

## **Chapter 6**

### **SUMMARY**

## **Introduction**

Dimethylsulphide (DMS,  $\text{CH}_3\text{SCH}_3$ ) is the major volatile sulphur compound produced biogenically and has a global average concentration in oceanic surface waters of 1.2 to 3.8 nM (Andreae and Crutzen 1997). It accounts for over 50% of the total biogenic sulphur entering the atmosphere annually, where it affects atmospheric processes and is involved in climate regulation (Andreae, 1990). DMS concentrations are determined by the balance between its production and biological and physical loss mechanisms (Gabric et al.1999), which are a part of a complex web of ecological and biogeochemical processes.

DMS is biologically produced in the ocean from enzymatic cleavage of dimethylsulfoniopropionate (DMSP), a compound synthesized by a wide variety of marine phytoplankton (Keller et al.1989). Dimethylsulfoniopropionate is a significant component of the organic matter that flows through the microbial food web. Its production is estimated to account for 3 –10% of the global marine primary production of carbon (Kiene et al. 2000), and its degradation supplies about 3 –10% of the carbon requirements of heterotrophic bacteria in surface waters (Simó et al. 2002). As stated by Simó (2001), a key to understand the dynamics of DMS + DMSP [(DMS (P))] is to find out whether DMSP utilization is widespread among bacterioplankton or is a characteristic of only a few phylotypes.

Studies on DMS (P) are sparse and focused on spatial distribution (Shenoi and Kumar 2007). The only biologically related study available is the one that describes the association of dinoflagellates with DMSP in Zuari estuary (Shenoy and Patil, 2003). Hence, in continuation, the present work examines the link between the distribution of DMS (P) to abiotic and biotic variables in the adjacent bay, at Dona Paula on a temporal scale both at short diurnal and long annual scales.

Dona Paula bay is semi-enclosed and located near the mouth of the Zuari estuary on the west coast of India. In order to understand the influence of biological and physicochemical variables that govern the distribution of DMS (P), the study has the following objectives.

#### **Objectives of the study**

The objective of this study was to

- To estimate the concentration of DMS and DMSP in Dona Paula Bay.
- To gauge the influence of physico- chemical parameters on the concentration of DMS/DMSP.
- To understand the role of microbes in the assimilation and flux of DMS /DMSP.
- To identify the organisms involved (phytoplankton/ microbes) in the process.

## **Approach**

In order to meet the above objectives, both diurnal and monthly water sampling were done at Dona Paula Bay at the fixed site (15° 27`N, 73° 48` E).

All parameters related to DMS (P) were measured. The physico-chemical (temperature, salinity, pH, DO, nitrite, nitrate, phosphate, TOC) and biological parameters (Chl *a*, Phaeopigments, Primary Productivity, Phytoplankton, TC, TVC, CFU) were studied using standard techniques. The organic sulphur compounds were measured using GC and HPLC. Two bacteria populations, 'DMS' and 'DMSP' utilizers were enumerated by the Most Probable Number (MPN) method (Visscher et al. 1991).

The composition of the producers and utilisers were studied using standard manuals for identification. Molecular taxonomy like 16S rDNA sequencing was employed to identify the representative strains that showed strong affinity, and ability to utilize DMSP. Chemotaxis chamber was used to study the affinity of bacteria to DMSP. Detailed studies of these isolates to utilize DMSP and related compounds were carried out in the laboratory for reconfirmation of their ability to utilize this substrate and to also quantify the results.

In order to understand the interrelationships among the variables, the data sets were also subjected to statistical analyses (Spearman's rank correlation and Principal Component analyses) using the software STATISTICA 6 and Primer 6.

Some of the highlights are given below.

### **Diurnal Study**

- DMS concentrations ranged from 0.9 to 20.4 nM. It was 1.7 times (20.4 higher at dusk than at dawn (11.7nM). DMSP ranged from 3.69 to 84 nM and the highest value was recorded at 17:00 hr. DMS(P) concentrations showed diel variation with high values in the day and low at night.
- The average flux of DMS from the bay to the atmosphere was  $4.6 \mu\text{M m}^{-2} \text{d}^{-1}$ . and was dependent upon wind speed and DMS concentration. The highest flux was observed at 09:00 hr with a value of  $13.32 \mu\text{M m}^{-2} \text{d}^{-1}$  when the wind speed was  $3.6\text{ms}^{-1}$ .
- Phytoplankton density ranged from  $0.3 - 1.2 \times 10^5 \text{ cells l}^{-1}$  comprising of 99% of diatoms, 0.4% of dinoflagellates and other algae forming the rest.
- The total abundance of the bacteria was of the order  $10^9 \text{ cells l}^{-1}$ , TVC of the order  $10^8 \text{ cells l}^{-1}$  and CFU of the order  $10^6 \text{ cells l}^{-1}$ . The DMSP and DMS utilizers averaged  $0.78$  and  $0.68 \times 10^3 \text{ cells l}^{-1}$  during night and  $0.43$  and  $0.45 \times 10^3 \text{ cells l}^{-1}$  during the day respectively amounting to 0.0001 % of the TC.
- The DMS concentration was coupled to DMSP concentrations (Spearman's  $r = 0.67$ ,  $P < 0.01$ ). DMSP varied both with temperature (Spearman's  $r = 0.68$ ,  $P < 0.01$ ) and DO (Spearman's  $r = -0.61$ ,  $P < 0.01$ ). The high concentrations of DMSP during day

time closely followed that of Chl *a* concentrations. The DMSP concentrations were influenced by phaeopigments levels (Spearman's  $r = 0.67$ ,  $P < 0.01$ ). The DMSPU was influenced by Chl *a* concentrations (Spearman's  $r = 0.63$ ,  $P < 0.01$ ). The DMSU are influenced by the phytoplanktons and diatoms (Spearman's  $r = -0.97$ ,  $P < 0.001$ ).

- In the diurnal observations, the variables highly correlating in the first axis (PCA1) were DMS, DMSP, TC, TVC and DMSPU and explain 51.4 % of the variance. The second axis, PC2 (DMSU, DMSPU and Chl *a*, DO, TOC, and wind) explained 14.6 % of the variance. Thus DMS (P) concentration was influenced more the biotic than by the abiotic with the utilizers wielding more influence.

### **Annual Study**

- The annual variation was lower than the diurnal variation for DMS (P) and ranged from 1.2 – 22.2 nM for DMS and 0.69 to 15.8 nM for DMSP. The variations in other physico-chemical and biological variables were larger.
- The seasonal concentrations in their levels was monsoon season > post – monsoon > pre – monsoon season. The DMSP was four fold higher during the monsoon at 13.4 nM.
- The mean rate of DMS emissions into the atmosphere was  $9 \mu\text{M m}^{-2} \text{d}^{-1}$ . The average annual flux was more than double the diurnal average.

- Composition of major producers: Major components among the diatoms, *Chaetoceros* sp, *Coscinodiscus* sp and *Thalassionema* sp and major components among the dinoflagellates, were *Ceratium* sp, *Dinophysis* sp, *Noctiluca* sp and *Prorocentrum* sp.
- Composition of utilisers: Most of them belonged to *Pseudomonas* sp, *Xanthomonas* / *Flavobacterium* sp, *Vibrio* sp, *Moraxella* sp and *Bacillus* sp. The most proficient utilizers among these were further identified by 16S rDNA which established their identity as gamma proteobacteria, (*Alcanivorax dieselolei*), beta proteobacteria (3 strains belonging *Bordetella petrii*) and firmicutes (*Bacillus* sp). This study has extended the DMSP producing characteristic to new phylogenetic territory.

#### Experimental study:

Since DMSP is an environmental cue indicating the proximity of algae, tests were conducted to gauge the chemotactic ability of some of the above bacterial strains. Interestingly all the DMSP utilisers were motile, a trait useful for expressing chemotactic responses. They were attracted to the molecule exhibiting a gradation in chemotactic capability. While some strains were attracted to micromolar concentrations (*Bordetella petrii* #2 ), others were attracted to picomolar levels (*Alcanivorax dieselolei*).

## **Conclusion**

The foregoing studies clearly show that the diurnal variation is greater than the annual. The biological factors have a greater control over the variation of the organic sulfur compound as compared to the abiotic factors. Same set of factors bring about both the diurnal and annual variations. There is wide spectrum of phytoplankton producers and bacterial utilizers and the utilizers have a wide range of affinities to organic sulfur compounds. In addition to wind, the producers (source) and the utilizers (sink), influenced the flux of the radiatively important DMS. Consequently, the study region ie Dona Paula bay, is a significant source of biogenic sulphur with total emissions amounting to ca  $0.1 \text{ km m}^{-2} \text{ yr}^{-1}$ .



## **Chapter 7**

### **ACTION PLAN**

## **Future studies**

Temporal variation in DMS(P) from specialized ecosystems such as mangroves and coral reefs have to be looked into.

Work on the microbial population (phytoplankton and bacteria) and its specific activity linked to UV would further improve the understanding on diurnal variations DMS (P) not only in the bay but other ecosystems also

As the compound DMS is highly volatile, some probes/ instrument/technology could be developed to measure the in situ concentration on real time basis.

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