PHYTOPLANKTON AND BACTERIAL INTERACTION IN DMSP DYNAMICS IN DONA PAULA BAY

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DECLARATION

As required under the university ordinance 0.19.8 (iv), I state that the present thesis titled ***Phytoplankton and bacterial interaction in DMSP dynamics in Dona Paula Bay*** is an original research work and carried out by me at the National Institute of Oceanography, Dona Paula, Goa and that no part thereof has been published or submitted in part or in full, for any other degree or diploma in any university or institute. To the best of my knowledge the present study is the first comprehensive work of its kind from this study area.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed.

June 2015 SUNITA S. PANDEY

Dona Paula, Goa

CERTIFICATE

This is to certify that the thesis titled ***Phytoplankton and bacterial interaction in DMSP dynamics in Dona Paula Bay***, submitted by Ms. ***Sunita S. Pandey*** for the award of the degree of ***Doctor of Philosophy*** in ***Marine Sciences***, is based on her original studies carried out by her under my supervision for the partial fulfillment of the award of the Doctor of Philosophy, Department of Marine Science, Goa University. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any university or institution.

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Date: 29 June 2015

Place: Dona Paula

Dedicated to my loving family

"If I have seen further it is by standing on ye sholders of Giants"

~ Sir Isaac Newton

Contents

Acronyms

DMSP	Dimethylsulfoniopropionate
DMS	Dimethyl sulfide
DMS/P	DMS, DMSP
DMSPp	Particulate DMSP
DMSPd	Dissolved DMSP
DMSPt	Total DMSP (DMSPp + DMSPd)
DMSPu	DMSP utilizers
DMSPu-MPN	Most probable number of DMSP utilizers
DMSPu-CFU	Colony forming units of DMSP utilizers
Eh	Oxidation – reduction potential
µg	Micro gram
µM	Micro mole
mV	Milli Volt
mg	Milli gram
mL	Milli liter
mM	Milli mole
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
Tg	Terra gram
TOC	Total Organic Carbon
TIC	Total Inorganic Carbon

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Preface

Planet Earth is a complex, self-regulating and evolving system. The interactions of different spheres on the Earth at multiple levels enable the smooth functioning of this earth system. The Gaia theory invokes the self-regulating mechanism of the Earth system wherein the Earth functions as one organism to maintain the planet conducive for life. Reflections of the Gaia theory were envisaged in the challenging and controversial CLAW hypothesis by the landmark Nature paper of ***C****harlson,* ***L****ovelock,* ***A****ndreae and* ***W****arren in 1972. The hypothesis derives its name from the initials of each author and proposes that the volatile sulphur compound, dimethylsulfide (DMS) which is emitted from the oceans could help in increasing the albedo effect by subsequent oxidation and formation of cloud condensation nuclei in the atmosphere.*

This hypothesis provided a new direction and boosted the research on the marine sulfur cycle particularly the dynamics of DMS and its precursor dimethylsulfoniopropionate (DMSP), which was known to be a major sulphur compound produced by marine algae since 1948. The dynamics of both these compounds have been widely investigated ever since the introduction of CLAW hypothesis because of the possible role of DMS as an anti-greenhouse gas. The expanse of this research roughly covers the open oceans, coastal areas, polar ice zones etc. However, tropical estuarine ecosystems have not received much attention. It is also noteworthy that barring a few studies from France, Peru and Netherlands, the dynamics of DMS/P in the intertidal sediments have been hitherto overlooked.

The research work presented in this thesis was carried out with a broad aim to investigate the dynamics of DMS/P in the intertidal zone of tropical estuarine ecosystem. We have attempted to delineate the patterns of distribution of major DMSP producers and utilizers in the Dona Paula bay. The rhythms of DMS/P dynamics were studied at short term (tidal, diurnal) and long term (seasonal) scales. In addition, we have quantified the rate of DMSP production and utilization by phytoplankton and bacteria in field and environmental conditions and finally we elucidate the diversity of

major producers and utilizers of DMSP from this study area. Further, a short term study (at six hour interval for 5 days) in the post upwelling waters off Trivandrum was also carried out to understand the interactions happening in the coastal waters. The outline of the thesis is given below.

Chapter 1** presents a general **introduction** of the research topic and the scope of the thesis. **Chapter 2** comprises a comprehensive **review of published literature** in DMS /P research at international and national level. **Chapter 3** covers the description of **materials and methods** adopted for fulfilling the objectives of the thesis including the study area and sampling strategies. In **Chapter 4,** the **results** of our field observations in Dona Paula bay and laboratory mesocosm experiments are presented. The findings from our study are elaborated and **discussed** in **Chapter 5** of this thesis. The short term investigation carried out in the **coastal waters off Trivandrum** is separately presented in **Chapter 6**. In **Chapter 7,** we summarize the salient results, inferences and conclusions drawn from the study. We also add future scope of the research carried out. This is followed by end list **References, Appendix, and** a list of **Publications.

Introduction

In this chapter, we introduce the complex earth system interactions and how these interactions influence every aspect of this planet. Taking further from the Gaia hypothesis, we elaborate on the role of phytoplankton in ocean system regulation, specifically, stressing on their role in driving the marine sulphur cycle through dimethylsulfoniopropionate (DMSP) synthesis. We elaborate on the distribution of DMSP in marine algae, its functions, and its degradation into dimethyl sulfide (DMS) and other by-products. The role of bacteria is crucial in DMSP catabolism to liberate DMS. The interaction of phytoplankton and bacteria that drives DMS dynamics forms the crux of this chapter. We also describe the previous work on the distribution of DMS and DMSP in the marine and estuarine ecosystems stressing upon the fact that intertidal ecosystems have been hitherto neglected. In the light of available background information from previous research, we introduce the purpose of this thesis work, the problems addressed, and the possible outcomes from this research.

The earth system is a complex, self-regulating and evolving system in which different spheres viz. the atmosphere, biosphere, hydrosphere, and lithosphere interact with each other. The earth functions are a result of such interactions happening unceasingly at multiple levels. From the tiniest microbes to the gigantic life forms, all organisms interact with each other and cause various types of positive and negative effect on the environment. The Gaia theory proposes that this self-regulating system involves the atmosphere, the biosphere, the hydrosphere, and the pedosphere and suggested that the Earth functions as an organism to maintain homeostasis to keep the planet suitable for life. More than 70% of the earth is covered by the oceans, which comprise a large part of the hydrosphere. The oceans influence the transformation of energy and materials important to the climate system. The earth, with its atmosphere, greenhouse gases, ocean, life, winds, and currents all interact to produce our climate. DMS is a part of the Earth's ocean-atmosphere feedback loop, a climate stabilizing mechanism,

moderating temperatures on the Earth. **C**harlson **L**ovelock **A**ndreae and **W**arren proposed the CLAW (derived from initials of each author) hypothesis which invokes the self-regulating mechanism of the oceans in response to global warming. This response is facilitated through the production of DMS in the marine systems and its subsequent ventilation and oxidation to form sulfate aerosols in the atmosphere, which act as cloud condensation nuclei and increase the albedo effect.

1.1 Role of Phytoplankton in DMS/P variability

Phytoplankton are photosynthetic eukaryotes that are responsible for about 50% of photosynthesis on Earth. They serve as the base of the marine food web when they are consumed by higher eukaryotes. Besides playing a pivotal role in food web dynamics they are also central to the climatic processes. Marine phytoplankton is thought to play an important role in the Earth's energy balance. Previously, a direct negative forcing of climate (*i.e.* a reduction in the amount of incoming solar radiation absorbed by the ocean system) was believed to be caused by an increase in the amount of solar radiation returned to the atmosphere and space through backscattering by spatially extensive and highly reflective coccolithophore (phytoplankton) blooms in the ocean (*e.g.* Tyrell *et al*., 1999). However, this effect was found to be marginal and not enough to perturb global climate (Gondwe *et al*. 2001). Further, they are also responsible for production of a tertiary sulfonium compound DMSP which is the major precursor to DMS, a gas that may be linked to local climate regulation through aerosol production and cloud formation.

DMSP is produced in high concentration by certain species of marine algae and plant halophytes (Yoch, 2002 and references therein). The synthesis of DMSP originates from methionine in both plants and algae (Gage *et al.*, 1997). Though cellular concentration of DMSP varies among taxa, in marine algae it is synthesized from methionine to serve as a compatible solute, for osmoregulation, chemical signaling and excess sulphur regulation (Malin and Kirst, 1997; Wolfe *et al.,* 1997; Stefels, 2000). The species ability to form DMSP decreases in the following order Coccolithophores > Phaeocystis > Dinoflagellates > Diatoms (Liss *et al.,* 1993, Malin & Turner, 1993). Recently, juvenile corals have been added to the list of DMSP producers in response to

alleviated thermal stress (Raina *et al.,* 2013). DMSP may act as neutral solute in some micro-algal species in response to salinity changes (Kiene *et al.,* 1996). In cold environments DMSP may act as a cryoprotectant, (Sunda *et al.,* 2002) or just as an algal exudate as a part of overflow mechanism (Laroche *et al.,* 1999) or in nitrate-limited situations (Jones and Storey 1981). Recent studies suggest that DMSP may also play a role in improving the tolerance to variable carbonate chemistry (Archer *et al.,* 2013 and references therein), and help the algae to tolerate high solar radiation or iron deficient water by scavenging free radicals (Turner *et al.,* 1988). However, UV radiation could inhibit bacterial DMSPd (dissolved DMS) and DMS consumption rates (Slezak *et al*., 2007). In addition to marine algae *Wollastonia biflora*, the salt marsh grass *Spartina,* and some species of *Saccharum* (sugarcane) also produce DMSP. Tropical corals have been described as one of the most important benthic sources of DMSP and DMS in the coastal zone. However, most data are from the Great Barrier Reef (GBR), Australia (Raina *et al.,* 2009, 2013).

DMSP synthesis by marine photoautotrophs accounts for about 50 x 10^{12} moles of sulfur per year. DMSP synthesis is also important in the carbon cycle because each molecule of DMSP contains five atoms of carbon; hence its production is estimated to account for 3–10% of the global marine primary production (Kiene *et al.,* 2000). Its degradation supplies about 3–10% of the carbon requirements of heterotrophic bacteria in surface waters (Simó *et al.,* 2002). Concentrations of particulate DMSP (DMSPp) in the ocean are extremely variable, ranging from 5 to >300 nM, primarily due to strong spatio-temporal variability in phytoplankton species composition and biomass. In contrast, dissolved DMSP (DMSPd) concentrations were found to be relatively low, typically in tens of nM. However, concentrations as high as 200 nM have also been recorded in open ocean regions due to sudden release of DMSPd into the water at the end of an algal bloom.

DMS emissions from the surface ocean to the atmosphere range from 0.5 to 1.0 x 10^{12} moles per year (Kettle and Andreae, 2000). DMS and DMSP concentration are generally higher in coastal and shelf waters than in the open oceans, and highest

concentrations have been recorded during blooms of specific phytoplankton taxa, notably the prymnesiophytes *Phaeocystis pouchetii* and *Emiliania huxleyi,* and the dinoflagellate *Gyrodinium aureolum* (Barnard *et al.,* 1984, Turner *et al.*, 1988, Malin *et al.,* 1993, Matrai & Keller 1993).

1.2 Role of bacteria in DMS/P variability

Heterotrophic bacteria are ubiquitous scavengers that utilize organic carbon produced by diatoms and other autotrophs, thereby remineralizing a large portion of organic matter back to CO_2. Because of their abundance and high functional diversity, marine bacteria drive the biogeochemical cycles of most biologically relevant elements (carbon, nitrogen, sulfur, phosphorus, iron). Such interactions play a fundamental role in the ecology of marine food webs. Understanding interactions between bacteria and phytoplankton is crucial for studying oceanic nutrient fluxes and biogeochemical cycles. There are many mechanisms for production of DMS from DMSP; however, the most significant processes involved are algal senescence, bacterial activity, phytoplanktonic enzymes and zooplankton grazing (Dacey & Wakeham 1986, Nguyen *et al.*, 1988, Belviso *et al.,* 1990, Kiene & Service 1991, Wolfe & Kiene 1993, Kwint & Kramer 1995). Microbial action can work both directly on the algal cell, and indirectly in the water column, in the gut of predators, and on faecal material (Nriagu and Holdway, 1989; Wakeham and Dacey, 1989; Kiene, 1990). Viruses are also being recognized as an important contributor to cell mortality and ultimately DMS or DMSP release (Malin *et al.,* 1994).

Most DMSP catabolism occurs in marine bacteria by various mechanisms. Many strains use different pathways to demethylate DMSP to methylmercaptopropionate (MMPA) which does not liberate DMS. The dissolved DMSP can undergo at least 2 different metabolic transformation pathways, one yielding DMS and the other yielding methanethiol (Todd *et al.,* 2007, Todd *et al.* 2009, Howard et al. 2011). The marine bacterioplankton are capable of demethylating DMSP to a large extent due to the presence of the gene *dmdA (*Howard et al 2002, 2006). The pathway adopted by marine bacteria is thought to be controlled by the sulphur requirements of the cell (Kiene & Linn 2000). The other factors are the availability of DMSPd or other S-containing substrates

to meet the S demand for protein synthesis (Pinhassi *et al.,* 2005). Both could ultimately control the amount of DMS that can be produced, which in turn constrains the sea to-air DMS flux (del Valle *et al.,* 2012). Kieber *et al.,* (1996) investigated the rate of DMS emission and suggested that it depends directly on the concentration of DMS in surface water. Closely tied food web dynamics and physical factors such as air–sea exchange, water column mixing and photochemistry control the DMS concentration in surface water.

Only ~1% of the total DMS produced is released in to the sea-air boundary layer which eventually oxidizes to various compounds such as sulphur dioxide, dimethyl sulfoxide (DMSO), dimethyl sulfone, methanesulfonic acid and sulfuric acid and finds its way into the atmosphere. DMS is also produced naturally by bacterial transformation of DMSO waste that is disposed of into sewers, where it can cause environmental odour problems. The sulfate aerosols in the atmosphere act as cloud condensation nuclei. As per CLAW hypothesis, through this interaction with cloud formation, the massive production of atmospheric DMS over the oceans may have a significant impact on the Earth's climate.

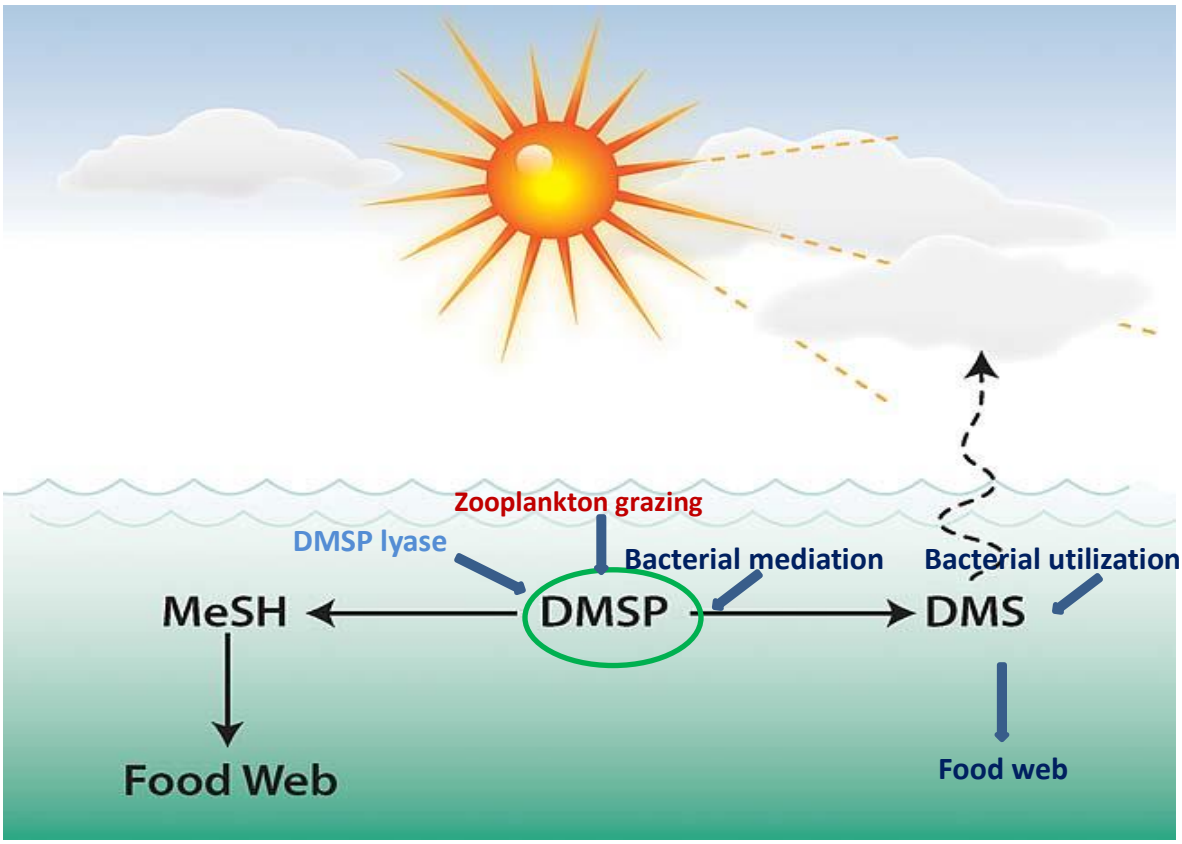


Figure 1: Schematic diagram of marine DMS cycle

(Image modified from: http://www.redorbit.com/news/science/2046995/)

1.3 Studies from coastal and estuarine ecosystems

Researchers have highlighted the role of estuaries and their plumes in the open sea as important sources of atmospheric DMS (Iverson *et al*., 1989; Turner *et al.,* 1996; Simo *et al.,* 1997). Although innumerable studies have been done on various aspects of the dynamics of DMS and DMSP in the global oceans, fewer reports are available from estuarine ecosystems and intertidal sediments.

The estuarine ecological environments are complex and highly variable compared to other marine environments. Tropical estuaries are characterized by clear dry and wet seasons and hence could be potential contributors of DMS and DMSP. The inflows of both sea water and fresh water provide high levels of nutrients in both the water column and sediment, making estuaries among the most productive natural habitats in the world. Intertidal beaches are dynamic environments, where the tidally generated

water movement and the associated processes of deposition and re-suspension of sediment affect the biotic components viz. diatom composition and distribution. In addition, hydrodynamic processes carry planktonic diatoms present in the ambient water to the intertidal sediment. The tidal wave also helps in mixing of saline and fresh water thus causing variation in salinity, which supports a variety of life in the estuarine ecosystem. The diversity of organisms that live in the estuary is limited by the harsh conditions. Temperature, salinity and light levels vary along the length of an estuary, and organic accumulation is common. Variations in salinity are affected by numerous influences, including temperature, dissolved gases, density and viscosity. Intertidal region of estuarine beach sediments are known to undergo physicochemical variations with tidal fluctuations. Semi-diurnal movement of tidal water alters biotic and abiotic environments in these intertidal sediments over short time intervals (Alongi, 1998).

Studies on such tropical estuarine sediments are very few, though DMS/P flux and variability have been probed from different habitats all over the globe. Nedwell *et al.* (1994) have stated that DMS and DMSP concentration in the North Sea sediment could be 1000 fold higher than in the overlying waters. Nevertheless, the concentrations were as low as 48nM in the sandy sediments. DMSP and DMS concentrations in the pore water of intertidal sediments of Ellewoutsdijk in the Westerschelde Estuary, The Netherlands were generally below or just above detection limit (Berjeigk *et al*., 2002), In the sediment pore water of upwelling area off Peru up to ~120nM DMS were detected (Andreae 1985). It was also noted that degradation of DMSP and DMS in sediment slurries was quite fast.

The Indian subcontinent experiences precipitation mainly from South West (SW) monsoon winds from June to September resulting in about 2700mm of rainfall (Chauhan *et. al.* 2011). Zuari estuary located along the central west coast of India is strongly influenced by the southwest monsoon and the changes associated with its onset have marked effects on the phytoplankton community, food web, and production (Devassy and Goes 1988; Bhattathiri *et. al.* 1976). All these factors make this ecosystem complex and highly variable as compared to other marine environments,

thus playing a pivotal role in the flux of various compounds to the atmosphere and to the sea. Also, intertidal sediments usually contain a high amount of DMSP and therefore represent environments with a potentially high emission of DMS (Bergeijk *et. al*. 2002). They contain high numbers of microorganisms that are involved in the production and consumption of DMSP and DMS (Jonkers *et*. *al.* 1998).

From the Indian context, Shenoy and Patil (2003, 2007) investigated variations in DMS and DMSP in bottom and surface seawater in Zuari estuary for a year. Mitbavkar and Anil (2006) focused on the diatoms community and their diversity at different tidal levels of the intertidal sediments of Dias beach. Kumar *et al.* (2009) also covered observations for a year in Dona Paula bay and related these parameters with other environmental variables along with 2-4 hourly observations to appreciate diel changes.

1.4 Aims and objectives of the present work

The present work focusses on the effect of tides and seasons on the dynamics of DMS/P of surfwater and sediment of the intertidal region. An attempt was made to study the phytoplankton and bacterial interaction in DMSP dynamics of Dona Paula bay situated at the mouth of Zuari River, which opens into the Arabian Sea. We examined the temporal variations in DMS/DMSP particularly focusing on the influence of tides and interactions of abiotic and biotic variables in the intertidal zone of Dona Paula bay. We also probed the semi-diurnal behavior of tide on the intertidal sediment and surfwater for similarities and differences. Further, the patterns of distribution of DMS, DMSP and its major producers and utilizers were elucidated. As DMS/P dynamics is governed by various environmental variables, an attempt was made to ascertain the major drivers throughout the seasons. The study included field measurements of DMS, DMSP and other relevant parameters that are known to affect the production of DMSP and the flux of DMS. The contribution of DMS from this study area in different seasons was estimated in terms of sea – air DMS flux. The field observations were complemented by laboratory experiments to get insights in to the role of major nutrients on the DMSP dynamics in mesocosm. Given the intricate interactions of abiotic and biotic factors in the intertidal zone of estuaries, it would be relevant to examine the fate of DMSP in order to understand the DMS emissions from these environments to the atmosphere.

Thus from this study we get an overview of the role of phytoplankton and bacteria in production of DMSP and release of DMS from the intertidal zone of a tropical estuarine beach. We also establish that intertidal beaches are potential sources of DMS to the atmosphere.

The study had the following objectives:

1. To delineate the patterns of distribution of phytoplankton and bacteria in sediment and surf water.
2. To quantify the rates of DMSP production by phytoplankton communities in relation to different environmental parameters in field and laboratory conditions.
3. To quantify the rates of DMSP utilization by bacteria in relation to different environmental parameters in field and laboratory.
4. To understand the diversity of major producers and utilizers.

We also made time-series observations at 6 hour interval for 5 days in the coastal waters off Trivandrum to get insights into the DMS/P dynamics in post upwelling waters.

Review of Literature

In this chapter, we present an extensive review of relevant literature on DMS/P research. We begin with the introduction of DMSP, its origin, function, and distribution in marine algae followed by a brief description of DMSP synthesis in marine algae. The role of phytoplankton and bacteria with reference to their interaction in governing the dynamics of DMSP and the subsequent flux of DMS to the atmosphere is the major highlight of this chapter. The pathways of DMSP synthesis and degradation to form DMS/methanethiol are also described. We also cover the diversity of DMSP producing phytoplankton and DMSP utilizing bacteria in the marine ecosystem. A section on DMS studies from the Indian waters highlights the major outcomes from the Indian context. The contribution of various researchers in terms of estimating DMS flux to the atmosphere is presented. We also touch upon the modelling studies. We have tried to identify some major lacunae in this research area and propose a few aspects that need to be addressed in future.

2.1 DMSP – distribution and functions

DMSP is a tertiary sulfur compound produced in high concentrations by certain species of marine algae and plant halophytes for the regulation of their internal osmotic environment, for cryoprotection and/or as an antioxidant (Yoch, 2002). This zwitterionic metabolite is found in marine phytoplankton, seaweeds, and some species of terrestrial and aquatic vascular plants (Yoch 2002 and references therein) and juvenile corals (Raina *et. al.,* 2013). Intracellular concentrations of DMSP in high DMSP producers range from 100 to 500 mM. Other phytoplankton groups, such as Prasinophytes, diatoms and cyanobacteria generally produce low amounts of DMSP, intracellular concentrations ranging from 1 to 100 mM (Keller *et al.,* 1989, Stefels 2000). Keller *et al.,* (1988, 1989) have established that dinoflagellates are significant sources of DMSP. Most recently, Caruana and Malin (2014) confirmed the role of dinoflagellates as

significant DMSP producers in their extensive study. In addition to marine algae *Wollastonia biflora*, the salt marsh grass *Spartina,* and some species of *Saccharum* (sugarcane) also produce DMSP. Most data from the Great Barrier Reef, Australia indicate that tropical corals are one of the most important benthic sources of DMSP and DMS in the coastal zone (Raina *et al.,* 2009, 2013). DMSP was first reported by Challenger and Simpson (1948) in an extract of the red algae (*Polysiphonia lanosa*) and subsequently Challenger (1959) discovered that DMSP reacts with cold aqueous alkali to produce DMS and acrylic acid in a 1:1 ratio. Kiene *et al.,* (1996) have reported that some microalgal species contain a high percentage of intercellular DMSP. This compound may act as a neutral solute that reacts minimally with the contents of the cell while protecting it from drying out, or in the cell's response to salinity changes. Laroche *et al.,* (1999) reviewed various studies and suggested that exudation may also be as important as cell autolysis in the release of DMSP during *Phaeocystis* blooms, although it has rarely been considered as a DMSP liberation mechanism before. Malin and Kirst, (1997); Wolfe *et al.,* (1997); Stefels, (2000) presented evidences for the synthesis of DMSP from methionine by an unicellular algae as a compatible solute and showed that it played important physiological roles in osmoregulation, chemical signalling and excess sulfur regulation. Wyn Jones and Storey (1981) studied the nitrogen analogue of DMSP, glycine betaine, and concluded that these were involved in the osmoregulation of certain plant cells.

DMSP and its degradation products: acrylate, DMS and DMSO also act as anti – oxidants in marine phytoplankton protecting photosystems from oxidative damage caused by hydroxyl radicals and other reactive oxygen species (Sunda *et. al.,* 2002). It has also been suggested that DMSP acts as an antioxidant to tolerate high solar radiation or iron deficient water (Turner *et al.,* 1988). More recently, Husband and Kiene (2007) reported that field populations of *S. alterniflora* contained DMSO and had higher DMSO: DMSP ratios in roots and senescent leaves, suggesting *in vivo* oxidation of DMSP to DMSO and a possible antioxidant function for DMSP in these higher plants. Nitrate and phosphate limitation may also influence the levels of DMSP in phytoplankton (Stefels and van Boekel 1993). However, UV radiation could inhibit the consumption rates of bacterial DMSPd and DMS (Slezak *et al.,* 2007). Archer *et al.,* (2013) have

shown that intracellular DMSP may play a role in improving tolerance to variable carbonate chemistry. DMSP has also been implicated in influencing the taste and odour characteristics of various products. For example, although DMSP is odourless and tasteless, it is accumulated at high levels in some marine herbivores or filter feeders. Increased growth rates, vigour and stress resistance among animals cultivated on such diets have been reported (DeBose *et al.,* 2008, Nevitt *et al.,* 1995).

2.2 DMSP – synthesis and catabolism

The synthesis of DMSP originates from methionine in both plants and algae (Gage *et al.,* 1997) by the following pathway

Methionine ⟶ 4-methylthio-2-oxobutyrate ⟶ 4-methylthio-2-hydroxybutyrate ⟶ 4 dimethylsulfonio-2-hydroxybutyrate ⟶ DMSP

The cleavage of DMSP under different conditions releases DMS in the seawater. Turner *et al.,* (1988) suggested that DMSP is likely to be released into seawater when algae lyse or leak due to osmotic stress, zooplankton grazing, or other processes. While some marine algae (including *Phaeocystis antarctica*) can directly catalyze the breakdown of intracellular DMSP to DMS, field studies indicate that most of DMSP cleavage is due to bacterial metabolism of the dissolved DMSP (Kiene *et al.,* 2000; Simo and Pedros - Alio, 1999). Various studies (Toole *et al.,* 2006; Galí *et al.,* 2011; Lizotte *et al.,* 2012; Miles *et al.,* 2012) highlight that light-driven processes are key to the biogeochemical cycling of DMS and its precursor DMSP. The degradation of DMSP to release DMS was primarily attributed to bacterial mediation (Reed, 1983; Keller, 1988). However, recent work with laboratory cultures suggests that phytoplankton may also be an important source of DMS due to biological reduction of DMSO in aerobic marine environments (Spiese *et al.,* 2009). This process has been observed in a variety of phytoplankton and bacterial species as well as in natural plankton communities (Hatton and Wilson, 2007; Simo *et al.,* 1998; Simo and Vila-Costa, 2006; Vila-Costa *et al.,* 2008).

Dimethylsulfide (DMS) production from DMSP has long been associated with the activity of the enzyme DMSP lyase according to the following reaction:

Equation 1

$$(CH_3)_2S^+CH_2CH_2COO^- \rightarrow CH_3SCH_3 + CH_2=CHCOO^- + H^+$$

DMSP DMS Acrylate

The other major pathway to degrade DMSP is the demethylation (equation 2 and 3) to produce 3-mercaptopropionate (3-MPA) or the intermediate 3-methiolpropionate (MMPA) which can be demethiolated to yield methanethiol (reaction 4) by microbes in anoxic sediments and in seawater (Yoch, 2002).

Equation 2

$$(CH_3)_2S^+CH_2CH_2COO^- \rightarrow CH_3SCH_2CH_2COO^- + XCH_3$$

DMSP MMPA

Equation 3

$$CH_3SCH_2CH_2COO^- \rightarrow HSCH_2CH_2COO^- + XCH_3$$

MMPA 3-MPA

Equation 4

$$CH_3SCH_2CH_2COO^- \rightarrow CH_3SH + CH_2=CHCOO^{-*}$$

MMPA MeSH Acrylate

In the second and the third equation, the nature of the X-CH_3 compound depends of the organism producing methanethiol. It has been shown that it was methyl-tetrahydrofuran in the majority of bacteria studied such as *Ruegeria pomeroyi* (Reisch *et al.,* 2011), *Pelagibacter ubique* or *Silicibacter pomeroyi* (Reisch *et al.,* 2008).

In the last reaction, the product marked with an asterisk is suspected but not proven to be the other product of demethiolation. Methanethiol appears to be particularly important as a source of sulfur for marine bacteria and perhaps some eukaryotic

organisms. Moreover, atmospheric DMS is rapidly oxidized to aerosol sulfates via methanesulfonic acid (CH_3SO_3H) and sulfur dioxide (SO_2). These sulfur-containing aerosols can absorb and scatter incoming radiation, altering global radiation budget and finish by falling to earth in acid rain. These mechanisms are represented in Equation 4.
Most if the DMSP catabolism occurring in the sea is due to different types of marine bacteria (Kiene and Taylor, 1988; Zubkov *et al.,* 2001). The pathway adopted by marine bacteria is thought to be controlled by the sulfur requirements of the cell (Kiene & Linn 2000). According to the 'S' demand hypothesis of Vila Costa *et. al.,* (2014) DMSP assimilation rates should increase when total bacterial activity is stimulated. Many strains use different pathways to demethylate DMSP to methylmercaptopropionate (MMPA) which does not liberate DMS. In another study from coastal waters of Alabama, Kiene (1996) concluded that DMSP degradation was primarily by bacterial size fractions (<1 μM). Also, the alga-associated, i.e., particulate DMSP (DMSPp), when released into the marine environment as dissolved DMSP (DMSPd), serve as a link between primary production and the microbial population, as it can be readily degraded by chemoheterotrophic bacteria (Kiene, 1990).

Detailed studies on DMSP uptake and cleavage have been carried out on isolates from each α-, β-, and γ-subclasses of the *Proteobacteria* (Yoch, 2002). In α-proteobacterial isolate (strain LFR), DMSP uptake preceded production of DMS and the accumulation of DMSP in the cytosol confirmed the intracellular location of the lyase. In the β-subclass isolate *Alcaligenes faecalis* M3A, the DMSP lyase and acrylase were shown to be extracellular in spite of same metabolism. The DMSP lyase and acrylase of the γ-subclass isolate *Pseudomonas doudoroffii* were shown to be only intracellular (Gonzalez *et al.,* 1996). The first DMSP-degrading microbial isolate able to produce DMS, methanethiol, 3-methiolpropionate, or to accumulate DMSP were all of undetermined phylogeny. However, a study of culturable lignin-utilizing bacteria by Gonzalez *et al.,* (1996) indicated that certain phylogenetic groups might be rich in DMSP-degraders. Jonkers *et al.,* (1999) were the first to report the complete oxidation of dimethyl sulfide (DMS) to sulfate by an anoxygenic, phototrophic purple sulfur bacterium *Thiocapsa roseopersicina*. DMSP lyases have been purified from four

species of bacteria; having a K_m for DMSP in the range of 0.5 to 2.25 mM. All these values are many orders of magnitude higher than the levels of DMSPd found in oceanic waters and sediments that range from 2 to 20 nM (Yoch 2002 and references therein).

Ansede *et al.,* (2001) proposed a putative model that best fits the experimental data regarding the pathway of DMSP and acrylate metabolism in α -proteobacterium, strain LFR. They suggest the growth of LFR strains on acrylate versus that on glucose stimulates the rate of acrylate metabolism eightfold, indicating that it acts as an inducer of acrylase activity. Cantoni and Anderson (1956) demonstrated that the products of DMSP cleavage catalyzed by partially isolated lyase from *Polysiphonia janosa* are DMS and acrylic acid. A bacterial isolate from the subdivision of the Roseobacter subgroup obtained from the coastal waters suggested that this class of bacteria represented a prominent lineage of DMS producers and play an important role in the marine sulfur cycle (Gonzalez *et al.,* 1999). Curson *et al.,* (2011) have described our current understanding of the genetic basis of these processes in several classes of bacteria. They provide molecular insights into earlier biochemical and physiological studies on DMSP catabolism particularly the remarkable diversity of this process in terms of the organisms that undertake it, the types of enzymes that they use and the ways that they regulate the process.

2.3 DMS distribution

DMS was found to be universally present in seawater (Lovelock *et al.,* 1972). They detected that this compound rather than H_2S from the coastal waters and mud flats was the most abundant sulfur compound. They also suggested that DMS was the missing gaseous sulfur compound that enabled the steady state flow of sulfur between marine and terrestrial environment, making DMS emission a key step in the global sulfur cycle.

DMS is thought to play a role in climate regulation through its atmospheric oxidation products methane sulfonic acid and sulfuric acid and to act as a carrier of sulfur from the marine to the terrestrial environment. The sea-to-air flux represents 50% of the global biogenic sulfur flux to the atmosphere (Verlancar and Desai, 2005). It has been suggested that oceanic DMS emissions may act as a biological climate feedback mechanism (Charlson *et al.,* 1987) and recent modeling studies have predicted climate-

dependent changes in the marine DMS cycle (Bopp *et al.,* 2003; Bopp *et al.,* 2004; Cameron-Smith *et al.,* 2011; Gabric *et al.,* 2001; Gabric *et al.,* 2004; Vallina and Simo, 2007). On the basis of its concentration and turnover, dimethylsulfide (DMS) is one of the most important biogenic sulfur compounds in the marine environment. It accounts for over 50 % of the total biogenic sulfur (1011 kg S) entering the atmosphere annually, and about 90 % of the DMS originating from marine sources (Andreae 1990). Some of the highest marine DMS concentrations are reported from polar-regions, where water temperatures below 01°C (Kettle *et al.,* 1999),

The rate of DMS emission depends directly on the concentration of DMS in surface water, which is controlled by complex production and removal processes that are closely tied to food web dynamics and physical factors such as air–sea exchange, water column mixing and photochemistry (Kieber *et al.,* 1996). DMS is formed largely in the oceans and much of it is released to the atmosphere, an estimated 38–40 Tg yr^{-1}, thus contributing up to 40 million metric tonnes per annum (Lomans *et al.,* (2002). DMS concentrations in seawater vary considerably both spatially and temporally and coastal and shelf waters often contain higher concentrations of volatile sulfur than open oceans (Holligan *et al.,* 1987) with open oceans emitting DMS in the range of 0.5 to 1.0 x 10^{12} moles per year (Kettle and Andreae 2000). DMS concentrations in oligotrophic region can reach concentrations of 5–12 nM, even though phytoplankton biomass and DMSP levels there are low. In salt marsh sediment, base hydrolysable DMS of ca. 200 µM has been reported (Kiene 1988). However, a recent study by Quinn and Bates (2011) shows that a direct biological control of dimethylsulfide over cloud condensation nuclei does probably not exist and that sources of these nuclei to the marine boundary layer and the response of clouds to changes in aerosol are much more complex. Nevertheless, these aspects need to be probed more intensively before the postulated role of DMS can be ruled out.

2.4 Interactions between biotic and abiotic variables

Various researchers have established the significance of phytoplankton and bacterial interactions as important drivers of DMS/P dynamics in the marine ecosystem. DMSP in marine algae not only depend largely on phytoplankton taxa, but also environmental

conditions which can cause significant variability in intracellular DMSP content. Due to its biogenic origin, the fate of DMSP is controlled by the factors influencing the biological community (Simo *et al.,* 1997). The biological drivers of DMSP dynamics viz. phytoplankton and bacteria are known to exhibit short and long term rhythms and are influenced by major nutrients (C, N, P, and Si) and micro nutrients (Fe, Zn, Mn, Co) chemistry.

Anderson *et al.,* (2001) studied the positive correlation between chlorophyll a and bacterial numbers in DMSP-producing phytoplankton blooms where intracellular DMSP can range from 0.01 to 100 mM and they suggest that DMSP and acrylate could be an important carbon source for bacterioplankton. Otte *et al.,* (2004) suggested that when DMSP is released from marine algae, following grazing or viral attack, they become available for subsequent microbial catabolic conversions, some of which release DMS. Andreae & Barnard (1984) compared the range of chlorophyll and DMS in several cases and found a close resemblance between the vertical distribution of DMS and chlorophyll a in marine water columns. This suggested that phytoplankton was responsible for the emission of DMS. Coastal phytoplankton assemblages in river plumes are typically dominated by diatoms, which are known to have relatively low DMSP per unit biomass thus corroborating the species – specific nature of DMSP (Keller *et al.,* 1989). The DMSP synthesizing ability of different phytoplankton groups have been estimated by normalizing DMSP concentrations to chlorophyll and a ratio of $\geq$100 nM mg^{-1} is typical of oligotrophic open ocean waters, whereas in coastal, estuarine or ice-edge waters it ranges from 3–30 nM mg^{-1} (Kiene *et al.,* 2000). The change in ratio indicates seasonal shift in the phytoplankton community composition (Vogt *et al.,* 2008). Studies from oligotrophic and coastal waters in temperate to subtropical zones exhibit a strong seasonal trend with maximum DMS concentrations measured in summer which was ~2 months later than the maximum concentration of DMSP, and Chl a is at its annual minimum (e.g. Dacey *et al.,* 1998, Uher *et al.,* 2000). Simó & Pedrós-Alió (1999) have called it the "DMS summer paradox". In another study from the Mediterranean coast, Vallina and Simo (2007) observed strong seasonal trend in DMS where incident solar irradiance correlated with surface water DMS concentrations. Cerquira and Pio (1999) in their study from intertidal mudflats of Canal

de Mira in Portugal reported strong seasonal variations in the DMS emission rates and attributed the summer peaks in DMS emissions to ambient temperature. However, in laboratory cultures of *Navicula* and *Nitzschia*, in terms of DMSP production to cold temperature no significant response was observed (Kasamatsu *et al.,* 2004).

More recently, Arnold *et al.,* (2013) in their laboratory mesocosm study on *Emiliania huxleyi* cultures reported that slight increase in temperature decreases the solubility of DMS in the water and leads to higher flux to the air. Recent study by Varaljay *et al.,* (2012) suggested that the DMSP degradation correlated with environmental variables like primary production, photosynthetically active radiation, DMSPp, and DMS concentrations. They conclude that SAR11 bacterioplankton dominate DMSP cycling in the upper ocean oligotrophic North Pacific Subtropical Gyre with lesser but consistent involvement of other members of the bacterioplankton community. Dacey and Wakeham (1986) suggested that grazing by zooplankton caused an increase in DMS output from phytoplankton cultures and they added that it might be an important mechanism of DMS and DMSP release in the sea. The production rates were approximately 24 times higher than those of phytoplankton alone, giving rise to DMS concentrations of about 200 nM which would be sufficient to support the growth of DMS-utilizing bacteria. They have shown that when herbivorous zooplankton is introduced into algal cultures considerably more DMS is produced. The autolytic processes related to algal senescence and zooplankton grazing also releases DMSP in the water column or sediment pore water (Dacey and Wakeham 1993).

2.5 Mesocosm experiments

Various researchers have investigated the production of DMSP by marine phytoplankton in cultures (Bucciarelli *et al.,* 2013; Kasamatsu *et al.,* 2004; Keller *et al.,* 1989; Matrai and Keller 1994; Keller and Bellows 1996; Sheets and Rhodes 1996, and Keller *et al.,* 1999). DMSP production has been shown in axenic cultures of various benthic diatoms isolated from intertidal sediments, including from the Schelde estuary (van Bergeijk & Stal 1996, Jonkers *et al.,* 1998). Nitrogen limitation may stimulate DMSP production, because it would lead to replacement of nitrogen-containing

osmolyte such as glycine betaine and proline, by DMSP (Andreae 1986, Turner *et al.,* 1988). This was confirmed by several studies (Turner *et al.,* 1988, Gröne & Kirst 1992, Keller *et. al.,* 1999). Cleavage of dissolved DMSP to DMS has been observed in marine bacterial cultures and aerobic seawater incubations (Dacey & Blough 1987, Wakeham *et al.,* 1987, Kiene 1990, Kiene & Service 1991, Taylor & Gilchrist 1991, Ledyard *et al.,* 1993) and has also been documented in an axenic *Phaeocystis* sp. as well (Stefels & van Boekel 1993).

Marine bacteria play a major role in the degradation of DMSP via DMS production and demethylation (Gonzalez *et al.,* 1999, 2000; Kiene and Linn, 2000) and this depends on the composition of the microbial community (Iverson *et al.,* 1989). The pathway adopted by marine bacteria is thought to be controlled by the sulfur requirements of the cell (Kiene & Linn 2000). The other factors are the availability of DMSPd or other S-containing substrates to meet the S demand for protein synthesis (Pinhassi *et al.,* 2005). Both could ultimately control the amount of DMS that can be produced, which in turn constrains the sea to-air DMS flux (del Valle *et al.,* 2012).

The availability of heavy metals has profound influence on the phytoplankton species and subsequently on the community structure. Iron (Fe) is a crucially essential element for the growth of phytoplankton and bacteria (Loper and Buyer, 1991) and plays an important role in some host–bacteria interactions (Mila *et al.,* 1996). It is also required for nitrogen fixation and reduction of nitrate, nitrite and sulfate. Laboratory studies involving metal ion buffers pointed to the possible role of deficiencies in one or more bioactive trace metals (Fe, Mn, Co, Ni, Cu and Zn) and this may play a role in controlling the production of phytoplankton in the oceans (Anderson and Morel, 1982, Sunda *et al.,* 1995). Little attention has been paid to the possible limitation of aquatic bacterial growth and metabolism by available iron. Studies have found that the addition of Fe2+ stimulated the bacterial growth. In addition to phytoplankton, bacteria also appear to benefit from increased iron concentrations. Iron is a cofactor of several enzymes and acts in transport processes and redox reactions. In addition, iron is required for a variety of functions in microorganisms that grow under aerobic conditions. It is important to note that the direct impact of iron-enrichment has not yet been studied

on DMSP metabolism kinetics. But several other compounds have been tested like acetylene, a known inhibitor of numerous enzyme reactions (Charlson *et al.,* 1987) and at different gradients of NaCl (Reisch *et al.,* 2008).

Low iron concentrations apparently affected the iron-rich electron transfer system. As yet, little attention has been paid to the possible limitation of aquatic bacterial growth and metabolism by available iron. The metabolic requirements of heterotrophic and other bacteria for iron have long been recognized (Berman, 1993). For example, the IronEx (II) experiment in the North Eastern Equatorial Pacific documented increase in bacterial abundance in presence of iron along with phytoplankton numbers though it was not clear whether iron addition directly or indirectly stimulated bacterial growth (Cochlan 2001). The effect of iron on the heterotrophic bacterial community was characterized in Sub arctic Ecosystem Response to Iron Enrichment Study, in the high nutrient low chlorophyll region of the Northeast subarctic Pacific, during July 2002. The study showed that there were significant changes in the bacterial dynamics in the iron-fertilized patch compared to the unfertilized patch (Wong and Crawford 2006). Few studies examining iron limitation of heterotrophic bacteria have reached contradictory conclusions. Pakulski *et al.,* (1996) found that biomass production of heterotrophic bacteria in the Southern Ocean (Gerlache Strait) was stimulated by addition of iron, whereas Church *et al.,* (2000) found that organic carbon but not iron additions stimulated bacterial production in experiments conducted along a transect south of Tasmania to the Antarctic Polar Front.

2.6 DMS/P research in coastal and estuarine ecosystems

Although tropical estuarine beach ecosystems are dynamic environments, where the tidally generated water movement and the associated processes of deposition and re-suspension of sediment affect the biotic components, diatom composition and distribution (Mithbavkar PhD thesis, 2003), studies focusing on DMS/P variability and flux are scarce. Belviso *et al.,* (2006) have highlighted the role of vertical flux as a source for sediment DMSP in northern Europe. Though the variability and flux of DMS from open oceans has been covered widely, studies from tropical estuaries and

intertidal sediments are few. However, researchers have highlighted the role of estuaries and their plumes in the open sea as important sources of atmospheric DMS (Iverson *et al.,* 1989; Turner *et al.,* 1996; Simo *et al.,* 1997). DMS and DMSP concentration in the North Sea sediment could be 1000 fold higher than in the overlying waters. Nevertheless, the concentrations were as low as 48 nM in the sandy sediments (Nedwell *et al.,* 1994).

DMSP and DMS concentrations in the pore water of intertidal sediments of Ellewoutsdijk in the Westerschelde Estuary, The Netherlands were generally below or just above detection limit (Berjeigk *et al.,* 2002) on the other hand, a maximum of ~120nM DMS was measured in the sediment pore waters of upwelling area off Peru (Andreae, 1985). They also noted that degradation of DMSP and DMS in sediment slurries was quite fast. Kulkarni *et al.,* (2005) have also recorded higher concentration of DMSP in the seawater during high tide in a salt marsh in South Carolina, USA. Studies by van der Maarel & Hansen (1997) have shown that in anoxic intertidal sediment, the demethylation of DMSP indirectly or directly leads to the formation of methane. Visscher *et al.,* (2003) have noted that this could be due to methylation of sulfide formed by the reaction of low-molecular-weight organic carbon and biogenic hydrogen sulfide derived from sulfate reduction. Belviso *et al.,* (2006) suggest that DMS in the sediment originates from DMSP sedimentation from top, and DMS production from the sediment-water interface below. Further, they state that the distribution of DMS/P concentrations in sediments of North Sea reflected the distribution of sedimentary organic matter (1998). Studies by Levine *et al.,* (2012) indicate that carbon availability may not be the primary process regulating bacterial DMS production.

From the Indian waters, Shenoy and Patil (2003, 2007) examined the monthly variations in DMS and DMSP in bottom and surface seawater in Zuari estuary for a year and reported high temporal variations in DMS and DMSP with maximal concentrations during the southwest monsoon coinciding with a dinoflagellate bloom. Rodekar, and Wagh, (2000), in their study on planktonic diatoms of the Zuari estuary, Goa (west coast of India) concluded that minimum number of diatoms was recorded during monsoon and

maximum during post monsoon and pre-monsoon months. Solitary forms were found in abundant quality, while colonial forms were few. Occasional blooms of *Chaetoceros* sp. were also observed. The diatoms of Dona Paula bay have been covered in detail in Mitbavkar and Anil (2006) Kumar *et al.,* (2009) also covered observations for a year in Dona Paula bay and related these parameters with other environmental variables along with 2-4 hourly observations to appreciate diel changes and found that the diel variation of DMSPt (total DMSP) was influenced more by biological variables than hydrographic parameters. However, to the best of our knowledge the influence of tides on the dynamics of DMS/P in the intertidal ecosystem has not been studied. Also, no attempt has been made to explore the role of intertidal beaches in terms of DMS flux to the atmosphere from the tropical estuarine environment.

2.7 Questions that need to be addressed

The review by Quinn and Bates (2011) suspected the impact of DMS on global climate as there is a lack of evidence for oceanic DMS production on climate-relevant time scales. It is known that generally 1% of total DMSP production by phytoplankton results in a DMS flux to the atmosphere, and the relation between DMSP production and DMS yield varies with geography. Consequently, the flux of DMS also varies as elaborated in the following table:

Table 1: Flux of DMS from different geographical locations

Area	**Average flux ($\mu molSm^{-2}d^{-1}$)**	**Reference**
North East Pacific Ocean	1.6	Wong *et al.,* (2005)
North Pacific Ocean	2.3	Sharma *et al.,* (1999)
West North Pacific Ocean	3.4	Berresheim *et al.,* (1991)
North Sea	3.84	Turner *et al.,* (1996)
Arctic Polar Ocean (Summer)	2.0	Leck and Persson (1996)
Amsterdam Island	2.1	Nguyen *et al.,* (1990)

South China Sea	5.5	Yang (2000)
East China Sea	3.4	Yang *et al.,* (2000)
Arabian Sea	3.5	Shenoy and Kumar (2007)
Bay of Bengal	9.63	Shenoy and Kumar (2007)
Central Indian Ocean	3.5	Shenoy and Kumar (2007)
Indian Ocean (total)	5.42	Shenoy and Kumar (2007)

The conditions under which DMSP production is stimulated are not yet clear. Plenty of evidence for seasonal and diel variability in DMSP related to light is available in literature. Moreover, sparing a few studies, time-series data for DMS/DMSP variability is not available.

Predictive modelling remains main concern as the major source areas like coastal and ice zones are poorly represented in Earth System Models (ESM). Very few DMS climatology models are available. The first and most widely accepted model was the K00 model by Kettle and Andreae 2000 which was based on 17000 data points. Most recently, Lana *et al.,* (2011), predicted flux of ~23 Tg sulfur from the sea to the atmosphere derived from 47000 data points. However, they also stressed that many regions including the Southern Indian Ocean are poorly represented in the global database. A preliminary estimate by Cameron-Smith *et al.,* 2011 predicted up to 150% increase in average flux of DMS to the atmosphere from particular regions of the Southern Ocean (Cameron-Smith *et al.,* 2011).

To meet some of the limitations of predictive modelling, it is also essential to have a DMSP and DMS database coupled with environmental parameters in different marine niches. This can be achieved by more intense and dedicated time-series observations covering diel, seasonal, annual and decadal scales. Also, the widely used models have a very high resolution (~200 km), which are suitable to open ocean grids and may not be able to justify the variability in the coastal areas.

Materials & Methods

In this chapter, we begin with the description of our study area, followed by detailed sampling strategy. We elaborate on the methods used for measuring environmental parameters which are relevant to DMS/P dynamics in the intertidal ecosystem along with DMS/P, producers (chlorophyll a and phytoplankton abundance) and utilizers (TC, TVC, MPN and plate counts) of DMSP. Further, we provide details on the estimation of sea–air flux and sediment–pore water diffusive flux of DMS. α and β diversity indices of phytoplankton have been included. To ascertain the identities of the most abundant utilizers, we performed biochemical tests and 16S rDNA sequencing of the selected dominant isolates. We also made an attempt to estimate the abundance of genes responsible for demethylation (*dmdA*) and DMS production (*dddR, dddD*) by Fluorescent In-situ Hybridization (FISH) in the surfwater and sediment. To complement the field observations, we also performed mesocosm experiments to measure the influence of macro (nitrate and phosphate) and micro (iron) nutrients on the production of DMSP by selected diatom isolates and utilization of DMSP by indigenous bacterial community. Experimental set up is described here while the composition of medium is appended. In the final paragraph, we provide information on the software used for data analysis.

3.1 Study area

The study area chosen is a sand flat at Dias Beach (15° 27′ N; 73° 48′ E), near Dona Paula Bay, surrounded by the Zuari estuary. This beach is about 200 m in length and is sheltered and protected on both sides by rocky cliffs (Figure 2).

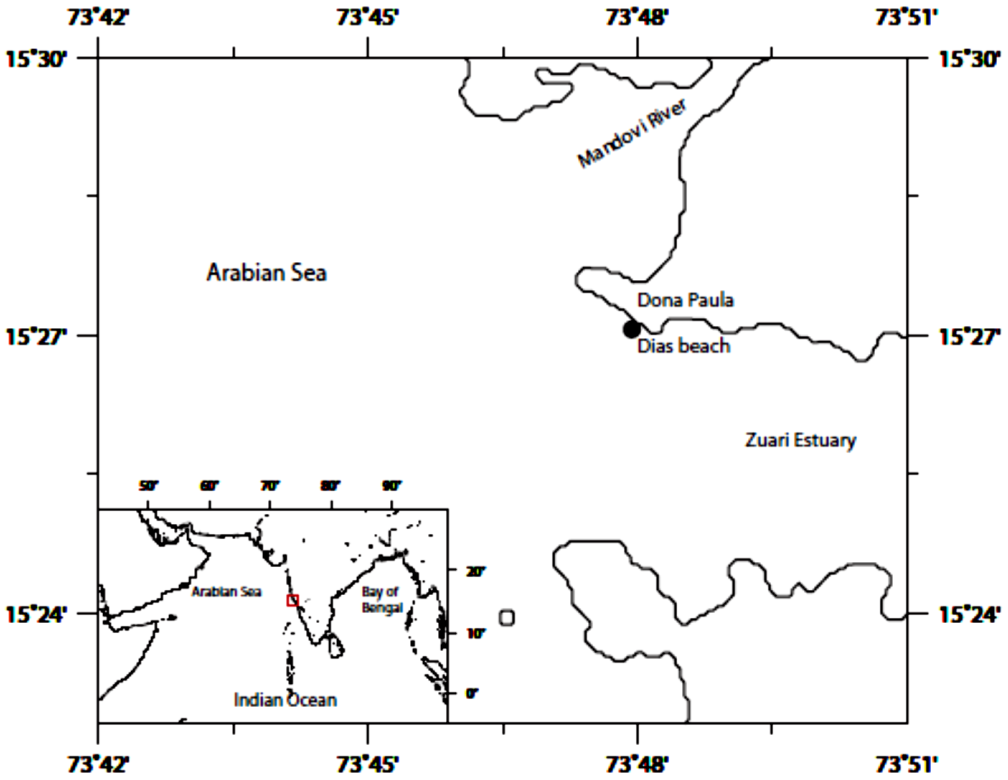


Figure 2: Study area, Dona Paula bay

3.2 Sampling strategy

Surfwater and sediment samples were collected in triplicates from the intertidal zone of the beach covering successive high tides and low tides every month from March 2010 to December 2011(except August & September 2011; Table 2). The monthly sampling dates were fixed after taking the tides into consideration and included spring and neap tides of each month. Surfwater samples were collected in acid washed PVC bottles and sediment samples (0–5 cm) were collected using a hand-corer with an inner diameter of 4.5 cm. All the samples were brought to the laboratory in an icebox for further analysis. Interstitial water samples were collected by removing the sediment (15 cm) with a shovel and allowing the water to collect. The water samples were allowed to stand for a few seconds to allow sand particles to settle.

Table 2: Sampling frequency

Month	Sampling frequency	No. of samples (Surfwater)	No. of samples (Sediment)
March'10	4	4	4
April'10	12	12	12
May'10	6	6	6
June'10	4	4	4
July'10	6	6	5
August'10	6	6	6
September'10	6	6	5
October'10	6	6	6
November'10	6	6	6
December'10	6	6	6
January'11	6	6	6
February'11	6	6	6
March'11	6	6	6
April'11	6	6	6
May'11	6	6	6
June'11	6	6	6
July'11	6	6	6
August'11	0	0	0
September'11	0	0	0
October'11	6	6	6
November'11	6	6	6
December'11	6	6	6
Total		122 X 3(replicates) = 366	120 X 3(replicates) = 360

3.3 Sample analyses

3.3.1 Environmental variables

Data on the tides at Dona Paula were accessed from the Indian Tide table (2010, 2011). Temperatures of the surfwater and sediment were recorded at the study site using a mercury bulb thermometer, and salinity was measured with the help of a hand held refractometer (ATAGO 2442- W01) which was calibrated to zero with distilled water prior sampling.

Light intensity was measured using TES digital Lux Meter. Samples for dissolved oxygen estimation were fixed on site with Winkler A and Winkler B reagents and further analysed in the laboratory. pH of the samples were measured in the laboratory using a

pH meter (Elico, LI614) after calibrating it with the standard buffers of pH 7 and 9.2 (HiMedia) respectively within few minutes of sample collection. For Eh measurements, the electrode was dipped slowly into the sediment cores and allowed to stabilize for 60 s. The direct reading obtained by mV meter was taken in triplicate and the mean values were determined. Before the measurements, the Eh electrode was rinsed with distilled water and calibrated with oxidation reduction potential (ORP) standard solution to ensure accurate readings.

Estimation of nutrients such as ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), phosphate (PO_4-P) and silicate (SiO_3) were carried out by standard procedures (Parsons *et al*. 1984). Total inorganic carbon (TIC) was analysed using a UIC CM 5014 Coulometer with $CaCO_3$ as a standard and total carbon with an NCS 2500 elemental analyser (Patience, et al 1990), cross-checked with a UIC CM 5014 Coulometer. Total organic carbon (TOC) was determined indirectly by subtracting the values of TIC from total carbon (Gupta and Jauhari, 1994).

3.3.1.1 Dissolved Oxygen (DO)

The chemical determination of oxygen concentration is based on the method first proposed by Winkler (1888) and modified by Strickland and Parsons 1968, Grasshoff *et al*. 1983.The basis of the method is that the oxygen in the water sample is made to oxidize iodine ion to iodine quantitatively; and the amount of iodine generated is determined by titration with a standard thiosulfate solution. In a strong alkaline medium, the DO of the water sample reacts with manganese hydroxide (II) and oxidizes it to manganese hydroxide (III). When the precipitate is well settled the samples are acidified to pH 2.5 to liberate the manganese ion, which is a strong oxidizing agent in an acidic medium. The iodide is oxidized liberating free iodine which forms a complex with excess iodide ions. In the final step, the iodine complex is titrated against a standard thiosulphate solution using starch as indicator.

The samples were collected to the brim in glass bottles, fixed with reagents Winkler A (0.5 mL) and Winkler B (0.5 mL) (Please refer Appendix for details) and immediately

mixed after stoppering. Care was taken to avoid air bubbles while collecting water samples. Precipitate was allowed to settle and stored in dark until subsequent analysis in the laboratory by titration with standardized thiosulphate solution. The values were expressed as mL L^{-1}.

3.3.1.2 Ammonia

Ammonia concentration was measured by spectrophotometric method as mentioned in Parsons *et al* 1984, where in the sample was treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside that acts as a catalyst. The blue indophenols formed with ammonia are measured at 885nM, spectrophotometer (UV – 2450, Shimadzu).

3.3.1.3 Nitrite

The determination of nitrite is based on method of Strickland and Parsons (1968). Nitrite reacts with sulfanilamide in an acid solution resulting in a diazonium compound. This is then coupled with N-(-1-Napthyl1)-ethylenediamine dihydrochloride to form a colored azo dye, the extinction of which can be measured spectrophotometrically at 543 nM.

3.3.1.4 Nitrate

Nitrate in seawater was quantitatively reduced to nitrite by heterogeneous reaction involving cadmium - mercury amalgam. When the sample is passed through the reductor column, nitrate is quantitatively reduced to nitrite and the nitrite in the sample passes unchanged. Nitrite is subsequently measured by colorimetry via formation of an azo dye. Hence the measured absorbance is due to the initial nitrite in sample and nitrite obtained after reduction of nitrate. Therefore a correction is made for any nitrite initially present in the sample. During the reduction stage a buffering agent is added to the sample to maintain a stable pH. Absorbance was measured against distilled water at 543 nM. Calibration curve was obtained with known amounts of nitrate standards to get the concentration–absorbance relationship.

3.3.1.5 Phosphate

The method is based on the reaction of the phosphate ions with acidified molybdate reagent to give phospho-molybdic acid which is then reduced to a highly colored molybdenum blue compound by a suitable reducing reagent i.e. ascorbic acid. The absorbance of the compound is measured by spectrophotometer at 885 nM.

3.3.1.6 Silicate

The determination of reactive silicate is based on the method of Strickland and Parson (1984). A sea water sample is allowed to react with ammonium molybdate under conditions which result in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate complexes. A reducing agent of metal and oxalic acid is added and silicomolybdate is reduced to a silicomolybdous acid with a blue color, the absorbance of which is measured spectrophotometrically 885 nM.

3.3.2 Dimethylsulfoniopropionate and Dimethyl sulfide

DMSP was measured by cold alkaline hydrolysis method after its conversion to DMS (Turner *et. al.,* 1990, Kumar *et. al.,* 2009) In brief, 22 mL of sample was fixed in headspace vial with 2 mL of 10 N NaOH (pH 14) and kept overnight for complete conversion of DMSP to DMS. These samples were then introduced in a purge and trap equipment (Telydene, Tekmar) and purged with nitrogen for 11 min for complete removal of DMS from the sample which was subsequently cryo-trapped and later transferred to Gas Chromatograph (Shimadzu, 2010) attached to the purge and trap equipment. The GC was equipped with a flame photometric detector set at 150 °C, Chromosil 330 column was used for separation and was set at 40 °C. The detection limit for the GC was 0.05 nM. Calibration standards were analysed using the same protocol with DMSP salt procured from Research Plus, USA (Visscher *et al.,* 1992). DMS was measured directly from the sample by purging with nitrogen gas for 11 minutes without addition of base. For measuring DMSP from the sediments, slurry of 1 g sediment was prepared in autoclaved, filtered seawater. Further processing of the sample was same as with surfwater.

3.3.3 DMS flux

The sea–air flux of DMS is expressed according to Liss and Merlivat (1986) as follows:

$\underline{Flux_{(DMS)} = K_w . \Delta DMS}$

where K_w = piston velocity / gas exchange coefficient (cm h^{-1})

Δ DMS = DMS concentration difference across the air – sea interface.

$\underline{Kw = \{(0.333* u) + (0.222 * (u)^2\} X (Sc/600)^{-0.5}}$ (Nightingale *et. al.* (2000)

where u = averaged wind speed at 10m height (m s^{-1})

$\underline{Sc \text{ (Schmidt number)} = 2674.0 – 147.12 (T) + 3.726 (T)^2 – 0.038 (T)^3}$ (as calculated by Saltzman *et al*, 1993)

Flux of DMS from sediment to pore water

The diffusive flux of pore water gas from sediment is calculated by Fick's law of diffusion

$J = -\phi D_s (dc/dz)$; where J = diffusive flux, ϕ = porosity, D_s = sediment diffusion co-efficient, dc/dz = concentration change for the gas with depth

$\underline{\text{For seawater, }} \mathbf{D_{DMS} \text{ (in } cm^2 sec^{-1}) = 0.020 \exp(-18.1/RT)}$**, (Saltzman et al 1993)**

Where $R = 8.314 \times 10^{-3}$ kJ $mole^{-1}$ K^{-1}, *T* is temperature in Kelvin.

$\underline{\text{For sediment,}}$

$Ds = D_{DMS} / [1 + n (1- \phi)]$

3.3.4 Producers

3.3.4.1 Chlorophyll *a* and Phaeophytin

Chlorophyll *a* was estimated based on the fluorometric method by Yentsch and Menzel (1963). A known amount of water sample (250 mL) containing chlorophyll pigments was filtered in diffused light through glass fiber filter (Whatman GF/F). Pigments concentrated on the filter were extracted for >24 h in ice cold 90% (v/v) acetone and was determined fluorometrically using Turner Designs fluorometer 10-000R at an excitation wavelength of 430 nM and an emission wavelength of 670 nM. Standardization was carried out by using chlorophyll *a* standards (Sigma, USA). For estimation of phaeopigments, the above sample was acidified with two drops of 1.2 M

HCl and determined fluorometrically. Both chlorophyll *a* and phaeopigment values are expressed as µg l^{-1}.

For estimating chl a and phaeopigments content in the sediment, 1 gram sediment was mixed with 10mL of 90% acetone (v/v) and the pigments were extracted in ice cold conditions as followed for seawater. Both chlorophyll *a* and phaeopigment values are expressed as µg g^{-1} wet sediment.

3.3.4.2 Phytoplankton abundance and identification

Phytoplankton abundance was estimated by Utermohl's method. In brief, 250 mL water samples were fixed with Lugol's solution (Hasle, 1978). Lugol's iodine was added in a ratio of 1:100 parts of the seawater sample. Prior to microscopic analysis, samples were concentrated to 5–10 mL by carefully siphoning the top layer with a tube covered by 11 µm Nitex filter on one end. For estimating the abundance of phytoplankton in sediment, 1 g of wet sediment was taken and diluted with 10 mL of sterile seawater and fixed with Lugol's iodine solution and later visualized under microscope. Phytoplankton cell density was estimated by using Sedgwick Rafter Cell. Replicates of the samples were counted and average values were taken into account for the calculation. The total number of phytoplankton present in a liter of water sample was calculated using the formula

$N = n \times v \times 1000 / V$

Where, N is the total number of phytoplankton cells per liter of water filtered,

n = average number of phytoplankton cells in 1 mL of plankton samples,

v = volume of plankton concentrate (mL),

V = Volume of total water filtered.

Generic and species identification of phytoplankton was done by their gross morphology, special structures and shape according to the keys provided by Subramanyan (1946, 1968), Subramanyan and Sarma (1961), Lebour (1978), Desikachary and Ranjithadevi (1986), Desikachary and Prema (1987), Desikachary *et al.* (1987), Tomas, (1997).

3.3.4.3 Phytoplankton diversity indices

We measured alpha diversity (a) as richness, by the Shannon-Wiener Index.

Shannon-Wiener Index denoted by H = -SUM [(pi) × ln(pi)]

SUM = summation

pi = proportion of total sample represented by species i

Divide no. of individuals of species i by total number of samples

S = number of species, = species richness

Hmax = ln(S) Maximum diversity possible

E = Evenness = H/Hmax

Evenness (Shannon–Wiener/log (richness)),

We calculated beta diversity (b) of the phytoplankton encountered in all samplings following Whittaker (1960)

(bw = (S/a) - 1, S = total number of the species in the system, a = average species richness).

3.3.5 Utilizers

3.3.5.1 Total Counts (TC)

Samples for total bacterial counts were fixed immediately with buffered formalin and stored at 4 °C upon arrival to the laboratory. Bacterial abundance was determined by acridine orange direct count method (Hobbie *et al.* 1977). In brief, water samples (2 mL) and sediment (1 g) preserved with 2% (final concentration) buffered formalin were stained with acridine orange (Hi-Media, Mumbai) (final concentration 0.01% w/v) for five minutes before filtering it through 0.22 um black stained Nuclepore filter (Whatman Asia Pacific, Singapore). Samples were enumerated at 1000X magnification in an Olympus (BX61) epifluorescence microscope, using a 515 nm barrier filter and at least 10 fields of >30 bacteria per field. Bacterial abundance is expressed as numbers per liter or numbers per gram dry sediment.

3.3.5.2 Total Viable Counts (TVC)

For estimating TVC in water and sediment samples the method of Kogure *et al.* (1984) was followed. Briefly, samples were incubated at room temperature for 8 h with nalidixic acid (0.02% w/v), piromidic acid (0.001% w/v), pipemidic acid (0.01%w/v) (Sigma, USA) and yeast extract (0.01%). Incubation was terminated by the addition of buffered formalin (final concentration 2%). Samples were stained with acridine orange (as mentioned for TC) and observed under epifluorescence microscope. Only swollen and elongated cells were enumerated as viable bacterial cells and their counts are expressed as numbers per liter or numbers per gram dry sediment.

3.3.5.3 Utilizers of dimethylsulphoniopropionate - MPN method

DMSP utilizing bacterial abundance was estimated by MPN method (hence forth denoted as DMSPu–MPN) by serially diluting the samples and inoculating them in mineral medium supplemented with 100 uM DMSP. Modified mineral medium of Kumar *et al*. (2009) and Visscher *et al*. (1991, 1992) was used for enumerating the 'DMSP' utilizers by Most Probable Number (MPN) method. The medium consisted of the following (in g L^{-1}): NaCl (25.0), NH_4Cl (0.2), $CaCl_2.2H_2O$ (0.225), KCl (0.2), $MgCl_2.6H_2O$ (0.2), anhydrous KH_2PO_4 (0.02), and anhydrous Na_2CO_3 (2.0), supplemented with vitamin B12 (20 pg L^{-1}), $FeSO_4.7H_2O$ (1 mg L^{-1}), trace element solution (1 mL L^{-1}). The pH of the medium was adjusted to 7.5. This medium was prepared in distilled water and supplemented with 1 uM of DMSP salt. MPN of DMSP utilizers were determined after 12 weeks of incubation in the dark according to Bacterial abundance is expressed as numbers per liter or numbers per gram dry sediment. All tubes were also examined for DMS production by estimating DMS concentration by gas chromatography.

3.3.5.6 Utilizers of dimethylsulphoniopropionate - Surface plate method

For the enumeration of colony forming units of DMSP utilizers (henceforth denoted as DMSPu – CFU) surfwater and sediment samples (12 each) were serially diluted in autoclaved seawater blanks. 100 µL (10 or 100 fold dilution) of the sample was surface plated on and subsequent plating on Mineral Media agar plates supplemented with 10 uM of DMSP salt. The plates were incubated at ambient temperature 28 ± 2 °C for 48 h and the DMSP utilizers were expressed as Colony Forming Units (CFU mL^{-1}, g^{-1})

3.3.5 Bacterial Identification

3.3.5.1 Biochemical characteristics

The most abundant DMSP utilizers retrieved from the agar plates were isolated based on their morphological characters and microscopic evaluation. The isolates were stored in NA slant tubes at 4 °C for taxonomic identification and characterization. The physiological and biochemical properties were examined according to standard methods. The bacterial isolates were identified up to generic level following the scheme of Oliver (1982).

Following biochemical tests were performed on the selected isolates:

a. Gram Staining (Gram, 1884)
b. KOH test
c. Motility (Wet Mount method)
d. Oxidase Test (Kovac's 1956)
e. Catalase Test
f. MOF Test (Hugh and Leifson's oxidation- fermentation test, 1983)

Please refer to Appendix for details.

3.3.5.2 16S rDNA sequencing and identification of bacterial isolates

The bacterial isolates were further subjected to 16S rDNA sequencing. In brief, total genomic DNA of the bacterial isolates was extracted by the phenol-chloroform method modified from Ausubel *et al.* (1987) and guanindine thiocyanate method by Pitcher et al (1989). The small-subunit 16S rRNA gene (0.5 g) was amplified using 10 pmol of each of the two primers 27F and 1492R in a total volume of 50 uL containing 0.25 uL Taq DNA polymerase, 4dNTP mix, 5 uL of master AMP Taq PCR buffer, pH 8.8, 25 mM $MgCl_2$, 12.75 µl sterile water. The amplification of the gene was carried out in a hot – air rapid thermocycler programmed for 40 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 1 min and extension at 72 °C for 1 min and final extension of 5 minutes at 72 °C. The amplified DNA was separated on 1% agarose gel and eluted for purification using QIAGEN PCR purification kit using their protocol. The purified products were outsourced for sequencing.

3.4 Abundance of DMSP degradation and DMS producing genes by CARD FISH

The abundance of DMSP utilizing genes in the sediment and surfwater was estimated by the Catalyzed Reporter Deposition – Fluorescent In-Situ Hybridization (CARD–FISH) method Pernthaler (2002, 2007). To permeabilize the cells, filters were treated with lysozyme (37 ºC, 1 h) and then with Achromopeptidase (37 ºC, 30 min). HRP labelled probes for the DMSP degradation genes dddDf (5′-ACCAACGTCATTGCAGGACC-3′) and dddDr (5′-TGTGCGTGTTCTTCCGGTG-3′), dddRf (5′-GGCGCGCAGCCAGTTCAG-3′) and dddRr (5′-GGCTATGAGGAGGGCTGG-3′) (Raina et al 2009) and dmdAf (5'-ATTGCCGACTCGGATGTTCT-3') and dmdAr (5'-CAAGAAGGTCAAACATGGCAAAC-3') (Varaljay et al 2010) were used. Counterstaining of CARD-FISH preparations was done with 4,6-diamidino-2-phenylindole (DAPI,1 µg mL^{-1}). In this method, the cells positive for the presence of the target genes (stained by Cy3) as well as total bacterial cells (stained by DAPI) can be visualized by epifluorescence microscopy. Filters were counted manually (1000X magnification) under Olympus (BX61) microscope.

3.5 Mesocosm experiments

3.5.1 Effect of Iron amendment on DMSP production by phytoplankton community

250 mL of whole seawater was incubated in laboratory mesocosm to study the effect of 10 mM Fe on the DMSP production by phytoplankton community. Appropriate controls were maintained for reference. Light and temperature conditions were maintained at ambient levels.

3.5.2 Effect of nutrient amendment on DMSP production by phytoplankton cultures

Four isolates of diatoms, identified at the generic level, were isolated and cultured in f/2 media in laboratory mesocosm and further studied for DMSP production in presence of macro and micro nutrients (1 uM P, 10 uM NO_3, 10 mM Fe). Appropriate controls were maintained for reference. Light and temperature conditions were maintained at ambient levels.

Media for phytoplankton growth: f/2 medium (Guillard and Ryther 1963) (Please refer Appendix)

3.5.3 Effect of nutrient amendment on DMSP Utilization by bacterial community

DMSP utilization by indigenous bacterial community was checked under varying nutrient concentration (1 uM P, 10 uM NO_3, 10mM Fe). The bacterial suspension was inoculated into 20 mL headspace vials containing mineral medium amended with DMSP (10 µM). All the experiments were conducted in triplicates. DMSP utilization was measured after conversion to acrylate by alkaline treatment. Appropriate controls were maintained for reference. Light and temperature conditions were maintained at ambient levels.

3.6 Data Analysis

Microsoft excel data analysis package (2010) was used for preliminary data analysis. *SURFER*11 was used for plotting the graphs in the Chapter 6. Multivariate analyses were performed using the statistical package Stat Soft Inc. STATISTICA for Windows version 6 and PRIMER version 6 (Clarke and Warwick, 1994).

Results

In this chapter, we present the salient findings of the field observations and mesocosm experiments. We looked for short term/tidal rhythms (March–May 2010) and seasonal rhythms (March 2010 – December 2011) in DMS/P and allied variables in the field. The section on field observation is presented accordingly. Within each section, we describe the environmental, biological parameters, DMSP, DMS, and DMS flux from the study area. This is followed by details of results from statistical analyses (correlations and principal component analyses) which highlight the interactions and ascertain the drivers of the DMS/P dynamics in the study area. After statistical analyses, we move on to the identification of dominant DMSP utilizers from surfwater and sediment by biochemical tests and 16S rDNA sequencing. We also present the results of CARD-FISH for estimating abundance of DMSP degrading genes. This is followed by the results from mesocosm experiments carried out for measuring the influence of macro (nitrate and phosphate) and micro (Iron) nutrients on the DMSP production and utilization by phytoplankton and bacteria respectively in the laboratory.

4.1 Field – Tidal Observations:

4.1.1 Environmental variables:

The tide data for the study area was accessed from the Indian Tide Table 2010. The height of low tides ranged from 0.05 to 1.64 m and high tides ranged from 0.87 to 2.24 m during the study period. The average of all high and low tides during sampling along-with the highest and lowest tide heights are given in Table 3. Data collected during high and low tides during regular spring and neap tides did not show remarkable difference. These variations had mixed influence on the physico-chemical and biological parameters. On few occasions the parameters significantly varied with the tide while on other hand no clear relationship was found.

Table 3: Characteristics of tide during the March – May 2010

	High tide (m)	Low tide (m)
Average	1.79	0.68
Highest	2.24	1.64
Lowest	0.87	0.05

Tidal influence on temperature was not discernible. Temperature of sediment (T2) was slightly higher than that of surf water (Table 4). Salinity varied from 26–34 in surfwater and 29–35 in sediment. The fluctuation in salinity and temperature in both surfwater and sediment were generally synchronized. Average pH of surfwater (7.85) was higher by 0.17 than sediment (7.68). Ammonium concentrations varied from below detection limit to 0.06 µM in surfwater and from 0.01 to 0.06 µM in sediment. Nitrate and silicate concentrations were higher in sediment pore water as compared to surfwater and higher values generally synchronized with high tide. Average nitrite and phosphate concentrations were marginally higher in surfwater. The dissolved oxygen values in pore water usually followed a tidal rhythm, with generally higher values during high tide and ranged from 4.21 to 4.65 mL^{-1}. In surfwater, maximum dissolved oxygen was not too low at 3.66 mL^{-1} during high tide; however, no clear tidal synchronization was observed. (Figure 3)

Table 4: Average values of physicochemical and biological variables in intertidal sediment and surf water along with their sd values

Variable	Surf water ± sd		Sediment ± sd	
Light (lux)	4.40E+05	± 0.01	4.40E+05	± 0.01
Temperature (°C)	30.75	± 1.75	31.13	± 2.09
Salinity	30.67	± 1.97	33.32	± 1.43
pH	7.86	± 0.24	7.86	± 0.24
Eh (mV)	-	-	-101.18	± 14.09
DO (ml L^{-1})	4.40	± 0.51	2.83	± 0.98
Nitrate (µM)	0.71	± 0.11	1.57	± 0.56
Nitrite (µM)	0.31	± 0.18	0.56	± 0.15
Phosphate (µM)	0.10	± 0.04	0.24	± 0.14
Silicate (µM)	3.89	± 1.31	7.28	± 1.34
Ammonia (µM)	0.02	± 0.009	0.02	± 0.01
TOC (µMml^{-1}, g^{-1})	244.75	± 150.75	440	± 155
DMSP (nM)	5.13	± 1.69	51.95	± 14.82
DMS (nM)	1.38	± 0.68	6.75	± 2.11
Chlorophyll *a* (µg L^{-1}, g^{-1})	2.00	± 0.18	5.40	± 2.44
Phaeophytin (µg L^{-1}, g^{-1})	0.36	± 0.19	1.07	± 0.7
Phytoplankton (cells L^{-1}, g^{-1})	3.46E+05	± 3.31	4.72E+05	± 3.05
TC(cells ml^{-1}, g^{-1})	4.81E+06	± 1.53	1.36E+07	± 2.08
TVC(cells ml^{-1}, g^{-1})	9.20E+05	± 2.24	4.67E+04	± 2.61
CFU(cells ml^{-1}, g^{-1})	3.56E+04	± 2.11	2.98E+04	± 2.92
MPN(cells ml^{-1}, g^{-1})	5.47E+03	± 2.56	4.77E+03	± 2.96
DMSP:DMS	4.71	± 2.47	7.69	± 4.99

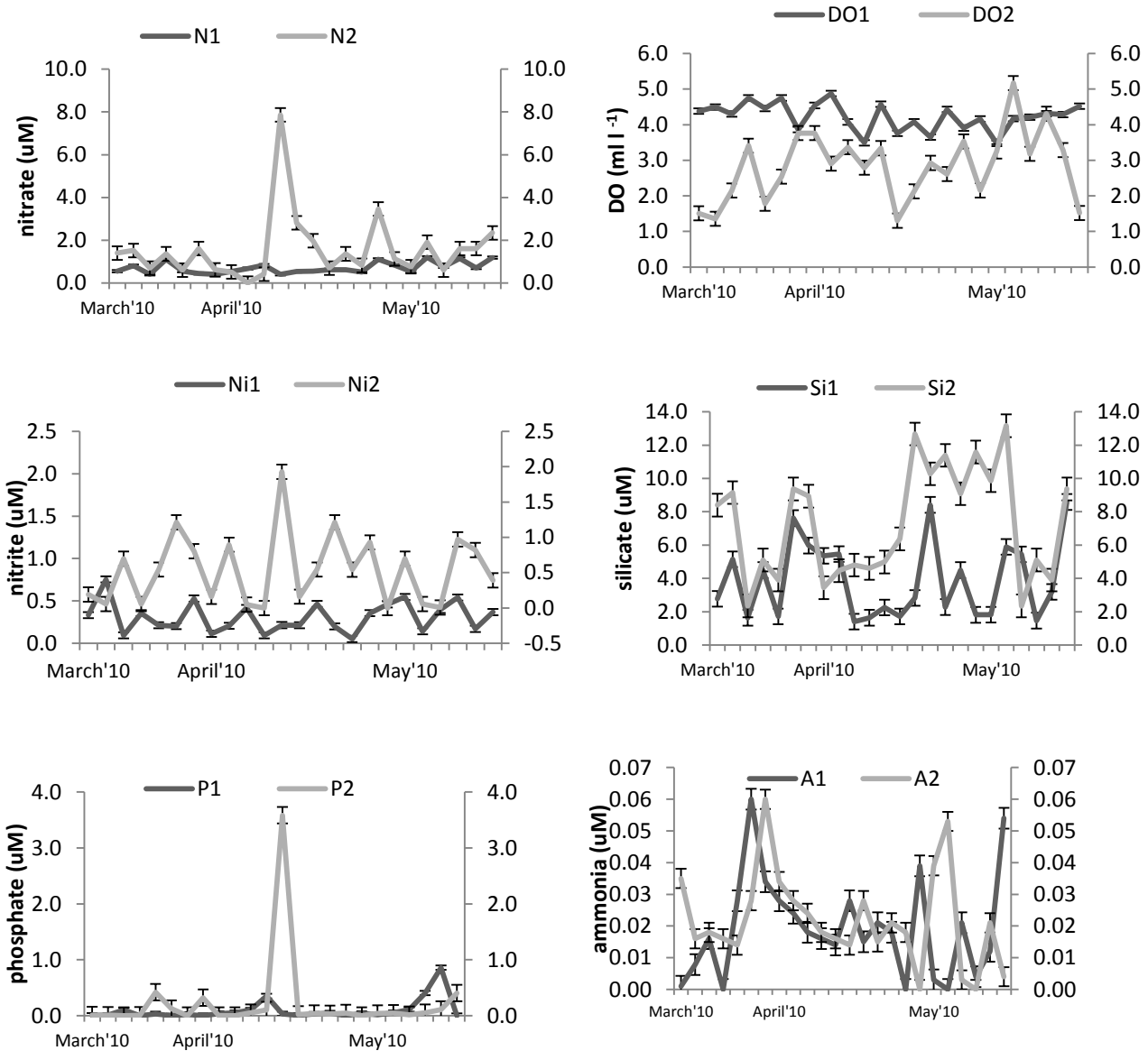


Figure 3: Variability in temperature in surfwater and sediment (T1, T2), salinity in surfwater and sediment (S1, S2), nitrate in surfwater and sediment (N1, N2), dissolved oxygen in surfwater and sediment (DO1, DO2), nitrite in surfwater and sediment (Ni1, Ni2), phosphate in surfwater and sediment (P1, P2), silicate in surfwater and sediment (Si1, Si2), ammonia in surfwater and sediment (A1, A2)

4.1.2 DMS/P

The average DMSP concentration was ~10 times higher in the sediment (51.95 nM) than in surfwater (5.13 nM) and usually synchronized with high tide levels during the study period (Figure 4). The corresponding average DMS concentrations were 6.75 nM and 1.09 nM in sediment and surfwater, respectively (Figure 5). Maximum concentration of DMSP and DMS were 72.51 nM and 10.95 nM respectively in sediment while in surfwater the highest values were 8.06 and 2.33nM respectively during low tide. In sediment, DMS generally varied with tide height with higher values during high tide. This trend was not very conspicuous in surfwater.

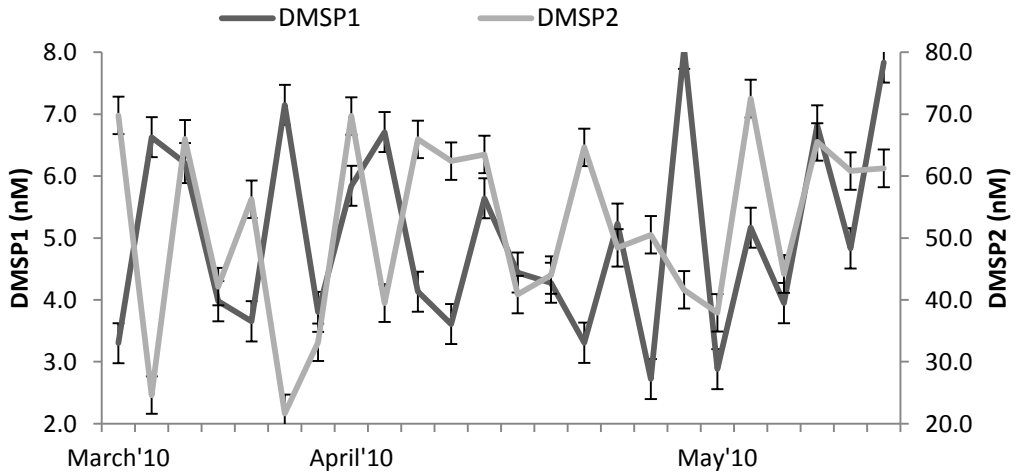


Figure 4: Variability in DMSP in surfwater (DMSP1) and sediment (DMSP2)

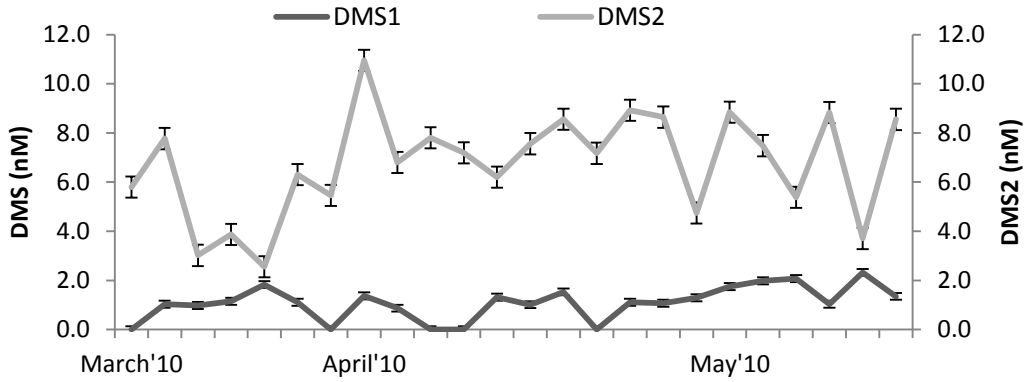


Figure 5: Variability in DMS in surfwater (DMSP1) and sediment (DMSP2)

4.1.3 DMS flux

The sea–air flux of DMS depended on the wind speed which was <3.5 ms^{-1} during the study period. The average flux was highest in the month of April at 2.57 µM $m^{-2}day^{-1}$ when the wind speed ranged from 0 to 5.5 ms^{-1}. In sediment, the diffusive flux of DMS from sediment to porewater varied from 0.08 to 0.343 µM $m^{-2}day^{-1}$.

4.1.4 Producers

4.1.4.1 Chlorophyll a and Phaeopigments

Phytoplankton biomass estimated as chlorophyll *a* (Chl *a*) followed contrasting patterns during the study period in surfwater and sediment. In the initial phase i.e. March till mid-April, the low tide and high tide values in surfwater did not synchronize while mid-April onwards till the end of May, the Chl *a* concentrations followed a tidal rhythm. In sediment this trend was just the opposite as Chl *a* concentration followed a tidal rhythm in March till mid – April after which we see an inverse trend (Figure 6).

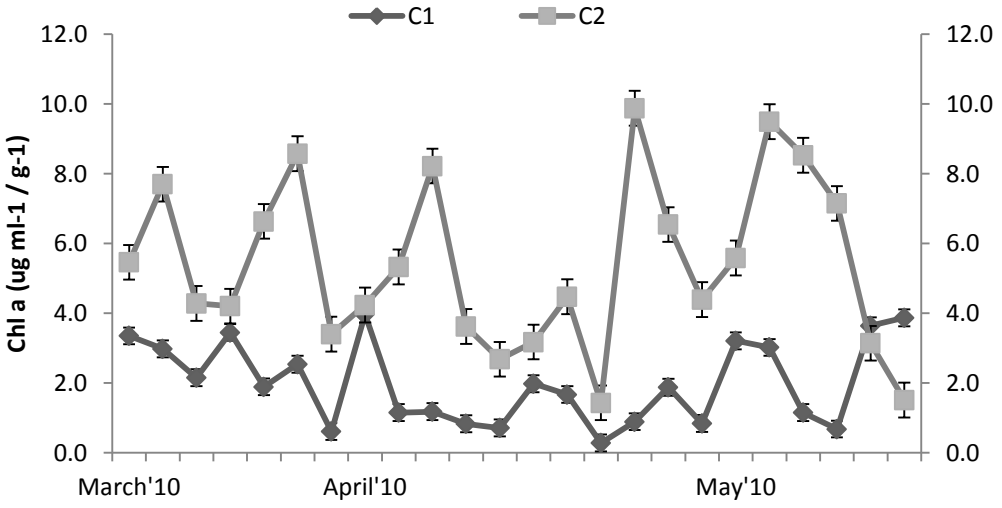


Figure 6: Variability in chlorophyll a in surfwater (C1) and sediment (C2)

4.1.4.2 Phytoplankton abundance

Phytoplankton abundance was higher in the sediment as compared to surfwater. The values were generally higher during high tide in the surfwater while in sediment the trend was inverse.

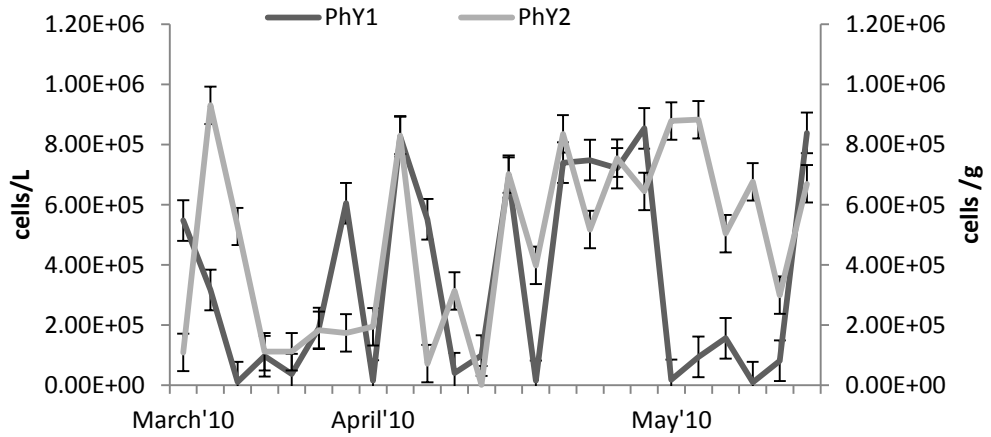


Figure 7: Variability in phytoplankton abundance in surfwater (P1) and sediment (P2)

Diatoms dominated the phytoplankton diversity in the surfwater and sediment during the study period. In surfwater, 34 species (23 centric, 11 pennate) of phytoplankton were found and the highest phytoplankton diversity was represented by three diatom species viz. *Navicula (33%), Odontella (12%),* and *Thalassiosira (7%)*. Dinoflagellates formed around 1 – 2% of the population.

The sediment harbored 30 species of diatoms (11 centric, 19 pennate) belonging to 20 genera. Pennate diatoms were dominant in terms of abundance. The most abundant pennate diatoms were *Amphora* and *Navicula*, whereas, *Thalassiosira* was the dominant centric diatom.

4.1.5 Utilizers

4.1.5.1 Total counts of bacteria

Total direct counts (TDC) of bacteria in both sediment and surfwater were generally constant and did not show any particular trend. In surfwater, the average TDC and varied between 0.78 - 3.6 × 10^7 cells mL^{-1}. The TDC was one order of magnitude higher for the sediment and varied between 0.459 - 9.94 × 10^8 cells g^{-1} dry weight (Figure 8).

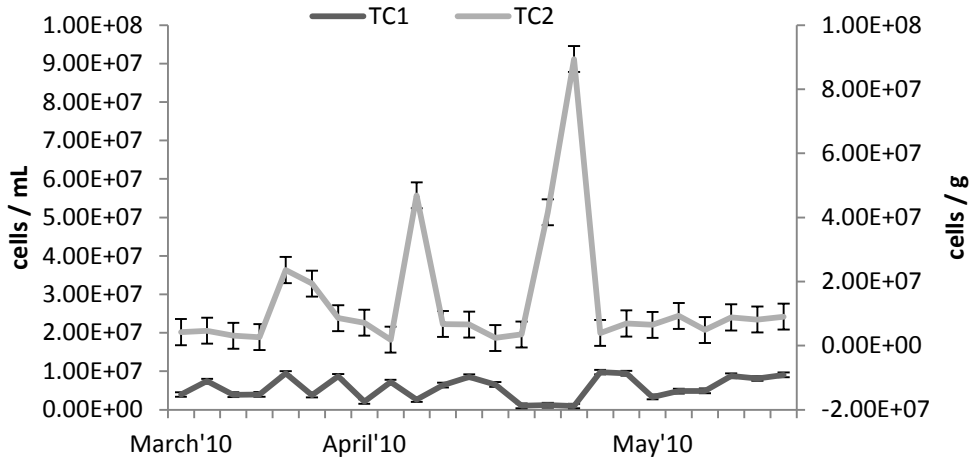


Figure 8: Variability in total bacterial counts in surfwater (TC1) and sediment (TC2)

4.1.5.2 DMSPu – MPN, CFU

MPN of DMSP utilizers varied from 0.108 – 9.38 × 10^3cells mL^{-1} in surfwater. The values were generally constant and did not exhibit any tidal influence (Figure 9). In sediment, although no clear pattern was seen in the MPN of DMSP utilizing bacteria, the CFU of DMSP utilizing bacteria followed a tidal rhythm and the values were generally higher during low tide. The highest MPN was recorded at 4.16 × 10^6 cells g^{-1} during high tide and least during low tide at 5.17 × 10^5 cells g^{-1}. DMSPu - CFU averaged to 4.07 × 10^4 cells mL^{-1} in surfwater and 4.56 × 10^7 cells g^{-1} in sediment (Figure 10).

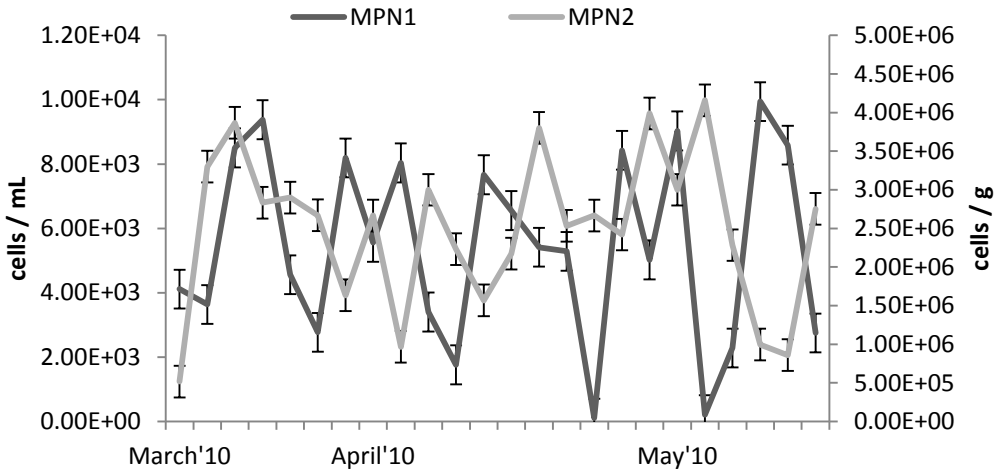


Figure 9: Variability in DMSPu - MPN in surfwater (MPN1) and sediment (MPN2)

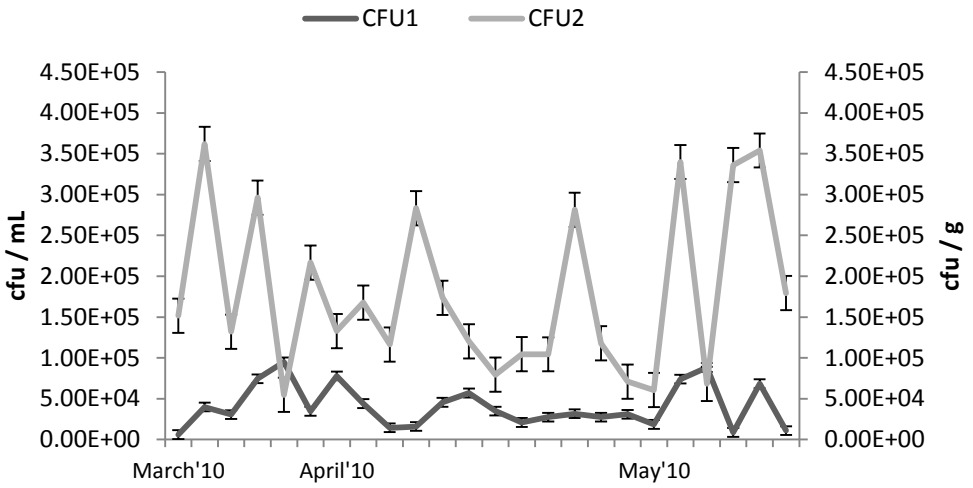


Figure 10: Variability in DMSPu - CFU in surfwater (CFU1) and sediment (CFU2)

4.1.6. Statistical analyses

4.1.6.1. Interrelationships tested by Spearmans rank r correlation:

To investigate the potential environmental drivers of DMSP dynamics, linear relationships between a suite of physical, chemical and biological factors were tested between the variables in surfwater and sediment. Table 5 highlights the interrelationships during high and low tide for producers, utilizers and chemical factors in surfwater and sediment respectively.

a. Surf water

DMSP concentration positively correlated with the total bacterial abundance. The DMS concentrations were usually higher during the day time resulting in a positive relationship of DMS with light intensity ($r = 0.664$, $p < 0.05$). The precursor DMSP co-varied with phosphate ($r = 0.730$, $p < 0.05$). DMSP concentrations followed a non-linear trend with tide height which was elucidated by polynomial regression curve of 3rdorder.

Phytoplankton abundance was in sync with both DMSP and DMS measurements. It also related positively with the silicate concentration and total viable bacteria (Table 3a). The chlorophyll values correlated with high tide ($r = 0.750$, $p < 0.05$) while light related with chlorophyll only during low tide and was responsible for 50% of the variation. It was noted that abundance of DMSP utilizing bacteria (MPN) increased during low tide ($r = -0.606$, $p < 0.05$). As the number of DMSP utilizing bacteria is comparatively low during

high tide, the DMSP concentrations are higher during high tide ($r = 0.478$, $p < 0.05$). During low tide, abundance of viable bacteria (direct viable counts) was positively influenced by phytoplankton abundance (43%). About 45% of these viable bacteria could account for the variation in the abundance of CFU.

b. Sediment

Tidal influence in DMS variations was evident from the linear correlation between tide height and DMS ($r = 0.471$, $p < 0.05$). Similar trend was seen in salinity and DMS variation ($r = 0.697$, $p < 0.05$). During our study, Chl *a* and phaeopigments showed a very strong positive correlation ($r = 0.83$ $p < 0.05$) in both sediment and surfwater. Phytoplankton abundance showed a significant non-linear relationship with DMSP and DMS when the sediment surface was exposed during low tide. Total viable counts of bacteria related with MPN ($r = 0.587$, $p < 0.05$).

Table 5: Significant correlations tested by Spearman's rank correlation during high tide (HT) and low tide (LT)

Correlating variables		Surfwater			Sediment		
		Overall	**HT**	**LT**	**Overall**	**HT**	**LT**
DMS	Tide	x	X	*0.431*	0.472	0.571	X
	Temperature	0.461	X	0.575	x	x	X
	Salinity	x	0.675	*0.578*	0.513	x	X
	Light	0.569	X	X	x	x	X
	Silicate	x	X	X	x	x	0.743
	Chlorophyll a	x	*0.431*	X	x	x	X
	Phytoplankton	*0.686*	X	X	x	0.546	0.643
DMSP	Tide	x	X	X	x	*0.516*	X
	DO	0.583	X	X	x	0.713	X
	Ammonia	x	0.598	X	x	x	X
	TC	x	0.622	X	x	x	X
	Phosphate	x	X	0.730	x	x	X
	Phytoplankton	*0.634*	X	X	x	x	0.573
Chlorophyll a	Tide	x	0.750	X	x	x	x
	Light	x	X	0.711	x	x	x
	Silicate	x	0.797	x	x	x	x
	Phaeopigment	x	- 0.704	x	0.830	0.791	0.853
	Phosphate	-0.402	X	x	x	x	x
	TC	x	X	x	x	x	0.615
Phytoplankton	Silicate	x	X	0.721	0.413	x	x
	TVC	x	X	0.657	x	x	-0.776
MPN	Tide	-0.497	X	-0.606	x	x	x
	pH	0.405	X	x	x	x	x
	TVC	x	X	x	x	x	0.587
	Silicate	x	X	x	x	0.608	x
	Nitrite	-0.684	X	x	-0.684	x	x
CFU	Tide	x	X	x	x	x	-0.645
	TVC	x	0.671	x	x	x	x
	Phaeopigment	x	X	x	x	x	-0.601

4.1.6.2 Principal Component Analysis (PCA)

a. Surfwater

The data were subjected to multivariate exploratory analyses to study the effect and relevance of various parameters to DMS/P dynamics. In surfwater, PCA revealed that all the factors fell into five major components (Table 6). About ~55% variation in the observations was explained by four components. Salinity (-0.249) light intensity (-0.26) and dissolved oxygen (0.20) grouped together as important physico-chemical factors of PC1 which showed their combined effect on phytoplankton (0.277), total viable bacterial abundance (-0.204) and DMS concentration (-0.335). The prominent variables were DMSP (0.47), DMS (-0.203), dissolved oxygen (0.311), temperature (0.299), ammonium (0.22) and TC (0.215).

b. Sediment

The sediment ecosystem was more complex since the variables segregated into six components for explaining only about ~60% of the variability (Table 7). PC1 was dominated by tide (- 0.389), Chl *a* (-0.339), temperature (- 0.27), DMSP (-0.218) and DMS (0.211). Variables like salinity (- 0.105), Eh (-0.276) nitrite (0.153), silicate (0.183) along with MPN (0.131) and TVC (0.115) featured in PC1. These factors seem to have a combined influence on the DMS/P concentration in the sediment. In PC2, salinity was the most important factor (-0.319) influencing DMS (-0.355) concentration and phytoplankton abundance (- 0.252), biomass (0.257) and CFU (0.376). (Table 7)

Table 6: Principle component analysis - Surfwater

Eigenvalues			
PC	Eigenvalues	%Variation	Cum.%Variation
1	4.15	17.3	17.3
2	3.53	14.7	32
3	2.99	12.5	44.4
4	2.62	10.9	55.4
5	2.36	9.8	65.2

Eigenvectors					
(Coefficients in the linear combinations of variables making up PC's)					
Variable	PC1	PC2	PC3	PC4	PC5
Tide	-0.097	0.134	-0.167	0.424	-0.238
Temp	-0.088	**0.299**	0.173	0.076	-0.134
pH	-0.104	0.115	0.278	0.006	0.373
Salinity	**-0.249**	0.112	-0.177	-0.031	-0.313
Light (lux)	**-0.26**	0.063	-0.004	-0.115	-0.272
DMSP	-0.049	**0.47**	-0.011	-0.095	0.023
DMS	**-0.335**	**0.203**	0.079	-0.137	-0.039
DMSP:DMS	-0.098	0.428	-0.032	-0.097	0.124
Phytoplankton	**0.277**	0.124	-0.119	0.183	0.197
Chl a	-0.4	0.011	0.035	0.182	0.132
DMSP:Chla	**0.355**	0.162	0.009	-0.179	-0.246
Phaeo	-0.234	-0.068	-0.194	-0.241	0.323
DO	**-0.201**	**0.311**	-0.117	-0.001	0.112
Nitrate	-0.094	0.175	0.176	0.303	-0.028
Nitrite	0.076	0.041	0.249	0.237	0.218
Phosphate	-0.036	0.146	0.325	-0.294	-0.191
Silicate	0.006	0.148	-0.226	0.289	-0.047
Ammonia	0.097	**0.222**	-0.369	-0.048	0.143
TC	0.2	**0.215**	0.327	0.094	0.14
TVC	**-0.204**	-0.122	-0.242	-0.286	0.172
MPN	-0.003	-0.008	0.354	-0.243	0.163
CFU	-0.117	-0.103	0.205	-0.127	-0.38

Table 7: Principle component analysis - Sediment

Eigenvalues			
PC	**Eigenvalues**	**%Variation**	**Cum.%Variation**
1	5	20.8	20.8
2	2.75	11.5	32.3
3	2.44	10.1	42.5
4	2.14	8.9	51.4
5	1.96	8.1	59.5

Eigenvectors					
(Coefficients in the linear combinations of variables making up PC's)					
Variable	PC1	PC2	PC3	PC4	PC5
Tide	**-0.389**	-0.13	0.177	-0.291	0.034
Temp	**-0.27**	0.077	0.083	-0.204	-0.382
pH	-0.024	0.119	-0.353	0.193	-0.265
Salinity	**-0.105**	**-0.319**	-0.026	-0.222	-0.136
Eh	**0.179**	0.072	0.236	0.348	0.087
Light (lux)	**-0.23**	0.063	-0.004	-0.115	-0.272
DMSP	**0.218**	-0.104	0.239	-0.405	0.077
DMS	**-0.211**	**-0.355**	0.266	0.072	-0.003
DMSP:DMS	0.286	0.218	-0.157	-0.312	0.113
Phyto	-0.148	**-0.252**	-0.046	0.165	-0.205
Chl a	**-0.339**	**0.257**	0.02	-0.092	0.148
DMSP:Chl	0.273	-0.361	0.136	-0.07	-0.131
Phaeo	**-0.321**	0.223	-0.191	-0.096	0.091
DO	-0.041	0.196	0.436	-0.134	-0.079
Nitrate	0.091	-0.089	0.136	-0.004	0.013
Nitrite	**0.153**	0.053	0.215	0.301	-0.04
Phosphate	0.056	**-0.209**	-0.349	0.154	0.101
Silicate	**-0.183**	-0.195	0.191	0.291	0.006
Ammonium	-0.033	0.176	0.3	0.215	0.232
TC	-0.084	-0.063	0.121	-0.027	0.282
TVC	**0.115**	-0.051	-0.086	0.02	0.553
MPN	**-0.181**	-0.125	-0.074	-0.193	0.219
CFU	0.061	**0.376**	0.196	-0.041	-0.081

4.2 Field – Seasonal Observations:

4.2.1 Environmental variables

Surfwater temperature was lowest during monsoon and there was a marginal difference between the average temperature in monsoon and post-monsoon. Pre-monsoon was the hottest season. Temperature of sediment did not show significant seasonal variation with an average at 26.08 °C during monsoon and 27.76 and 28.5 °C during pre-monsoon. During monsoon, the average temperature recorded was 28 °C in sediment.

Average salinity measured in surfwater and porewater during pre and post-monsoon season was 31 and 28 respectively. During monsoon, salinity fluctuated from 13.66 to 24.66. In sediment, the highest salinity was recorded in the pre-monsoon season at 36.04 while the least was during monsoon at ~ 18 and increased to ~32 during the post-monsoon.

In surfwater, highest average pH was recorded during pre-monsoon at 7.8 followed by 7.57 during post-monsoon. During monsoon the pH was comparatively low and averaged at 7.25. In sediment, pH was highest in the post-monsoon season at an average of 8.04 followed by pre-monsoon value of 7.47 and the lowest during monsoon at 7.37.

All nutrients were generally higher in pore water during pre-monsoon while in monsoon it was higher in seawater. The molar concentration of nutrients was in the order silicate >nitrate> phosphate> nitrite. As in seawater, the trend of distribution of nutrients in pore water was same. Phosphate concentration was marginally higher in porewater during monsoon and post-monsoon while during pre-monsoon it was higher in surfwater (1.05 µM). Ammonium concentrations varied from below detection limit to 0.06 µM in surfwater and sediment during pre-monsoon. During monsoon, the ammonium concentration in the surfwater and sediment increased considerably to 1.25 and 1.01 µM, respectively. Nitrate concentrations were higher in sediment pore water as compared to surfwater in both pre and post monsoon. Silicate concentrations were dynamic and followed similar trend in surfwater and sediment with highest values during post-monsoon followed by monsoon and least during pre -monsoon.

The dissolved oxygen concentrations in surfwater ranged from 4.40 mL^{-1} during pre-monsoon to the lowest average of 2.54 mL^{-1} during monsoon and slight increase at 2.86 mL^{-1} during post-monsoon. In sediment pore water, DO was less than in surfwater through the seasons. The pre and post monsoon values were almost similar at 2.8 mL^{-1} in both seasons while during monsoon, average DO was 2.62 mL^{-1}. Please refer table 8.

Table 8: Seasonal variation in environmental variables in intertidal sediment and surf water along with their sd values

Variable	Pre-monsoon		Monsoon		Post-monsoon	
	Surfwater	Sediment	Surfwater	Sediment	Surfwater	Sediment
Light (lux)	18194.44 ± 0.0		11134.167 ± 0.06		24194.44 ± 0.0	
Temperature(°C)	30.75 ± 1.75	27.76 ± 0.0	25.33 ± 0.15	26.08 ± 0.02	26.33 ± 0.12	28.5 ± 0.02
Salinity	30.67± 1.97	36.42 ± 0.04	20.67± 3.21	18.22 ± 0.53	28.33 ± 2.15	32 ± 0.54
pH	7.86 ± 0.24	7.47 ± 0.04	7.25 ± 0.14	7.37 ± 0.07	7.54 ± 0.32	8.04 ± 0.04
DO (mL L^{-1})	4.40 ± 0.51	2.8± 0.98	2.54 ± 0.68	2.62 ± 0.42	2.86 ± 1.01	2.74 ± 0.48
Nitrate (µM)	1.15 ± 0.11	1.57± 0.56	8.8 ± 0.23	3.66 ± 0.04	2.71 ± 0.88	3.5 ± 0.84
Nitrite (µM)	0.41 ± 0.18	1.12 ± 0.15	1.13 ± 0.18	2.35 ± 0.14	0.84 ± 0.21	0.8 ± 0.35
Phosphate (µM)	0.10 ± 0.04	1.02 ± 0.14	3.57 ± 0.4	2.63 ± 0.01	2.60 ± 0.08	1.4 ± 0.63
Silicate (µM)	3.89 ± 1.31	7.83 ± 0.48	11.1 ± 2.31	9.92 ± 0.07	12.32 ± 2.54	11.33 ± 0.91
Ammonium(µM)	0.02± 0.009	0.02 ± 0.01	1.25 ± 0.09	0.04± 0.01	1.01 ± 0.02	1.42 ± 0.02
DMSP (nM)	9.467± 2.16	49.19± 3.11	20.98 ± 4.1	269.66± 9.5	12.734 ± 1.1	60.34±2.11
DMS (nM)	1.583± 0.09	5.79± 1.5	6.711± 1.8	8.49± 2.66	7.64± 2.39	4.14± 0.15
Phytoplankton (cells l^{-1}, g^{-1})	3.15×10^{5}± 1.2	6.83×10^{5}± 1.8	3.45×10^{5}± 1.56	5.02×10^{5} ± 1.22	4.67×10^{5} ± 1.1	1.15×10^{6} ± 0.98
Chlorophyll (ug l^{-1}, g^{-1})	1.41± 0.18	5.05± 0.5	1.31± 0.2	7.21± 0.36	2.19±0.05	6.05± 1.2
DMSPu - MPN (cells ml^{-1}, g^{-1})	4.77×10^{3} ± 1.1	3.7×10^{3}± 0.9	4.91×10^{3}±1.02	4.87×10^{5}± 0.6	5.20×10^{3}± 1.2	4.49×10^{4}± 1.3
DMSPu - CFU (cells ml^{-1}, g^{-1})	1.97×10^{4} ± 1.1	4.86×10^{4}± 0.8	1.82×10^{4}±0.9	5.33×10^{5}± 0.2	2.86×10^{4}± 0.5	5.06×10^{4}± 0.12

4.2.2 DMSP, DMS

DMS and DMSP exhibited a seasonal pattern with highest values during monsoon in surf water and sediment of Dona Paula bay.

In surfwater, DMSP concentrations were highest during the monsoon season (June–September) at an average of ~21 nM followed by post and pre-monsoon (Figure 11). The concentration was lowest in the pre-monsoon season and averaged at 9.46 nM. The DMS concentrations were lowest during the pre-monsoon season at 1.58 nM. The corresponding DMS levels varied from non-detectable limits to 1.98 nM during pre-monsoon. During the monsoon, average DMS concentrations ranged from 4.63 nM in June to maximum value of 19.38 nM in August. During post-monsoon, the variability was similar and averaged at 6.7 nM.

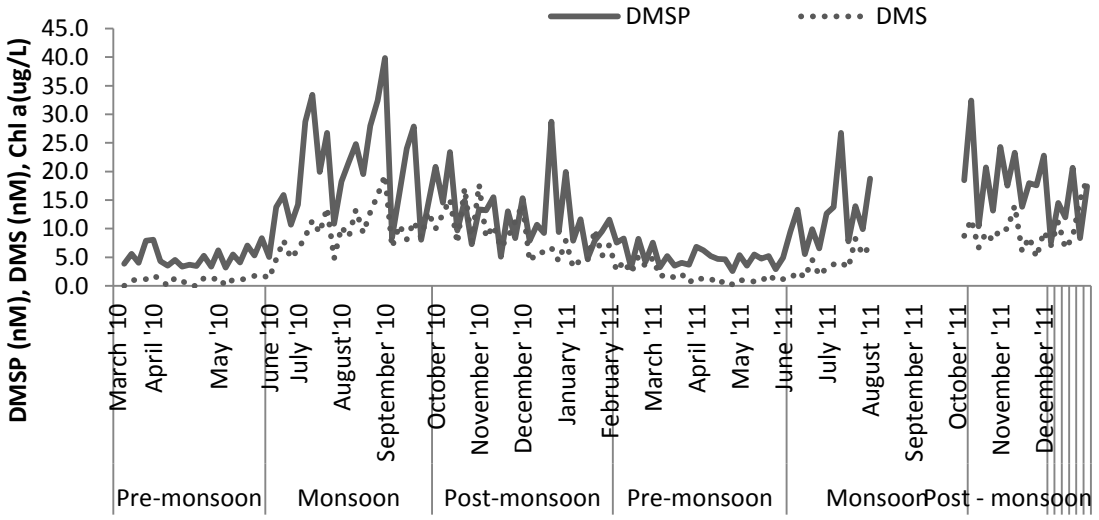


Figure 11: Seasonal variability in DMS, DMSP in surfwater of Dona Paula bay

In sediment, DMS/P concentrations exhibited a strong seasonal pattern (Figure 12). On an average DMSP was ~5 times higher in the intertidal sediment than surfwater of Dona Paula bay during pre and post monsoon. During monsoon, total DMSP concentration was ~10 times higher in sediment than in surfwater. The highest values were recorded during this season and the average was 269.66 nM. Though DMSP was way higher in the sediment, the DMS concentrations were not significantly high.

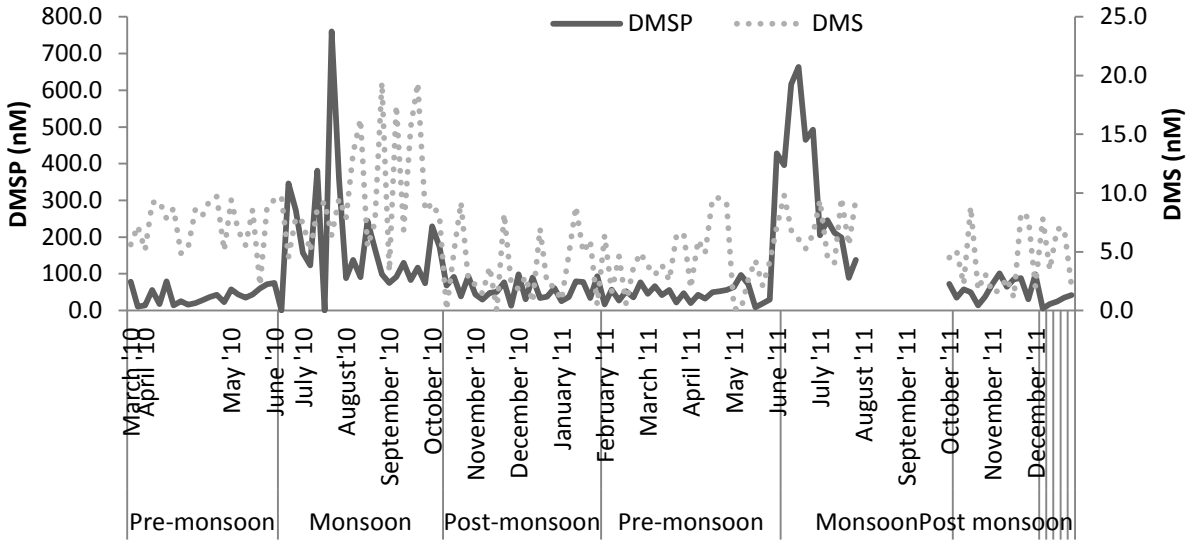


Figure 12: Seasonal variability in DMS, DMSP in sediment of Dona Paula bay

4.2.3 DMS flux

The estimated sea–air flux of DMS was highest during the monsoon season where it ranged from 0.3 to 60.91 µM $m^{-2}d^{-1}$ (Figure 13). The post-monsoon average value was almost 10 times higher (18.93 µM $m^{-2}d^{-1}$) than the pre-monsoon (2.29 µM $m^{-2}d^{-1}$). During the pre-monsoon season, DMS concentrations in many samples were below detection limit, and varied from 0 to 2.31 µM $m^{-2}d^{-1}$. Wind speed varied from 0.2 m s^{-1} to 3.4 ms^{-1}.

DMS sediment to pore water diffusive flux varied from 0.08 to 0.21 µM $m^{-2}day^{-1}$ during the pre-monsoon season. During monsoon higher values ranging from 0.14 to 0.48 µM$m^{-2}day^{-1}$ were estimated. During post-monsoon the values were not too less as compared to monsoon ranging from 0.09 to 0.41 µM $m^{-2}day^{-1}$.

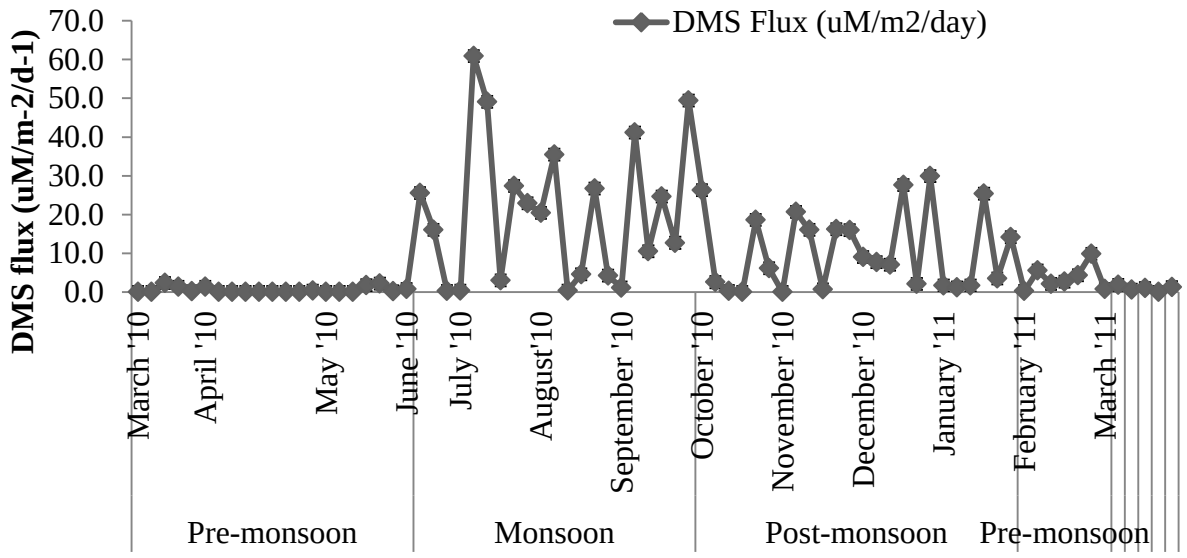


Figure 13: Seasonal variability in sea - air DMS flux in Dona Paula bay

4.2.4 DMS/P production rate

DMSP and DMS were produced by the phytoplankton at an average rate of 0.06 nM hr^{-1} and 0.02 nM $hr^{-1,}$ respectively in the surfwater in non-monsoon season. In sediment, the average DMSP production rate was 0.054 nM hr^{-1} while DMS was produced at the rate of 0.079 nM hr^{-1}. During monsoon, DMSP was produced at the rate of 0.8 nM hr^{-1} while DMS was at 0.24 nM hr^{-1} in surf water. The DMSP and DMS production rates were much higher in sediment at 5.94 nM hr^{-1} and 0.06 nM hr^{-1} respectively during this season. In laboratory experiment carried out to measure the production and utilization of DMS, the calculated turnover time of DMS was faster in sediment (2.27 h) than in surfwater (4.16 h) (Table 9).

Table 9: DMS turnover in surfwater and sediment on Dona Paula bay

	Experiment Turnover rate (nM hr^{-1})		**Turnover time (h)**
	Production	Utilization	
DMS (Surfwater)	0.496	0.24	4.16
DMS (Sediment)	0.518	0.44	2.27

4.2.5 Producers

4.2.5.1 Chlorophyll a and Phaeopigments

In surfwater, chlorophyll content exhibited a seasonal pattern with higher values during post-monsoon followed by pre-monsoon while during monsoon, the average was lowest. Intriguingly, sediment had a different pattern with highest values during monsoon season followed by post-monsoon and least during pre-monsoon. Phytoplankton abundance also exhibited a seasonal pattern; the abundance was least during monsoon 5.02 × 10^5 cells g^{-1} which gradually increased in the post monsoon phase and averaged to1.15 × 10^6 cells g^{-1}. Intermittent peaks were recorded during pre-monsoon with an average value of 6.83 × 10^5 cells g^{-1} (Figure 14, 15).

4.2.5.2 Phytoplankton abundance

Diatoms dominated the phytoplankton diversity in the surfwater as well as sediment during the study period. In surfwater, phytoplankton abundance was in the order of 10^5 L^{-1} through the seasons. However, we encountered marginal rise in the numbers during post monsoon season (average 4.57 × 10^5 cells L^{-1}) from 3.45 × 10^5 cells L^{-1} during monsoon. The lowest abundance was during pre-monsoon at an average of 3.15 × 10^5 cells L^{-1} (Figure 14, 15).

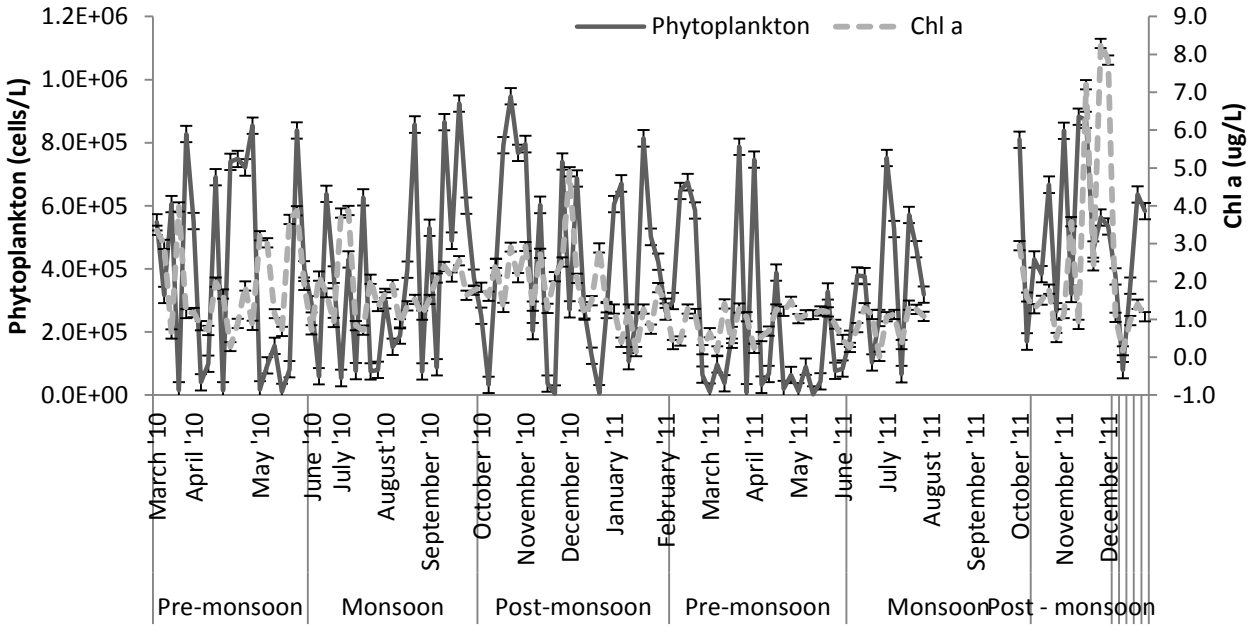


Figure 14: Seasonal variability in phytoplankton abundance and chl a in surfwater of Dona Paula bay

The trend in variability of phytoplankton in sediment was similar to surfwater with highest values during post-monsoon (Figure 15)

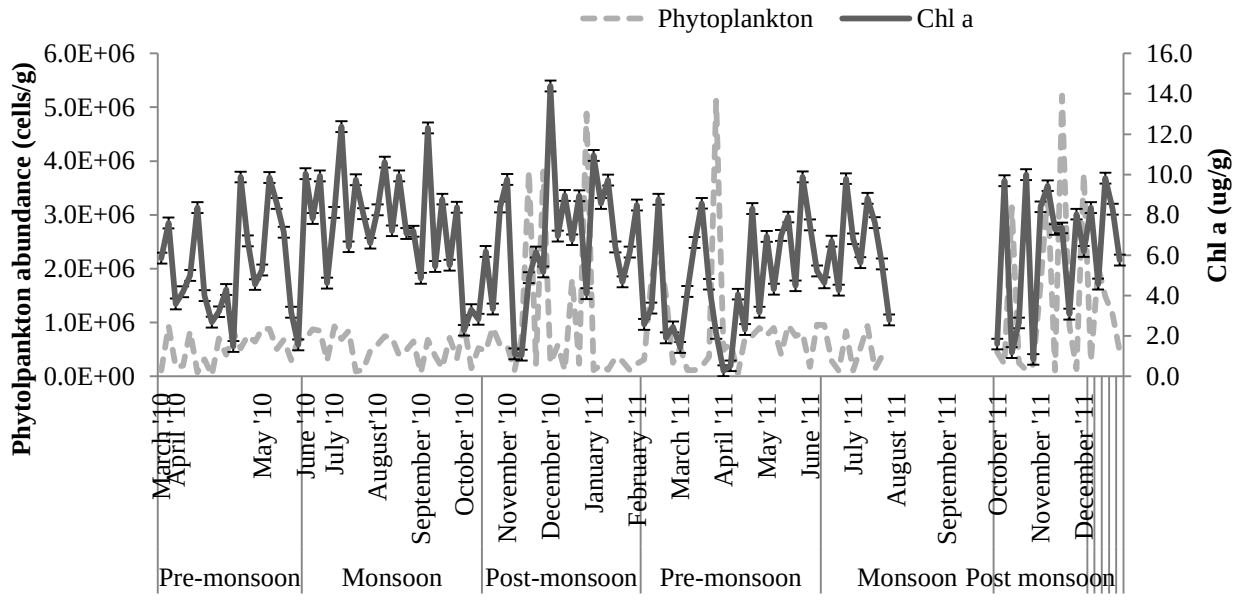


Figure 15: Seasonal variability in phytoplankton abundance and chl a in sediment of Dona Paula bay

Surfwater harbored 36 species of diatoms (21 centric and 15 pennate). The results indicate that the phytoplankton diversity was dominated by three diatom species viz. *Navicula (33%), Odontella (12%),* and *Thalassiosira (7%).* in the pre-monsoon season. During monsoon, two entirely different diatom species, *Rhizosolenia (21%)*, and *Fragilleria (9%)* dominated the surf-water samples.

During post-monsoon, *Navicula (33%), Coscinodiscus (12%),* and *Thalassiosira (7%)* were the most abundant diatom species. However, the number of dinoflagellates increased by an order in few samples and was represented by *Protoperidium* and *Gymnodium* species. *Other phytoplankton species were Coscinodiscus, Thalassiothrix, Grammatophora, Navicula trevelyana, Nitzschia closterium, Rhopalodia, Synedra, Calonies, Epithemia, Protoperidinium, Cymbella, Pinnularia, , Diatoma, Asterionella Nitzschia, Amphora, Naviculairidis, Hantzschia, Fragillaria, Navicula cancellata.*

The ratio of Diatom: Dinoflagellate was constant (4:1) during pre-monsoon and monsoon and changed to (7:3) during post-monsoon season. Thus the dinoflagellate abundance increased from 20% to 30%.

The sediment harbored 30 species of diatoms (11 centric, 19 pennate) belonging to 20 genera. Pennate diatoms were dominant in terms of abundance. The most abundant pennate diatoms were *Amphora* and *Navicula*, whereas, *Thalassiosira* was the dominant centric diatom. The most abundant phytoplankton in surfwater and sediment are enlisted in table 10 and 11 respectively.

Table 10: Phytoplankton diversity in surfwater

Pre-monsoon	Monsoon	Post-monsoon
Navicula (33%),	*Rhizosolenia (21%)*	*Navicula (29%)*
Odontella (12%),	*Fragillaria (9%)*	*Coscinodiscus (17%)*
Thalassiosira (7%).	*Skeletonema (3%)*	*Thallasiosira (9%)*

Table 11: Phytoplankton diversity in sediment

Pre-monsoon	Monsoon	Post-monsoon
Navicula (22%),	*Nitzschia (26%)*	*Navicula (29%)*
Amphora (18%),	*Dinoflagellates (7%)*	*Chaetoceros (11%)*
Thalassiosira (7%).	*Coscinodiscus (3%)*	*Dinoflagellates (6%)*

4.2.5.3 Phytoplankton diversity indices

In sediment, species richness and evenness (Shannon–Weinner) exhibited a seasonal trend as the value was lowest during pre–monsoon (1.05, 0.87) and it gradually increased to 2.97 and 2.81 during monsoon. The highest value was encountered during

post–monsoon season at 3.11 and 3.01 respectively. Overall, the α diversity index was higher in the surfwater at 3.5 as compared to 2.43 in sediment.

4.2.6 Utilizers

4.2.6.1 Total bacterial counts

During pre-monsoon, the average total bacterial count was one order lower in the surfwater and varied between 0.78 and 3.6 × 10^7 cells mL^{-1}. The total bacterial counts in the sediment varied between 0.459 and 9.94 × 10^8 cells g^{-1} dry weight. During monsoon, bacterial counts in surfwater were generally stable and ranged from 1.51 to 8.6 × 10^7 cells mL^{-1} the total bacterial counts in sediment varied between 1.75 × 10^6 and 11.2 × 10^8 cells g^{-1} dry weight. The bacterial counts in both surfwater and sediment increased in the post monsoon season to 6.52–9.11 × 10^8 cells mL^{-1}. Bacterial abundance in sediment was highest during this season at 7.8 – 9.45 × 10^9 cells g^{-1}

4.2.6.2 DMSPu–MPN, CFU

DMSP utilizing bacterial abundance did not show any significant seasonal pattern. Most Probable number (MPN) of DMSP utilizers varied from 0.108 to 9.38 × 10^3 cells mL^{-1} in surfwater. In sediment average MPN of DMSP utilizing bacteria ranged from 3.71 × 10^3 in pre-monsoon to 4.87 × 10^4 cells g^{-1} during monsoon. Though negligible, the bacterial abundance decreased during post -monsoon season to 4.49 × 10^3 cells mL^{-1}. The DMSPu-CFU varied from 4.86 × 10^4 to 5.33 × 10^4 cfu mL^{-1} during pre-monsoon and post-monsoon, respectively. Lowest values were recorded during pre-monsoon season at 4.86 × 10^4cfu mL^{-1} (Figure 16).

In sediment, the DMSPu–CFUs were generally lower during the monsoon season, though no particular trend could be elucidated (Figure 17).

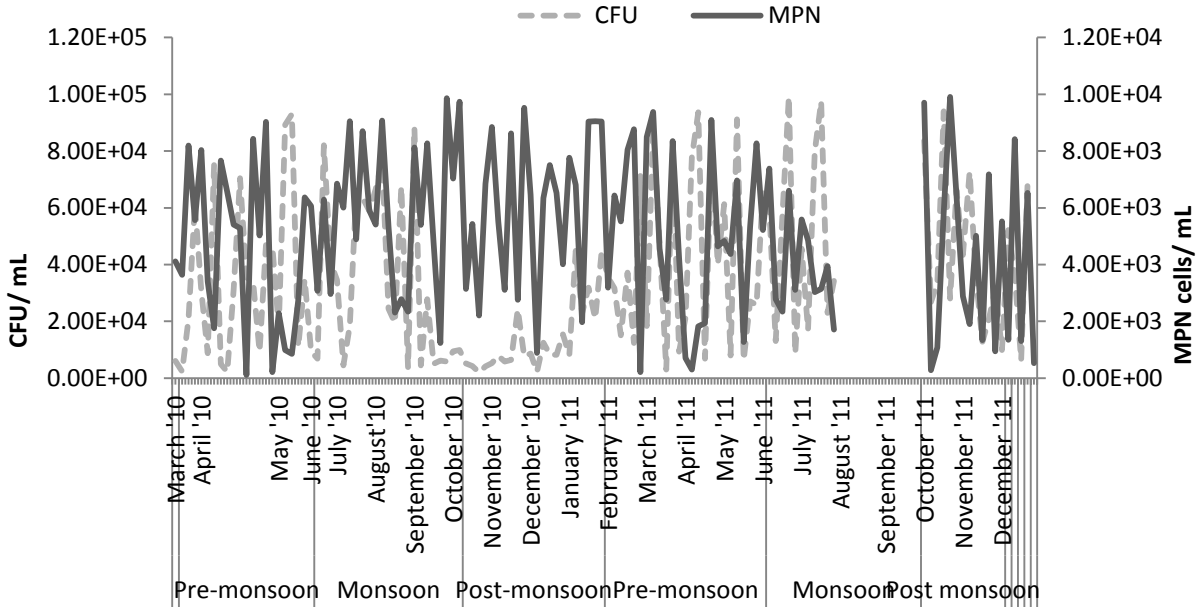


Figure 16: Seasonal variability on DMSPu – CFU and MPN in surfwater of Dona Paula bay

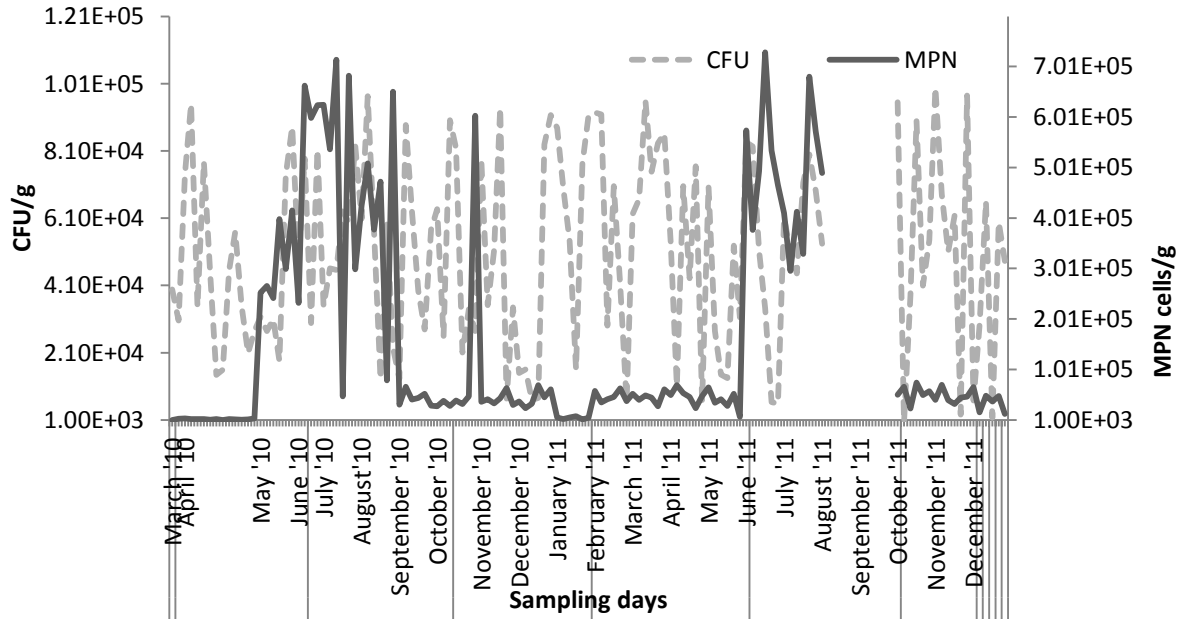


Figure 17: Seasonal variability on DMSPu – CFU and MPN in sediment of Dona Paula bay

4.2.7 Statistical analyses

4.2.7.1 Interrelationships tested by Spearman's rank correlation

a. Surf water

In surfwater, the physicochemical factors relating with DMS varied in different seasons. During pre-monsoon, factors that directly influenced the DMS concentration were temperature, light intensity and chl a ($r = 0.569, 0.461, 0.415$ at $p < 0.05$) (Table 10). DMSP values associated positively with phytoplankton and temperature while it was negatively influenced by light. The producers were linked positively with nitrate but negatively with phosphate. (Table 12)

During monsoon, among the variables examined, light intensity, temperature and salinity were the major constraints for DMS variability. Irrespective of the season, light intensity was responsible for ~25% of the variation in DMS concentrations. The temperature of surfwater related to the DMS concentrations positively in all the three seasons, but the strength of correlation was marginally higher in the pre-monsoon season as compared to the other two (spearman's rank r = 0.461during pre-monsoon > 0.456 during post-monsoon > 0.433 during monsoon). Salinity correlated positively with the DMS concentrations only during the monsoon season ($r = 0.513$, $p < 0.05$) which explained about 23% of the variation.

During post-monsoon, temperature and light continued to influence DMS. The association of chl a and DMS turned inverse during this season. DMSP related positively with nutrients and phytoplankton, light temperature and salinity. The viable counts of bacteria related negatively with DMSP.

b. Sediment

In sediment, we see seasonal pattern in the influence of physico-chemical variables on DMS variability. During pre-monsoon, tide, temperature and salinity correlated with DMS. The precursor DMSP correlated well with the abundance and biomass of producers (phytoplankton and chl a, which in turn were influenced by nutrients during this season. Phytoplankton also affected the bacterial numbers in sediment (Table 13).

During monsoon physico-chemical variables like light, salinity, and phosphate were related to DMS. Also, the utilizers of DMSP (CFU) linked positively with the DMS content in the sediment. The association of DMSP with the phytoplankton continued during monsoon. The influence of nutrients on phytoplankton was discernible. The dependence of bacteria (TC, MPN) on phytoplankton was evident from the correlations.

During post-monsoon, the interactions were similar to those during monsoon where light, salinity, chl *a* and phosphate were linked to DMS. DMSP related only to chl *a* and not phytoplankton. The dependence of phytoplankton on nutrients (nitrite and phosphate) was significant. The abundance of DMSP utilizers (MPN) was also linked to the abundance of producers.

Table 12: Significant interrelationships in surfwater tested by Spearman rank correlation

Correlating variables		Pre-monsoon	Monsoon	Post-monsoon
DMS	Light	0.569	-0.501	-0.541
	Temperature	0.461	0.456	0.433
	Salinity	*X*	-0.513	*X*
	Chlorophyll a	0.415	0.384	-0.429
DMSP	Phytoplankton	0.634	0.513	0.498
	Light	-0.569	-0.584	0.321
	Temperature	0.513	-0.461	0.484
	Salinity	*X*	0.615	0.463
Phytoplankton	Nitrate	0.657	0.584	0.629
	Phosphate	-0.402	0.389	0.481
	TVC	0.639	0.574	-0.388

Table 13: Significant interrelationships in sediment tested by Spearman rank correlations

Correlating variables		Pre-monsoon	Monsoon	Post-monsoon
DMS	Light	Ns	-0.63	-0.63
	Tide	0.49	ns	ns
	Temperature	-0.54	ns	ns
	Salinity	-0.36	0.42	0.43
	Chlorophyll a	X	X	0.43
	Phosphate	X	-0.55	-0.51
	CFU	X	0.57	X
DMSP	Phytoplankton	0.43	0.506	X
	Chlorophyll a	0.51	0.43	0.41
	Temperature	X	X	X
Phytoplankton	Nitrate	-0.36	-0.35	Ns
	Nitrite	-0.57	0.43	0.44
	Phosphate	X	X	0.49
	TC	0.49	0.37	X
	MPN	X	-0.48	-0.53

4.2.7.2 Principal component analysis

a. Surf water

In surfwater, PCA revealed that all the factors fell into five major components. Here we present the factors loadings of PC1 and PC2. Important variables during pre-monsoon that grouped together were DMS, light, temperature, DO, DMSP, producers (phytoplankton) and bacteria (MPN and TC). During monsoon, the significant variables driving DMS variability in this ecosystem were light, temperature, salinity, phytoplankton nutrients and DMSP (Table 14). During post-monsoon, the significance of phytoplankton declined and the DMS variability seemed to be governed by light, temperature, nitrate and MPN.

Table 14: Principal component analysis of variable in surfwater

Variables in PC1 and PC2	Pre-monsoon	Monsoon	Post-monsoon
DMS	-0.335	-0.119	0.159
Light	-0.263	0.145	0.228
Temperature	-0.249	-0.256	0.231
Dissolved Oxygen	0.20	-	-
Phytoplankton	0.277	-0.338	-
TC	0.204	-	-
Salinity	-	-0.332	-
Nitrate	-	0.482	-0.147
MPN	-0.181	-	-0.191
DMSP	0.218	-0.205	-

b. Sediment

In the sediment, both producers and utilizers of DMSP featured with DMS, tide, temperature and salinity influencing the DMS variability during the pre-monsoon season. During monsoon, temperature and salinity grouped together with phytoplankton and MPN to influence DMSP and DMS. Similar to previous seasons, temperature continued to have high factor loading during post monsoon and grouped together with light, salinity and nitrate to influence DMS concentrations (Table 15).

Table 15: Principal component analysis of variables in sediment

Variables in PC1 and PC2	Pre-monsoon	Monsoon	Post-monsoon
DMS	-0.335	-0.109	0.159
Light	-		0.228
Tide	0.334	-	-
Temperature	-0.113	-0.112	0.231
Phytoplankton	0.277	-0.338	-
TC	0.204	-	-
Salinity	-0.114	-0.332	0.256
Nitrate	-	-0.482	-0.147
MPN	-0.181	0.461	-0.191
DMSP	0.218	-0.205	-

4.3 Identification of DMSP utilizing bacteria

4.3.1 Biochemical tests

About 74 isolates (40 from surfwater and 34 from sediment) which were major DMSP utilizers were obtained from mineral medium plates supplemented with 100 µM DMSP over the study period. The DMSP utilizing bacteria were identified by biochemical tests up to the generic level. Most of the isolates were Gram negative motile rods.

The culturable diversity of the surfwater and sediment was mainly affiliated to heterotrophic groups of bacteria namely: Alcaligenes, Pseudomonas, Flavobacterium, Moraxella gp, Vibrio etc. Gamma- Proteobacteria dominated the surfwater and sediment. Firmicutes were also present in significant numbers (3% in surfwater and 7% in sediment) (Table 16).

Table 16: Bacterial identity of dominant culturable fraction by biochemical tests

Total isolates = 74	**Surfwater (40)**	**Sediment (34)**
Alcaligenes (Proteobacteria; Betaproteobacteria)	19%	12%
Pseudomonas sp, (Proteobacteria; Gammaproteobacteria)	12%	10%
*Flavobacterium*sp, (CFB group)	7%	10%
Marinobacter,(Proteobacteria; Gammaproteobacteria)	6%	3%
Xanthomonas, (Proteobacteria; Gammaproteobacteria)	3%	-
Vibrio sp, (Proteobacteria; Gammaproteobacteria)	3%	5%
Moraxella sp, (Proteobacteria; Gammaproteobacteria)	2%	-
Bacillus sp.(Firmicutes)	3%	7%

- 100% = 10^5 L^{-1} in water, 10^5 g^{-1} in dry sediment

4.3.2 16S rDNA sequencing

16S r DNA sequencing revealed that *Alcaligenes* sp were most abundant in surfwater (19%) while *Salinicola salarius* dominated the sediment (22%). Firmicutes formed about 34% of the population in sediment (Table 17).

Table 17: Bacterial identity of dominant culturable fraction by 16S rDNA sequencing

	Surfwater (40)	**Sediment (34)**
Alcaligenes sp (Proteobacteria; Betaproteobacteria)	19%	-
Pseuodomonas aeruginosa (Proteobacteria; Gammaproteobacteria)	12%	-
Salinicola salarius (Proteobacteria; Gammaproteobacteria)	-	22%
Virgibacillus sp (Firmicutes)	-	9%
Lentibacillus sp (Firmicutes)	-	14%
Pauscibacillus sp (Firmicutes)	-	11%

- 100% = $10^5 L^{-1}$ in water, $10^5 g^{-1}$ in dry sediment

4.3.3 *CARD–FISH* for DMSP utilizing genes

Potential demethylation and DMS production were estimated by enumerating the *dmdA* and *dddR* genes respectively in sediment and water dilutions. Results clearly show that the dddR gene was enumerated at 10^2 to 10^3 g^{-1} in sediment and 10^2 mL^{-1} in water. The corresponding dmdA genes were in the order 10^2 to 10^3 g^{-1} in sediment and 10^2 mL^{-1} in surfwater.

4.4 Laboratory mesocosm experiments

4.4.1 Effect of Iron on DMSP production by phytoplankton community

Laboratory experiment to study the effects of Fe on DMSP production by phytoplankton community was carried out with appropriate controls for Fe and phytoplankton. Iron enrichment brought about 3 times increase in phytoplankton growth and DMSP production (Figure 18). Iron induced maximum DMSP concentration of around 450 nM after three days (E1+). Without Fe (C1-) the DMSP concentration also reached a maximum after three days and peaked at only 70 nM. Diatoms decreased from 80% at the beginning of the experiment to 40% towards the end. However, in the absence of phytoplankton, the iron-enrichment does not have any impact on DMSP dynamics. In E2+ and C2-, (with and without phytoplankton) the highest concentrations recorded were of 2.1 and 2.5 nM after three and seven days respectively. But the concentration quickly decreases after seven days probably because of the bacterial consumption.

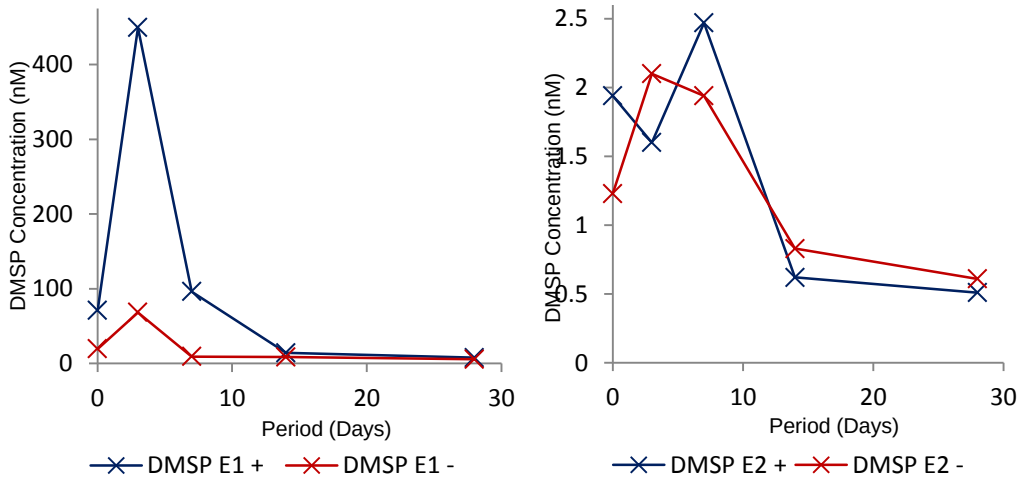


Figure 18: Effect of iron amendment on DMSP production by phytoplankton community

4.4.2 DMSP production by diatom cultures

We made an attempt to measure the influence of selected environmental variables on the DMSP production by near axenic diatom cultures and their utilization by bacteria in laboratory mesocosm. The abundant diatoms were isolated and cultured in f/2 medium amended with (1 µM Phosphate, 10 µM Nitrate, 10 mM Iron) and maintained under near axenic conditions for the experiments to measure DMSP production. The concentration

chosen for the experiment was near ambient. Appropriate controls were maintained for each set of experiments.

Different phytoplankton showed varied phases of growth and peak attainment with respect to nitrate and phosphate (macronutrient) and iron (micronutrient).

4.4.2.1 Effect of Nitrate on DMSP production

Cultures supplemented with 10 µM nitrate showed maximum growth in terms of cell numbers. Maximum response was seen with *Coscinodiscus* sp., the abundance of which was highest at 5×10^3 cells L^{-1}. On day 13, DMSP concentration also followed a similar trend; the highest concentration was measured when the phytoplankton population collapsed on day 20 (Figure 19).

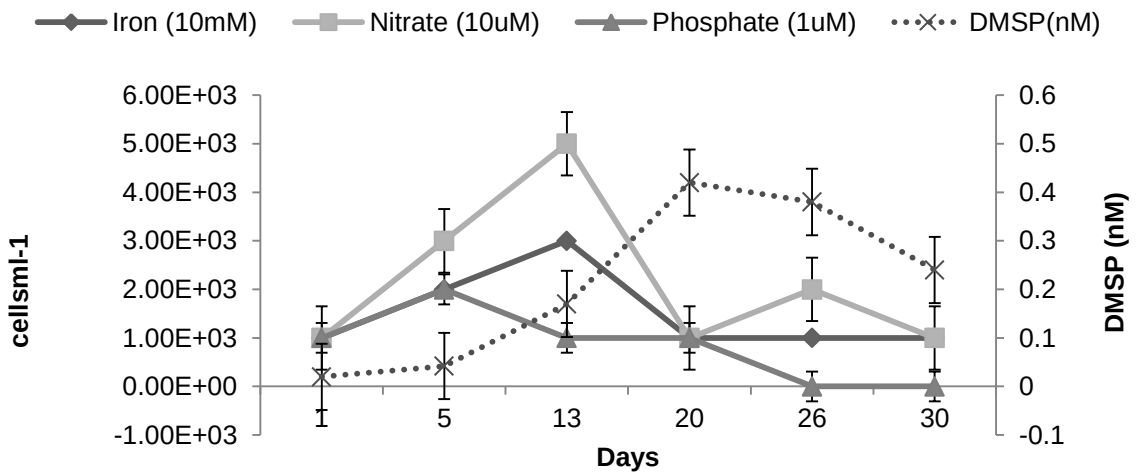


Figure 19: Varied response of *Coscinodiscus* sp to different nutrient amendments

NB: Experiments have been conducted separately

4.4.2.2 Effect of Phosphate on DMSP production

The addition of phosphate to the growth medium did not have significant effect on the phytoplankton abundance. Maximum response was seen with the isolate *Thallasiosira pseudonana* which peaked at 8×10^3 cells L^{-1} (Figure 20).

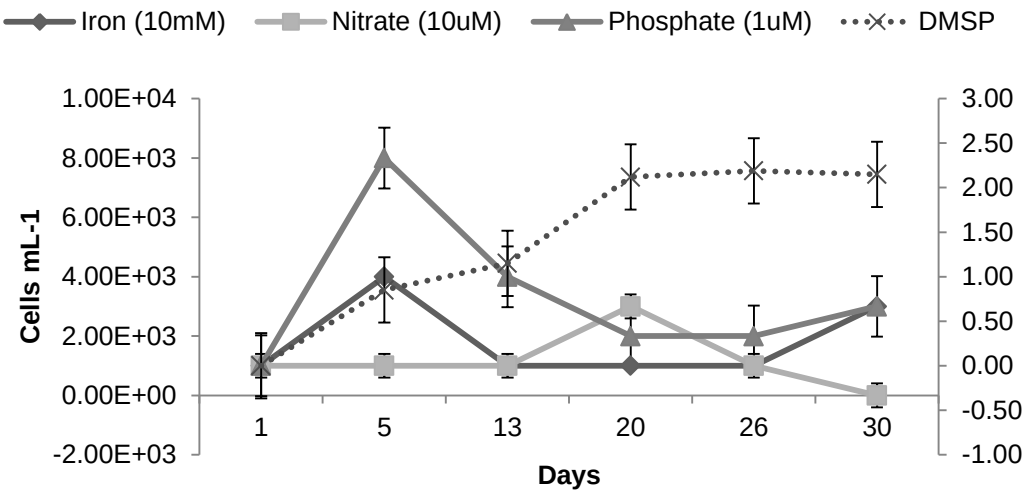


Figure 20: Varied response of *Thallasiosira* sp. to different nutrient amendments

NB: Experiments have been conducted separately

4.4.2.3 Effect of Iron on DMSP production

Iron amendment brought about significant increase (×3) in the cell numbers and DMSP concentration in the phytoplankton cultures (Figure 21). *Thallasiosira* sp. was most affected by the iron amendment in the mesocosm and peaked only on day 20 at 5×10^3 cells L^{-1}.

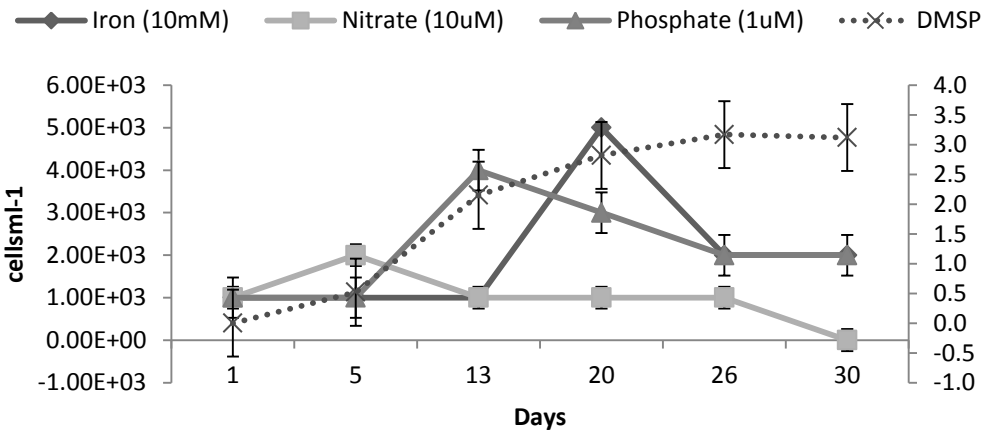


Figure 21: Varied response of *Thallasiosira* sp. to different nutrient amendments.

NB: Experiments have been conducted separately

Interestingly, the diatom *Nitzschia closterium* sp attained two peaks in response to iron amendment. The first was on day 5 at 6×10^3 cellsL^{-1} and secondary maxima on day 20 after which the cell numbers declined. It showed a steady increase in phosphate amended medium and attained peak on day 26. (Figure 22).

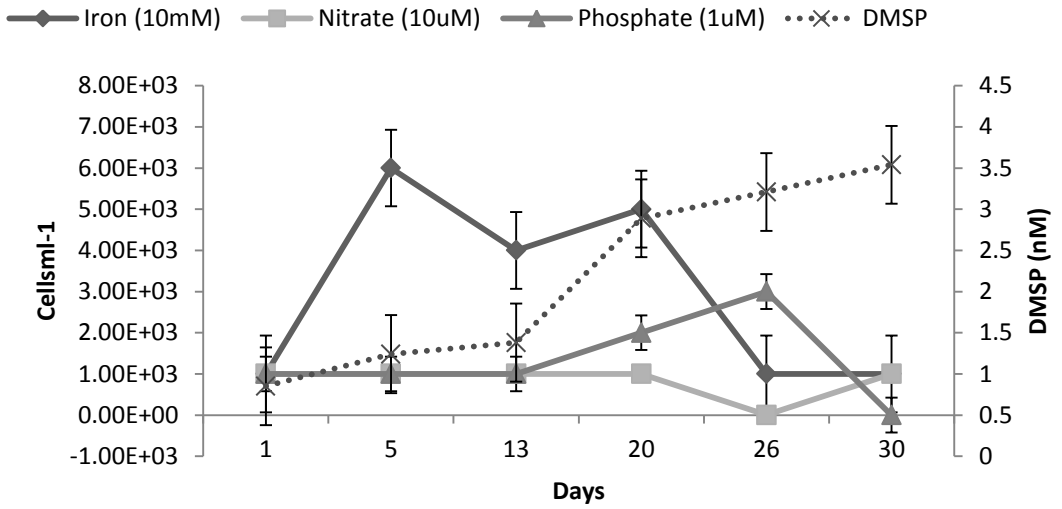


Figure 22: Varied response of *Nitzschia closterium* sp to nutrient amendment

NB: Experiments have been conducted separately

4.4.2.4 Change in DMSPt

DMSPt concentrations were as low as 0.2 nM in the cultures and increased with increase in cell abundance. Maximum DMSPt i.e. 3.03 nM was measured in *Thallasiosira* culture on day 26 when the abundance was highest at 15×10^3 cells mL^{-1} in iron amended medium. Thus the most significant effect on the diatom was seen with iron which indirectly affects the DMSPt concentration of the medium.

Increase in DMSPt in phosphate amended medium was steady in all the cultures with concentrations ranging from non-detectable levels to 0.5 nM. Highest values were recorded in *Thallasiosira pseudonana* culture on day 5.

Amendment of nitrate (10 µM), phosphate (1 µM) and iron (10 mM) to phytoplankton community of seawater showed that iron elicited maximum response in DMSP production (450 nM) on the 3rd day.

Similar experiments with individual cultures also showed that iron had maximum influence on *Thallasiosira sp.* which responded the best with peak on 20th day with a maximum DMSP production of 2.15 nM on day 25. However, another isolate of *Thallasiosira pseuodonana* responded best to phosphate amendment and peaked on day 13 with maximum DMSP production of 2.8 nM on day 20.

Nitrate amendment brought about maximum response in *Coscinodiscus sp*. which attained peak on 13th day and resultant DMSP peak at 2.54 nM on 20th day.

4.4.3 DMSP utilization by bacterial community

The bacterial community did not show much variation in the initial days in the presence of DMSP as the carbon source. The cell abundance was at its peak on day 7 at 1.48 × 10^7 cells mL^{-1} which coincided with the first lowest concentration of DMSP at 4.34 µM and second highest DMS concentration at 2.98 nM. DMSP concentration was lowest on day 30 (1.65 nM) while DMS attained its maximum on the same day at 3.47 nM. A sharp decrease in concentration of DMSP was observed till day 7. Thus the microbial community gradually utilized the DMSP and converted it to DMS (Figure 23).

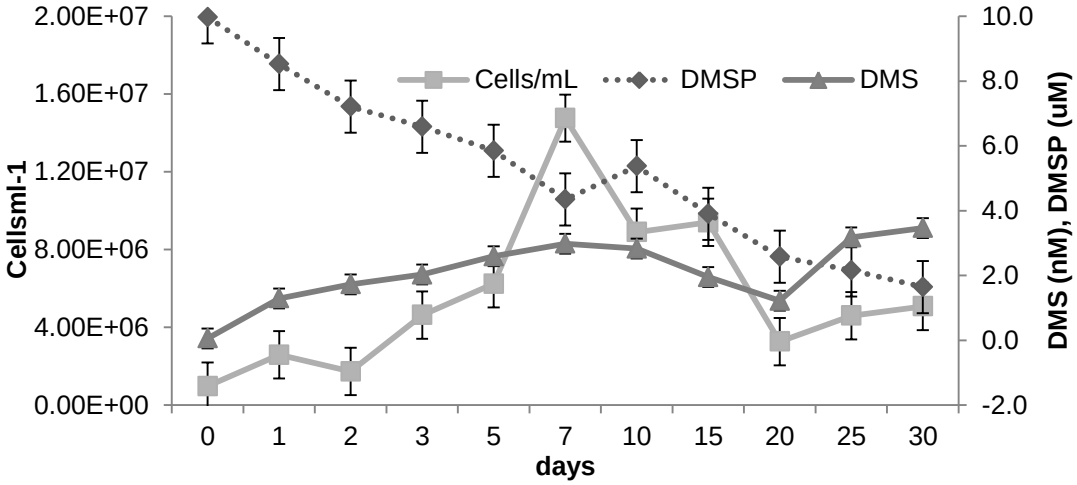


Figure 23: Utilization of DMSP by microbial community

4.4.3.1 Effect of Nitrate on DMSP utilization

Nitrate amendment in the media resulted in the gradual utilization of DMSP and build-up of DMS by the microbial community (Figure 24). The concentration of DMSP was usually less when the cell abundance was high. DMS concentration varied from 1.59 (day 5) to 3.47 nM (day 30). Cell abundance varied from 1.92 – 4.14 × 10^6 cells mL^{-1}.

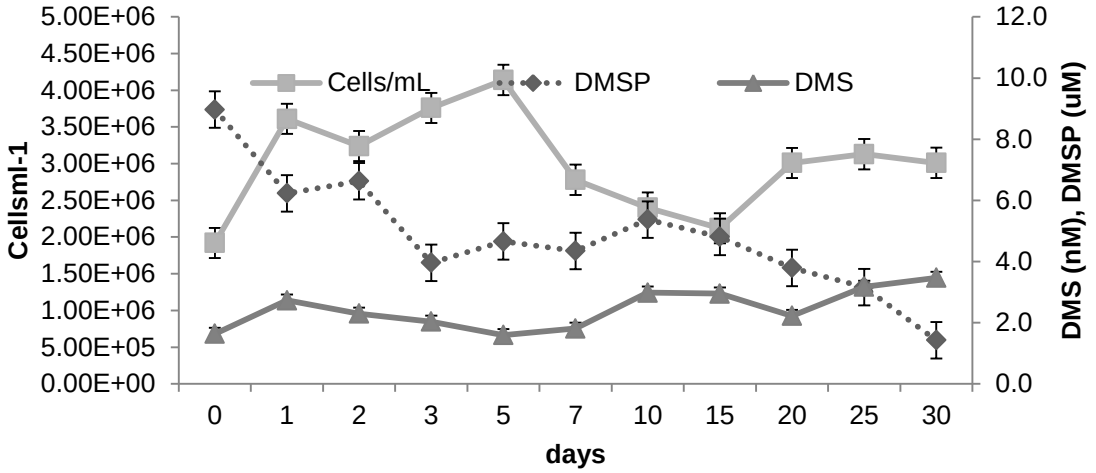


Figure 24: Effect of nitrate amendment on DMSP utilization by microbial community

4.4.3.2 Effect of Phosphate on DMSP utilization by bacterial community

Phosphate amendment showed maximum fluctuation in the cell numbers. DMSP concentration ranged from 6.14 to 7.8 µM and peaked at regular intervals on day 1, 7 and 20. The lowest concentration of DMSP (6.14 µM) measured on day 2 coincided with DMS maxima at 5.48 nM. However, the bacterial cell numbers were highest at 2.73 × 10^7 cells mL^{-1} on day 5 and crashed to its minimum value on day 7 (1.21 × 10^7 cells mL^{-1}) and gradually increased till day 25 before crashing again on day 30 (Figure 26).

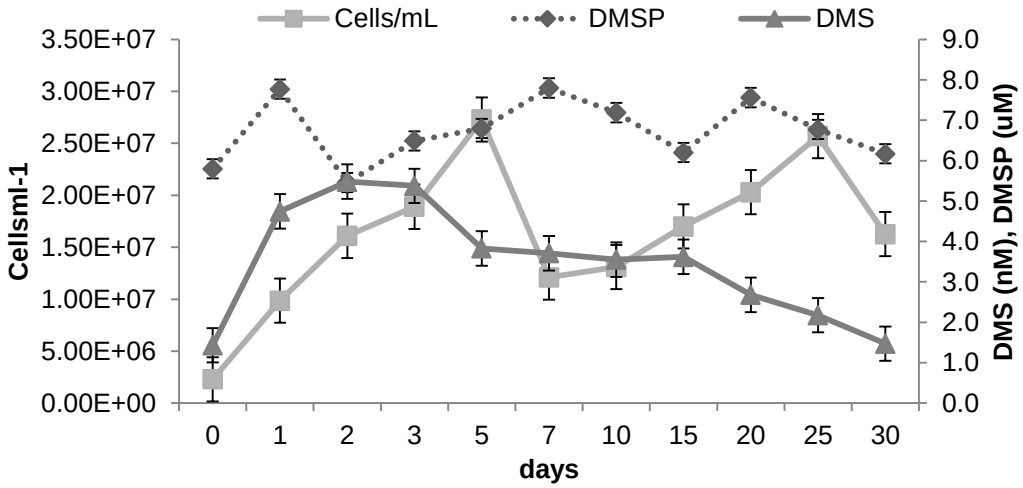


Figure 25: Effect of phosphate amendment on DMSP utilization by microbial community

4.4.3.3 Effect of iron on DMSP utilization by bacterial community

In presence of 1mM iron, the bacterial cell numbers underwent four cycles of bloom and crash at regular intervals with peaks on day 1, 5, 10, and 25. Similar trend was seen in the levels of DMSP concentration though overall the values decreased from 7.80 µM on day 1 to 4.24 µM on day 30. The trend in the evolution of DMS was more or less simple with gradual decrease from 3.39 nM on day 1 to 0.51 nM on day 30 (Figure 27).

Amendment of nitrate (10 µM), phosphate (1 µM) and iron (10 mM) on DMSP utilization by heterotrophic bacterial community showed that only iron could induce highest DMS release in the medium (3.39 nM) when DMSP concentration was 5.42 µM and coinciding with the first peak of cell growth.

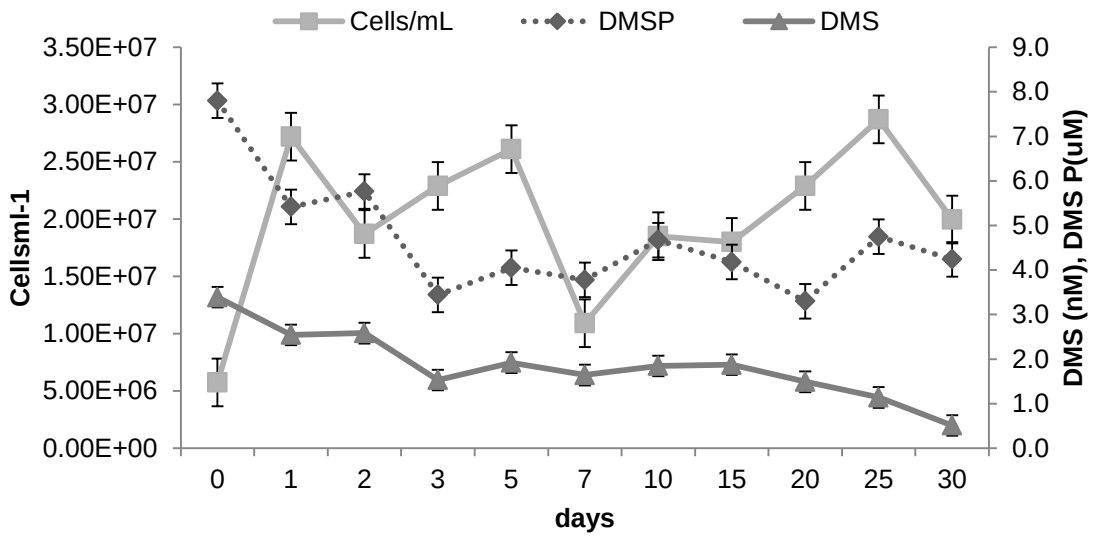


Figure 26: Effect of iron amendment on DMSP utilization by microbial community

Discussion

In this chapter, we discuss the temporal variability in DMSP dynamics and the allied variables during the study. Further, we elaborate on the interactions between abiotic and biotic variables particularly DMS/P and its producers and utilizers during the short term (tidal, diurnal) variation. A section is dedicated to long term (seasonal) variability in DMS/P and the interactions that drive these dynamics. The main focus of this chapter is phytoplankton and bacterial interaction in driving DMSP dynamics at short and long term scales. The diversity of producers and utilizers is discussed in subsequent sections. This is followed by discussion on laboratory mesocosm experiments on DMSP production by phytoplankton and utilization by bacteria. This chapter ends with a summary of salient findings from this study.

Coastal ecosystems are characterized by various interactions between environmental and biotic variables at multiple levels, which in turn govern the functioning of the ecosystems. Intertidal zones are niche ecosystems where tidal height and tidal amplitude affect light penetration, nutrient concentration, temperature, salinity and pH (Nixon and Pilson, 1983). To the best of our knowledge, this is the first study which evaluated the interaction of physico-chemical factors in the DMS/P dynamics in the intertidal zone of tropical estuarine beach during semi-diurnal tidal cycles. Our results suggest that though the same factors drive the DMS/P dynamics both in surfwater and sediment, their interaction and influence is diverse. Also, on a broader scale, these interactions are different in different seasons.

5.1 Tidal variability

5.1.1 Environmental variables

Dona Paula bay experiences semi-diurnal tides and the average tide height during spring tide is ~2.3 m (Shetye *et al.,* 1995; Sundar and Shetye, 2005), while average neap tide is within the range of ~0.7 m. The wave height in this area is >1.5 m from

June–August and <0.7 m during October–April (Chandramohan *et al.,* 1997). The study area (Figure 1) is characterized by strong seasonal variations and experiences strongest tidal currents of about ~25 cm s^{-1} during the pre-monsoon months (Bhaskar *et al.,* 2000). The variations in tide height had mixed influence on the physico-chemical and biological parameters and on few occasions the parameters significantly varied with the tide while on other, no clear relationship was found during this study.

The periodic and predictable flood produced by tides affects interstitial water levels and chemistry (Alongi, 1998). In our study the nutrient concentrations were generally higher in the sediment porewater than surfwater. Wang *et al.,* (2011) have also reported higher concentration of nutrients in pore water as compared to overlying waters in tidal marshes. The synchronization of high nitrate and silicate values with high tide suggested that these nutrients were brought in by seawater. TOC values measured in this study are comparable to the previous studies from this area by Bhaskar *et al.,* (2000) and Kumar *et al.,* (2009). They have reported an average carbon concentration of 981 ± 371 µg Cg^{-1} dry sediment and 472.12 ± 248 µM in seawater from the same area. They attributed the low organic carbon concentration (approximately 0.1% of dry sediment) to the consumption of the sedimented carbon at the sediment water interface or re-suspension/advection before its final burial. The organic carbon concentrations presented here are less than those observed from the Mandovi estuary where they ranged from 1.04 to 32.77 mg Cg^{-1}(Nasnolkar *et al.,* 1996). Various researchers have investigated temporal and spatial variability in porewater nutrients while few have reported short term changes linked to tidal cycle and exposure time (Trimmer *et al.,* 1998, Usui *et al.,* 1998, Kuwae *et al.,* 2003).

Several studies (Bradley and Morris 1990; Middelburg *et al.,* 1996 and references therein) have recognized the importance of intertidal areas as major sites of accumulation.of sediment associated organic matter and contaminants. Denitrification coupled to nitrification (Jorgensen 1983) is an important biochemical loop in sediments which causes enhanced mineralization and turnover of nitrogen compounds (Jorgensen 1983; Nielsen 1993) and represents an important nitrogen sink in coastal ecosystems.

During our study the concentration of nitrate was high during low tide, which can be attributed to the inhibition of nitrate reduction and stimulation of nitrification by the increased oxygen supply following high tide (Usui et al., 1998).

Nitrite and phosphate peaks synchronize; they increase at high tide and decrease at low tide perhaps due to physical input by tidal influence. Ward *et al.,* (1984), suggest that the transformations in nitrogen in seawater are biologically mediated and are carried out by organisms ranging from bacteria to large metazoans. The decrease in nitrate concentration when there is an increase in nitrite concentration could be suggestive of nitrate reduction. Senescent planktonic algae and aggregates are rapidly colonized by bacteria (Smith *et al.,* 1995; Simon *et al.,* 2002). The negative relationship between chl a and DO observed during this study was intriguing since DO is consumed for the decomposition of dead and decaying organic matter, a major portion of which is contributed by phytoplankton. However, active respiration by plankton-associated viable counts could also be one of the contributing factors.

5.1.2 DMS/DMSP

Very few studies have been carried out focusing on DMS/P variability in the intertidal sediments, none of which report of any tidal influence. In the present study, we found significant tidal pattern in DMS concentration in sediment but not in surfwater. The DMS concentrations measured in the surfwater during this study are comparable to the previous studies from this area (Shenoy *et al.,* 2003, Kumar *et al.,* 2009). In our study, the association of physico-chemical factors like temperature, light, salinity was more discernible with the surfwater DMS. Salinity has been reported to influence DMS production (Yoch 2002, Stefels *et al.,* 2007). Also, surfwater DMS was influenced by salinity irrespective of the tide, while sediment exhibited this characteristic only during high tide when the sediment was submerged under water indicating that the effect is due to flood waters. This could possibly be due to the influence of salinity which stimulates DMS production by algae in field (Hu *et al.,* 2005) and in laboratory cultures (Vairavamurthy *et al.,* 1985).

DMSP measurements in surfwater are in agreement with the earlier study from this area (Kumar *et al.,* 2009) and correlated well with both chl *a* and phytoplankton abundance in surfwater in our study. Similar and comparable observations have been made by Shenoy and Kumar (2007) along the west coast of India. The concentrations of DMSP in the present study are also close to those reported from other coastal waters (Bates *et al.,* 1987; Michaud *et al.,* 2007).The nutrients (phosphate and silicate) correlated well with the phytoplankton, which are the producers of DMSP thus implying an indirect influence on the DMS/P variability. The positive correlation of DMSP and phytoplankton abundance further corroborated this inference. However, Nedwell *et al.,* (1994) have stated that DMS and DMSP concentration in the North Sea sediment could be 1000 fold higher than in the overlying waters. Nevertheless, the concentrations were as low as 48 nM in the sandy sediments.

Andreae *et al.,* (1985) reported a sharp peak in DMS concentration in sediment porewater of the Peru upwelling area at 2–5 cm below the sediment surface and suggest that DMS is released within the upper few centimeters of the sediment and removed by biological consumption and diffusion into the overlying water. The maximum value for DMS in the sediment pore water of the upwelling area off Peru at ~120 nM, (Andreae, 1985) are higher than the present values.

Bergeijk *et al.,* 2002 observed that DMSP and DMS concentrations in the pore water of intertidal sediments of Ellewoutsdijk in the Westerschelde Estuary, The Netherlands were generally below or just above detection limit. They also noted that degradation of DMSP and DMS in sediment slurries was quite fast. Studies by van der Maarel & Hansen (1997) have shown that in anoxic intertidal sediment, the demethylation of DMSP indirectly or directly lead to the formation of methane. The association of DMS with phytoplankton abundance in sediment in the intertidal region of Dona Paula bay suggested that the phytoplankton was the source of DMS. Although intertidal sediments were rich in DMSP content, the corresponding DMS concentration was only marginally higher than in surfwater. Studies by Bergeijk *et al.,* (2002) suggest that the low flux of DMS from intertidal sediments is caused by relatively high turnover of dissolved DMSP and DMS and a relatively low turnover of particulate DMSP in intertidal sediments.

Kulkarni *et al.,* (2005) also recorded higher concentration of DMSP in the seawater during high tide in a salt marsh in South Carolina, USA. Though phytoplankton are the major source of DMSP, and consequently DMS, not many researchers have found significant link between phytoplankton and DMS in marine ecosystems, (Simo *et al.,* 1999, Stefels *et al.,* 2007 and references therein). During our study, the measured DMS linked consistently with phytoplankton abundance in sediment suggesting phytoplankton to be the main source of DMS. The high DMSP concentrations during high tide could probably be due to comparatively lower abundance of DMSP utilizing bacteria during high tide. Also, the higher DMSP: Chl a ratio in sediment (9.62) than in surfwater (2.57) suggests the presence of DMSP rich phytoplankton in the sediment. The sediments were 10 times richer in DMSP, and 5 times in DMS values than surfwater. The high DMSP content in sediment could be attributed to vertical flux from the seawater (Belviso *et al.,* 2006). We suspect that the demethylation pathway of DMSP could be dominating the sediment ecosystem, leading to the low measured DMS coupled with abundance of DMSP rich dinoflagellates in the sediment. The possibility of faster degradation of DMS could not be ruled out (Bergeijk *et al.,* 2002). Also, demethylation of DMSP to form methane (van der Maarel & Hansen, 1997) could also be responsible for low DMS in spite of high DMSP and these aspects need to be investigated in future.

The diurnal trends observed in DMSP in surfwater suggest the association of phytoplankton (which is the source of DMSP) which exhibit diurnal behavior. This was further supported by the high chlorophyll a values measured during the day. The utilizers of DMSP were higher during the night in both sediment and surfwater which explains the high abundance of DMS during this time. The results of the mesocosm experiments which were carried out to measure the utilization rate of DMS in sediment and surfwater indicate faster utilization of DMS (0.44 nM hr^{-1}) in sediment than in surfwater (0.24 nM hr^{-1}). As the surface sediment was used for this mesocosm experiment, this observation made is in concurrence with Belviso *et al*., (2002) where they found higher utilization of DMS/DMSP under aerobic conditions. However, in-spite of the high utilization rate, the net DMS content in the sediment is higher, thus making it potential source of DMS to the atmosphere. The estimated diffusive flux of DMS (0.04 µM $m^{-2}d^{-1}$) from the sediment to the porewater could explain the probable locking of

DMS within the sediment ecosystem. Since we did not measure the other degradation products of DMSP viz. methanethiol and acrylic acid, the definite fate of DMS/DMSP in these intertidal sediments cannot be presented.

5.1.3 Phytoplankton

The hydrodynamic processes like waves and tides influence the structuring of the microphytobenthic diatom community in intertidal areas (Steele and Baird 1968; de Jonge and van Beusekom 1995). During our study, both producers and utilizers in surfwater showed signature of tidal influence. The higher concentration of chl a in surfwater during high tide suggest that the measured chl a was mostly contributed from the seawater during high tide and due to resuspension of sediment dwelling phytoplankton, while the higher abundance of phytoplankton during low tide is due to the sediment dwelling diatoms. The contrasting pattern in distribution of chl *a* and phytoplankton abundance during this study seem to be influenced by the physico-chemical processes, tidal variations in salinity induced by mixing of marine water of low chlorophyll content with chlorophyll-rich riverine water (Fast 1993). Like phytoplankton, chl *a* concentration followed a tidal pattern with higher values during low tide in the sediment and vice versa in surfwater. Similar trend was observed in phytoplankton abundance in both surfwater and sediment which could be due to the phenomenon of phytoplankton getting physically suspended in the water during flood tide.

Stress and turbulence generated by waves may cause re-suspension of surface sediments and their associated micro-phytobenthos and consequently lower microalgal biomass (Baillie and Welsh 1980; de Jonge and van den Bergs 1987; Delgado *et al.,* 1991; de Jonge 1992; de Jonge and van Beusekom 1992, 1995). The sediment-dwelling diatoms form a major component of the microphytobenthic community in the intertidal region (Meadows and Anderson 1968; Round 1979). A study in Elbe estuary by Bernat *et al.,* 1994 showed that suspended particulate matter content and salinity were the main factors controlling biological processes in the maximum turbidity zone of the estuary and salinity fluctuation caused a decrease in phytoplankton biomass.

DMSP:Chl *a* ratio was higher in sediment (9.62) than in surfwater (2.57) suggestive of presence of DMSP rich phytoplankton in the sediment. Uher *et al.,* (2000) have reported high average DMS:Chl *a* ratio (nM DMS mg Chl *a)* of 45 ± 35 in July 1995, and an average of 7.4 ± 6.0 along the European eastern continental margin in September 1994. They concluded that the high difference in the DMS:Chl ratios for coastal and shelf regions cannot be attributed to regional differences. Visscher *et al.,* (1994), Van Bergeijk & Stal (2002), Jonkers *et al.,* (1998) have observed that the vertical distribution of chl *a* corresponded with the vertical distribution of DMSP in benthic intertidal systems.

5.1.4 Bacteria

The DMSP produced in these sediments and other systems acts as a substrate to the bacteria present. However, the attempt to study the bacterial population utilizing the DMSP has been scanty (Visscher *et al,* 1992, 1993). Total direct counts (TDC) of bacteria in both sediment and surfwater were significantly influenced by tide usually showing higher values during high tide.

Most Probable number of DMSP utilizers varied from 0.108–9.38 × 10^3cells mL^{-1} in surfwater. The abundance of DMSP utilizers varied with tide showing higher values during low tide and vice versa. In contrast, with sediment DMSP utilizing bacteria generally synchronized with tidal amplitude and the maximum abundance was recorded at 4.16 × 10^6cells g^{-1} during high tide and least during low tide at 5.17 × 10^5cells g^{-1}. Our values exceed those reported earlier from this area (Kumar *et al.,* 2009) by two orders and are comparable to those reported by Niki *et al.,* (1997) from a eutrophicated bay of Japan. Marine bacteria assimilate dissolved DMSP which can contribute up to 100% of the sulfur and 15% of the carbon requirements of the bacterioplankton community (Kiene and Linn 2000, Simo *et al.,* 2001). The retrievable counts of DMSPu–CFU did not show any variation with tide. It is probable that there is very little influence of the tides on the variation of bacterial retrievability. However, the phytoplankton abundance is significantly correlated with the retrievable counts suggesting the dependence of DMSP utilizing bacteria on the phytoplankton abundance. Though MPN method was

useful for monitoring changes in DMSP utilizing population, it may sometimes underestimate the true bacterial population due to the selectivity of the media (Niki *et al.,* 1997).

5.1.5 Interactions and probable drivers of DMS/P dynamics

The producers and utilizers of DMSP interact at different levels to influence the DMSP dynamics in an ecosystem. During our study, phytoplankton affected the abundance of viable bacteria in both surfwater and sediment albeit in a contrasting manner. The positive influence (43%) in the surfwater can be explained by the well-known fact that phytoplankton exudates could support bacterial proliferation and their dynamics are closely linked in coastal environments (Rooney-Varga *et al.,* 2005). About 45% of these viable bacteria could account for the variation in the abundance of the DMSP utilizers (CFU). In contrast, this correlation was negative in the sediment suggesting that either phytoplankton are outcompeting bacteria or the phytoplankton cells are producing compounds which inhibit bacterial growth in the sediment. Some phytoplankton produce antimicrobial compounds, thereby inhibiting bacterial growth (Rooney Varga *et al.,* 2005). Alternatively flagellates/ciliates could prey upon viable bacteria (Fernandes *et al.,* 2012). Another probable reason for this could be the presence of heterotrophic dinoflagellates particularly *Protoperidinium sp* which formed about (1–2%) of the sediment population. Also, the bacterial abundance could be inhibited by the antimicrobial compounds produced by some phytoplankton (Rooney Varga *et al.,* 2005). In sediment, the strong positive correlation of chl *a* and phaeopigments suggests the presence of easily degradable phytoplankton products for bacterial utilization. The positive correlation of chl *a* with total bacterial abundance also suggests the dependence of bacterioplankton on phytoplankton biomass

Cloern (1996) have mentioned that strong correlations between bacterial abundance and phytoplankton primary productivity are not always observed in nature because bacterioplankton populations are influenced by losses to micro-zooplankton (protozoan) grazers or bacterial viruses. The non-linear interactions observed in phytoplankton abundance and DMSP, DMS in the current study could be due to the interactions

between bacteria, phytoplankton and zooplankton. The role of dinoflagellates is crucial in studying DMSP distribution as a dinoflagellate cell holds about five times more DMSP than a diatom cell (Jean et al., 2006). The intracellular DMSP content of *Ceratium* spp is found to be 9.8 pM $cell^{-1}$ and *Protoperidinium* spp to be 14.7 pM $cell^{-1}$ (Jean *et al.,* 2006). Studies by Simo *et al.,* (2002) and Zemmelink *et al.,* (2006) suggest that the rate of DMSP synthesis is linked to photosynthesis, showing a diurnal signal of particulate DMSP. They reported higher concentration of DMSP during the day and the increase was due to increased level of particulate DMSP. Studies have demonstrated that the DMSP containing dinoflagellates are active diel migrants (Belviso *et al.,* 2000). Hence, the changes in planktonic composition affected the DMS/P levels in these waters. This taxon specific dependence is the main reason for the poor DMS v/s chl *a* correlations. Though, correlation has been found between chl *a* and DMSP (Townsend and Keller, 1996), which is in agreement with the algal origin of this compatible solute in marine pelagic systems.

The role of marine bacteria in the degradation of DMSP via DMS production and demethylation (Gonzalez *et al.,* 1999, 2000; Kiene and Linn, 2000) is very important and the composition of the microbial community decides the route of DMSP degradation (Iverson *et al.,* 1989). In this study, it is possible that high DMSP concentrations during high tide were linked to comparatively lower abundance of DMSP utilizing bacteria (DMSPu) during high tide. As the number of DMSPu is comparatively low during high tide, the DMSP concentrations are higher during high tide ($r = 0.478$, $p < 0.01$). Due to bacterial DMSP demethylation and DMS consumption processes, only a small percentage (1–2%) of DMSP produced by marine phytoplankton is ventilated to the atmosphere as DMS (Bates et al., 1994; Kwint and Kramer, 1996). The significant positive correlation between MPN of DMSP utilizers and dissolved oxygen suggests the participation of aerobic bacteria. The involvement of aerobic bacteria in DMSP utilization is further supported by positive correlation between DMSPu–CFU and DO.

There is scarcity of information on tidal influence on microbial loop functioning at diel time scale from estuarine waters (Hyun *et al.,* 1999; Kolm and Andretta, 2003). Some studies report that at night, bacterial biomass is reduced by heterotrophic nanoflagellate

predation (Wikner et al., 1990; Solic and Krstulovic, 1998) which exceeds production. However, the heterotrophic nanoflagellates top down control on bacteria on a short time scale (from diel to daily) is disputed (Puddu et al., 2000; Iriate *et al.,* 2003).

Multivariate analyses of the data showed that during our study, both producers and utilizers of DMSP featured in PC1, with high factor loadings suggesting the linkage between physical, chemical and biological factors in DMSP dynamics in the sediment. Chemical factors i.e. nitrate, nitrite, ammonium, Eh and DMSP associate together indicating their strong collective influence on this ecosystem. The influence of different physical parameters on source and sink of DMSP varied in surfwater and sediment. Based on observations and statistical analyses, it is evident that the physical forcing affects the properties of sediment more than surfwater. The abundance of the phytoplankton is mainly governed by changes in salinity in the sediment pore water.

Statistical analyses of the variables in this study highlight the importance of physical parameters like salinity and light which affect the phytoplankton abundance and biomass. These factors in turn influence the DMS concentrations and its flux from the intertidal beach. Based on observations and statistical analyses, it is likely that the semi-diurnal behaviour of tide affects the DMS/P dynamics in surfwater and sediment either directly or indirectly.

5.2 Seasonal variability

5.2.1 Environmental variables

The westcoast of India is influenced by Southwest Monsoon winds which is characteristic of the Indian subcontinent and plays an important role in governing the hydrography and climate (Shetye *et al.,* 1995, Qasim and Sen Gupta 1981). Dona Paula bay is situated in the Zuari estuary and earlier studies have addresses the seasonality in hydrography and biological productivity of this area (Shenoy and Patil 2003, Patil and Anil 2011). Due to the onset of monsoon, we saw strong seasonal trend in salinity and nutrients. The fluctuation in salinity was highest during the monsoon, due to influx of rainwater and riverine fresh water. The higher values of nutrients during monsoon could be attributed to river discharge in the estuary.

5.2.2 DMSP/ DMS

Intra-seasonal variation in DMSP, DMS was higher than the inter-seasonal variability. The highest values of DMSP were recorded during the monsoon season in both surfwater (8.08–39.85 nM) and sediment (74–759.95 nM). DMSP also showed secondary peaks during the post monsoon season varying from 4.77 to 28.73 nM in surfwater and from 13.28 to 98.31 nM in sediment. The pre-monsoon values were least varying from 3.13 to 8.08 nM in surfwater and from 10.48 to 78.60 nM in sediment. DMS levels varied from non- detectable limits to 1.98nM during pre-monsoon. During the monsoon, average DMS concentrations ranged from 4.63nM in June to a maximum of 19.38 nM in August. During post-monsoon, the variability was similar and averaged at 6.7 nM. Our results are in agreement with the study carried out by Shenoy and Patil (2003) where they reported that significant variability in DMS/P with maximal concentrations occurring in the southwest monsoon season in the Zuari estuary. Concentrations of DMSPp typically range from 5 to 300 nM depending on factors such as phytoplankton biomass and species composition (Iverson *et al.,* 1989; Malin *et al.,.* 1993). In open ocean regions, DMSPd concentrations as high as 200 nM have been observed (Malin *et al.,* 1993). The DMSPd pool is generally smaller (1–50 nM) than the particulate pool, but is believed to be more dynamic, with turnover times estimated to be in the order of hours in subtropical waters (Kiene 1996; Ledyard and Dacey 1996).

Various studies suggest seasonal variation in DMSP and DMS in North Sea coastal and shelf waters [Turner *et al.,* (1988, 1989), Kwint and Kramer (1996)]. This study shows that the drivers of DMSP dynamics are influenced by tide but the impact varies with seasons. Berjeigk *et al.,* (2002) have reported seasonal variation in DMSP concentration and concluded that diatoms comprise an important source of DMSP in intertidal sediments of Schelde estuary. Belviso *et al.,* (2006) suggest that DMS in the sediment originates from DMSP sedimentation from top, and DMS production from the sediment-water interface below. Further, they state that the distribution of DMS/P concentrations in sediments of North Sea reflected the distribution of sedimentary organic matter (1998). The amounts of DMS and DMSP have been measured previously from different marine sediments (Visscher *et al.,* 1991, 1994, Van Bergeijk & Stal 1996, Jonkers *et al.,* 1998a) and coral reef pore waters (Broadbent *et al.,* 2002). The DMSP produced in these sediments have been shown to act as a substrate to the bacteria present. The three main sinks of DMS are: consumption by bacteria, photo-oxidation into dimethylsulphoxide (DMSO; Brimblecombe & Shooter1986, Kieber *et al.,* 1996) and ventilation to the atmosphere.

5.2.3 Phytoplankton

Chl a and phytoplankton abundance in both surfwater and sediment exhibited a clear seasonal pattern. Changes in salinity, in addition to nutrient availability, affect the diatom community (Patil and Anil 2008) while the germination of dinoflagellate cysts are known to be affected by factors like temperature, salinity and light conditions (Kremp and Anderson 2000), thus driving the seasonal cycling between pelagic and benthic domains. Environmental variables play a significant role in dynamics of phytoplankton biomass and abundance. The increase in number of dinoflagellates in the post-monsoon could be linked to higher availibility of nutrients. Generally, the concentrations of nitrite, phosphate and silicate were lower during the pre-monsoon along with high temperature, salinity, and bacterial abundance. These factors could affect the phytoplankton community composition. Diatoms dominated the study in all the three seasons. Similar results have been reported by Rodekar, and Wagh, (2000), in their

study on planktonic diatoms of the Zuari estuary, Goa where minimum number of diatoms were recorded during monsoon and maximum during post monsoon and pre-monsoon months.

5.2.4 Bacteria

The DMSPu did not show a conspicuous seasonal trend as the numbers varied marginally. The highest values of MPN were recorded during monsoon probably due to the availability of high DMSP as well as organic matter from the land runoffs. We did not see any specific trend in the distribution on utilizers in the surfwater and sediment as they are general heterotrophic bacteria which use DMSP as a source of carbon and sulphur.

5.2.5 Interactions and probable drivers of DMS/P dynamics

Based on the observations and statistical analyses, the interactions in the intertidal sediment seem to be more complex and diverse. The phytoplankton (producers) and bacteria (utilizers) are major drivers of DMS variability as they act as source and sink of DMSP and are limited by environmental variables. Various researchers have stressed on the role of physical forces such as UV radiation dose and depth of mixing layer as important drivers of DMS variability in oceanic surface waters (Simo´ and Pedros-Allio 1999; Toole and Siegel 2004; Vallina and Simo´ 2007). In our study, among the variables examined, light intensity, temperature and salinity were the major constraints for DMS variability.Tides have a crucial role to play in the dynamics of fauna and flora dwelling on beach sediments. The effect of this physical forcing could be more evident during the pre-monsoon seasons when the effects of other factors are less discernible. During pre-monsoon, the producers and utilizers of DMSP and DMS show a definite tidal pattern. However, during monsoon, the estuary is more dynamic and there is a drastic change in the salinity and nutrient flux and hence the impact of these parameters is more significant than that of tide during the monsoon. Based on observations and statistical analyses, we infer that the influence of tide is masked by the hydrographic changes caused due to monsoon in sediment.

Irrespective of the season, light intensity was responsible for ~25% of the variation in DMS concentrations. This is in agreement with Vallina and Simo 2007 where incident solar irradiance correlated with surface water DMS concentrations. Cerquira and Pio (1999) in their study from intertidal mudflats of Canal de Mira in Portugal reported strong seasonal variations in the DMS emission rates and attributed the summer peaks in DMS emissions to ambient temperature. More recently, Arnold et al., (2013) in their laboratory mesocosm study on *Emiliania huxleyi* cultures reported that slight increase in temperature decreases the solubility of DMS in the water and leads to higher flux to the air. Salinity correlated negatively with the DMS concentrations only during the monsoon season ($r = -0.513$, $p < 0.05$) which explained about 26% of the variation. The negative correlation of salinity with DMS suggests higher DMS content in low saline waters which was formed due to the mixing of freshwater and seawater from the Arabian Sea. The temperature of surfwater related to the DMS concentrations positively in all the three seasons, but the strength of correlation was marginally higher in the pre-monsoon season as compared to the other two ($r = 0.461$during pre-monsoon > 0.456 during post-monsoon > 0.433 during monsoon). Intriguingly, the high values of DMS during monsoon coincided with the low chl a concentration which is possibly due to the higher abundance of dinoflagellates during this season. Our results confirm the significant temporal variation in DMS and species dependency in the study area. Studies by Barranguet *et al.,* (1997) showed that during spring and autumn blooms the microphytobenthic biomass of a tidal flat was dominated by diatoms while in summer cyanobacteria and Euglenophyceae members also coexisted Similar observations have been noted by Bergeijk *et al.,* (2002) from intertidal sediments. Shenoy and Patil (2003) observed the same phenomena in the Zuari estuary in 2000. All year they recorded values of DMSP concentration less than 10 nM except for the month of July when the abundance of phytoplankton cells was highest. For a concentration of 10^5 cells of dinoflagellates per liter and 9×10^5 cells of diatoms per liter, they recorded a concentration in DMSP of more than 400 nM.

In this study, DMSP related with phytoplankton abundance in all the three seasons however the strength of the correlations varied. This could be attributed to the fact that DMSP is species specific. A very clear shift in the salinity, temperature and

concentration of nutrients was discernible during the monsoon which affected the phytoplankton species composition and consequently the DMSP levels in the surfwater. Also, physicochemical parameters have more profound influence as compared to pre-monsoon. Negative correlation between utilizers and silicates is suggestive of the presence of silicate solubilizing bacteria. An increase in DMSP utilizing bacteria with decrease in salinity indicates the availability of DMSP as substrate for bacteria in the less saline ambient water. During the post-monsoon, the intermittent peaks of DMS coincided with the abundance of dinoflagellates in the surfwater. It is known that dinoflagellates are affected by the nitrate and nitrite concentration. Alkawri and Ramaiah (2010) in their study of dinoflagellates from the west coast of India have established that nutrients have the great influence on dinoflagellate abundance in our study area. Keller *et al.,* (1988, 1989) have established that dinoflagellates are significant sources of DMSP. Most recently Caruana and Malin (2014) in their extensive study confirmed the role of dinoflagellates as significant DMSP producers. In the present study too, there was a predominance of *Alexandrium minutum* at 0–6.12 × 10^2 cells L^{-1} during monsoon and 0–58 cells L^{-1} during post-monsoon. The other dinoflagellate encountered during these seasons was *Prorocentrum* sp. in the range 0–3.83 × 10^2 cells L^{-1} during monsoon and 0–19 cells L^{-1} during post–monsoon. Both these genera are known to produce high amount of DMSP (Caruana and Malin, 2014). Other DMSP producing genera in the samples were *Dinophysis* sp*.* and *Scripsiella* sp*.* Various studies (Nguyen *et al.,* 1988, Keller *et al.,* 1989, Leck *et al.,* 1990, Matrai & Keller 1994) suggest that the particulate DMSP concentration in seawater shows seasonal fluctuations which correlate well with algal densities of certain species.

Bacterial abundance related well with the phytoplankton indicating the dependency on the phytoplankton exudates which of which DMSP could be a part TVC of bacteria are only one order less than their total counts. Bacterial numbers are low in these sediments because of higher fraction of sand content. Fernandes *et al.,* (2012) have also reported similar observations and attributed these high numbers to the influence of the plant exudates and continuous nutrient replenishment from the surf waters. Thus in the present study, the linear correlations explain the contribution of tide and other factors on the abundance of viable counts (r = 0.48), total utilizers (r = -0.41), and

DMSP producers (r = 0.55) at p <0.01 in the two seasons. Principal component analysis of the data revealed that light intensity and temperature continue to drive the ecosystem in all the three seasons in surfwater. However, in sediment each season is driven by different environmental variable viz. tide in pre-monsoon, salinity in monsoon and light and temperature during post-monsoon.

Various researchers have shown that biological turnover of DMS is the dominating process in determining DMS concentration in marine surface waters (Kiene & Bates 1990, Kiene & Service 1991, Kiene 1992, Wolfe & Bates 1993, Bates et al., 1994). From our study we found that turnover time for DMS is sediment is half of that in surfwater suggesting the faster utilization of DMS in the sediment. This could also be attributed to the higher abundance of DMSP utilizing bacteria in the sediment leading to marginally higher DMS concentration in sediment in spite of high DMSP.

We investigated microbial production and utilization of DMSP in freshly collected estuarine samples. During non-monsoon, DMSP and DMS were produced by the phytoplankton at an average rate of 0.06 nM hr^{-1} and 0.02 nM hr^{-1} in the surf-water, and sediment, respectively. In sediment, the average DMSP production rate was less at 0.054 nM hr^{-1} while DMS production was more at the rate of 0.079 nM hr^{-1}. During monsoon, DMSP was produced at the rate of 0.8 nM hr^{-1} while DMS at 0.24 nMhr^{-1} in surf water. Even in sediment, the DMSP production rate was much higher at 5.94 nM hr^{-1} while DMS was produced at the rate of 0.06 nM hr^{-1}. This is in concurrence with the field measurements of DMSP and DMS from surfwater and sediment. We infer that the high DMSP and lower DMS production rates in sediment could possibly be the reason for locking of the DMSP in the sediment by either demethylation pathway and or DMS utilization by indigenous microbial community. Also, the enumeration of dmsp demethylating genes and dms producing genes indicate the dominance of demethylating group of microbes in the sediment. DMSP degradation, are generally orders of magnitude higher in intertidal sediment ecosystems than in open seawater. Visscher *et al.,* (2003) have noted that this could be due to methylation of sulfide formed by the reaction of low-molecular-weight organic carbon and biogenic hydrogen sulfide derived from sulfate reduction.

Probing of DMSP degrading genes in the Dona Paula bay revealed that demethylating genes were abundant in the sediment and were higher by one order of magnitude than surfwater. Though preliminary, we infer that it is likely that the high concentration of DMSP encountered in sediment is being demethylated leading to comparatively less DMS production. Recent study by Varaljay *et al.,* (2012) suggest that the DMSP genes correlated with environmental variables like primary production, photosynthetically active radiation, particulate DMSP, and DMS concentrations. They conclude that SAR11 bacterioplankton dominate DMSP cycling in the upper ocean oligotrophic North Pacific Subtropical Gyre with less but consistent involvement of other members of the bacterioplankton community. Studies by Levine *et al.,* (2012) suggest that it is the combination of genetic and biochemical variables which could modify the 'bacterial switch' hypothesis where the prevalence of different bacterial DMSP degradation pathways is regulated by a complex set of factors like carbon, temperature, and UV-A dose.

Ecosystems are complex and dynamic hence it is difficult to ascertain the specific drivers of DMS variability in natural samples. From this study we see that common variables interact at different levels viz. short term (tidal) and long term (seasonal) scales to wield their impact on the variability of DMS flux from the intertidal beach. The influence of physico-chemical factors like temperature and salinity is important in governing the flux of DMS from this ecosystem even at short term intervals. The influence of tides is restricted to pre-monsoon season.

5.2.6 Sea – air DMS flux

The calculated sea-air flux of DMS was highest during the monsoon season where it ranged from 0.3 to 60.91 µM $m^{-2}d^{-1}$. The average flux during this season was almost 10 times higher (24.75 µM$m^{-2}d^{-1}$) than the pre-monsoon (2.29 µM $m^{-2}d^{-1}$). In the post-monsoon season, the DMS flux was comparable to the value during monsoon (18.93 µM $m^{-2}d^{-1}$). The peaks in DMS flux were due to higher DMS concentrations and wind speed which varied from 0.2 ms^{-1} to 3.4 ms^{-1}. During the pre-monsoon season, as DMS concentrations in many samples were below detection limit, the flux varied from 0 to

2.31 µM $m^{-2}d^{-1}$. The annual mean flux of DMS from the surfwater of tropical intertidal beach of Dona Paula was 15.32 µM $m^{-2}d^{-1}$. During the southwest monsoon, the DMS flux was as high as 60.91 µM $m^{-2}d^{-1}$. These values exceeded the earlier reports on intertidal sediment where the flux varied from 0.96–1.92 µM $m^{-2}d^{-1}$ (Berjeigk *et al.,* 2002). The present values are also higher than the annual averages e recorded for the coastal Arabian Sea (µM $m^{-2}d^{-1}$) and the open ocean 3.8 µM $m^{-2}d^{-1}$ (Shenoy *et al.,* 2 007).

5.3 Diversity

5.3.1 Phytoplankton

Intertidal estuaries contain high numbers of microorganisms that are involved in the production and consumption of DMSP and DMS (Jonkers *et al.,* 1998a). The taxonomic composition of phytoplankton population, the physiology and nutrient status of these populations play a crucial role in ascertaining the DMS/P variability in the marine ecosystem (Simo and Dachs, 2002, Keller *et al.,* 2004). The sediment-dwelling diatoms form a major component of the micro phytobenthic community in the intertidal region (Meadows and Anderson 1968; Round 1979). During this study, diatoms dominated the phytoplankton diversity in the surfwater as well as sediment. Similar observations have been reported by Mitbavkar & Anil (2006).

As phytoplankton are governed by environmental factors like salinity, temperature, nutrients etc., monsoon may disturb the biotic community by either altering the levels of available nutrients or by influencing mortality (Shea *et al.,* 2004). It may also influence the community structure through effects on physiological processes such as cell division or by disorienting organisms (White 1976). There is considerable input of nutrients in coastal water bodies through precipitation and run-off originating from terrigeneous sources during monsoon. These factors along with drastic fluctuations in salinity due to influx of freshwater bring about major variations in the DMSP producers and utilizers. The sediment harbored 30 species of diatoms (11 centric, 19 pennate) belonging to 20 genera. Variations in salinity influenced the abundance of diatoms in the sediment ($r = 0.439$, $p < 0.01$).

Earlier studies from the Arabian Sea (Sawant and Madhupratap 1996, Subrahmanyan and Sarma 1961) have reported clear seasonal trend in the abundance and composition of phytoplankton. Our results from the intertidal zone are similar to those reported by Mithbavkar and Anil (2006), Subrahmanyan and Sarma (1967) where they noted the dominance of Dinophyceae members during post-monsoon.

5.3.2 Bacteria

This study reveals that Dona Paula bay is dominated by DMSP utilizing bacteria belonging to the gamma *Proteobacteria* group. The abundance is always higher in the sediment. In surfwater, the most abundant DMSP utilizers were *Alcaligenes* and *Pseuodomonas aeroginasa*, which are widely found in soil and water and dominated the surfwater in all the seasons during this study. Both these species are gram negative bacteria belonging to the gamma Proteobacteria group and are known to catabolize DMSP to form DMS (deSouza and Yoch 1995). *Bordetella* sp was also found in high abundance, similar to previous reports from this study area (PhD thesis Kumar S S 2009).

Sediment was dominated by *Salinicola salarius* which belongs to Halomonaceae, group of heterogeneous, heterotrophic, Gram-negative rods with oxidative metabolism (Haba *et al.,* 2010). This organism is found in saline and intertidal waters (Kim *et al.,* 2007) and it is possible that the fluctuating salinity conditions in the intertidal zone facilitate their abundance in the sediment. It was not surprising to find Firmicutes represented by *Virgibacillus* sp, *Lentibacillus* sp and *Pauscibacillus* sp which are Gram-positive in high numbers in the sediment as they are known to be obligate aerobes or facultative anaerobes. Also, these organisms are known to produce cysts under stress conditions. Based on the ecological characteristics of these organisms, their abundance in intertidal sediments is obvious.

5.4 Mesocosm experiments

5.4.1 DMSP production

Field observations showed that there could be certain pertinent environmental factors especially nutrients that could trigger higher production of DMSP/DMS in communities. Iron limitation is the most talked about driver of primary productivity in open oceans and a recent study by Buccirelli *et al.,* (2013) observed that under iron limitation, there is increase in DMSPp and DMSPt.

Our experiments clearly show that though individual cultures respond differently to different nutrients, iron is an important nutrient and a major driver for cell growth and DMSP production in selected diatom cultures and community from Dona Paula bay. Our investigation on the influence of nitrate, phosphate and iron enrichment on DMSP synthesis showed that Iron amendment triggered the maximum response in terms of DMSP synthesis and cell abundance in *Thallasiosira sp.* This could possibly be due to the role of DMSP as an antioxidant as proposed by Sunda *et al.,* (2002) from their study on coastal diatom species like *Thalassiosira pseudonana.*

Though diatoms are not considered to be very good producers of DMSP in nature, altering the limiting factors have positively affected the synthesis of DMSP (Bucciareli *et al.,* 2003, Sunda *et al.,* 2002, Kettles *et al.,* 2014). *T. pseudonana* is one such diatom which has received maximum attention for its role in DMSP production. Also, it is an excellent model organism in biological studies as its entire genome has been sequenced (Ambrust *et al.,* 2004). Under nutrient deplete conditions, enhanced level of DMSP in this organism has been found (Bucciarelli and Sunda 2003). More recently, Bucciarelli *et al.,* 2013 have shown that Iron limitation decreases the growth rate of the oceanic diatom *T oceanica.*

Another abundant diatom in our study area is Coscinodiscus sp. which showed maximum response in terms of cell numbers to nitrate amendment. There was no significant rise in DMSP levels under laboratory conditions. We also investigated DMSP synthesis in pennate Diatom, *Nitzschia closterium sp* which unlike Thallasiosira responded maximum to Iron amendment, but only in terms of cell abundance. Similar

results were seen in Navicula sp where maximum cell growth was triggered in nitrate amended medium. Thus, we concur with Keller *et al.,* (1989) that DMSP production is highly species specific. The enrichment of macronutrients (nitrate and phosphate) triggers cell growth which is not always accompanied by increased DMSP synthesis in these diatoms. Spielmeyer and Pohnert (2012) concluded that DMSP content of *Skeletonema marinoi* is influenced by multiple parameters like nitrogen levels, diurnal rhythm, growth phase and limitation of other nutrients such as silicate.

Similarly, the phytoplankton community also responded the best to iron enrichment in terms of DMSP concentration which increased 3 times than on 0 day. The increase in DMSP was associated with the hike in the dinoflagellate abundance on day 3, which are known producers of DMSP (Keller *et al.,* 1988, Caruana and Malin, 2014). The IronEx studies in the Pacific Ocean have also revealed such a behavior in the phytoplankton community wherein the phytoplankton standing stock bloomed dramatically over a period of 6 days following iron additions (Landry *et al.,* 2000).

5.4.2 DMSP utilization

Similar to phytoplankton, the micro-nutrient iron also plays a prominent role in inducing high proliferation and activity. The metabolic requirements of heterotrophic and other bacteria for iron have long been recognized (Berman, 1993). Our mesocosm experiments showed that iron enrichment promoted DMSP utilization by bacterial community resulting in gradual production of DMS over 30 days.

Amendment of iron (10 mM), phosphate (1 µM), nitrate (10 µM), on DMSP utilization by heterotrophic bacterial community showed that iron induced highest DMS (3.39 nM) release in the medium when DMSP concentration was 5.42 µM and coinciding with the first peak of cell growth. Also, in presence of iron, the bacterial population underwent for cycles of bloom and crash. This indicates that the microbial community probably recovers from the crash by recycling the nutrients in the medium. Also, there is a steady decline in the DMS concentration. This suggests that the DMS evolved is also consumed by the microbial community to meet their carbon and sulphur demands as highlighted by various researchers (Kiene *et al.,* 1999, Simo *et al.,* 2000).

In contrast to iron, phosphate amendment triggered maximum growth in terms of cell abundance which was not accompanied by DMS release. In agreement with our findings, Tortell *et al.,* (1996) had showed that iron deficiency decreased the growth efficiency of 3 of the 5 bacterial strains they tested. Low iron concentrations apparently affected the iron-rich electron transfer system. As yet, little attention has been paid to the possible limitation of aquatic bacterial growth and metabolism by available iron. Thus it is important to note that Iron limitation equally affects the growth efficiencies of bacterial assemblages.

A study focusing on short term variation in DMS/P dynamics was needed to provide more insight into the cycling of this complex compound as the drivers of coastal ecosystems respond even to short term variations. These short term variations reflect the changes in the biota of the estuarine ecosystem over tidal cycles. Consequently, tidal forcing directly governs the DMSP dynamics in the intertidal sediments during pre-monsoon and indirectly during monsoon. The tidal effect gets masked by the influence of lowered salinity and increased nutrients during monsoon. Thus the source and sink of DMSP are affected differently in different seasons in tropical estuarine beach sediment. Though previous research has shown that DMS concentrations in the surface waters exhibit a considerable spatial and temporal variation (Kettle *et al.,* 2000 and references therein) our study has been able to provide an insight into drivers responsible for the seasonal variation in DMS concentration in the intertidal sediments of tropical estuary for the first time. Therefore the influence of different physical parameters on source and sink of DMSP and DMS/P is varied in different seasons. Tide and temperature have strong positive influence on DMS during the pre-monsoon study. While the influence of chemical factors likes salinity, nitrate, nitrite on DMSP is pronounced during monsoon season.

From our study, we conclude that the tropical estuarine intertidal sediments are potential sources of DMS/P and the semi-diurnal behavior of tides influences their variability during pre-monsoon season. This study also shows that the drivers of DMSP dynamics are influenced by tide but the impact varies with seasons. During pre-monsoon, the producers and utilizers of DMSP show a definite tidal pattern. However,

during monsoon, the estuary is more dynamic and there is a drastic change in the salinity and nutrient flux. It is well established that diatoms are the major source of DMSP in intertidal sediments. Previous studies from this area have concluded that the most abundant DMSP producers in the Dona Paula bay are diatom (Shenoy *et al.,* 2003, 2007). From our study we delineate the seasonal pattern of distribution of these DMSP producers in the sediment and surfwater of Dona Paula bay. A shift in diversity and abundance of diatoms was observed from pre-monsoon to monsoon and post monsoon season. Phytoplankton respond to the changes in nutrients and light intensity and their numbers and diversity vary as per variations in physico-chemical variables. Zooplankton grazing is also a crucial factor governing DMSP concentrations and phytoplankton abundance.

DMS/P dynamics in the coastal waters off Trivandrum

In this chapter we present the findings of our time-series observations carried out in the coastal waters off Trivandrum, located on the south west coast of India. We begin with a brief introduction on the phenomenon of coastal upwelling and elaborate on its effects on biological processes with impetus on DMS/P dynamics. We provide information on the study area, sampling strategies and parameters analysed.

6.1 Introduction

Coastal upwelling in the Arabian Sea is an annual phenomenon occurring during summer monsoon (Banse, 1968; Sankaranarayanan *et al.,* 1978). This phenomenon bring nutrient rich, cold subsurface water into the euphotic zone, which results in high biological productivity, making the Arabian Sea one of the most productive areas in the world (Gardner *et al.,* 1999; Prasannakumar *et al.,* 2001; Wiggert *et al.,* 2005). Upwelling along the west coast of India appears to start in the southern regions (ca 9^{o} N) with the onset of summer monsoon in May-June and intensifies in July-August. It gradually proceeds north and has been observed up to 15′ N (Banse, 1959, 1968, 1984; Ramamirtham and Jayaraman, 1960; Sankaranarayanan *et al.,* 1978). An upsurge of nutrients from the bottom to the surface triggers a series of cascading effect on the trophic levels of coastal ecosystem. The biological processes of these ecosystems are largely controlled by the presence of phytoplankton, which make up the base of marine food web, responsible for CO_2 fixation. They form the basis of the food chain and subsequently influence the higher organisms viz. zooplankton and fishery production.

An upwelling zone exhibits both short term and long term temporal changes in nutrient uptake, productivity rates and community structure of phytoplankton in response to the rapid changes in nutrient fields (Habeebrehman *et al.,* 2008). The dynamics of DMS/P in a coastal upwelling region is intriguing due to the influence of change in chemistry on

the biota of the ecosystem. In this study we monitored a few pertinent abiotic and biotic variables to understand the dynamics of DMSP in coastal area post upwelling, over a short time series along Trivandrum coast, India. Apparently the variations in this compound are influenced by the grazers during the post upwelling period.

6.2 Study area

Sampling was carried out along the south west coast of India (Off Trivandrum) (Figure 27). Sea water samples were collected onboard FORV Sagar Sampada 282 in November 2010.The study consisted of Time Series observation at 50 m depth for five days at 6 h interval.

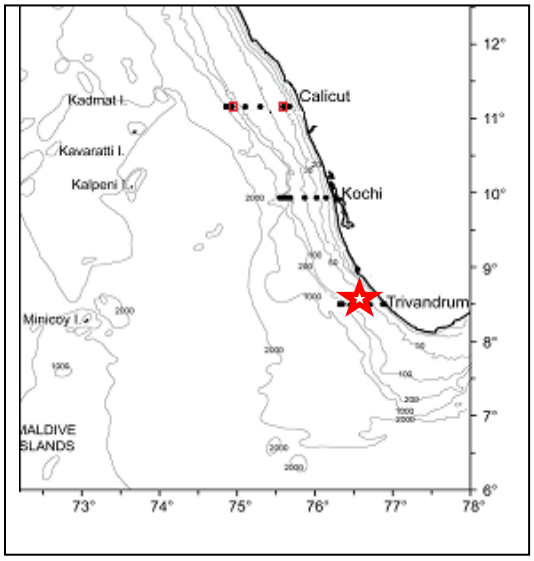


Figure 27: Study area and sampling location

6.2.1 Sample collection and analyses

Water samples were collected from the selected depths (5, 10, 25 and 45m) by using 10 L Niskin bottles. The sub samples were collected for the analysis of different parameters like DMSP, nutrients, chlorophyll, phaeopigments, phytoplankton. Details of the methods are given in Chapter 3. Meso zooplankton samples were collected in vertical haul from near bottom to the surface at different depth intervals using a multiple plankton net (MPN). Biomass of zooplankton was estimated as displacement volume after removing the large gelatinous organisms. These samples were then preserved in 4% buffered formalin for further analysis. Subsamples obtained from the large samples

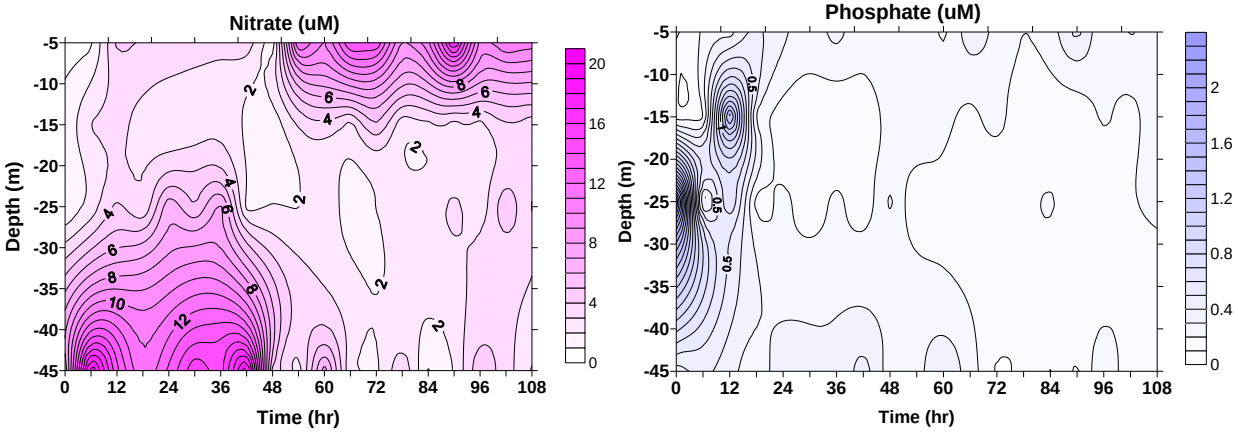
using a Folsom plankton splitter. was placed on Bogorov counting chamber and meso zooplankton were enumerated using stereo zoom microscope (Nikon SMZ1000, 10 × magnification.)

6.3 Results and Discussion

6.3.1 Environmental variables

Seawater temperature varied from 28.7 to 27.2 °C with an average value of 27.9 °C and did not show much fluctuation throughout the time series study. Average salinity was 34.87 and ranged from 33.17 to 35.46. The dissolved oxygen in the water column ranged from 1.19 to 4.68 mL L^{-1} and exhibited a uniform pattern without any significant vertical variation.

Nitrate concentration ranged from 0.77–20.02 µM. In the beginning of the time series, higher concentration of nitrate was measured at deeper depths i.e. 45 m while on the 5th day the surface concentration on nitrate was higher (Figure 28a). Phosphate concentration fluctuated between 0.08 to.2.368 µM at the beginning of our observations, the highest concentration of phosphate was measured at 25 m depth. However it gradually decreased from the water column towards the end of the time-series (Figure 29b).



a b

Figure 28: Nitrate (a) and phosphate (b) distribution in the water column

6.3.2 DMS, DMSP

Average DMSPt concentration was 103.2 nM while DMS was 1.11 nM. Highest DMSP and DMS concentrations were in the subsurface waters at 25 m depth with an average value of ~297nM and ~3nM respectively. (Figure 29a, b). In the beginning of the time series, DMSP was concentrates at 15 m depth. Gradually this DMSP maximum shifted to 25 m and the highest concentration was measured at this depth at 42 h. Like DMSP, DMS was concentrated in the surface to 15 m depth in the beginning. We encountered DMS maximum at 25 m depth at 42, 78 and 90 h.

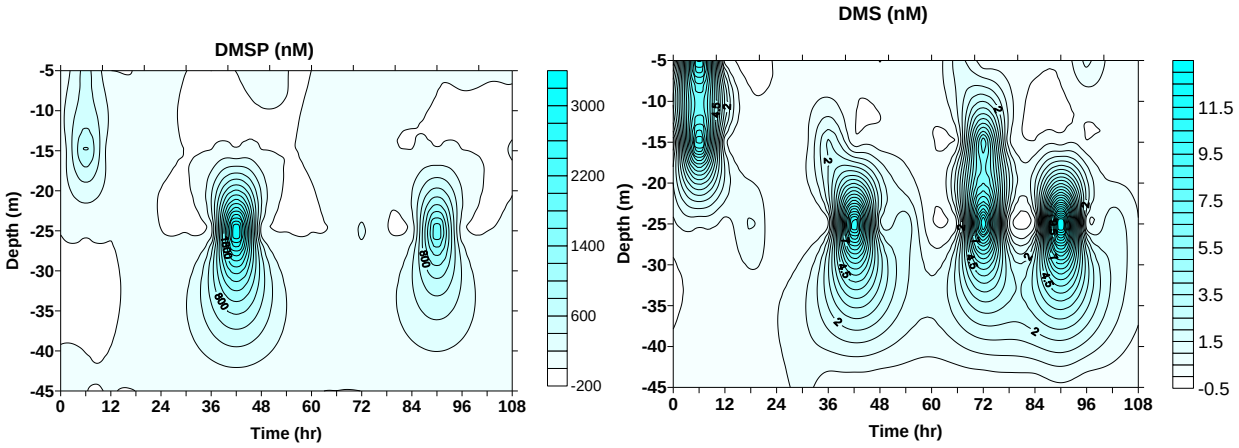


Figure 29: Variation in DMSP, DMS in seawater at different depths

6.3.3 Chlorophyll a and phytoplankton abundance

The chl *a* concentration ranged between 2.89 and 0.70 μgL^{-1} with an average of 1.39 μgL^{-1} (Figure 30). A total of 61 genera of phytoplankton comprising 40 diatoms and 21 dinoflagellates were identified. Diatoms dominated the phytoplankton community by contributing 78% followed by dinoflagellates which contributed 21% of the total abundance. Among the diatoms and dinoflagellates, *Psuedonitzchia* and *Gymnodinium* were the dominant genera. Average phytoplankton abundance was $1.17E \times 10^5\ L^{-1}$.

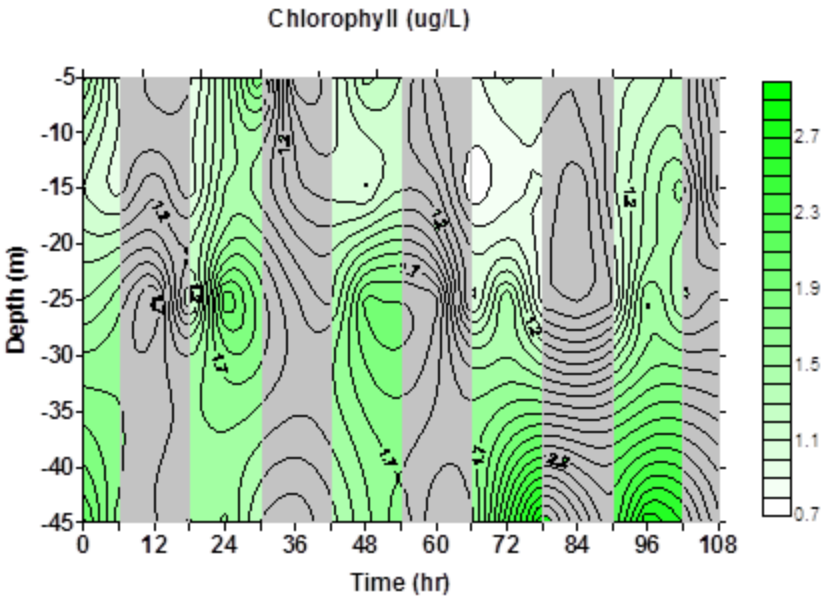


Figure 30: Chlorophyll a distribution at different depths

6.3.4 Zooplankton

Average number of zooplankton was 8.86 × 10^3 org m^{-3} of which copepod was dominant contributing about 80% of the abundance followed *by Oikopleura, Ostracods* and *Chaetognaths* (Figure 31).

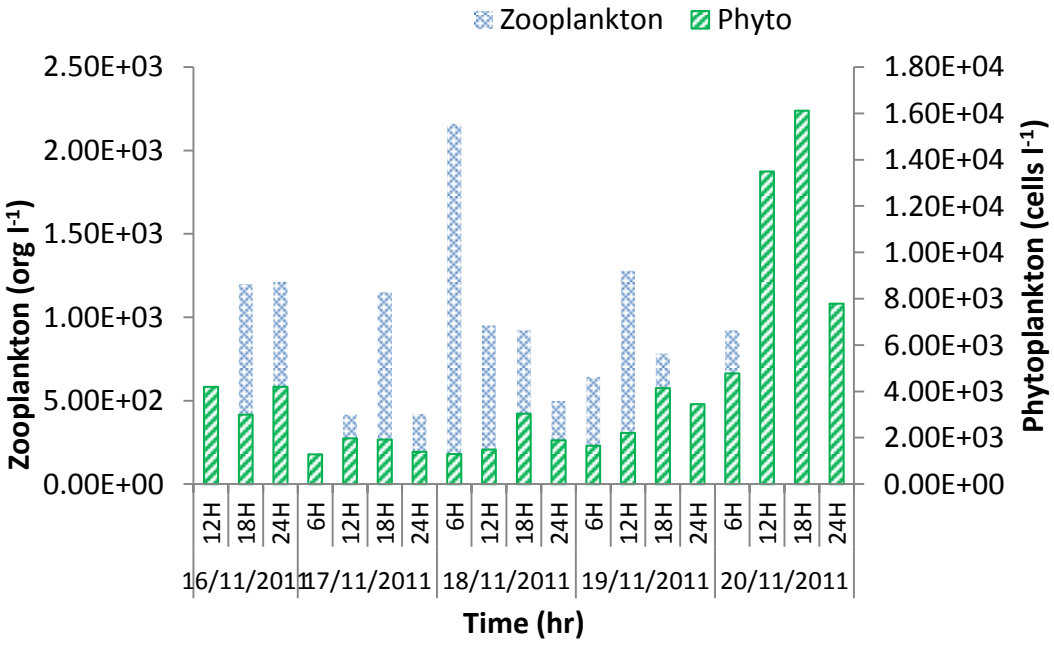


Figure 31: Variation in phytoplankton and zooplankton abundance

6.3.5 Interactions

Studies of the distributions of DMS and DMSP in the marine environment have shown that there is significant spatial and temporal variability of both compounds in surface waters of the oceans, where phytoplankton are generally highest (Holligan *et al.,* 1987,Turner *et al.,* 1988, Malin *et al*., 1993, Matrai & Keller 1993). However in our study the lowest abundance of phytoplankton at 4.18E × 10^3 L^{-1} was reported at this depth with dinoflagellates comprising ~25% of the population. Also, high vales of DMSP coincided with the abundance of dinoflagellates in the water column.

The phytoplankton abundance elevated significantly as a consequence of increase in the concentration of nitrate due to upwelling. Sonia *et al.* (2007) found negative correlation of nitrate and DMSP i.e. DMSPt and DMS concentrations increased with decreasing nitrate concentrations in Lawrence estuary. However, this link was not discernible in our study. Previous studies have reported this coincident low nitrate with high DMSP and DMS concentrations (Turner *et al.,* 1988; Leck *et al.,* 1990; Curran *et al.,* 1998). It has been hypothesized that phytoplankton adapted to conditions of low nitrogen would have evolved toward a high synthesis rate of a nitrogen-free osmolyte such as DMSP (Andreae, 1986). Phytoplankton abundance was negatively correlated with phosphate ($r = -0.61$, $p < 0.01$) which indicates the utilization of phosphate by the primary producers. This can be attributed to biological utilization of phosphate (Robin *et al.* 2010). Both DMS and DMSP showed significant positive relationship with phosphate ($r = 0.831, 0.938$, $p<0.01$) indicating significant role of phosphate as a nutrient for the DMSP producers.

In the present study, chl *a* did not show the significant correlation with the DMSP. However, since the biosynthesis of DMSP by phytoplankton is species-specific, there are usually only weak correlations between phytoplankton biomass, as indicated by chlorophyll a concentrations, and DMS or DMSP concentrations in sea water (Barnard et al. 1982, Bates & Cline 1985). Our findings agree with (Kwint & Kramer, 1996; Simo, Grimalt, & Albaiges, 1997; Townsend & Keller, 1996; Uher *et al.,* 2000) who found poor

or no correlations between chl *a* and DMS and DMSP, while some (Andreae, Andreae, & Schebeske, 1994; Matrai, Cooper, & Saltzman, 1996; Yang, Liu, Li, & Zhang, 1999) have reported significant correlations between chlorophyll and DMS and DMSP. Phaeopigment concentration which can be used as a grazing index (Legendre *et al.* 1993) showed no significant correlation with DMSP and DMS and ranged between 1.28–0.14 µg L^{-1} during the study. Our results agree with those reported by Guy *et al.,* (1996), which showed no significant relationship with DMSPd or DMS.

Zooplankton may play a significant role in the dynamics of DMS production in seawater. Leck *et. al.,* (1990) reported a positive correlation between the distribution of copepod biomass and DMS concentrations over an annual cycle in the Baltic Sea, (Guy *et. al.,* 1996). At the beginning of the time series, the zooplankton dominated the phytoplankton abundance while gradually the phytoplankton seems to recuperate from the grazing and their abundance increased. Phytoplankton abundance decreased with increase in abundance of zooplankton. This trend was supported statistically by negative correlation of phytoplankton abundance with zooplankton ($r = -0.586$, $p < 0.001$). It also correlated negatively with DMS and DMSP ($r = 0.944$, 0.861 $p < 0.001$ respectively) suggesting increase in DMS concentrations due to grazing activity of zooplankton (Levasseur et al., 1996; Wolfe & Steinke, 1996). Our result agree with the Vrede *et al.* (1999) which showed in the experimental study negative effect of zooplankton on phytoplankton biomass. Meso-zooplankton grazing probably plays an important role in DMS production in spring, in frontal regions, and in upwelling areas where the plankton community is dominated by larger phytoplankton cells *(*>5 µm) that are heavily grazed by meso-zooplankton (Cushing 1989, Legendre & Le Fevre 1989). Effect of meso-zooplankton grazing on DMS production may vary in time and space, probably in response to changes in the abundance and type of prey encountered in the marine environment (Guy *et. al.*, 1996).

6.4 Conclusion

Upwelling is a major event in the coastal ecosystems that brings about a series of effects some of which are immediate viz. drop in temperature, rise in salinity of surface

waters while others are reflected gradually, for e.g. increase in phytoplankton biomass subsequently triggering rise in zooplankton abundance and gradually fisheries. From our observations we conclude that the zooplankton play a major role in release of DMS from DMSP by feeding on the phytoplankton during post upwelling season. Thus DMS/P dynamics is top down controlled in this ecosystem. Further studies in different seasons and different phases of upwelling will help us to explore the role of these factors in DMS/P dynamics.

Summary

In the present thesis titled '***Phytoplankton and bacterial interaction in DMSP dynamics in Dona Paula Bay***' an attempt has been made to understand the intricate interactions of abiotic and biotic factors that drive the dynamics of DMS/P in the intertidal zone of Dona Paula bay. DMSP and DMS along with their producers (phytoplankton), utilizers (bacteria) and environmental variables were monitored over short term (tidal and diurnal) and long term (seasonal) in the Dona Paula bay from March 2010 – December 2011. To understand the driving factors in the water column, a short term study (at six hour interval for 5 days) was conducted in the post upwelling waters off Trivandrum located on the west coast of India. As tides constantly affect intertidal zones, the study hypothesizes that this parameter could have a major role on both producers and utilisers of DMSP through the seasons. Hence, it had the following objectives:

1. To delineate the patterns of distribution of phytoplankton and bacteria in sediment and surfwater.
2. To quantify the rates of DMSP production by phytoplankton communities in relation to different environmental parameters in field and laboratory conditions.
3. To quantify the rates of DMSP utilization by bacteria in relation to different environmental parameters in field and laboratory.
4. To understand the diversity of major producers and utilizers.

A sand flat at Dias Beach (15° 27′ N; 73° 48′ E), near Dona Paula bay, surrounded by the Zuari estuary was chosen as the study area. Surfwater samples were collected in acid washed PVC bottles and surface sediment samples were collected using a hand-corer with an inner diameter of 4.5 cm. All the samples were brought to laboratory in an icebox for further analysis. Interstitial water samples were collected by removing the sediment (15 cm) with a shovel and allowing the water to collect. The water samples were allowed to stand for a few seconds to allow sand particles to settle.

Temperatures of the surfwater and sediment were recorded at the study site using a mercury bulb thermometer, and salinity was measured with the help of a hand held refractometer. Light intensity was measured using TES digital Lux Meter. Samples for dissolved oxygen estimation were fixed on site with Winkler A and Winkler B reagents and further analysed in the laboratory. pH of the samples were measured in the laboratory using a pH meter (Elico, LI614) within few minutes of sample collection. Estimation of nutrients such as ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), phosphate (PO_4-P) and silicate (SiO_3) was carried out by standard procedures (Parsons *et al*. 1984).

DMSP was measured by cold alkaline hydrolysis method after its conversion to DMS (Turner *et. al.,* 1990, Kumar *et. al.,* 2009) by gas chromatography. Calibration standards were analyzed using the same protocol using DMSP salt procured from Research Plus, USA (Visscher *et al.,* 1992). Phytoplankton abundance was estimated by Utermohl's sedimentation method and Biomass (Chlorophyll a concentration) was measured by fluorometric method using a Turner designs fluorometer. Total direct bacterial counts (TC) and viable counts (TVC) were estimated by Acridine Orange Direct Count (AODC) method by Hobbie *et al.* (1977) and Kogure *et al.* (1983) respectively. The samples were serially diluted and inoculated in mineral medium supplemented with 100 uM DMSP. DMSP utilizing bacterial abundance was estimated by MPN method (Visscher *et al* 1990, Kumar *et al* 2009) and surface plate count method.

The salient findings of this study are enlisted below.

1. In surfwater, phytoplankton (abundance and biomass) followed a tidal pattern in the pre and post monsoon season with higher values during high tide in surfwater. The values were marginally higher in the post-monsoon (4.67×10^5cells L^{-1}) than in pre-monsoon (3.15×10^5cells L^{-1}) in surfwater.

2. In sediment, the phytoplankton abundance exhibited a seasonal pattern. Intermittent peaks were recorded during pre-monsoon with an average value of

6.83 × 10^5 cells g^{-1}. The lowest abundance was observed during monsoon at 5.02 × 10^5 cells g^{-1} which gradually increased in the post monsoon phase and averaged to 1.15 × 10^6 cells g^{-1}.

3. On the contrary, total bacterial numbers were highest during monsoon in both sediment and surfwater and were significantly influenced by tide usually showing higher values during high tide in sediment. However, during pre-monsoon, tide influenced the bacterial numbers only in surfwater. The sediment total bacterial counts for surface sediments during monsoon varied between 0.175 × 10^7–1.12 × 10^8 cells g^{-1} dry weight during low tide.
4. DMSP utilizing bacterial abundance did not show any significant seasonal pattern. The most probable number of utilizers varied from 3.71 × 10^3 in pre-monsoon to 4.87 × 10^3 cells mL^{-1} during monsoon. The bacterial abundance decreased during post -monsoon season to 4.49 × 10^3 cells mL^{-1}. The culturable fraction of DMSP utilizers varied from 4.86 × 10^4 - 5.33 × 10^4cfu mL^{-1}during pre-monsoon and post-monsoon respectively. Lowest values were recorded during pre-monsoon season i.e. 4.86 × 10^4cfu mL^{-1}.
5. Measurement of demethylation and DMS production were estimated by enumerating the *dmdA* and *dddR* genes respectively in sediment and water dilutions. Results clearly show that the *dddR* gene was enumerated at 10^2–10^3 g^{-1} in sediment and 10^2 mL^{-1} in water. The corresponding *dmdA* genes were in the order 10^2 and 10^3 in sediment and water respectively suggesting that the DMS releasing function is more prominent in the sediment.
6. However the rate measurements show that DMS flux is actually more in the sediment during monsoon suggesting there could be more players in the DMS flux from the sediment during this season.
7. During non-monsoon, DMSP and DMS were produced by the phytoplankton at an average rate of 0.06 nM hr^{-1} and 0.02 nM hr^{-1} in the surfwater, respectively. In sediment, the average DMSP production rate was less at 0.054 nM hr^{-1} while DMS was production was more at the rate of 0.079 nM hr^{-1}
8. During monsoon, DMSP was produced at the rate of 0.8 nM hr^{-1} while DMS was at 0.24 nM hr^{-1} in surfwater. Even in sediment, the DMSP production rate was

much higher at 5.94 nM hr^{-1} while DMS was produced at the rate of 0.06 nM hr^{-1}. During non-monsoon, the average of DMSP concentrations were 5.13 and 51.95 nM and that of DMS were 1.09 and 6.75 nM in surfwater and sediment, respectively

9. During this season significant relation of DMS with tidal amplitude ($r = 0.478$, $p < 0.01$) was noted in sediment but not in surfwater where the roles of other abiotic factors were more prominent. In sediment, tides influenced the chlorophyll *a* ($r = 0.412$, $p < 0.01$) and Most Probable Number (MPN) of DMSP utilizers ($r = -0.551$, $p < 0.01$). Thus tides impart direct influence on the DMS/P dynamics in the sediment and only indirectly in the surfwater.
10. During monsoon, the tidal influence is rather indirect and nonlinear. DMS concentration correlated negatively with salinity ($r = 0.651$, $p < 0.001$). Salinity and NO_2-N concentration were significantly influenced by tide (33.5% and 22% respectively). Tidal amplitude also affected 32% variation in DMSP utilizing bacterial population and 12% variation in producers of DMSP.
11. The biotic factors involved in DMSP dynamics are directly influenced by tide during non-monsoon. During monsoon, chemical factors like pH, DO, and nitrate influenced the producers and utilizers.
12. This is also supported by Principal Component Analysis which segregated the variables in to five major components with higher factor loadings for physical components like tide, salinity and temperature during the non-monsoon season while the influence of chemical parameters is more evident in during monsoon season.
13. Hence, tidal influence on the source and sink of DMSP varies with seasons which could result in seasonal variation in flux of Dimethyl sulphide from the intertidal sediments.
14. In contrast to coastal sediments, DMS/P variations in post upwelling waters off Trivandrum were apparently more driven by grazers during the study period thus suggesting that DMS(P) dynamics was top down controlled in waters off Trivandrum during this season. Average DMSP concentration in these waters was 103.2 nM while DMS was 1.11 nM. Highest DMSP and DMS concentrations

were in the subsurface waters at 25 m depth with an average value of ~297 nM and ~3 nM, respectively.

15. As field observations showed definite influence of parameters like nutrients on producers and utilizers, mesocosm experiments were conducted which showed that iron was the most important driver for producers.

16. Amendment of nitrate (10 µM), phosphate (1 µM) and iron (10 mM) to phytoplankton community of seawater showed that Iron elicited maximum response in DMSP production (450 nM) on the 3rd day.

17. Similar experiments with individual cultures also showed that iron had maximum influence on *Thallasiosira sp.* which responded the best with peak on 20th day with a maximum DMSP production 2.15 nM on 25th day. However another species of *Thallasiosira pseuodonana* responded best to phosphate amendment and peaked on day 13 with maximum DMSP production on day 20 (2.8 nM).

18. Nitrate amendment brought about maximum response in *Coscinodiscus sp*. which attained peak on 13th day and resultant DMSP peak on 20th day 2.54 nM.

19. The aforementioned experiments clearly show that though individual cultures respond differently to different nutrients, iron is an important nutrient and a major driver for cell growth and DMSP production in community and selected diatom cultures from Dona Paula bay.

20. Parallel experiments conducted with the utilizers i.e. bacteria also showed similar interesting results. Amendment of nitrate (10 µM), phosphate (1 µM) and iron (10 mM) on DMSP utilization by heterotrophic bacterial community showed that only iron could induce highest DMS release in the medium (3.39 nM) on day 1 when DMSP concentration was 5.42 nM which also coincided with the first peak of cell growth.

21. Similar experiments conducted with the bacterial isolates in dark conditions showed maximum utilization of DMSP in presence of iron.

22. These results further corroborated the influence of Iron in DMSP production by phytoplankton and utilization by bacteria.

23. Highest phytoplankton diversity was represented by three diatom species viz. *Navicula (33%), Odontella (12%),* and *Thalassiosira (7%).* in the pre-monsoon

season. During monsoon, two entirely different diatom species, *Rhizosolenia (21%)*, and *Fragilleria (9%)* dominated the surf-water samples. However, in the post monsoon season, the number of dinoflagellates increased by an order in few samples and was represented by *Protoperidium* and *Gymnodium* species. Other phytoplankton species were *Coscinodiscus, Thalassiothrix, Grammatophora, Navicula trevelyana, Rhopalodia, Synedra, Calonies, Epithemia, Protoperidinium, Cymbella, Pinnularia, Diatoma, Asterionella, Nitzschia, Amphora, Naviculairidis, Hantzschia, Fragillaria, Navicula cancellata.* The ratio of Diatom:Dinoflagellate was constant (4:1) during pre-monsoon and monsoon and changed to 7:3 during post-monsoon season. Thus the dinoflagellate abundance increased from 20% to 30%.

24. About 74 isolates which were major DMSP utilizers were obtained from mineral medium plates supplemented with 100 µM DMSP. The DMSP utilizing bacteria were identified by biochemical tests up to the generic level. They mostly belonged to Gamma Proteobacteria. The most abundant ones were as follows:

i.	*Alcaligenes* (Proteobacteria; Betaproteobacteria)	19%
ii.	*Pseudomonas* sp, (Proteobacteria; Gammaproteobacteria)	12%
iii.	*Flavobacterium*sp, (CFB group)	7%
iv.	*Marinobacter*, (Proteobacteria; Gammaproteobacteria)	6%
v.	*Xanthomonas*, (Proteobacteria; Gammaproteobacteria)	3%
vi.	*Vibrio* sp, (Proteobacteria; Gammaproteobacteria)	3%
vii.	*Moraxella* sp, (Proteobacteria; Gammaproteobacteria)	2%
viii.	*Bacillus* sp.(Firmicutes)	1%

16S rDNA sequencing revealed that *Salinicola salarius* belonging to the Proteobacteria; Gamma proteobacteria group was the most dominant DMSP utilizing bacteria in the intertidal sediment of Dona Paula bay while *Alcaligenes sp* dominated the surfwater.

Conclusion

1. The intertidal sediment is a complex ecosystem and the variability in DMS is driven by both tidal and seasonal influence.

2. Although the environmental variables in surfwater and sediment same, their interaction varies in both systems. The influence of tides is restricted to sediments and only during pre-monsoon while salinity strongly influences the system during monsoon. Temperature and light continues their effect through the seasons.

3. The source and sink of DMSP are differently affected in different seasons in tropical estuarine beach sediment.

4. Tides influenced the distribution of both phytoplankton and bacteria especially during the non-monsoon season. However, during monsoon, the impact of tide on both producers and utilizers of DMSP get masked.

5. The present findings also indicate that intertidal ecosystems could be as important a source of DMS as the open ocean and coastal waters contributing higher flux particularly during the monsoon.

6. The intra-seasonal variation in DMS flux from surfwater particularly during monsoon was greater than inter-seasonal variations.

7. In the coastal waters off Trivandrum, DMS/P dynamics is top-down controlled during non-monsoon. This study highlights this aspect for the first time.

Future scope

1. Detailed study on the complexity of the processes in the sediment will throw more light on the DMS/P dynamics in the sediments.

2. Measuring abundance of the other degradation products of DMSP viz. methanethiol and MMPA would provide better understanding of the DMS/P dynamics in the sediment.

3. Metagenomic analysis of the samples would provide detailed information about the diversity of the DMSP utilizing microbes in this ecosystem.

4. Equations for estimating direct sediment – air flux of DMS would be helpful in establishing the contribution of DMS from the intertidal sediments to the atmosphere.

5. Mathematical modeling approach can provide more insights on contribution of DMS to the atmosphere.

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Appendix

Outline of Appendix

Chemical variables
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Chemical variables
Dissolved oxygen –
1. Estimation of dissolved oxygen
Winkler's Reagent A

Manganese (II) chloride ($MnCl_2.5H_2O$)	60 g
Distilled water	100 mL

Winkler's Reagent B

Potassium iodide (KI)	60 g
Sodium hydroxide (NaOH)	32g
Distilled water	100 mL

Sulfuric Acid (50%)

Concentrated H_2SO_4	50 mL
Distilled water	50 mL

Sodium thiosulphate solution 0.01 N

$Na_2S_2O_3.5H_2O$	0.249 g
Distilled water	100 mL

Starch indicator solution

Starch indicator	1 g

Standard iodate solution

KIO_3.	0.3567 g
Distilled water	100 mL

Work flow:

1. Water samples were fixed in 125mL dissolved oxygen bottles without any air bubbles and Winkler's reagent (A and B) 0.5mL each was added. This was followed by addition of 3mL of 50% H_2SO_4 to dissolve the precipitate. The bottles were shaken vigorously till the precipitate homogenized
2. An aliquot of 50 mL was titrated against standardized thiosulphate solution till solution turned pale yellow. At this point 1mL of starch indicator was added and the solution was titrated till the blue color disappeared and the solution turned colorless.
3. The burette readings were noted. The titration was repeated three times and the mean titer value were calculated.

.4. For estimating reagent blank 50mL of distilled water was processed in the similar way as samples and titrated against 0.01N sodium thiosulphate solution.

4. For standardization of sodium thiosulphate, 10mL of standard iodate solution (0.01N) was added to 50mL D/W fixed with 0.5mL of Winkler's A and B solution each and the titration was completed as described above.

6. The concentration of dissolved oxygen is calculated as per the below mentioned formula and the values are expressed as mL/l.

$$O_2(\text{ml/l}) = \frac{(R - R_{b/k}) V_{IO_3} \cdot M_{IO_3} \cdot E}{(R_{Std} - R_{b/k})(V_b - V_{reg})} - DO_{reg}$$

R =	Volume (mL) used for sample
*R*Std =	Volume used for standard
*R*b/k =	Volume used for blank
*M*IO3 =	Molarity of standard KIO3 (M)
*V*IO3 =	Volume of standard KIO3
E =	5,598 mL O2/equivalent
*V*b =	Volume of sample bottle
*DO*reg=	oxygen added in reagents
*V*reg =	Volume of reagents

2. Estimation of ammonia
Reagents

NaOH (0.8mol):

NaOH	8g
Distilled water.	250 mL

Phenol Reagent:

Phenol	20 g
Ethanol	750 mL
Distilled water	400 mL
Sodium nitoprusside	150 mg

Trione reagent:

Trione	0.5 g

Tri sodium citrate solution:

Tri sodium citrate	120 g
NaoH	5 mL
Distilled water	250mL

Primary standard:

Ammonium nitrate	0.008 g
Distilled water	100 mL
Concentration: 1mL =	1 mM.

Working standard:

Primary standard solution	1 mL
Distilled water =	99 mL
Concentration: 1mL =	10 µM

Work flow:

1. A 10 mL working volume of the sample is taken in a test tube. In case of blank, 10 mL distilled water is used.

2. To this, 0.4 mL of phenol reagent is added

3. Then 0.2 mL of trisodium citrate buffer is added to the sample followed by 0.5 mL of trione reagent

4. The mixture is then mixed well and incubated in dark for 6 h.

5. Spectrophotometric readings are measured after 6 h at 630 nm.

Sr. no	Volume of sample (mL)	Volume of D/W	Phenol reagent (mL)	Tri sodium citrate buffer (mL)	Trione reagent (mL)	Total volume
Blank	0.0	10	0.4	0.2	0.4	11
1	0.0	10	0.4	0.2	0.4	11
2	0.0	10	0.4	0.2	0.4	11
3	0.0	10	0.4	0.2	0.4	11
4	0.0	10	0.4	0.2	0.4	11
5	0.0	10	0.4	0.2	0.4	11

Estimation of nitrites

Reagent:
Sulfanilamide solution:

Sulphanilamide	5 g
Concentrated. HCl	50 mL
Distilled water	300 mL

N-(1-Napthyl)-ethylenediamine dihydrochloride solution:

N-(1-Napthyl)-ethylenediamine dihydrochloride	0.5 g
Distilled water	500 mL

Primary nitrite standard:

Sodium nitrite	0.069 g
Distilled water	100 mL
Concentration: 1mL =	1 mM.

Secondary standard

Primary standard solution	1 mL
Distilled water.	99 mL
Concentration: 1mL =	10 µM

Working nitrite standard:

Secondary standard solution	1mL
Distilled water.	99 mL
Concentration: 1mL =	1µM

Work flow:
(a) A working sample of 50mL was taken in 125 mL flasks in triplicates.

(b) Sulfanilamide solution (1 mL) was added to each flask, mixed and allowed to react for 5–8 minutes.

(c) To this, 1mL of the N-(1-Napthyl)-ethylenediamine dihydrochloride solution was added and mixed immediately.

(d) The reagent blank was determined using distilled water as sample instead of seawater, exactly following the procedure for sample analysis in triplicates. Absorbance was measured by spectrophotometer at 540 nM.

Sr. no	Volume of sample (mL)	Volume of D/W	Sulphanilamide solution (mL)	NED solution (mL)	Total volume	
Blank	0.0	50	1.0	1.0	52	Measure absorbance at 540 nM
1	0.0	50	1.0	1.0	52	
2	0.0	50	1.0	1.0	52	
3	0.0	50	1.0	1.0	52	
4	0.0	50	1.0	1.0	52	
5	0.0	50	1.0	1.0	52	

Estimation of nitrates

Reagents

• Ammonium chloride buffer (Conc. buffer)

NH_4Cl	25 g
Distilled water	100 mL

• Diluent buffer:

Concentrated buffer	12.5 mL
Distilled water	500 mL

• Sulphanilamide reagent

Sulphanilamide	5 g
Concentrated HCl	50 mL
Distilled water	300 mL

• N-Naphthyl ethylene diamine reagent.

N-Naphthyl ethylene diamine	0.5 g
Distilled water	500 mL

Primary nitrate standard:

Sodium nitrite	0.0101 g
Distilled water	100 mL
Concentration: 1mL =	1 mM.

Working nitrate standard:

Primary standard solution	10 mL
Distilled water.	90 mL
Concentration: 1mL =	100 µM

Work flow

1. A cadmium column of 30cm length was prepared by using coarse cadmium powder (1–2 mm mesh size).
2. The flow rate of cadmium column was adjusted at approximately 10 mL per minute.
3. The column was recharged with 15mL of mercuric chloride solution and was rinsed thoroughly with Distilled Water (D/W).
4. Blanks were prepared with D/W (50 mL).

5. Each sample was poured through the cadmium column.
6. The first 20 mL flow through were collected in a measuring cylinder and discarded.
7. Rest 25 mL sample was collected and 0.5 mL of sulphanilamide and 0.5 mL ethylenediamine dihydrochloride solution was added to it were added
8. The absorbance was measured spectrophotometrically at 540 nM after 10 min.

Estimation of phosphates

Reagents:
Ammonium molybdate solution:

Ammonium paramolybdate	15 g
Distilled water	500 mL

Sulphuric acid solution

Sulphuric acid	250 mL
Distilled water	750 mL

Potassium antimonyl tartarate solution:

Potassium antimonyl tartarate	0.34 g
Distilled water	250 mL

Ascorbic acid solution:

Ascorbic acid	27 g
Distilled water	500 mL

Mixed reagent:

Ammonium molybdate solution	100 mL,
Sulphuric acid,	250 mL
Potassium antimonyl tartarate	50 mL

Standard stock solution:

Anhydrous potassium dihydrogen phosphate	0.816 g
Distilled water	1000 mL

Concentration: 1mL=0.6 uM

Secondary standard:

Primary standard	10.0 mL
Distilled water	990 mL
Concentration: 1mL=	0.06 µM

Work flow:
1. Take 25 mL of distilled water sample in conical flask labelled as blank.
2. In another set of labelled conical flasks 25 mL water sample was taken and 0.5 mL of mixed reagent was added to all the flasks. The contents were thoroughly mixed and kept standing for 10 minutes.
3. This was followed by 0.5 mL of ascorbic acid and the flasks were, mixed thoroughly and allowed standing for 15 minutes for complete formation of molybdenum blue complex.
4. Absorbance was measured in the spectrophotometer at 880 nM preferably within one hour of addition of reagents.

Sr. no	Volume of sample (mL)	D/W (mL)	Mixed reagent (mL)	Mix contents for 10 minutes	Ascorbic acid	Total volume (mL)
Blank	0.0	25	0.5		0.5	26
1	25	0.0	0.5		0.5	26
2	25	0.0	0.5		0.5	26
3	25	0.0	0.5		0.5	26
4	25	0.0	0.5		0.5	26

Estimation of silicates

Reagent:
Ammonium molybdate solution:

Ammonium heptamolybdate	38 g
Distilled water	300 mL

Sulphuric acid solution:

Concentrated. sulphuric acid	250 mL
Distilled water	750 mL

Oxalic acid:

Oxalic acid	10 g
Distilled water	100 mL

Ascorbic acid solution:

Ascorbic acid	28 g
Distilled water.	1000 mL

Mixed reagent:

Ammonium molybdate solution,	300 mL
Sulphuric acid solution	300 mL

Standard stock solution: (primary standard)

Sodium Hexafluoride silicate	0.19 g
Distilled water	1000 mL
Concentration: 1mL=	1 mM

Secondary standard:

Primary standard	10 mL

Concentration: 1mL=	100 µM
Distilled water	90 mL

Work flow:

1. In a plastic conical flask, 25 mL of distilled water sample was taken and labelled as blank.
2. In another set of labelled conical flasks 25 mL water sample was taken and 0.5 mL of mixed reagent was added to all the flasks. The contents were thoroughly mixed and kept standing for 10 minutes.
3. This was followed by 0.5 mL of ascorbic acid and 0.5 mL of Oxalic acid. The contents of the flasks were mixed thoroughly and allowed standing for 15 minutes.
4. Absorbance was measured by the spectrophotometer at 880 nM preferably within one hour of addition of reagents.

Sr. no	Volume of sample (mL)	D/W (mL)	Mixed reagent (mL)	Mix contents for 10 minutes	Ascorbic acid (mL)	Oxalic acid (mL)	Total volume (mL)
Blank	0.0	25	0.5		0.5	0.5	26.5
1	25	0.0	0.5		0.5	0.5	26.5
2	25	0.0	0.5		0.5	0.5	26.5
3	25	0.0	0.5		0.5	0.5	26.5
4	25	0.0	0.5		0.5	0.5	26.5

2. Biological variables
Chlorophyll

Reagents
Acetone (90%)

Acetone	90 mL
Distilled water	10 mL

Work flow:

1. 500 mL of the sample was filtered and the filter paper was placed in scintillation vials wrapped with aluminium foil to avoid the exposure to light.
2. 10 mL of 90% acetone was added into each scintillation vial and the filter paper was crushed with a glass rod for 10 seconds.
3. The samples were refrigerated for 18 hrs to allow the extraction of the pigments in the solvent.
4. Acetone (90%) served as blank
5. The fluorometer was allowed to warm up and stabilize for 30 minutes prior to use. Pigment concentration before and after acidification was measured using a Turner design's fluorometer and is expressed as ug/L of Chlorophyll *a* and Phaeophytin.

Media for phytoplankton growth:-

f/2 medium (Guillard and Ryther 1963)

1. $NaNO_3$ stock solution : $NaNO_3$ = 75.0 gL^{-1}.
2. NaH_2PO_4 stock solution : NaH_2PO_4 = 5.0 gL^{-1}.
3. Trace Metals stock solution:
   - Na_2EDTA: 4.36 gL^{-1}. l
   - $FeCl_3 \cdot 6H_2O$: 3.15 gL^{-1}.
4. Primary Metals Stocks (below) 1mL of each of the five
   - $CuSO_4 \cdot 5H_2O$: 1.0 g/100 mL
   - $ZnSO_4 \cdot 7H_2O$: 2.2 g/100 mL
   - $CoCl_2 \cdot 6H_2O$: 1.0 g/100 mL
   - $MnCl_2 \cdot 4H_2O$: 1.8 g/100 mL
   - $NaMoO_4 \cdot 2H_2O$: 0.63 g/100 mL
5. Vitamin Stock solution
   - Biotin:- 10.0 mL of 0.1 $mg \cdot mL^{-1}$ solution (1mg in 10 mL)
   - Vitamin B12:- 1.0 mL of 1.0 $mg \cdot mL^{-1}$ solution (1mg in 1mL)
   - Thiamine HCl:- 0.2 g

To make the final medium, add the following to autoclaved filtered seawater and make up the volume to 1000mL

- $NaNO_3$ Stock solution: 1.0 mL.
- NaH_2PO_4 Stock Solution - 1.0 mL
- Trace Metals Stock Solution- 1.0 mL
- Vitamin Stock Solution: 0.5 mL
- Primary metals stock: 5.0 mL

Bacterial variables –

1) Antibiotic Cocktail

Autoclaved distilled water	30 mL
Nalidixic acid	24 mg
Piromidic acid	12 mg
Pipemidic acid	12 mg
Sodium hydroxide	150 µl (saturated)

Use autoclaved sea water to dissolve the content and then filter sterilize

2) Yeast Extract

Yeast extract	0.3 g
Distilled water	30 mL
(Freshly prepared)	

3) Sulfide solution

Sodium sulfide	1 g
Distilled water	20 mL
(Freshly prepared)	

4) Buffered Formalin

Saturate available formalin with Hexamine and Borax. Filter sterilize and store at room temperature.

5) Acridine orange

Acridine orange powder	0.1 g
5% formalin solution	100mL

Filter through 0.22µm filter paper. Store in amber bottle in refrigerator

Media preparation:

Nutrient Broth	**gram/L**
Quarter strength	
Nutrient broth (Himedia)	2 g
Seawater	
pH 7.5	

Full strength	
Nutrient broth (Himedia)	8 g
Seawater	
pH 7.5	

Mineral medium (Visscher et al. 1992)

NaCl	25 gL^{-1}
NH_4Cl	0.2 gL^{-1}
$CaCL_2.2H_2O$.	0.225 gL^{-1}
KCl	0.2 gL^{-1}
$MgCl_2.6H_2O$	0.2 gL^{-1}
KH_2PO_4	0.02 gL^{-1}
Na_2CO_3	0.2 gL^{-1}
Vitamin, B12	0.002 gL^{-1}
$FeSO_4.7H_2$	0 0.001 gL^{-1}
HEPES Buffer	0.238 gL^{-1}
Trace element solution*	1 mL
pH 7.5	
Milli Q water	1000 mL
***Trace element solution**	
EDTA	0.50 gL^{-1}
$ZnSO_4.7H_2O$	0.01 gL^{-1}
$MnCl_2.4H_2O$	0.003 gL^{-1}
H_3BO_3	0.03 gL^{-1}
$CoCl_2.6H_2O$	0.02 gL^{-1}
$CuCl_2.2H_2O$	0.001 gL^{-1}
$NiCl_2.6H_2O$	0.002 gL^{-1}
Na_2MoO_4	0.003 gL^{-1}
Milli Q water	

Fluorescence In-situ Hybridization

<u>Reagents</u>

1. *Formaldehyde (8%)*

Dilute 1025 µL of 39% formaldehyde in 10 mL MilliQ water/

Filter sterilize before use

2. 10X PBS (stock)

Reagent	Quantity of the reagent	Total Volume (mL)
NaCl	0.8 g	10 mL
KCl	0.02 g	
Na_2HPO_4	0.144 g	
KH_2PO_4	0.024 g	

Adjust the pH to 7.4 Do not autoclave. Store at 4°C.
When required, prepare 1X PBS and autoclave before use.

3. Hybridization buffer:

Reagent	Concentration	Volume used (µL)	Quantity of the reagent
NaCl	5M	720	5.84g in 20 mL
Tris.HCl	1M	80	1.576 in 10 mL
Formamide	32%	1200	3.2mL in 10 mL
SDS	10%	4	10g in 100 mL
MilliQ water			2996 µL to make up the total volume to 5 mL

All the reagents are prepared in MilliQ water and autoclaved before mixing.
Volume required. per sample = 20 µL

4. Washing buffer:-

Reagent	Concentration	Volume used(µL)	Quantity of the reagent
NaCl	5 M	1020	5.84g in 20 mL
Tris.HCl	1 M	1000	1.576 in 10 mL
EDTA	0.5 M	500	1.86g in 10 mL
SDS	10%	50	10g in 100 mL
MilliQ water			47.43 mL to make up the total volume to 50 mL

Procedure:

I. Fixation of sediment samples and prehybridization

1. Fix 0.5 g of sediment sample in 5 mL of formaldehyde solution (1) for an hour.
2. Add 5 mL of 1X PBS and re-suspend the sample.
3. Centrifuge at 10,000 rpm for 5 min at 4°C, pour off supernatant.
4. To the pellet add 5 mL of a 1:1 mix of PBS/ethanol (store at -20°C for next step if required)
5. Re-suspend the sample and transfer 1 mL of aliquot to 5 mL of a 1:1 mix of PBS/ethanol.
6. Sonicate the aliquot for 15 sec at 40 MHz and filter 1mL in triplicates onto white membrane filters and wash 5X with autoclaved filtered sea water.
7. Air dry the filter preparations and store in petri dishes until hybridization.

II. Fixation of bacterial cells from culture and prehybridization

1. Take 1 mL of the culture in a 2 mL microfuge tube. Centrifuge at 10k rpm for 5 min at 4°C, pour off supernatant.
2. To the pellet add 1.5 mL of saline. Centrifuge at 10k rpm for 5 min at 4°C, pour off supernatant. Repeat this step twice.
3. Repeat steps 4–7 as above.

III. Hybridization on membrane filters

1. For hybridization mixtures, add 1 µl each of forward and reverse primer to 18 µl of hybridization buffer (Final concentration of each primer/probe in the working solution = 50 ng/µl)
2. Place membrane filter (refer I. 6) with samples on glass slides (the side having the cells facing upwards).*Filters can be cut into sections to fit the filter rim.*
3. Add a 20 µl of D/W on to hybridization chamber (Corning) and fix the slide holding the filter.
4. Add 20 µl hybridization mix on the filter and incubate at 46°C, 100 rpm in a shaking incubator for 3 hours.

IV. Washing of cells

1. Transfer filter sections into 10 mL of pre-heated washing buffer (37°C) in a beaker and incubate for 15 min at 48°C.
2. Pour washing buffer with filter into a petri dish. Pick filter sections and rinse them by placing them into a petri dish with distilled water (autoclaved) for 2 min. Then let them air dry on blotting paper with cells facing upward.

V. Mounting & microscopy

1. Samples are mounted with Vecta Shield mounting medium which contains DAPI.
2. Probe conferred fluorescence fades much more rapidly than DAPI fluorescence For counting, it is therefore safer to first quantify specifically stained cells in green excitation and subsequently all cells from the same field of vision in UV excitation. Filter 3 for DAPI and filter 4 for Cy3 signal.

Identity of bacterial isolates

Biochemical tests

1) Catalase Test

H_2O_2	3%
Distilled water	10 mL

2) Oxidase Test (1%)

N,N Dimethyl para Phenylene Diamine oxalate	0.1 g
Distilled water	10 mL

3) Oxidative/ Fermentative medium

1. Dextrose	1 g
2. Peptone	0.2 g
3. KH_2PO_4	0.03 g
4. Agar	1.5 g
5. Bromothymol blue	0.002 g
6. 50% Sea Water	100 mL

Combine 2, 3, 5 and check pH= 7.2 or more.
Then add 4 and boil to melt. Add 1.

4) Amylolytic Media

Nutrient Agar	28 g
Starch	2 g
50% Sea water	1000 mL
pH	7.5–7.8

Mix Well. Sterilize at 121°C for 15 minutes

5) Lipase Media

Peptone	10 g
NaCl	5 g
$CaCl_2$	0.1 g
Tween 80	10 mL
Agar	15 g
50% SW	1000 mL
pH	7.0–7.4

Tween 80 is autoclaved separately and added to medium before pouring.
Mix Well. Sterilize at 121°C for 15 minutes

Gram staining
Method
• Smear of the isolates was prepared on clean dry grease free slides.
• The smears were air-dried and heat fixed.
• They were then treated with crystal violet for 1 min followed by Gram's iodine for 1 min.
• The slide was then washed with decolorizing solution (ethyl alcohol) till the blue color disappears.
• Counter stained with safranin for 30 sec. The slide was then washed with water.
• Dried and observed under oil- immersion microscope.

KOH test
Method
- One drop of freshly prepared 3% Potassium hydroxide (KOH) was placed on a clean microscopic slide.
- A loop full of growth of organism from the plate was scraped and a dense suspension was made with KOH solution.
- Continuously mixed it for 60 seconds and the suspension was pulled up with sterile toothpick.
- String formation indicated Gram's negative and KOH positive, vice versa.

Oxidase test

Method
- A drop of oxidase reagent was placed on Whatmann filter paper no.1
- Isolates were taken using sterile toothpicks and placed on treated filter paper to check the presence of Cytochrome oxidase in the isolates.
- The observations were inferred from the following table

Catalase test:
Method

- This test was performed using 3 % hydrogen peroxide on a glass slide.
- Scrape the growth from a slant or plate with a non- metallic instrument.
- Suspend it in 3 % hydrogen peroxide on a slide.
- Examine for effervescence, presence of effervescence denotes catalase positive and absence denotes negative reaction.

MOF test:
Method
- The tubes containing the OF medium were stab inoculated with the cultures
- Incubated at 26 ± 2 C for 48 hrs.
- The observations were inferred from the following table.

Amylase test:
- Starch Agar medium was prepared and sterilized by autoclaving.
- It was poured into sterile petri-dishes and kept for surface drying at room temperature (28 ± 2° C).
- The culture was inoculated for 24 –48 hrs.
- Then Lugol's iodine was flooded over the culture and observed for the clearance zone indicating amylase production.

Lipase test:
- Tributyrin agar medium was prepared and sterilized by autoclaving.
- It was poured into sterile petri dishes and kept for a surface drying at room temperature (28 ± 2 °C).
- The culture was then inoculated onto the medium by spot inoculation method.
- The cultures were then incubated for 24–48 hrs.
- Presence of a clearance zone indicates lipid production.

Molecular methods
Extraction of DNA from bacterial cells:
1. Pellet out (minimum) 0.1g of Bacterial cells at 7000 rpm for 10 minutes at 4 °C.
2. The cells were washed thrice with 1 mL of Phosphate buffered saline (PBS) by centrifugation at 7000 rpm for 10 minutes at 4°C.
3. Then the pellets were suspended in 750µL of DNA extraction buffer (DEB) and then 250µL of 20% SDS was added.
4. The suspension was mixed by inversion and was incubated in water bath at 65 °C for one hour with gentle inversion at every 5 min interval.
5. Equal volume of Phenol: Chloroform: Isoamyl alcohol (PCI) mixture was added and mixed thoroughly for 5 min.
6. The above mixture was centrifuged at 10000 rpm for 15 min at 4 °C.
7. The clear aqueous layer was carefully transferred into a fresh sterile eppendorff tube without disturbing the other two bottom layers.
8. With this aqueous layer 500 µL of absolute ice cold ethanol was added and was incubated for 24hours at -20 °C.
9. After incubation the samples were pelleted out at 8000 rpm for 5 min at 4 °C.
10. The pellet was washed with 500 µL of 70 % Ethanol and air dried in dessicator.
11. Then 50µl of 1x TE buffer was added and was stored at -20 °C.

Quantification of DNA

The DNA extracted was then quantified using Thermo ScientificTM NanoDrop 1000 Spectrophotometer.

Agarose Gel Electrophoresis (AGE):

The crude DNA extracts after quantification was separated in 0.6% agarose gel by applying the electric field in a horizontal electrophoresis unit.

The steps used in carrying out the AGE were as follows:-

1. Agarose (0.8g) was suspended in 100 mL of 1X TAE buffer, heated in a microwave oven for 2.5min, poured in the gel casting tray and was allowed to solidify for 15 min at room temperature.
2. The gel was transferred to the horizontal electrophoresis unit and slowly the electrophoresis buffer (1X TAE) was poured into the buffer tank without any air bubble until it covers the gel surface and the comb was lifted carefully.
3. The sample of DNA (8µL of the DNA sample + 2µL of the Gel loading dye) was loaded in the wells using sterile pipette tips.
4. DNA ladder was prepared by mixing 2µl of Marker (supermix DNA ladder, Genie), 2µl of Gel loading dye and 6µl of 1X TAE buffer.
5. The gel was allowed to run for 2 hours and the power was disconnected when the tracking dye (Bromophenol blue front) reached 3/4th of the total gel length.

Staining and de-staining of the agarose gel and visualization of DNA:

The gel was taken out from the unit and was transferred into staining solution containing Ethidium bromide (0.5µgmL^{-1}) for 15min in the dark followed by de-staining solution (1X TAE Buffer) for 5 min and was visualized under UV transilluminator for bands. Thereafter, the photogragh was taken in Gel-Doc (Alphalmagar 2000).

Amplification of DNA by Polymerase Chain Reaction (PCR)

	Steps	**Volume required**	**Concentration**	**Time (Vortex/ incubation)**
1	Sterile protease, RNase, DNase free water	12.75 µL	-	-
2	Master AMP Taq PCR buffer	5 µL	10X	-
3	$MgCl_2$	7 µL	25 mM	-
4	Master AMP PCR enhancer	9 µL	10X	-
5	dNTP mix	4 µL	2.5 mM	Vortex for 3 seconds
6	Primers (forward and reverse)	5 µL each	10 pmol each	-
7	Master AMP Taq DNA polymerase	0.25 µL	-	-
8	DNA Template	2 µL	-	Vortex for 3 seconds
	Total volume of reaction mixture = 50 µL			

*PCR of the samples was carried out under following conditions:-

Initial Denaturation	94 °C	3 min
Denaturation	94 °C	1 min
Annealing	50 °C	1 min
Extension	72 °C	1 min
Final Extension	72 °C	5 min

Publications

1. *An appraisal of biological responses and network of environmental interactions in non-mining and mining impacted coastal waters*, Christabelle E G Fernandes, Ashish Malik, V K Jineesh, Sheryl O Fernandes, Anindita Das, **Sunita S Pandey**, Geeta Kanolkar, P P Sujith, Dhillan M Velip, Shagufta Shaikh, Samita Helekar, Maria Judith Gonsalves, Shanta Nair, P A LokaBharathi, Environmental Science and Pollution Research 04/2015; DOI: 10. 1007/s11356-015-4497 -4.

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6. *DMSP dynamics is tidally driven in tropical estuarine beach sediment of Dona Paula, Goa – India.* **Pandey Sunita**, P. A. LokaBharathi, Poster presentation in 5th International SOLAS Summer School held at Corsica, France from 29th August – 10th September 2011

Conference papers

1. Inter-seasonal variability in DMS/P in the tropical estuarine intertidal beach – Pandey Sunita Surendra and P A LokaBharathi, 4th National Conference of Ocean Society of India, Goa.
2. Adenosine triphosphate in the waters of West Coast of India -An indicator of living microbial biomass - Anindita Das, Christabelle EGF-C, Sheryl OF, Sunita Pandey, Dhillan Vellip and LokaBharathi P A, 4th National Conference of Ocean Society of India, Goa.

3. *Seasonal variability in DMS fluxes from tropical estuarine intertidal* surfwater – Pandey Sunita, P. A. LokaBharathi, 6th International DMSP symposium at the CSIC-ICM, Barcelona, Spain.

4. *Top down control of post upwelling waters off Trivandrum: Indications from variability in DMS(P)* - Pandey Sunita, Bhonsle Sneha, Manguesh Gauns, Ritu Kumari, P. A. LokaBharathi.

5. *Phosphate solubilizing bacteria: comparison between coastal and deep sea sediments* – Sushanta U. Biche, Pandey Sunita, Maria-Judith Gonsalves, P.A.LokaBharathi.

6. *Anthropogenic forcings modulate coastal ecosystems* - Christabelle E.G. Fernandes, Ashish S. Malik, Jineesh V.K., Sheryl O. Fernandes, Anindita Das, Pandey Sunita, Geeta Kanolkar, Sujith P.P., Samita Helekar, Maria Judith Gonsalves, Manguesh U. Gauns, Shanta Nair, Baban S. Ingole, P.A. Loka Bharathi.

Under review

1. *DMS (P) dynamics in intertidal zone of tropical estuarine beach* – **Pandey Sunita**, P A LokaBharathi - Under review

2. *Monsoon masks the impact of tide on the source and sink of DMSP in a tropical estuarine bay* – **Pandey Sunita**, P A LokaBharathi – Under review