

G model re-visited: Seasonal changes in the kinetics of sulphate reducing activity in the salterns of Ribander, Goa, India

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ABSTRACT

In order to evaluate the contribution of Sulfate Reducing Bacteria (SRB) to the carbon flux from marine salterns, sulfate reducing activity (SRA) was measured for a year (Sept 2000- Aug 2001) This covered the pre-monsoon period (Feb-May), the monsoon (June to Sept) and the post-monsoon (Oct-Jan) in the salterns of Ribandar, Goa, India. Sulphate reduction was examined to gain insights into the decomposition characteristics (kinetics) of organic carbon; this connection is based on the theory of stoichiometric coupling between anaerobic carbon degradation and sulphate reduction to sulphide. SRA was measured using radiotracer techniques. Spatially, recalcitrance of the substrates, as depicted by the reaction rate coefficient (k), increased with depth, except during the monsoons. The values ranged from 0.0061 during the pre monsoon to 0.06 month⁻¹ during the post-monsoon at 5-10 cm depth. The recalcitrance was highest at the surface during the monsoons at a k value of 0.014 month⁻¹. The anaerobic degradation of labile organic matter followed first order kinetics or G model during non-monsoon seasons when the substrate was limiting due to closure of the system to the addition of extraneous source of organic matter. However, the integrated recalcitrance or the reaction rate coefficient was much higher during the post monsoon at 0.642 month⁻¹ compared to 0.053 month⁻¹ during the pre-monsoon season. Interestingly, the reaction rate followed zero order kinetics when the substrate was non-limiting during monsoon due to exposure to external inputs.

INTRODUCTION

In estuarine and benthic environments, sulphate reducing bacteria (SRB) contribute significantly to the carbon flow of the ecosystem through sulphate reducing activity (SRA). Numerous detailed studies have clearly documented the importance of dissimilatory sulphate reduction for mineralization of organic matter in sulphate rich marine ecosystems (Jorgensen 1977; Skyring 1987). The carbon turn over can vary from very low to high rates, depending on the geographical location, quantity and quality of organic matter and SRA.

Rates of sulphate reduction in marine sediments are known to vary over eight orders of magnitude and this large variation is mainly due to changes in the amount and biological lability of the organic matter undergoing decomposition (Goldhaber and Kaplan 1974; Westrich 1983). The quality of organic matter is known to have a great influence on the SRA (Schubert et al.2000).

In this work, we answer the question how SRA responds to the organic matter dynamics in the salterns using a well known kinetic model, 'G' model (Westrich and Berner 1984). This explains the diagenesis of organic matter with variations in the rates of sulphate reduction over time. It is now increasingly realised that > 50% of the degradation of organic matter in coastal marine sediments is through sulphate reduction (Jorgensen 1982). Hence this process could be equally important in marine salterns where high sulphate and low oxygen concentrations are conducive for the SRA . In order to understand how these systems would respond when they alternate between a salty hyper-saline non-monsoon season, and a dilute hypo-saline monsoon season, a one year study was carried out in the Ribandar salterns, Goa (Kerkar and Loka Bharathi 2007). Interestingly, SRA followed first order kinetics during the non-monsoon seasons, when the availability of the organic matter was limiting. On the other hand, during the monsoons when the organic matter was non-limiting, the activity followed zero order kinetics. This paper highlights an important seasonal effect for the first time.

MATERIALS AND METHODS

Study Area

Mandovi and Zuari are the two main rivers of Goa that have their origin in the Western Ghats mountain range and meet the Arabian Sea on the west coast, meandering through a distance of about 50

km downstream. The study area includes the saltpans at Ribandar (Fig. 1) situated at 15^o 30.166 N and 73^o 51.245 E and occupies a total area of 12,329.12m² on the Mandovi river. The sites are subject to heavy annual rains (262cm) during the monsoon season. The salt pan is under the influence of semi-diurnal tides and the surrounding marshy land supports maritime mangrove vegetation, dominated by 3 species *Rhizophora mangle*, *Avicennia germinans* and *Laguncularia racemosa*.

The Solar salterns in the present study are man-made systems, in which the sea water is directed into an inlet pan through a sluice gate. From here the water flows by gravity to new condenser pans. The seawater is allowed to evaporate sequentially so that the brine becomes more and more concentrated with respect to total salts and NaCl. At 8-10% concentration, salts begin to precipitate. As the salt concentration approaches 40%, the brine is allowed to pass into the crystallizers where the salt is subsequently harvested. As the pre-monsoon and post monsoon seasons are the main periods for salt-processing, the salterns remain closed periodically after the sea water enters, till the salt is harvested. During the monsoons however, the sluice gates are raised, salinity decreases and the system remains open for four to five months. The manual restructuring of the salt pan accompanied by stirring, cleaning and removal of biofilm debris was mostly carried out in the months of December and August.

Sample Collection

Monthly samples were collected for a period of one year i.e. September 2000 to August 2001, so as to cover all the three seasons-pre-monsoon (Feb to May), monsoon (June to September) and post-monsoon (October to January). The overlying salt pan water was collected in sterile glass bottles. Sediment cores (0-15 cm) were collected in triplicates using a 1.5 inch diameter and 15 cm long graduated PVC hand-held corer. The cores were sealed at both ends with sterile core caps to prevent direct contact with air during transport to the laboratory and stored in an ice box for further physico-chemical and microbiological analyses.

Physico-Chemical Parameters

The physico-chemical parameters analyzed in the sediments cores were restricted to concentration of sulphide, Eh, total organic carbon, fatty acids and grain size analysis. Pore water was also collected by centrifugation and analyzed for density, salinity, and sulphate. The overlying water

was also analysed seasonally for the above parameters as well as temperature, pH and dissolved oxygen.

Salinity and density were measured using a hand refractometer, (S/Mill-E, ATAGO Co. Ltd., Japan) calibrated to zero with distilled water. Whenever salinity was above 100 psu, the sample was suitably diluted, with distilled water (1:5) and corrected for the dilution before being recorded.

The pH was measured using a digital pH meter (Thermo Orion model 420 A, USA) after calibration with standard buffers. A combination redox electrode (Orion Research) was used to check the redox potential of sediment cores using potassium ferrocyanide and potassium ferricyanide to standardize as outlined in the Orion Instruction manual with a minimum delay after collection. The sealed anoxic graduated core was opened and the redox was checked by introducing the electrode at 0-2, 2-5 and 5-10 cm. TOC was estimated by titrimetry according to the method of Allen et al. (1976), that involves complete wet oxidation of organic matter in the sample with chromic acid. The total organic carbon was expressed as percentage. Glucose was used as a standard. The carbon and nitrogen analysis was determined by combustion/gas chromatography (Carlo Erba NA500 analyzer) with a precision of $\pm 1.2\%$.

The sulphate content was estimated by turbidometry (Clesceri et al. 1998). Sulphide concentration in these samples was determined according to Pachmayr (1960), cited by Schlegel et al. (1964). The quantity of sand, silt and clay of each layer of sediments from each depth was determined using the 'wet sieving' method for sand and the 'pipette method' for silt and clay analyses (Day 1965; Carver 1971).

For a qualitative fatty acid analysis 10 gm of sediment samples was made into slurry in 20 ml of distilled water and 15 ml of chloroform. The chloroform layer was collected after centrifugation at 9000 rpm for 15 minutes. A total volume of 45 ml of the pooled chloroform (3 extractions) was dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure at 30 °C using a Rota vapor (Heidolph). The residue was dissolved in deuterated chloroform. The sample along with TMS (Tetramethylsilane) as internal standard, was analyzed by ¹H NMR on a Bruker Avance AMX 300 spectrophotometer with a spectral width of 16δ. Appropriate resolution enhancement was applied to optimize the separation of signals.

Enumeration of SRB and Estimation of SRA

SRB were enumerated on modified medium of Hatchikian, (1972) [NH_4Cl 2g; NaCl 2g, K_2HPO_4 0.2g; yeast extract 1g; agar 0.8%] prepared in 1L of sea water and supplemented with 60g of natural salt. The carbon sources included were sodium salts of lactate, acetate, butyrate, benzoate and formate at a final concentration of 1mM each, when used individually or at 0.2 mM each, when used in combination. SRB medium supplemented with 1 mM cocktail of 5 carbon sources listed above was distributed into screw capped tubes after adding 2.5 ml/L of 5% Na_2S and 5ml/L of 0.04% of FeSO_4 (acidified with 0.2ml of 0.1% of H_2SO_4). The tubes were incubated for 3-4 days to check for contamination and the maintenance of anaerobic conditions. Six sets of these tubes were inoculated with 0.5 ml of the suitably diluted sediment samples from different depths and three sets were incubated at room temperature for enumeration. The other three sets of tubes (for SRA) were incubated after adding 10 μl of sodium sulphate (1mCi, specific activity: 750 MBq/mg) from BARC, Mumbai. The same method was repeated with water samples from the salterns, where 1 ml of water samples were used for inoculation. SRA was estimated after 7, 14, 21, 28 and sometimes after 35 days by fixing 1 ml of the incubated liquid media in 10 ml of 2 % zinc acetate. following the radiotracer method, (King 2001) with slight modifications (as described below) and expressed as $\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{d}^{-1}$ for water and $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ for sediments.

In this ^{35}S isotopic method, the sediment cores were purged with oxygen free nitrogen gas and stored at 4°C till analysis. SRA of the sediment sample was measured *per se* at 2 different salinities (85 and 330 psu). Mini cores from the above intact cores were prepared corresponding to the three depths by removing the luer tips from plastic disposable syringes (2ml). Each section was homogenized anaerobically, by stirring under a nitrogen atmosphere in air tight plastic bags and immediately used for experiments. Aliquots of 10 μl (74 KBq) of $^{35}\text{SO}_4^{-2}$ (sodium sulphate) from BARC, Mumbai was injected into the homogenized section and allowed to become evenly distributed. SRA was terminated at the end of 24 and 48 hrs. Triplicate mini-cores (ca. 1g.) were incubated anaerobically in air tight bags with nitrogen gas for four different incubation times viz. 12, 24, 48 and 60 hrs for 85 psu and 4, 8, 12 & 16 days for 330 psu cores using 10 μl of $^{35}\text{SO}_4^{-2}$ for each slurry. Mini-cores (1 cm^3) from 0-2 cm of 330 psu intact cores were used for amended sediment studies : 0.4 ml of 0.2 mM concentrations of each of the following substrates: acetate, formate, butyrate, lactate and benzoate was mixed together in autoclaved sea water to a final slurry of 2 ml. The Radiotracer assay was carried out using single step

chromium reduction assay (King, 2001) using Perkin Elmer Wallac 1409 DSA liquid scintillation counter.

Sulphate reduction rate was calculated using the formula:

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

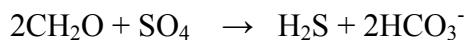
IDF = isotopic discrimination factor taken as 1.06

T = incubation time.

SRA was expressed as $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$

Depth integrated SRA was also calculated as a function of time and at two different salinities (85 and 330 psu)

G- type modeling, A simple first order model was the first kinetic model developed to describe the dependence of sulphate reduction rate in sediments on organic matter (Berner, 1964). An important assumption of this model is that rates of sulfate reduction can be related to overall rates of organic carbon decomposition according to the reaction:



The formula CH_2O refers to the solid phase organic detritus undergoing decomposition.

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 CH_2O is assumed to be decomposed at an overall rate directly proportional to its own concentration.

This can be expressed as,

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

Where,

G = is the concentration of metabolizable organic carbon that can be decomposed by the sulphate reducing community. k= first order degradation constant for decomposition via sulphate reduction, t = the time. The value for 'k' was calculated by dividing TOC (corrected for sediment density) by twice the SRA.

Thus G is indicative of the reactivity of the available organic carbon in this study.

Statistical analyses were carried out using MS Excel version 2000 to relate physico chemical and bacteriological parameters.

RESULTS

Physico-Chemical Parameters

The sediment displayed two different colors throughout the seasons, an upper dark brown and a deeper black layer. Salt pan temperatures varied from 25 to 36⁰C in the non- monsoon seasons and from 28 to 30⁰C in the monsoon season. The pH was generally alkaline with a maximum of 9.03 in the monsoons. The maximum average pore water salinity was 73.91 ± 31.16 at 0-2 cm, 38.9 ± 20.62 at 2-5 cm and 38.67 ± 18.2 at 5-10 cm during the premonsoon season. The lowest salinity was recorded at 2-5cm with an average value of 10.55 ± 4.86 during the monsoon season .(Table 1) However, the salinity of the overlying water showed a maxima of 330 to 400 psu during the salt manufacturing non- monsoon seasons. Consequently, the sulphate values were the highest in the overlying water ranging from 5.8 to 12.7 g.L⁻¹ in the premonsoon season (Table 2). The redox potential in the sediments, followed the general paradigm of increasing with depth. Maximum sulphide values up to $62 \mu\text{g} \cdot \text{g}^{-1} \pm 97.15$ were also recorded at the sub-surface (2-5cm) during the premonsoon season (Table 1).

Grain Size Analyses

The sediment at 0-2 cm was dominated by silt during the non monsoon seasons and sand at all the three depths during the monsoons. The clay fraction was however very prominent throughout the whole core in the pre-monsoon season (Table 3).

Total Organic Carbon

The total organic carbon content in the sediments was highly variable ranging from 1.28 to 2.94 % at 0 to 10 cm depth. A high concentration of organic carbon was observed in the monsoons at all the three depths sampled. The top layer and the bottom layer showed a relatively higher concentration of organic carbon than the intermediate section of the core. There was no definite decrease in the organic carbon concentration with depth in the salt pans throughout the year (Table1).

Fatty Acids

Qualitative NMR analyses of lipid extracts of sediment pore water samples showed that the number of different fatty acids increased from 4 at 0-2 cm to 5 at 5-10 cm on an average. The levels were also found to be higher in the deeper layers. The concentration of lactate and acetate was almost negligible in the 0-2 cm depth. In the deeper 2-10 cm, the concentrations of lactate, acetate, propionate and benzoate were relatively higher than those observed at 0-2 cm, except formate and isobutyrate (Data not shown). Fatty acid analyses also showed high concentrations of formate at the surface.

SRB Counts

The average agar shake counts were always in the order of 10^3g^{-1} regardless of the depth (Table 4). However, they generally decreased arithmetically with depth. SRB isolated from lower salinities grew homogeneously and faster in the medium as compared to those from higher salinities which formed flocs and aggregates. SRB as estimated by MPN method gave similar trends in distribution, but one order higher in abundance (Data not shown). Based on season, the retrieval of SRB was post-monsoon > pre-monsoon > monsoon. Maximum counts were obtained on formate at 0-2 cm, butyrate at 2-5 cm and lactate at 5-10 cm. Formate and lactate were preferred during the post-monsoon season and butyrate during the monsoon.

Sulphate Reducing Activity

SRA was calculated from the peak values of activity normalized for the number of days taken to reach the peak. These values relate to the other bacterial and physico-chemical parameters. Average SRA in the sediments ranged from a minimum of 5.3 in the monsoons to $520 \mu\text{g.g}^{-1}\text{d}^{-1}$ in the pre-monsoon season at 0-2 cm with average values of 307.7 ± 238 . Deeper down the maximum values were $152 \pm 98 \mu\text{g.g}^{-1}\text{d}^{-1}$ at 2-5 cm. However at 5-10 cm, maximum values of 198 ± 164 were observed during the monsoon season (Table 1). In water, the SRA ranged from 0.62 to $30 \mu\text{gml}^{-1}\text{d}^{-1}$ with a minimum activity during the monsoons and a maximum activity during the pre-monsoon season (Table 2).

Seasonal Variation in Integrated SRA Measured at Weekly Intervals

Integrated SRA values ranged from 0.001 to 35 $\mu\text{g. cm}^{-2}$ and showed that it followed first order kinetics, during pre and post-monsoon seasons. After 20-21 days of incubation, the levels of activity decreased but during the monsoon season the rise in the activity was continuous even after the 28th day suggesting that the substrate was not limiting even after 21 days (Fig 2).

Variation in Integrated SRA at 85 and 330 psu Using $^{35}\text{SO}_4^-$

The SRA values measured with $^{35}\text{SO}_4$ clearly showed increasing trends in SRA even after 48 hours in the mesohaline sediments at 85 psu showing zero order kinetics (Fig 3). In the hypersaline sediments at 330 psu the peak attainment was slower reaching a peak only at the end of 12 days and declining by the 16th day (Fig. 4) showing first order kinetics.

Contribution of SRA to Decomposition of Organic Matter

Table 5, gives seasonal variation in the degradation constant, k with depth. These values ranged from 0.0021 to 0.06 mo^{-1} . There was a definite increase in the k values with depth during the non-monsoon seasons (Table 5).

When these values were depth integrated, k remained generally low, except in September and January (Fig 5). In January the values were almost 10 times higher than February. Season-wise, the k value was low during pre-monsoon and monsoon season and high in the post-monsoon season. (Inset, Fig 5).

SRA and the C mineralization on an aerial basis in substrate amended sediments ranged from 271.5 to 2094 $\text{g Sm}^{-2}\text{y}^{-1}$ which is equivalent to 543 $\text{g Cm}^{-2}\text{y}^{-1}$ to 4188 $\text{g Cm}^{-2}\text{y}^{-1}$.

DISCUSSION

Sulphate Reduction Rates:

In the present study SRA has been measured both in natural and amended (with fatty acids) samples. We found it necessary to amend the hypersaline samples as the SRA was low. Hypersaline

conditions were prevalent during the pre-monsoon and post-monsoon seasons. However, amendment improved the SRA by only 19%. Hence SRA measured, gave an idea of the activity after supplementing with metabolizable substrates. Thus the measured SRA and its contribution to the degradation constant could be driven by the intrinsic levels of the substrates. The activities measured could be reflective of the actual rates prevailing in the hypersaline sediments as amendments seem to stimulate and improve the activity only marginally.

In the coastal sediments, the contribution of SRA to the turnover of organic matter may be very important. When the dissolved O₂ declines, dissimilatory sulphate reduction becomes prominent. The end products of SRA limit the availability of O₂ for heterotrophs and other higher organisms (Jorgensen 1982; Fenchel 1998). Sulphate dominates the dissolved electron acceptors in marine sediments in concentrations up to 28 mM. This concentration is greater than the sum of all other electron acceptors. (King 2001). The role of SRB could also be equally important in the hypersaline salt pans where sulphate is high (up to 12.6 g/l or 131 mM) and oxygen is low (minimum value of < 0.5 ml/l). In the present study, the SRA of the overlying waters of the salterns in Ribandar ranged from 0.62 to 30 $\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{day}^{-1}$, which is equivalent to 19.4 nM day⁻¹ to 963.31 nM day⁻¹. These values are much above those reported for the Black Sea, where the rates range from 0.08 nM day⁻¹ to 0.48 nMday⁻¹ (Albert et al. 1995). In sediments, the average SRA value was $120.53 \pm 97.3\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ during the monsoon to 307.70 ± 238

$\mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ during the pre-monsoon season in the 0-2 cm layer. This variation of 2 orders is not as high as that reported elsewhere. Numerous reports (Jorgensen 1977; Cohen et al. 1984; Skyring 1988) have indicated a great variability ($0.4 - 3000 \mu\text{g SO}_4^{-2}\cdot\text{cm}^{-3}\cdot\text{d}^{-1}$) in SRA of marine sediments.

High rates of SRA in Ribandar have been measured during the pre-monsoon months when salinity was high and SRB numbers were low. Lactate stimulated the activity but not acetate (Kerkar and Loka Bharathi 2007). There are reports which show that acetate metabolism by SRB is inhibited at salinities > 150-200 psu causing acetate to accumulate to high levels (Boone et al. 1993; Ollivier et al. 1994; Brandt and Ingvorsen 1997). Hence acetate degrading SRB are related inversely to SRA in the present study ($r = -0.74$) at 2-5 cm sediment depth. Other fatty acids like benzoate and butyrate could be detected only in the deeper layers suggesting negligible numbers of SRB degrading these substrates at these depths. On the contrary, formate degraders were frequently encountered at all depths whereas

formate was beyond detectable levels when estimated. It is therefore inferred that this metabolite could be actively produced and immediately utilized suggesting highly dynamic turnover.

The average value of SRA measured in fatty acids-amended samples ($3190 \text{ nMcm}^{-3}\text{d}^{-1}$) is almost comparable to the average SRA measured using unamended samples ($2050 \text{ nMcm}^{-3}\text{d}^{-1}$). The former values were 1.3 times higher than the SRA measured by the ^{35}S method (85psu) at the surface layers of the sediments. These values are 5 orders of magnitude greater than the rates given by Li et al. (1999) which were $0.5 \text{ nM.ml}^{-1}\text{day}^{-1}$. These authors have reported a seasonal variation with a maximum value of $13 \text{ nMml}^{-1}\text{day}^{-1}$. These changes in the rates of sulphate reduction have been linked with the sulphate concentrations given by Li et al. (1996). They reported highest values after a flood and the increased activity was attributed to the stimulation by both sulphate and organic carbon in the flood waters. A similar scenario can prevail in the monsoon season in the present site. However, in Ribandar, the maximum activity was noted during the pre-monsoon season. The TOC values of the surface sediments were minimal during this season. Hence the reasons attributed to relatively higher activity in the pre-monsoon may not be due to TOC or sulphate alone but to overall increase in salinity, leading to higher sulphate respiration rates. However, the positive influence of salinity on SRA has been found within a limited range of 60 to 120 psu. At salinities above 150 psu, sulphate reduction decreases (Kerkar and Loka Bharathi 2007).

Over a year, SRA ranged from 1.5 to $520.3 \mu\text{g.g}^{-1}\text{.day}^{-1}$ in the salt pan sediments (0-10 cm) which is equivalent to 46.8 to $16250 \text{ nMg}^{-1}\text{day}^{-1}$. These values are much above those reported for Lake Elisa in South Australia (Ollivier et al. 1994) and Great Salt Lake at Utah (Brandt et al. 1999) and lower than rates reported by Caumette et al. (1994) with values of $8200 \text{ nM cm}^{-3}\text{d}^{-1}$ in the hypersaline ponds of Salins-de-Giraud, France (summarized in Kerkar and Loka Bharathi 2007).

SRA did not show any significant relationship with many of environmental parameters in sediments. However, in the overlying waters, the relationship was negative with temperature, pH and dissolved oxygen. Spatially, SRA was highest at the surface perhaps due to gradients in sulphate concentration. The average sulphate concentration measured in the pore water of the three layers was 3.06, 1.89 and 2.27 g.l^{-1} respectively. Studies on pore water sulphate concentration by other researchers (Hordjik et al. 1985; Rudd et al. 1986) revealed steep sulphate gradients directly below the sediment surface, suggesting that the sulphate turnover is intense in the top few cm. In similar manner, Albert et

al. (1995) state that the sulphate reduction rate in sediments of the Black Sea was measurable in the upper 5-10 cm layers. This phenomenon of higher SRA being restricted to the surface sediments has also been reported for Arctic marine sediments (Ravenschlag et al. 2000). High SRA was reported in the top sediments up to 6 cm in the Mediterranean salterns especially during the summer months (Caumette et al. 1994). Jorgensen and Bak (1991) have emphasized that when SRA is higher at the surface than at the sub-oxic or anoxic zones below, the actual SRA could be much higher than that measured and therefore could be underestimated. The sharp zonation and restriction of SRA to the upper few cm are different in many marine types of sediment. Sulphate can penetrate much deeper into the sediments and thus the zone of SRA can reach even down to a depth of 10-20 cm or more (Jorgensen et al. 1990; Teske et al. 1998). The relatively higher SRA observed at the shallower depths thus appears to be quite common in marine environments. SRA could also be governed by the abundance of SRB among other factors.

Sulphate Reducing Bacteria

The abundance of sulphate reducing bacteria in the salt pans varied dynamically through out the year. The abundance in the sediments was in the order of 10^{2-5} g^{-1} . These bacterial numbers are comparable to the earlier studies on the mangrove and estuarine systems around Goa (Saxena et al. 1988, Loka Bharathi et al. 1991). Interestingly, bacterial density was also comparable to marine coastal waters and sediments of the temperate regions (Hines and Buck 1972; Nedwell 1978; Jorgensen 1978; Brandt et al. 2001, Kondo et al. 2007). However at the gene level high abundance of SRB in terms as dsr gene copy number of $10^8 \text{ cells ml}^{-1}$ from hypersaline lakes has been reported by Foti et al. (2007).

Hypersaline systems are interesting to study because of the array of physiological adaptations the organisms undergo to survive. Being the first study on Thalassohaline salt pans from a tropical area, the results of this study could not be compared to other similar tropical systems. Hence the results are discussed in the context of hypersaline ecosystems from temperate regions. Most of these temperate studies have dealt with ecological and eco-physiological aspects of sulfate reduction in hypersaline mats and few studies have covered sulfate reducing activity in aphotic hypersaline sediments (Oren 1998; Canfield and Des Marais 1993; Brandt et al. 2001; Sahm et al. 1999a). Brandt et al. (1999) and Sahm et al. (1999 b) have reported the abundance of SRB in the sediments or microbial mats to range from 10^5 to $10^8 \text{ cells.cm}^{-3}$, which is 2-3 orders higher than in this study. This difference in number may either be

due to regional differences than enumeration techniques used such as MPN. In spite of the limitation of shake agar technique, it was adopted in this study as this approach helps to isolate representative cultures. The medium was amended with low concentrations of 1 mM of mixed substrates to improve the retrievability. Also, natural salt from salterns was used for obtaining the desired salinity.

The abundance of SRB was more in the surface than in the deeper layers. Similar high numbers were reported in the surface layers of temperate salt pans irrespective of the different techniques used by authors (Vester and Ingvorsen 1998; Llobet-Brossa et al. 2002). Lactate and acetate oxidizing bacteria were the most abundant groups present in the salt pan. The lactate degraders were abundant at 0-5 cm depth and acetate degraders at 5-10 cm. The difference with depth in the distribution of different substrate utilizing bacteria was probably due to the differences in unbound and freely available fatty acids. Our qualitative analysis of the fatty acids in the sediments showed lactate and acetate were negligible in the 0-2cm depth suggesting that these could be available in the labile form and hence the preferred substrates. In the deeper layers the qualitative and quantitative distributions of fatty acids were higher than at the surface. SRB decreased with depth suggesting that the fatty acids in the deeper layers must be in bound form and therefore less bioavailable or that SRB capable of utilizing them were absent. In the present study, although the size of the SRB population did not correlate with the SRA in sediments (n=12), the relationship was significant when both sediment and water were examined together (n=16; $p < 0.05$). This could be because of higher solubility and diffusibility of fatty acids in water. Nevertheless, SRA was also independent of TOC. Hence, there could be other factors that govern the changes in SRA for example grain size distribution. In the present study, the silt-clay and sand clay was abundant in the pre-monsoon season when maximum activity was observed. During monsoon, sand fraction dominated all the three depths thus decreasing the SRB abundance and activity. Christian et al. (1983) reported that there was decreased anaerobic activity in the sandy zone due to lowered concentration of organic matter. Silt clay is more conducive to bacterial proliferation and activity. However, clay could be a deterrent for SRA. Wong et al. (2004) have attributed inhibition up to 70-90% in SRA due to high aluminum content in the clay fraction. In this study, the higher of concentration of silt during post monsoon supported higher abundance of SRB.

G- model Revisited

SRA in marine ecosystems, especially the coastal regions contributes to as much as 50% organic carbon turnover (Jorgensen 1982). Sulphate respiration accounts for 70 - 90% of total respiration in salt marsh sediments, where total sediment respiration rates are $2.5 - 5.5 \text{ g. cm}^{-2}\text{day}^{-1}$. Besides sulphate, organic carbon levels also influence SRA which is an important reaction for the production of reactive by- products and the regeneration of nutrients. Naturally occurring organic polymers are stated to have vastly different susceptibilities to bacterial attack. Jorgensen (1977) has stated that on an average 2 moles of organic carbon (C) are oxidized for every mole of sulphate reduced. Redfield's stoichiometry for organic matter oxidation by sulphate reduction is $C_{\text{org}}: \text{SO}_4^{-2} = 2.1$ (Volkov et al. 1998). Thus based on this relationship, the annual carbon mineralization rate estimated by SRA in sediments in the present study ranged from $543 \text{ g Cm}^{-2}\text{y}^{-1}$ during the monsoon season to $4188 \text{ g Cm}^{-2}\text{y}^{-1}$ during the pre-monsoon season. As the concentration and the range of availability of substrates is higher during the monsoon season, it would be befitting to assume mineralization using different stoichiometries where the ratio of sulphide produced per carbon mineralized is different from 1:2. Besides the mineralization rates that have been calculated in the present study would be an underestimate if for e.g. a direct and complete mineralization of phenol like compounds are considered.

The rates determined at two different salinities, in the Ribandar salterns during the peak salt manufacturing seasons were low (18.25 at 330 psu and $573 \text{ g C.m}^{-2}\text{y}^{-1}$ at 85 psu) (Kerkar and Loka Bharathi, 2007). The present values are much higher than that reported for Great Salt Lake at Utah (Brandt et al. 1999; Alongi et al. 1999 a, b). Porter et al. (2007) have also described SRA in hypersaline coastal pans across a salinity gradient. The depth integrated (up to 12 cm) rate varied from $6\text{-}241 \text{ mmol m}^{-2}\text{d}^{-1}$ and decreased down core in the present study. These high values observed in the salt pans could be due to high degradation coefficient of SRB. 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 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 in this study. The k values (signify the degree of recalcitrance) calculated from SRA and TOC ranged from $0.0021\text{-}0.086 \text{ mo}^{-1}$. We here have applied a single reactivity constant meant for anaerobic systems where k is the 1st order decay constant for decomposition via sulphate reduction. The constant k was calculated every month and the values of four months were calculated to represent a

season. As SRB are more adept in utilizing secondary metabolites present in anaerobic systems, we have used only a single k meant for more recalcitrant or less labile substrates. These values remained constantly low, reflecting high decomposibility of matter in these saltpans. Organic matter of marine origin is known to be more easily degradable than terrestrial. C:N ratio of around 11-17 encountered in the present study suggest that the influence is mixed from terrestrial and marine sources (Redfield et al. 1963). Further, during non-monsoon seasons, k values increased with depth suggesting increased recalcitrance in the sub-surface layers. In the present study, k values calculated based on seasons were $0.0532 \text{ month}^{-1}$ in the pre-monsoon and 0.6428 in the post monsoon. Such marked difference was not observed in the TOC values. However, k calculated on an annual basis was 3.2 y^{-1} . These values are much lower than the k value of 14 reported by Grill and Richards (1964) and the range 11 - 26 given by Otsuki and Hanya (1972) and those reported by Westrich and Berner (1984), which were around 24 y^{-1} i.e. approximately 2 month^{-1} . However, the k values of Schubert et al (2000) ranged from 0.001 to 0.01 mo^{-1} in the 0-20 cm sediment core, off the central Chilean coast. 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 regard to decomposition. Based on this concept, seasonal differences in k values can be explained by the series of changes, the organic matter undergoes once it enters the system during the monsoon season. The ratio of non-labile to labile fractions increases as the season progresses from the pre-monsoon to monsoon to post-monsoon. On the contrary, the particle-associated bacteria in the estuarine waters are more productive on labile particles during the post monsoon and more active- mineralizing on less labile particles during pre-monsoon (De Souza 2001). The difference could be attributed to the reverse trends in the physicochemical parameters of the salt pan sediments as compared to the surrounding estuarine and marine ecosystems. Sequential decomposition with depth was noticed even on short time scale studies covering 7 to 21 days. Over time, the k values increased with depth. The k progressively increased from 0.01 on the 7th day to 0.02 on the 14th day and 0.03 on the 21st day. (Results not shown) Hence, as Westrich and Berner (1984) have proposed, the kinetics of sulphate reduction in the saltpans follows a sequential decomposition. Schubert et al. (2000) suggest that the source and type of organic matter appear to play a role in the distribution of the sulphate reduction rate with depth. They also opine that the organic matter being degraded may be simply diluted by non-reactive components including terrestrially derived organic matter.

CONCLUSION

Kinetics of SRA varied depending upon the environmental parameters. During the pre-monsoon and post-monsoon when the system was open and closed intermittently, the substrate availability was limited. Consequently SRA was governed by the availability of the substrate. However, during the monsoons, the system was open to continuous pulses of substrates. During this season the availability of substrate was not only non-limiting but SRB numbers were also lower. Consequently, SRA in the hypersaline ecosystems of Goa follow first order kinetics during the non-monsoon seasons and zero order during the monsoons.

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Table 1. Seasonal variability in physico-chemical parameters and SRA of the sediment at different depths. (Average values and standard deviations).

	<u>Eh</u> (mV)	<u>TOC</u> (%)	<u>Salinity</u> (psu)	<u>Density</u>	<u>SO₄</u> (mg.L ⁻¹)	<u>S⁻²</u> (μg.g ⁻¹)	SRA (μgs.g.d ⁻¹)
0 to 2 cm							
premonsoon							
Av	-41.05	1.88	73.91	1.07	10.79	42.39	307.70
sd±	(15.95)	(0.44)	(31.16)	(0.04)	(4.55)	(44.41)	(238.46)
monsoon							
Av	-75.65	2.34	25.00	1.03	3.65	16.55	120.53
sd±	(43.85)	(2.17)	(22.67)	(0.02)	(3.31)	(13.79)	(97.37)
postmonsoon							
Av	-71.25	2.27	26.92	1.03	3.93	12.67	94.12
sd±	(53.59)	(0.46)	(21.97)	(0.02)	(3.21)	(7.21)	(68.45)
2 to 5 cm							
premonsoon							
Av	-57.18	1.28	38.90	1.04	5.68	62.10	115.84
sd±	(21.55)	(0.43)	(20.62)	(0.02)	(3.01)	(97.15)	(56.77)
monsoon							
Av	-67.25	2.07	10.55	1.01	1.54	10.73	152.23
sd±	(18.00)	(2.47)	(4.86)	(0.01)	(0.71)	(11.09)	(98.46)
postmonsoon							
Av	-75.68	2.34	28.41	1.03	4.15	10.23	53.05
sd±	(55.26)	(0.77)	(9.83)	(0.01)	(1.43)	(4.25)	(42.82)
5 to 10 cm							
premonsoon							
Av	-64.98	2.33	38.67	1.04	5.65	19.63	148.72
sd±	(29.73)	(0.29)	(18.20)	(0.02)	(2.65)	(15.39)	(179.10)
monsoon							
Av	-79.43	2.94	21.41	1.02	2.86	18.69	196.11
sd±	(62.04)	(2.40)	(11.92)	(0.01)	(1.67)	(6.77)	(163.90)
postmonsoon							
Av	-70.15	2.45	35.41	1.04	5.15	8.50	20.82
sd±	(32.94)	(0.49)	(16.57)	(0.02)	(2.40)	(5.52)	(16.14)

Table 2: Physicochemical parameters and sulphate reducing activity of overlying salt-pan water.

	Premonsoon	Monsoon	Postmonsoon
Temp (°C)	28 - 36	28 - 30	25 - 30
pH	7.7 - 8.9	8.1 - 9.03	7.3 - 8.4
Eh (mV)	-9. to- 59.1	-38 to- 268	-76 to-177
DO (ml.L ⁻¹)	0.38 - 0.7	0.24-3.83	0.05 - 2.5
Salinity (psu)	80 - 400	5. - 21	10. - 330
Density	1.06 - 1.2	1.003-1.02	1.01 - 1.04
SO ₄ (g.L ⁻¹)	5.8 - 12.7	0.14 - 1.6	0.7 - 4.1
S ⁻² (mg.L ⁻¹)	0.09 - 1.63	0.19 - 0.9	0.05 - 12.7
SRA (μg.ml.d ⁻¹)	2.8 - 30	0.62 - 3.2	4.3 - 17.4

Table 3. Seasonal variation in the percentage of sand silt and clay in sediments.

	Sand	Silt	Clay
Pre-monsoon			
0-2 cm	14.55	45.38	40.07
2-5 cm	46.19	8.69	45.12
5-10 cm	49.34	3.12	47.54
Monsoon			
0-2 cm	60.7	12.8	26.4
2-5 cm	35.14	30.66	34.2
5-10 cm	48.04	21.5	30.04
Post-monsoon			
0-2 cm	4	93.5	2.5
2-5 cm	25	68.5	3.0
5-10 cm	31	67.0	3.0

Table 4 : Seasonal and spatial variability of sulphate reducing bacteria (SRB) on different substrates

Substrates	Counts x 10 ³ .g ⁻¹ of dry sediment								
	0 to 2 cm			2 to 5 cm			5 to 10 cm		
	Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
SRB L	1.86	0.90	10.66	2.26	0.73	3.33	2.31	0.56	4.08
SRB F	2.23	0.26	22.73	2.45	0.35	2.67	0.77	0.57	1.57
SRB A	2.01	0.46	18.97	1.81	0.76	4.05	3.58	0.21	1.81
SRB Bz	8.59	1.50	8.41	1.13	0.12	2.16	2.31	0.94	1.41
SRB Bu	1.20	0.72	6.87	1.41	0.93	5.24	0.77	0.94	3.41
Av. Count	3.17	0.77	13.52	1.81	0.58	3.49	1.95	0.49	1.67

L=lactate; F=formate; A=acetate; B_z = benzoate; Bu=butyrate

Table 5. Depth-wise and seasonal variation of K values (month⁻¹).

Depth	Pre-monsoon	Monsoon	Post-monsoon
0-2 cm	0.00205	0.014896	0.00673
sd.±	(0.00187)	(0.024)	(0.00588)
2-5 cm	0.00206	0.003384	0.04009
sd.±	(0.00106)	(0.00328)	(0.06553)
5-10 cm	0.00610	0.006782	0.06038
sd.±	(0.00531)	(0.00786)	(0.08648)

Fig. 1. Study area and sampling location.

Fig. 2: Seasonal integrated sulphate reduction (0-10 cm) as a function of time showing first order rate law for organic matter decomposition.

Fig.3. Integrated sulphate reduction (0-10 cm) as a function of time showing zero order rate law for organic matter decomposition at 85 psu .

Fig 4. Integrated sulphate reduction (0-10 cm) as a function of time showing first order rate law for organic matter decomposition at 330 psu.

Fig 5. Monthly variation in integrated K values; Inset: Seasonal values.

Fig. 1.

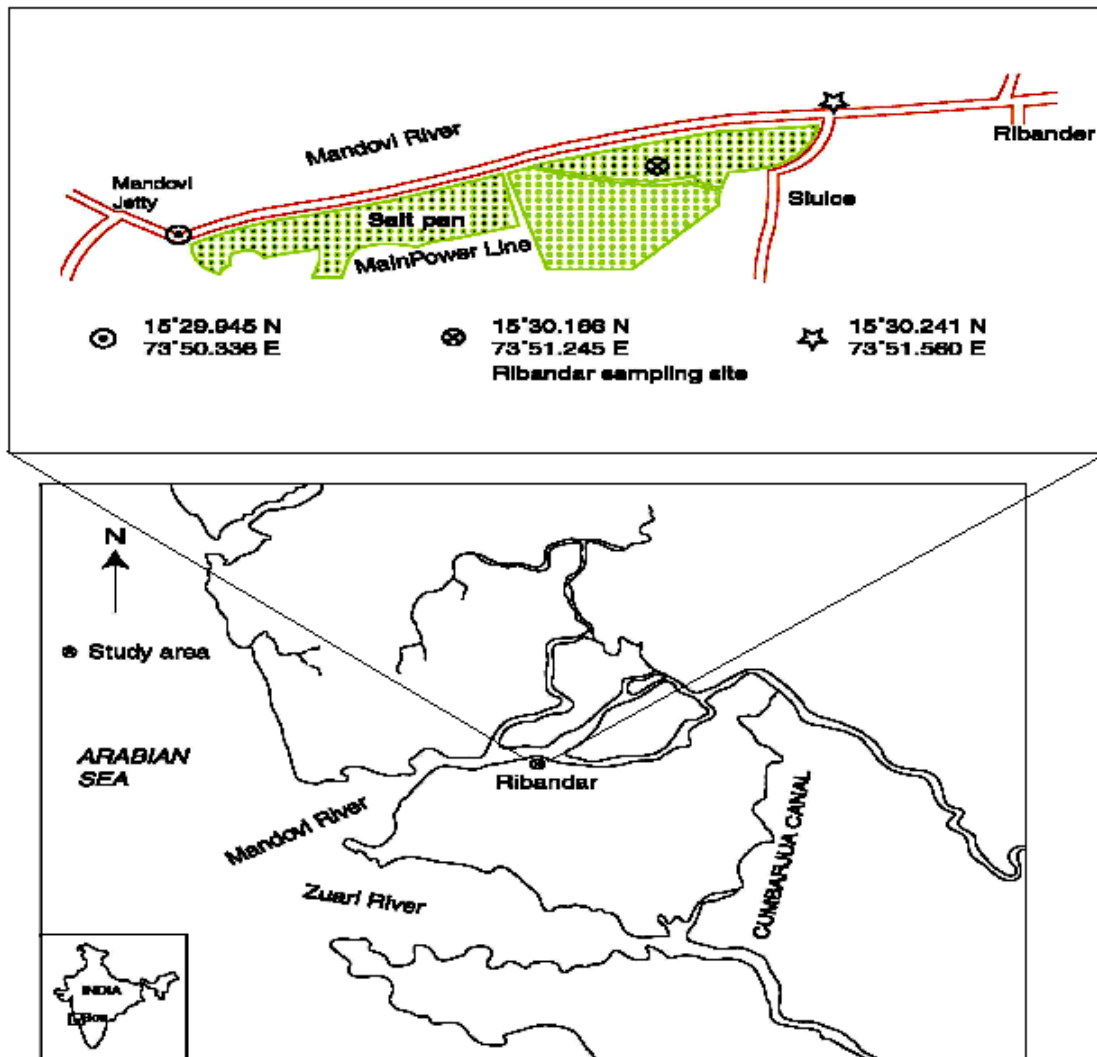
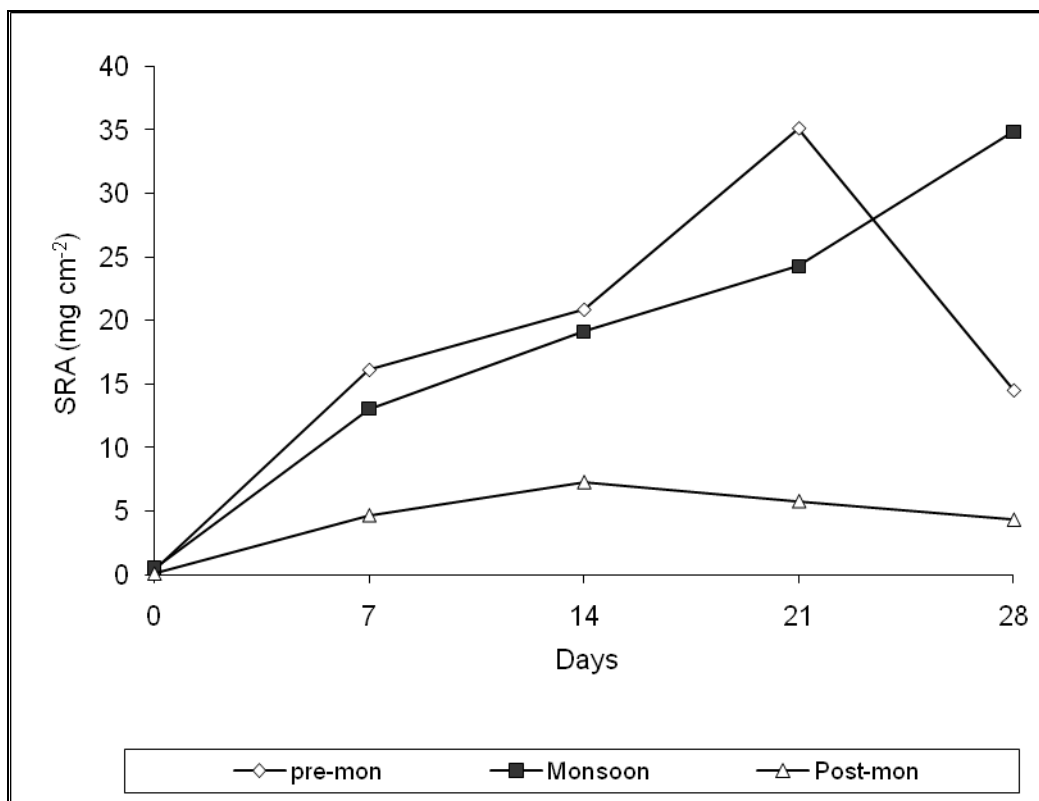


Fig 2



Standard deviations (\pm sd)

days \ season	0	7	14	21	28
Premonsoon	0.035	1.198	0.625	3.663	2.252
Monsoon	0.036	0.788	1.196	1.208	2.963
Postmonsoon	0.012	0.52	0.524	0.583	0.448

Fig. 3.

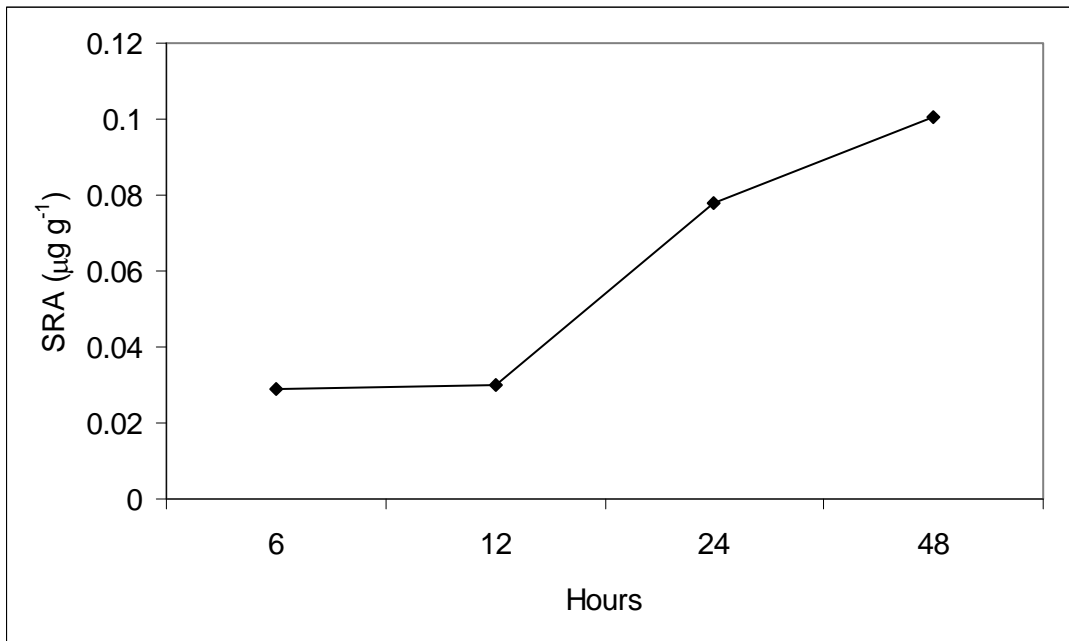


Fig. 4.

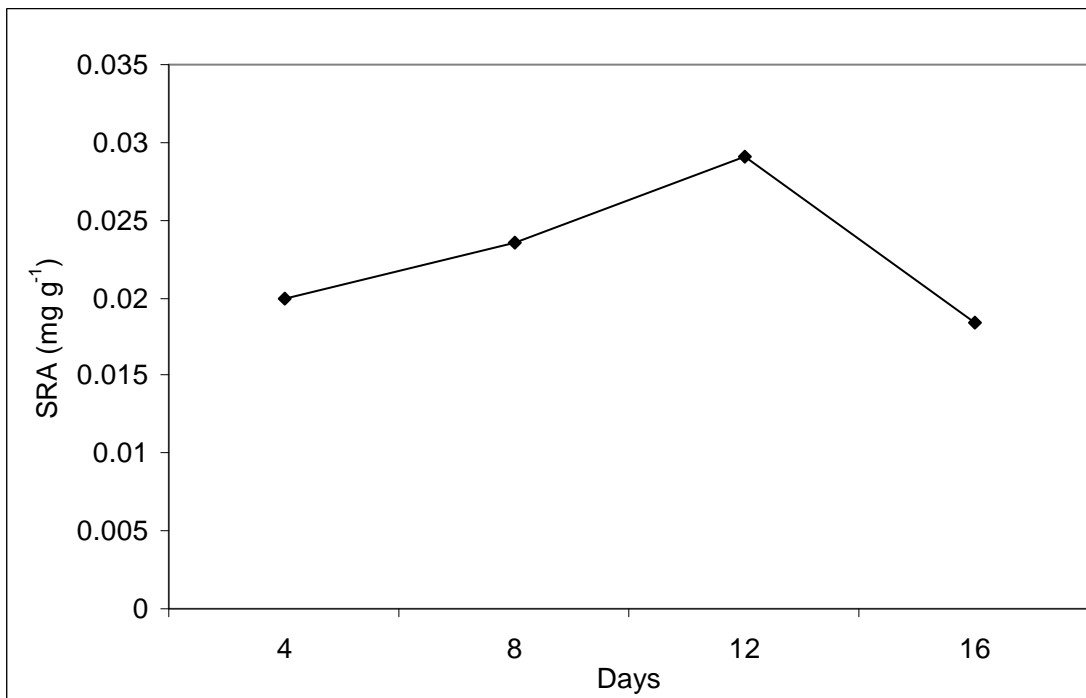


Fig. 5.

