positively influenced by lipids (r²=0.249, p<0.05), LOC (r²=0.310, p<0.01) thiosulfate (r²=0.487, p<0.001) and their activity (r²=0.362, p<0.01) Fig.3. During monsoon season, TIC positively influenced both SOB population (r²=0.550, p<0.001) and SOA (r²=0.467, p<0.001). SOA also related to thiosulfate (r²=0.281, p<0.05) and negatively correlated with depth (r²=-0.216, p<0.05) and protein content (r²=-0.226, p<0.05). During the post-monsoon season, the SOB population (r²=-0.249, p<0.05) and its activity negatively correlated with depth (r²=-0.343, p<0.01). Similarly, SOB (r²=0.286, p<0.05) and SOA was positively influenced by thiosulfate (r²=0.366, p<0.01). SOA was also negatively influenced by sulfide concentration (r²=-0.312, p<0.01), and it was positively influenced by sulfate concentrations (r²=0.327, p<0.01). Sulfur-oxidizing bacterial abundance synchronized with its activity (r²=0.473, p<0.001) (Fig 3).

Analysis of variance showed that though there was no significant change in the SOB abundance between the three seasons, SOA showed significant variation between seasons (Table.2).

The principal component analysis (PCA) was performed to reveal which factors most significantly influence the sulfur-oxidation activity. For interpretation >0.2 values were considered for PCA.

PC1 which mostly accounted for Eh, carbohydrates, LOM, TOM, TOC, TIC, sulfate, TC, TVC and cell specific sulfur oxidation rate were brought about 26% variation during pre-monsoon. While PC2 included parameters like SOB, SOA, sulfide, thiosulfate, sulfate which brought about 20% variation (Table 3).

In the monsoon season, 33% variation in PC1 was brought about mainly by physicochemical and biochemical parameters like, temperature, pH, Eh, carbohydrates, lipids, LOM, LOC, TOC, TOM, and bacterial parameter like TVC. In the post monsoon season, 33% variation in PC1 was caused mainly by biochemical parameters like, carbohydrates, protein, lipids, LOM, LOC, and sulfate along with bacterial parameter like TC and TVC. However, a 21% variation in PC2 was mainly due to sulfide, thiosulfate SOA, SOB and cell specific activity (Table 3).

Discussion:

Sulfur-oxidizing bacteria significantly correlated with lipids ($r^2=0.249$, $p<0.05$) thus contributing to the lipid pool in mangrove sediments. This is also corroborated by the studies of Jiang et al. (2012) in Ordos Basin, China where sulfur-oxidizing bacteria like *Beggiatoa*, *Thioploca* and other are known to have significantly contributed to the lipid pool. Significant amount of 16:1 ω 7c and 18:1 ω 7c fatty acids were detected wherever the SOB was dominant. Jacq et al. (1989) suggested that lipids are used as carbon substrates for the metabolism of sulfur-oxidizing bacteria.

During the monsoon season, the sulfur-oxidizing bacteria correlated ($r^2=0.467$, $p<0.001$) with total inorganic carbon indicating the uptake of inorganic carbon by sulfur-oxidizing bacteria (Luther et al., 2011). This also suggests that, sulfur-oxidizing bacteria are involved in oxidation or hydrolysis of inorganic sulfur compounds (Friedrich et al., 2001; Kelly et al., 1997). In the pre-monsoon and post-monsoon season SOB positively correlated with thiosulfate ($r^2=0.487$, $p<0.001$, $r^2=0.366$, $p<0.01$), indicating thiosulfate being used as an energy source and inorganic carbon as a carbon source for the chemolithotrophic sulfur-oxidation activity (Grote et al., 2017). Similarly, Grimm et al. (2008), Sorokin (2003), Das et al. (1996), Houghton et al. (2016) stated that, SOB is able to oxidize thiosulfate and also, they have the metabolic flexibility according to different geochemical conditions. They can oxidize sulfide, thiosulfate and elemental sulfur compounds and thrive in marine environment and identified as an obligate aerobic chemolithomixotroph (Takai et al., 2004). *Thiobacillus* and *Thiomicrospira* spp. are reported as widespread in marine sediments (Robertson and Kuenen, 1992; Brinkhoff and Muyzer, 1997) and actively participate in the sulfur oxidation in different mangrove environments. (Varon-Lopez et al., 2014; Pascal et al., 2014; Jean et al., 2015).

Bacterial oxidation by chemolithoautotrophs plays a significant role in the formation of fresh organic matter (Howarth, 1984). The sulfur-oxidizing bacteria positively correlated with labile organic matter suggesting that SOB was able to liberate energy from the fixed organic matter and produce fresh organic biomass. Howarth (1984) stated that, the combined cycle of anaerobic decomposition by sulfate reduction as reduced sulfur compounds and production of new organic

carbon by sulfur-oxidation helps to recover relatively new organic matter from throughout the sediments and deliberate the energy as living biomass near the sediment surface where it can be readily grazed by animals. Kelly (1982) also stated that, sulfur-oxidizing bacteria were able to catalyse the oxidation process from the fixed organic biomass, to fix CO₂ and produce new organic biomass with about 15-35 % of the energy released upon re-oxidation of sulfide along with organic matter degradation.

The average SOA during pre-monsoon was 1.66 mM, while in the monsoon season the activity decreased to 0.92 mM, and during post- monsoon it increased by 2.3 times. This suggests it to play a role in detoxification in the mangrove ecosystem (Grote et al., 2012) more so during the post-monsoon season. The SOA activity was influenced by thiosulfate during all three seasons. The activity significantly correlated with thiosulfate during monsoon ($r^2=0.281$, $p<0.05$) and post-monsoon season ($r^2=0.366$, $p<0.01$), with a stronger influence in the pre-monsoon season ($r^2=0.756$, $p<0.001$). This suggests that, thiosulfate acts as an important energy source (González-Toril et al., 2006) for sulfur-oxidizing activity during all the seasons but more importantly during the pre-monsoon season. Friedrich et al. (2001) reported the SOB to have the thiosulfate-oxidizing multi-enzyme complex which aids in the oxidation of different sulfur compounds. Sorokin et al. (2001) reported high rates of oxidation of sulfide and thiosulfate by sulfur-oxidizing bacteria from soda lakes under alkaline conditions. Also others (Harrison, 1984; Jannasch et al., 1991) reported oxidization of sulfide, thiosulfate, and various polythionates by some chemotrophic sulfur oxidizers under neutral and acidic conditions.

The sulfur-oxidation activity positively correlated with sulfate ($r^2=0.327$, $p<0.01$) during post-monsoon, suggesting that the sulfur compounds were oxidized to sulfate by sulfur-oxidizing activity (Larkin, 1980; Nelson and Jannasch, 1983; Jorgensen and Revsbech, 1983; Lee Eun et al., 2000). While in the monsoon and pre-monsoon season no such relationship was observed. However, SOA in the monsoon and post-monsoon season related to thiosulfate. Seasonal changes, significantly influenced SOA. Maximum sulfur-oxidation activity was observed during the post-monsoon season, which also showed a significant variation with that of monsoon season ($p<0.000$). These changes brought about by seasonal variations have a significant influence on microbial mediated enzymatic activities in this ecosystem. Similar observations were also reported by Wang et al. (2018). Thus, SOA and the bacterial community play an important role in remineralizing inorganic compounds.

The sulfur oxidation process is known to fix CO₂. According to Anderson et al. (1987) the ratios of net CO₂ fixation by thiosulfate oxidation was 0.3:1 and 0.58:1, corresponding to thermodynamic efficiencies of 16% and 31% respectively. Using Anderson et al. (1987)

thermodynamic efficiencies in our studies, 0.47 mg C m²/ day at 16% and 0.9 mg C m²/ day at 31 % efficiencies of CO₂ was fixed by thiosulfate oxidation with nitrate as an electron acceptor during microbial sulfur oxidation. Maximum carbon fixation took place in the post-monsoon and least in the pre-monsoon season indicating the mangroves ecosystem to be a better sink of carbon during the former than the latter (Fig.4). While Howarth (1984) reported that, the pure cultures of chemoautotrophic *Thiobacilli* from coastal marine sediments were able to fix 0.65 mg C m² d⁻¹ and 11 mg C m² d⁻¹ and used 21 to 37 % as the efficiency of bacteria as reported earlier by Kelly (1982). These efficiencies are 3 to 6 times higher, probably due to the growth of these pure cultures was more favorable than the conditions found in the stratified water bodies (Jørgensen, 1982). Using Anderson et al. (1987), where 2 sulfide =1 thiosulfate, in our study, 2.09 mM thiosulfate is oxidized during post-monsoon which amounts to 4.18 mM of sulfide being detoxified.

Conclusion:

This study elucidates the role of sulfur-oxidizing bacteria and their activity in the mangrove ecosystem in three different seasons. The sulfur-oxidizing bacterial activity and population play a vital role during all seasons and is influenced by the studied parameters, in remineralizing the inorganic compounds, release energy from the fixed organic biomass, which is used by other bacterial communities. Sulfur-oxidizing bacteria and their activity were mainly influenced by total inorganic carbon in monsoon, thiosulfate in the pre-monsoon and sulfide in post-monsoon season. Besides the SOA detoxifies sulfide and aids in the assimilation of CO₂. During the post-monsoon season the mangrove system serves as a better carbon sink as mediated by sulfur-oxidation process. Thus, the sulfur-oxidation activity helps to reduce impact of anthropogenic activity, environmental pollution and promotes sustainable mangrove ecosystem especially during the post-monsoon season as compared to other seasons.

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Legends to figures and Tables

Figures:

Fig.1. Map showing geographical location of the sampling station.

Fig.2. Seasonal average and standard deviation (n=20) of sulfur-oxidizing bacterial abundance and its activity during the three seasons.

Fig.3. Scatter plots indicating the influence of environmental variables on sulfur-oxidizing bacterial abundance and its activity during the pre-monsoon, monsoon and post-monsoon seasons (n=20).

Fig.4. Box-whisker plot showing net carbon dioxide fixation by thiosulfate oxidation during the three seasons.

Tables:

Table.1. Average and standard deviation values (n=20) of physicochemical parameters along with sulfur oxidizing bacterial abundance and activity during the pre-monsoon, monsoon and post-monsoon seasons.

Table 2. ANOVA table for assessing influence of seasonal variations and with depth on sulfur oxidizing bacteria (SOB) and its activity (SOA)

Table.3. Principal component analysis of physicochemical, bacterial parameters during the three seasons.

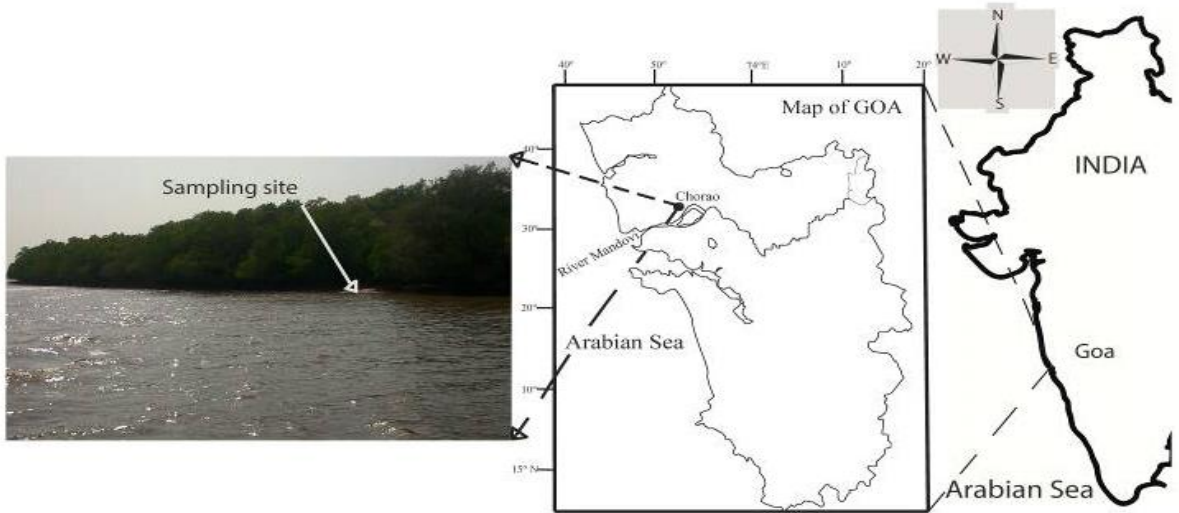


Fig.1. Map showing geographical location of the sampling station.

Fig 1

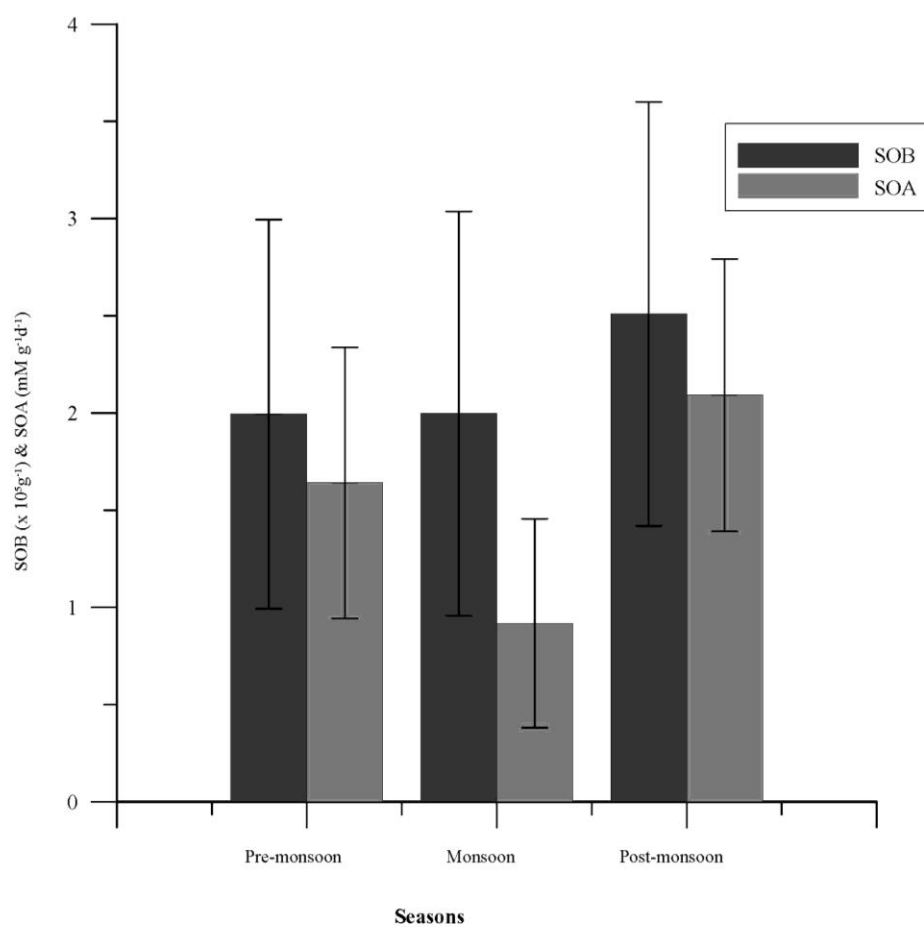


Fig. 2. Seasonal average and standard deviation (n=20) of sulfur-oxidizing bacterial abundance and its activity during the three seasons.

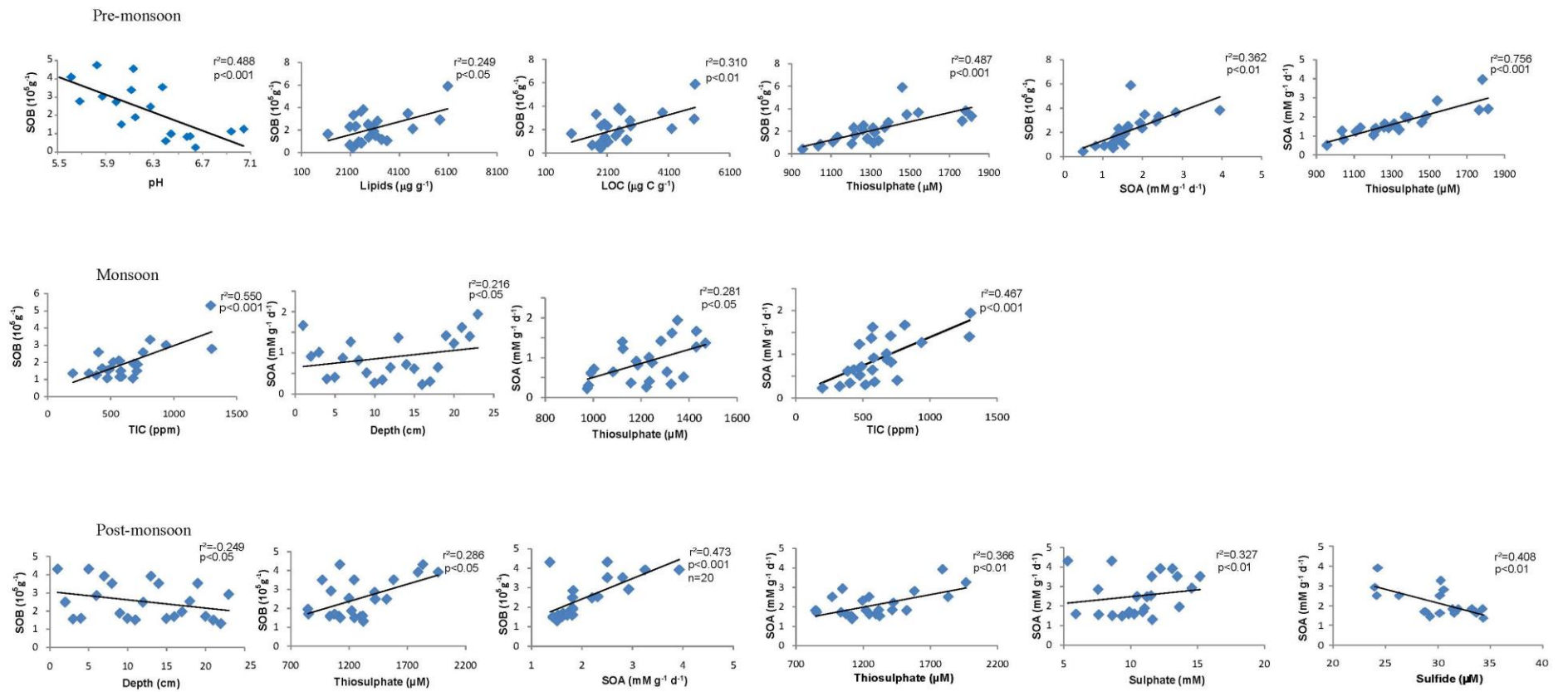


Fig.3. Scatter plots indicating the influence of environmental variables on sulfur-oxidizing bacterial abundance and its activity during the pre-monsoon, monsoon and post-monsoon seasons (n=20)

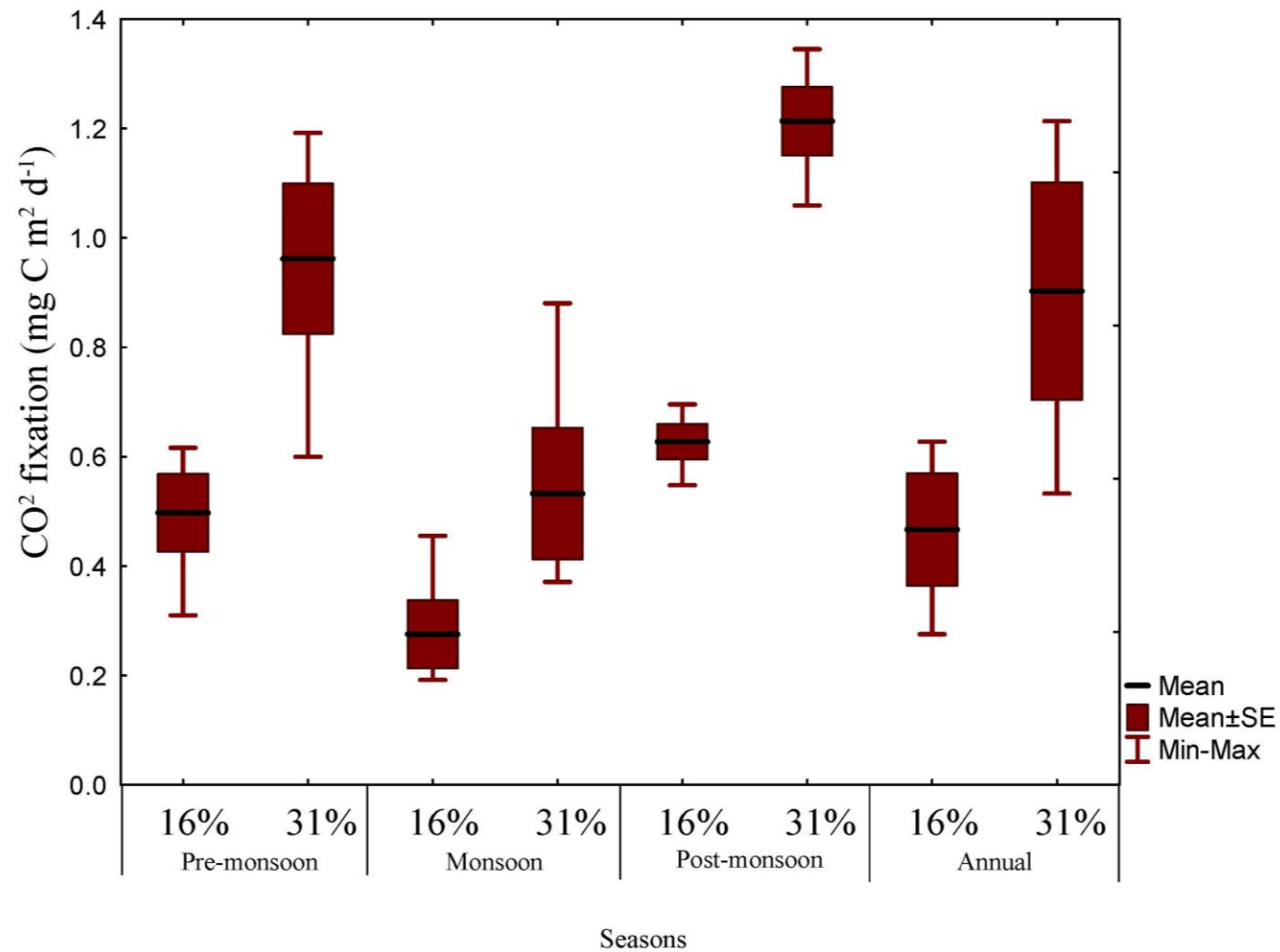


Fig. 4. Box-whisker plot showing net carbon dioxide fixation by thiosulfate oxidation during the three seasons.

Table.1. Average and standard deviation values (n=20) of physicochemical parameters along with sulfur oxidizing bacterial abundance and activity during the pre-monsoon, monsoon and post-monsoon seasons.

Parameters	Pre-monsoon Avg \pm SD	Monsoon Avg \pm SD	Post-monsoon Avg \pm SD
Temp*	27.19 \pm 0.95	26.89 \pm 0.76	27.01 \pm 1.03
pH	6.36 \pm 0.31	6.86 \pm 0.32	6.92 \pm 0.31
Eh	38.34 \pm 10.00	8.57 \pm 13.21	4.24 \pm 11.00
DO (mg ml ⁻¹)	3.86 \pm 0.24	4.67 \pm 0.40	3.53 \pm 0.21
Carbo* (mg g ⁻¹)	2.41 \pm 0.69	2.43 \pm 1.21	2.02 \pm 0.96
Protein (mg g ⁻¹)	0.77 \pm 0.63	0.58 \pm 0.60	2.26 \pm 1.02
Lipids (mg g ⁻¹)	3.04 \pm 1.21	1.36 \pm 0.91	1.52 \pm 1.23
LOM (mg g ⁻¹)	6.23 \pm 1.80	4.3 \pm 1.99	5.80 \pm 1.73
LOC (mg C g ⁻¹)	2.56 \pm 1.05	1.24 \pm 0.75	2.05 \pm 1.08
TOC (%)	5.87 \pm 1.69	7.72 \pm 2.63	7.20 \pm 2.95
TIC (%)	0.05 \pm 0.03	0.06 \pm 0.02	0.08 \pm 0.03
TOM (%)	10.10 \pm 2.90	13.29 \pm 4.53	12.38 \pm 5.08
Sulfide (mM)	0.76 \pm 0.38	0.69 \pm 0.19	0.98 \pm 0.11
Thiosulfate (mM)	1.32 \pm 0.24	1.22 \pm 0.16	1.28 \pm 0.32
Sulfate (mM)	15.85 \pm 4.80	10.30 \pm 3.22	10.67 \pm 2.67
TC (x10 ⁷ g ⁻¹)	10.92 \pm 2.43	15.00 \pm 2.59	11.21 \pm 3.57
TVC (x10 ⁶ g ⁻¹)	7.85 \pm 2.83	11.63 \pm 1.61	8.66 \pm 3.27
SOB (CFU x 10 ⁵ g ⁻¹)	1.99 \pm 1.47	2.00 \pm 1.04	2.51 \pm 1.09
SOA (mM g ⁻¹ d ⁻¹)	1.66 \pm 0.70	0.92 \pm 0.54	2.09 \pm 0.70
CSA (nM cell ⁻¹ d ⁻¹)	0.97 \pm 4.10	0.52 \pm 0.36	0.90 \pm 0.21

Table 2. ANOVA table for assessing influence of seasonal variations and with depth on sulfur-oxidizing bacteria (SOB) and its activity (SOA)

<i>Parameters</i>	<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
SOB	Pre vs Mon*	0.103	1	0.103	0.071	0.7913	4.098
	Within Groups	55.388	38	1.458			
	Pre vs Post	1.687	1	1.688	1.116	0.2973	4.098
	Within Groups	57.449	38	1.512			
	Mon vs Post	2.626	1	2.627	2.314	0.1365	4.098
	Within Groups	43.127	38	1.135			
SOA	Pre vs Mon	5.217	1	5.2175	13.470	0.0007	4.098
	Within Groups	14.717	38	0.3875			
	Pre vs Post	2.036	1	2.037	4.170	0.0481	4.098
	Within Groups	18.558	38	0.488			
	Mon vs Post	13.773	1	13.774	35.343	0.0000	4.098
	Within Groups	14.809	38	0.390			

*Pre-Pre-monsoon, Mon-Monsoon, Post-Post-Monsoon

Eigenvalues			Eigenvalues			Eigenvalues			
Pre Monsoon	%Vari'n	Cum.%	Monsoon	%Vari'n	Cum.%	Post-Monsoon	%Vari'n	Cum.	
1	5.14	25.7	25.7	6.63	33.2	33.2	6.54	32.7	
2	4.04	20.2	45.9	3.25	16.2	49.4	4.14	20.7	
3	3.59	17.9	63.8	3.12	15.6	65	2.6	13.0	
Variable	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Depth	0.071	0.095	-0.123	0.139	-0.372	-0.283	-0.080	-0.423	0.076
Temp	0.024	-0.078	-0.446	-0.289	0.066	-0.181	-0.204	0.133	0.406
pH	0.190	-0.132	-0.302	0.221	0.121	0.170	0.104	-0.105	-0.165
Eh	-0.268	0.041	0.077	-0.327	-0.080	-0.006	-0.067	0.218	0.257
Carbo	-0.310	-0.142	0.092	-0.285	0.008	0.211	-0.251	0.283	0.057
Protein	-0.068	0.233	-0.375	-0.166	-0.239	-0.140	0.342	0.057	0.148
Lipids	-0.158	0.244	-0.320	-0.269	0.237	-0.243	0.323	-0.103	0.263
LOM	-0.310	-0.142	0.092	-0.285	0.008	0.211	-0.251	0.283	0.057
LOC	-0.140	0.294	-0.338	-0.295	0.108	-0.262	0.363	-0.026	0.195
TOC	0.295	0.167	0.243	0.316	0.044	-0.028	-0.187	-0.158	0.395
TIC	0.370	0.103	0.043	0.174	0.301	-0.278	0.014	0.175	0.187
TOM	0.294	0.168	0.243	0.316	0.044	-0.027	-0.188	-0.158	0.395
Sulfide	-0.197	-0.294	-0.066	-0.132	0.105	-0.385	-0.235	-0.264	-0.117

Thios	-0.136	0.388	0.061	-0.104	0.351	-0.206	-0.080	0.319	-0.178
Sulfate	0.231	0.328	0.032	-0.082	0.258	0.172	0.280	0.142	-0.170
TC	0.289	-0.128	-0.095	-0.049	0.047	0.423	0.327	0.122	0.148
TVC	0.236	-0.056	-0.398	-0.277	0.195	0.217	0.325	0.061	0.300
SOA	-0.090	0.359	0.128	0.150	0.497	0.013	0.103	0.341	-0.169
SOB	-0.187	0.392	0.006	0.124	0.152	-0.250	-0.053	0.349	0.062
CSA*	0.206	-0.111	-0.030	0.102	0.323	0.183	0.149	-0.204	-0.188

Table.3. Principal component analysis of physicochemical, bacterial parameters during the three seasons.

*Note: Temp= temperature, carbo=carbohydrate, LOM=labile organic matter, LOC=labile organic carbon, TOC=total organic carbon, TOM=total organic matter, TIC=total inorganic carbon, TC=total counts, TVC=total viable counts, thios=thiosulfate, SOA=sulfur-oxidizing activity, SOB=sulfur-oxidizing bacteria, CSA= cell specific activity.