

of lysozyme is through binding and cleavage of cell wall polysaccharide by the enzymatic action. Even though *M. tuberculosis* cell wall contains mainly cord factor and other lipids, it also contains polysaccharides such as lipoarabinomannan and lipomannan. In ATB, the bacterial load harbouring in the system may be high, which may induce the system to produce more lysozyme to encounter the *M. tuberculosis* pathogen.

In the present study, a trend towards a decreased CFU of *M. tuberculosis* pretreated with plasma of either NHS or ATB patients was observed. One of the mechanisms for this decreased CFU may probably be due to the damage/injury caused by the enzymatic action of lysozyme on the carbohydrate moieties of the cell wall of *M. tuberculosis*, along with other enzymes and proteins of the plasma (which may also be detrimental to *M. tuberculosis*).

The stimulatory effect of 4 h plasma pretreated with live *M. tuberculosis* may be due to the release of some of the carbohydrate moieties such as lipoarabinomannan, lipomannan and some other cell wall components due to the action of lysozyme and other enzymes on live *M. tuberculosis*, which may be immunogenic and stimulatory to lymphocytes.

The present study suggests that lysozyme in the plasma as well as in cells/tissues may be detrimental to *M. tuberculosis* and to other pathogens. Moreover, lysozyme along with other host enzymes and proteins may play an important role in innate immunity against *M. tuberculosis* infection.

1. Andrew, P. W. *et al.* (eds), in *Mononuclear Phagocytes: Physiology and Pathology*, Elsevier Science Publishers B.V. (Biomedical Division), London, 1985, pp. 311–334.
2. Philips, D., *Sci. Am.*, 1966, **215**, 78–90.
3. Li, Y. M., Tan, A. X. and Vlassara, I., *Nat. Med.*, 1995, **1**, 1057–1061.
4. Takada, K., Ohno, N. and Yadomae, T., *Infect. Immunol.*, 1994, **62**, 1171–1175.
5. Tanida, N., Ohno, N., Yadomae, T., Matsuura, M., Kiso, M. and Hasegawa, A., *J. Biochem.*, 1992, **112**, 616–623.
6. Marolia, J. and Mahadevan, P. R., *Indian J. Lepr.*, 1984, **56**, 776–783.
7. Selvaraj, P., Kannapiran, M., Reetha, A. M., Uma, H., Xavier, T. and Narayanan, P. R., *Int. J. Tuberc. Lung Dis.*, 1997, **1**, 265–269.
8. Near, K. A. and Lefford, M. J., *J. Clin. Microbiol.*, 1992, **30**, 1105–1110.
9. Arya, S. C., *J. Clin. Microbiol.*, 1993, **31**, 2836–2838.
10. Osserman, E. F. and Lawlor, D. P., *J. Exp. Med.*, 1966, **124**, 921–952.
11. Boyum, A., *Scand. J. Clin. Lab. Invest.*, 1968, **21**, 31–50.
12. Selvaraj, P., Uma, H., Reetha, A. M., Xavier, T., Prabhakar, R., Narayanan, P. R., *Indian J. Med. Res.*, 1998, **107**, 208–217.

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## *Noctiluca* blooms in Port Blair Bay, Andamans

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**Moderate to intense blooms of the dinoflagellate *Noctiluca scintillans* occurred in Port Blair Bay, Andamans during June and July of 2000. The blooms occurred thrice in succession after short periods of waning. The bloom that came about second in the sequence, between 11 and 20 July 2000, gave us an opportunity to investigate the changes in water characteristics associated with the event. Results indicate that variations in dissolved oxygen concentration were fairly small, whilst nutrient levels and plankton compositions were dramatically altered. Primarily, the bloom led to exclusion of other phytoplankton, also limiting zooplankton prevalence to essentially copepods. The bloom resulted in exceptionally high levels of chlorophyll *c*, which exceeded levels of chlorophyll *a* by several folds. Nutrient levels were characterized by decrease of nitrate during active periods of the bloom, and sharp increase in phosphate, particularly during the waning phase.**

BLOOMS of the dinoflagellate *Noctiluca scintillans* have been reported from Indian waters by Prasad<sup>1</sup> in Palk Bay, Subrahmanyam<sup>2</sup> and Katti *et al.*<sup>3</sup> in the Arabian Sea, Sargunam, Rao and Nair<sup>4</sup> in Kalpakkam coastal waters and more recently, by Naqvi and coworkers<sup>5</sup> off Cochin. In only one case has fish kill been reported<sup>5</sup>, although the authors were cautious not to overrate the severe fish mortality as a direct consequence of the *Noctiluca* bloom.

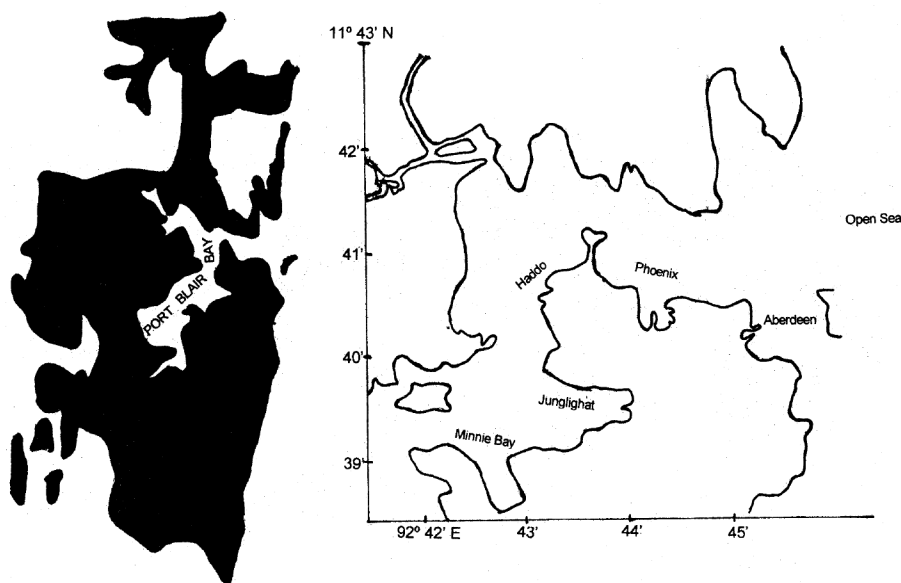
An intense bloom of *Noctiluca* was first noticed in the waters of Port Blair Bay during the second week of June 2000. The bloom imparted light to the vivid green colour of the coastal waters. The bloom persisted for several days, waning eventually by the end of the month. In course of time, the bloom reappeared twice during July 2000. This communication addresses the changes in coastal water characteristics associated with the event, with particular emphasis on the bloom that occurred between 11 and 20 July 2000.

The Port Blair Bay extends as a narrow stretch in a northeast to southwest direction (Figure 1). Six station locations spread over the entire Bay, as indicated in the figure, were considered for the primary sampling done on 12 June 2000. Further investigations were limited to

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**Table 1.** Surface water characteristics data for 12 June 2000 from six sampling stations in Port Blair Bay. Stations are listed from left to right in the order of their position in the northeast to southwest course.

Parameter	Open sea	Aberdeen	Phoenix	Haddo	Junglighat	Minnie Bay
Water temperature (°C)	29.5	29.5	29.2	29.1	29.5	29.3
Salinity (‰)	33.6	33.5	33.1	33.1	32.3	32.1
pH	8.1	8.1	8.2	8.2	8.1	8.3
Dissolved oxygen (mg l <sup>-1</sup> )	5.5	5.5	5.2	4.9	4.7	4.8
Nitrite (µmol l <sup>-1</sup> )	0.35	0.29	0.51	0.79	0.47	0.58
Nitrate (µmol l <sup>-1</sup> )	0.60	2.03	2.04	3.41	1.85	2.22
Phosphate (µmol l <sup>-1</sup> )	0.44	0.36	0.36	0.52	0.33	0.48
Silicates (µmol l <sup>-1</sup> )	10.7	6.6	12.4	10.3	5.6	7.7



**Figure 1.** Location of Port Blair Bay and the sampling stations.

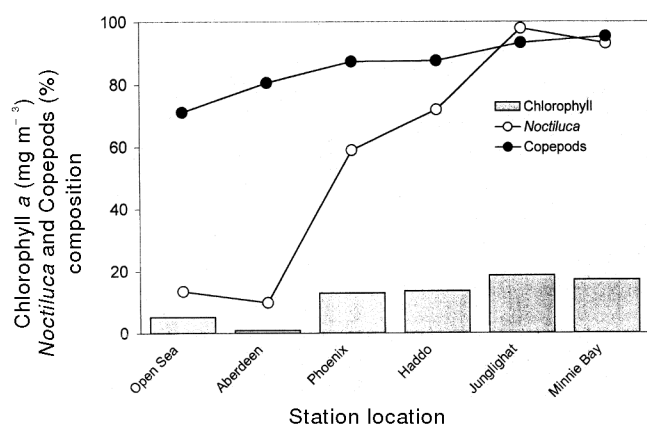
the Minnie Bay area, which is the farthest end of the Port Blair Bay. Surface water samples were taken from onboard a fishing vessel, *MV Ramkali*, using a clean plastic bucket and/or a water sampler (Hydrobios, Kiel). The sampling schedule was so programmed as to ship the samples to the field lab within 30 min of collection.

Water temperature and pH were measured and samples for dissolved oxygen were fixed on board the vessel. Estimates of *Noctiluca* cell numbers were made using water samples of volume 50 to 500 ml, depending on the bloom intensity. Plankton nets (Heron type, mouth area 0.197 m<sup>3</sup>) were also towed for an examination of associated organisms. Plankton estimates were made using a CETI trinocular microscope in conjunction with a video monitor. Nutrients were determined in accordance with standard methods<sup>6,7</sup> using a JASCO 7800 spectrophotometer. Chlorophyll was measured following the trichromatic method<sup>7</sup>.

Results of the preliminary investigation made at the six station locations on 12 June 2000 are presented in Table 1. These results, listed in the order of the station

positions in the northeast to southwest course, correspond to the period of an intense bloom that occurred first in the series. Over the entire Bay, surface water temperature varied from 29.1 to 29.5°C and pH 8.1 to 8.3. There was a decrease in salinity at the innermost point of Port Blair Bay, by 1.5 units, compared to open sea. Decrease in dissolved oxygen values followed a similar gradient, but the variations were quite small. Nutrient distribution, on the contrary, did not reveal any marked gradient, although the levels were on the higher side of the concentrations measured during the year.

The data in Figure 2 for 12 June 2000 show chlorophyll *a* values and the relative numerical abundance of *Noctiluca* and copepods at the different station locations. Together, the data point out to a distinct gradient with respect to the distance from the entry point of Port Blair Bay. In other words, the data suggest that the intensity of the bloom was highest at the innermost point, viz. the southwest portion of Port Blair Bay. Also note that the relative occurrence of *Noctiluca* and copepods correlated positively. *Noctiluca* cell numbers in the

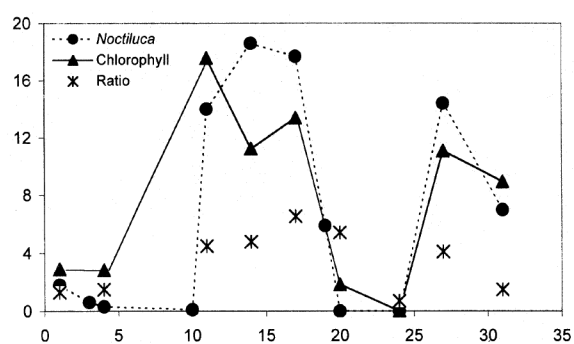


**Figure 2.** Chlorophyll and plankton data at various points in Port Blair Bay on 12 June 2000.

innermost portions of the Bay ranged from  $1.5$  to  $2.3 \times 10^4$  cells  $l^{-1}$ . Neither the cause for the bloom nor the point in time of its origin could be assessed on the basis of the above observations. Nevertheless, these results prompted a continuous monitoring of the water characteristics in the Minnie Bay region.

Figure 3 shows the variations in *Noctiluca* cell numbers and chlorophyll *a* concentrations in Minnie Bay area during July 2000. The data sets for the period 1 to 10 July 2000 correspond to the phase between the first and second blooms. During this period when *Noctiluca* cells were extremely low, chlorophyll *a* ranged about  $2.9$  mg  $m^{-3}$  while the ratio of chlorophyll *a* to *c* was about 1.4. The re-appearance of *Noctiluca* bloom led to high values of chlorophyll *a* (up to  $17.6$  mg  $m^{-3}$ ). More significantly, levels of chlorophyll *c* surpassed those of chlorophyll *a*, by as much as 4.5 to 6.5 times. Dissolved oxygen concentrations, measured intermittently during peak bloom, were not drastically reduced, touching a low of  $4.2$  mg  $l^{-1}$ . The bloom showed signs of waning by 20 July to eventually recur by 27 July on a moderate intensity.

Rainfall and nutrient data for July 2000 are given in Figure 4. As shown in the figure, the period prior to bloom #2 was characterized by significant amounts of rainfall. Levels of nutrients at the beginning of the bloom on 11 July 2000 could be perceived to be much higher than at the beginning of the month, possibly due to the inputs associated with the rains. The onset of the bloom led to a reduction in nitrate and an increase in phosphate levels. The increase in phosphate concentration was particularly marked during the waning phase. Nutrient levels dropped to insignificant values after the waning phase. Note from Figure 3 that the beginning of bloom #3 on 27 July 2000, in sharp contrast to bloom #2, corresponded with the exhaustion of nutrients in the waters.



**Figure 3.** Plankton and chlorophyll data for July 2000.

No fish kill was encountered during the blooms, but the event led to exclusion of most plankton. Some phytoplankton species still persisted in small numbers, regardless of the bloom intensity. Notable among them were *Ceratium* spp., *Coscinodiscus eccentricus* and *Thalassiothrix longissima*. Zooplankton prevalence, on the other hand, was limited exclusively to copepods whilst the blooms were most active.

Microscopic examination of live *Noctiluca* cells showed the presence of innumerable motile green flagellates, as noted earlier<sup>2-4</sup>. *Noctiluca* cells were circular-to-bean shaped and measured 250–400  $\mu m$  in diameter. The cells possessed a flagellum that exhibited noticeable twists. The function of the flagellum is generally considered to support buoyancy of the organism rather than active swimming movements<sup>8</sup>.

The mechanism by which chlorophyll *c* values exceeded those of chlorophyll *a* by several folds is rather unclear. It is generally considered that domination of chlorophyll *c* over *a* points to degraded chlorophyll in the waters and rather inactive conditions of phytoplankton<sup>9</sup>. It is also possible that such high values resulted from bacteriochlorophyll, as *Noctiluca* is known to ingest bacteria at remarkably high rates<sup>10</sup>.

Consistent with the observations by Sargunam *et al.*<sup>4</sup>, dissolved oxygen was not drastically lowered as a consequence of the *Noctiluca* bloom. As regards nutrients, Katti *et al.*<sup>3</sup> pointed to little variations before and after the bloom. However no records were presented. Sargunam *et al.*<sup>4</sup> measured only minor increments in phosphate during active periods of the bloom as opposed to the present data. However, the reduction in phosphate to non-detectable levels when the bloom waned is coherent with this work. Montani *et al.*<sup>11</sup> have reported sharp increase in phosphate during *Noctiluca* blooms as noted in the present work. These authors have measured, in patches containing high numbers of *Noctiluca*, phosphate concentrations 25 times higher than those in ambient seawater. We consider that nutrient variations in the present work appear more conspicuous than in earlier *Noctiluca* reports because the blooms were

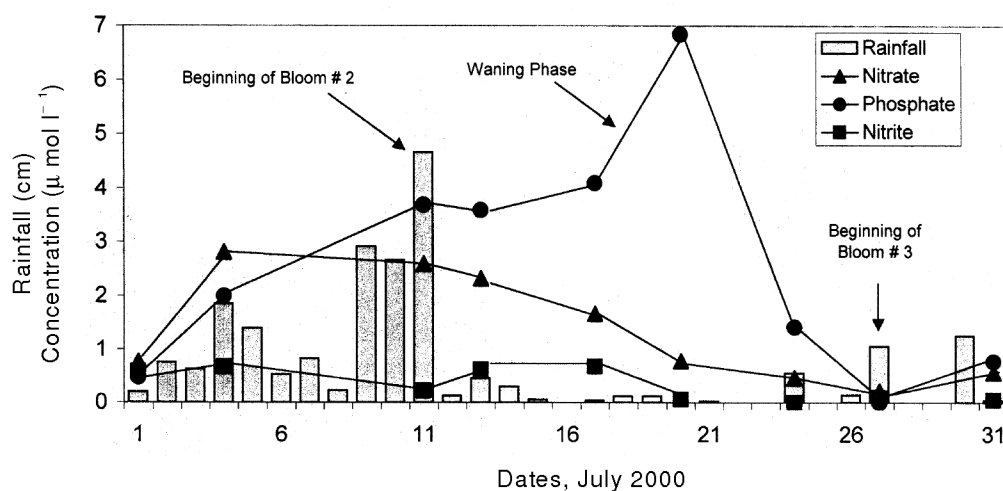


Figure 4. Rainfall and nutrient data for July 2000.

repetitive, permitting an unambiguous progression in the monitoring programme.

To our knowledge, this is the first technical account of plankton blooms from the Andamans. The *Noctiluca* bloom observed in this work was somewhat unique, because it appeared thrice in a time-period of two months. The precise cause for the blooms remains unclear; nevertheless the data in this work suggest that the origin of *Noctiluca* blooms was not always concurrent with nutrient inputs into Port Blair Bay.

1. Prasad, R. R., *Proc. Indian Acad. Sci.*, 1953, **31**, 41.
2. Subrahmanyam, R., *Proc. Indian Acad. Sci.*, 1954, **B39**, 118.
3. Kattii, R. J., Gupta, T. R. C. and Shetty, H. P. C., *Curr. Sci.*, 1988, **57**, 380–381.
4. Sargunam, C. A., Rao, V. N. R. and Nair, K. V. K., *Indian J. Mar. Sci.*, 1989, **18**, 289–290.
5. Naqvi, S. W. A., George, M. D., Narvekar, P. V., Jayakumar, M. S., Shailaja, M. S., Sardesai, S. Sarma, V. V., Shenoy, D. M., Naik, H., Maheshwaran, P. A., Krishnakumari, K. Rajesh, G., Sudhir, A. K. and Binu, M. S., *Curr. Sci.*, 1998, **75**, 543.
6. Grasshoff, K., Ehrhardt, K. and Kremling, M., *Methods of Seawater Analysis*, Verlag Chemie, Weinheim, 1983.
7. Strickland, J. D. H. and Parsons, T. R., *A Practical Handbook of Seawater Analysis*, Fisheries Research Board, Canada, Ottawa, 1972.
8. Elbrächter, M. and Qi, Y-Z., in *Physiological Ecology of Harmful Algal Blooms* (ed. Anderson, M.), Springer Verlag, Berlin, 1998, pp. 315–335.
9. Desai, B. N., Ram, M. J., Abidi, S. A. H. and Nair, R. *Maharashtra*, 1984, **17**, 79.
10. Kirchner, M., Sahling, G., Uhlig, G., Gunkel, W. and Klings, K. W., *Sarsia*, 1996, **81**, 45–55.
11. Montani, S., Pithakpol, S. and Tada, K., *J. Mar. Biotechnol.*, 1998, **6**, 224–228.

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## *In vitro* micropropagation of *Lippia alba*

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**The study has given a method for rapid multiplication of *Lippia alba* cv. Kavach, the perennial shrub that protects soil erosion when grown on slopes and whose leaves yield a linalool-rich essential oil. Multiple shoots were induced *in vitro* from shoot tips of *L. alba* on Murashige and Skoog (MS) medium containing 2 µg/ml 6-benzyl adenine. The stem nodal segments derived from *in vitro*-grown shoots also gave multiple shoots on the medium of the same composition. The shoots readily rooted upon transfer to basal MS medium. The rooted *in vitro* raised plants established well on soil following acclimatization. The essential oil profiles and morphology of the micropropagated plants were identical to the normal vegetatively propagated plants.**

THE genus *Lippia* belongs to family Verbenaceae and consists of nearly 200 species of herbs, shrubs and small trees widely distributed in tropical to semitemperate areas of the American, African and Asian continent. *Lippia alba* and some other species have been reported to be used in traditional medicine<sup>1</sup> and pest control in food grains. Plant extracts from *L. alba* have also been reported to possess cytostatic properties<sup>2</sup>. Medicinal and cytostatic properties of this herb may primarily be

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