While dense blooms of phytoplankters are known to occur frequently, the intensity of the present bloom appears to be unprecedented. Their numbers were so great that it was impossible to haul a plankton net beyond a few sec, since the net was getting completely clogged within about 15 s. Therefore, numerical counts were made from surface water samples collected at various points in the Gurpur and Netravathi estuaries and in the inshore waters at stations extending from Someswar in the south to Panambur in the north. A single sample was also obtained from Malpe about 50 km further north. The samples in the sea were taken from the 8–30 m contour lines. The number of *Noctiluca milaris* in these samples varied from $1.6 \times 10^4/m^3$ to $7.55 \times 10^9/m^3$. The maximum density was noticed in the 18–20 m depth zone off the bar mouth at Mangalore.

This rare phenomenon has been reported only twice before, by Prasad[3] in Palk Bay and by Subramanyan[4] in the Arabian Sea. Both noticed the association of *Noctiluca milaris* with a green flagellate. Subramanyan[4] identified this as *Protoeuglena* and described the association of these two organisms as more of a symbiotic nature than of parasitism or saprophytism. The present case appears to be a recurrence of the phenomenon observed about three and a half decades ago, but with greater intensity. The persistent green discoloration of the water aroused the curiosity of fishermen and the public as to its possible effects. While it is known that both these organisms do not secrete any toxins, a possible danger could be from the serious depletion of oxygen, resulting from the death and decay of these dense blooms. Normally the dissolved oxygen level in the region varies from 3 to 5 ml/l, but this went down to 0.2 ml/l after the occurrence of these blooms. However, no large-scale fish mortality was noticed in these areas. This was due to the fact that the fishes largely avoided this oxygen-deficient zone.

The density of the blooms was found to vary over the period. An examination of the hydrobiological data of the region before and after the occurrence of these blooms has not shown any drastic change, particularly with respect to the important nutrients. It is, therefore, difficult to deduce the reason for the mass production of these two organisms. The only difference noticed was that the surface temperature of these waters was about 2.4°C higher as compared to that of the moderately clear waters. A more detailed examination is in progress.

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EFFECT OF NUTRITIONAL STRESS ON CAPSAICIN PRODUCTION IN IMMOBILIZED CELL CULTURES OF *CAPSICUM ANNUUM*

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IMMOBILIZED plant cell cultures are ideally suited for production of some secondary metabolites[1]. Nutritional factors required for growth and production of secondary metabolites in plant cells have been known to be different. There are some reports on the development of induction medium for secondary metabolites[2,3]. In this communication the influence of nutritional stress factors for induction of capsaicin (an alkaloid) in immobilized *Capsicum* cells has been investigated. Capsaicin is used in food formulations as a spice and in pharmacy for preparing tincture, liniments and plaster[4].

Seeds of *Capsicum annuum* var. selection[1], obtained from the Indian Institute of Horticultural Research, Bangalore were germinated aseptically on moistened Whatman No. 1 filter paper in 5 cm petri dishes. Callus was raised from 7-day-old seedlings on Murashige and Skoog's medium[5] (MS) containing 3% sucrose and 2 mg/l 2, 4-Dichlorophenoxy acetic acid and 0.5 mg/l kinetin. Callus was maintained on the above medium (termed as growth/control medium) by subcultures at a 30 day interval. Cell suspension was raised from callus and maintained on growth medium.

One gram of the cells were immobilized by mixing with 2% (w/v) sodium alginate and extruded[6] into $CaCl_2$ solution (1.35 g/150 ml) using sterilized pas-

ture pipettes to get a bead size of 4 mm. Beads were washed with sterile water and incubated at 25 ± 2°C in 40 ml of control or experimental medium on a rotary shaker at 80 rpm under continuous illumination of 3000 lux. Medium and beads were harvested at weekly interval for capsaicin analysis by the paper chromatography method[7] using Gibb's chromogenic reagent. Data were recorded as an average of 5 replicates. In the experimental media, nitrates (potassium nitrate and ammonium nitrate) or phosphates (potassium dihydrogen orthophosphate) or sugar (sucrose) were eliminated separately and all other nutrients and hormones were added as in control.

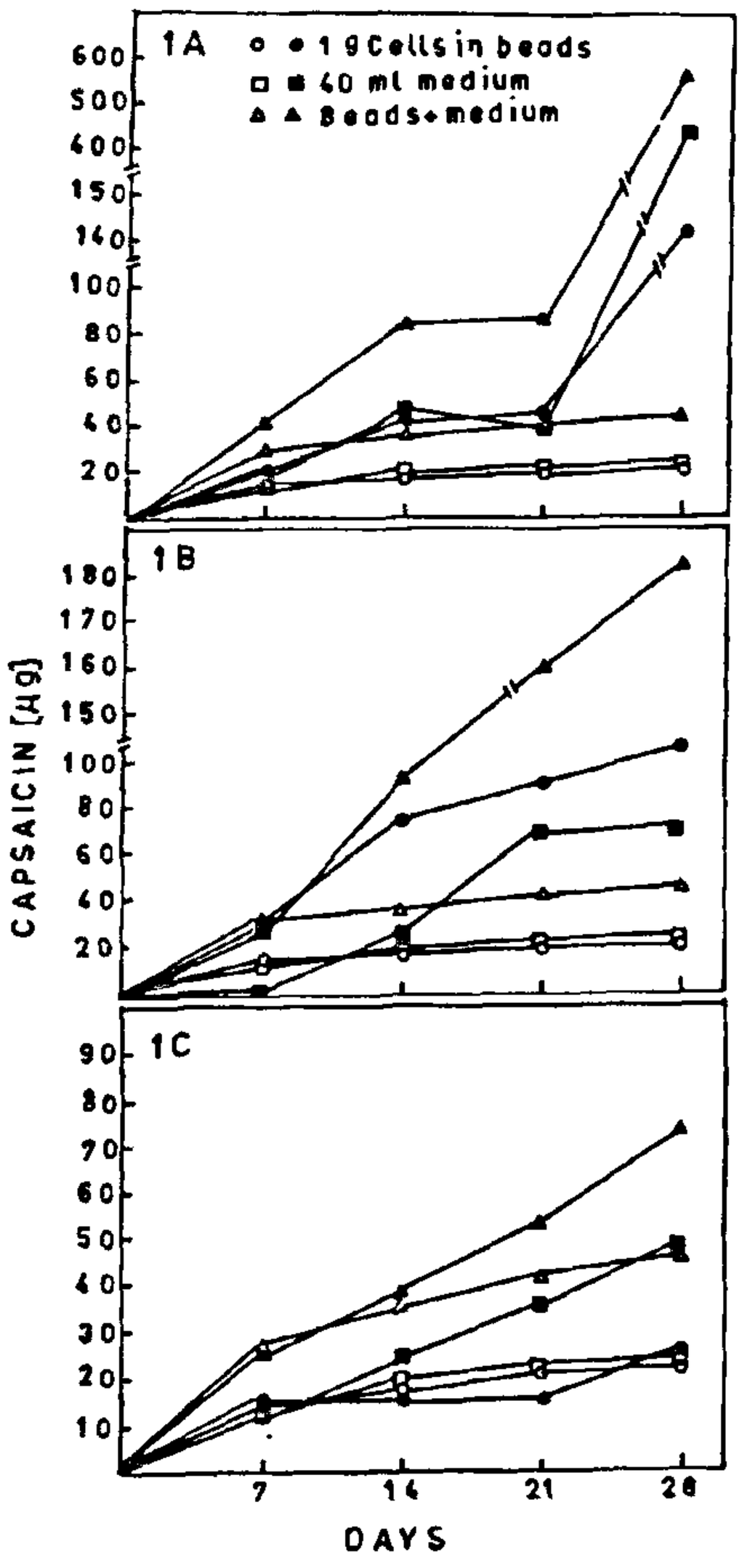

Figure 1A–C. Capsaicin production by immobilized *Capsicum* cells in control and nutrient stressed media. **A.** Nitrogen stressed; **B.** Phosphate stressed, and **C.** Sugar stressed; ○ □ △ — Control; ● ■ ▲ — Experimental.

Cells which were not immobilized did not produce capsaicin as reported by Lindsey and Yeoman[8]. However, on entrapment with alginate there was progressive capsaicin production. Control produced 26.5 μg capsaicin per g fresh weight of immoblized cells at the end of 7 days which increased to 46.9 μg on 28th day (figure 1A). Capsaicin leached out of cells into the medium both in control and in treated samples.

Of the three experimental media, the nitrate eliminated ones showed maximal capsaicin production and leaching into the medium (figure 1A). Capsaicin production increased 13-fold over the control with a total production of 593 μg per g of cells at the end of 4 weeks. Elimination of the phosphate (figure 1B) and sugar (figure 1C) induced 4-fold and 1.5-fold increase over the control respectively. Similar results have been reported for other metabolites[3,9,10].

Nutritional stress factors are known to arrest the growth and channel the precursors for secondary metabolite synthesis[3]. This is also facilitated by physical entrapment of cells[1]. Capsaicin production improves in immobilized cells with the addition of precursors[11]. The present study shows that immobilization of cells in induction medium with nitrate as stress factor will benefit scaling up of this system. Excretion of capsaicin into the medium facilitates continuous production and use of immobilized cells for longer period.

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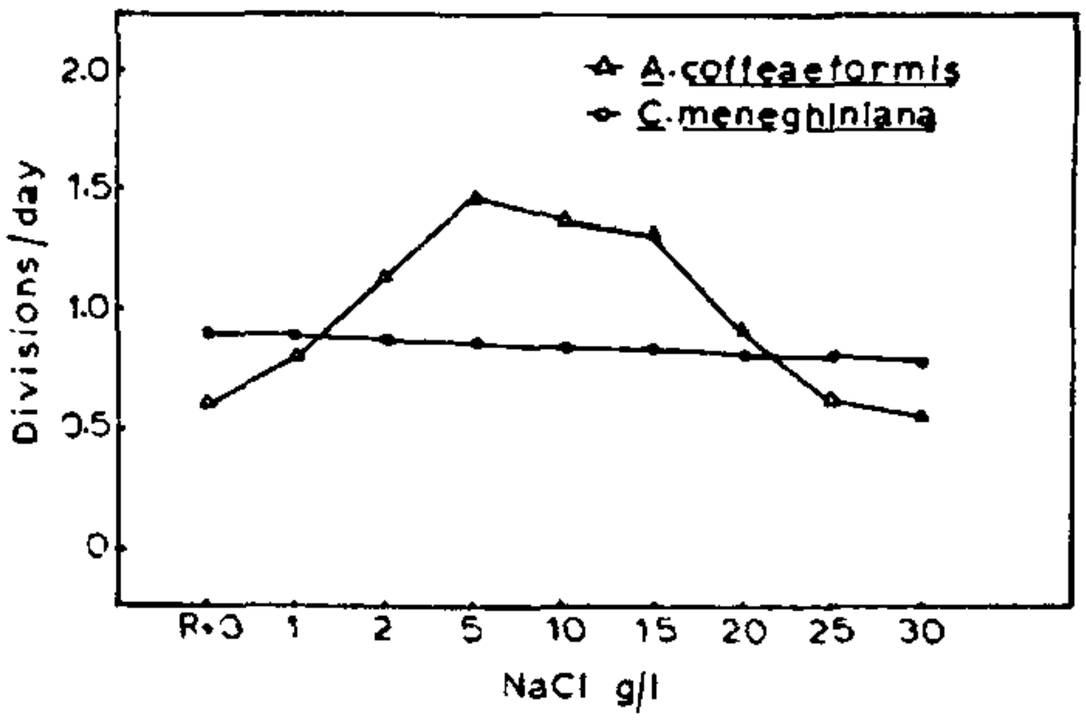

Figure 1. Division rates of *A. coffeaeformis* (Agardh) Kütz. and *C. meneghiniana* Kütz. in media of different salinities.

DIATOM ABUNDANCE IN THE ESTUARIES

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THE Cooum and the Adyar river estuaries in Madras are bar protected at their bay mouths and are substantially influenced by freshwater inputs only during monsoon seasons. During summer, shallow bars formed at the bay mouths prevent free flow of water and evaporation can then increase their salinity by as much as 60‰. One may reasonably expect and, in fact, laboratory studies have generally confirmed that diatoms in such biotopes are well adapted to live under fluctuating salinity conditions[1].

Cyclotella meneghiniana Kütz. and *Amphora coffeaeformis* (Agardh) Kütz. occur in the Cooum and the Adyar estuaries where salinity varies from almost freshwater to very near that of the sea[2,3]. We have isolated a number of clones of the two diatoms from upstream of the estuaries, where there is no tidal influence; from the estuaries themselves; and also from several places in the Bay off Madras. The behaviour of these clones in relation to salinity of the medium has been studied in the laboratory. For comparison, clones isolated from a garden pond have also been included in the study.

Cyclotella meneghiniana Kütz.

Isolates of *Cyclotella meneghiniana* Kütz. when grown in different NaCl (1–30 g/l) amended Reimann medium[4] grew well at all salinities and there was no need to 'train' the diatom to tolerate different levels of NaCl. The typical behaviour of a single isolate is illustrated in figure 1. Cells were transferred from freshwater Reimann medium to different salinities (1–30 g/l) NaCl added to Reimann medium and again from media of high to low salinity. Transfer of cells from either Reimann medium to low salinity ranges or from high to low salinity increased their dimensions due to auxospore formation. The increase of average cell diameter obtained in different salinities of one isolate is shown in table 1.

In the Coum and the Adyar estuaries there are two periods of auxospore formation: once during monsoon months, when there is large freshwater influx from the river; and again on the return of saline conditions when freshwater inflow stops and the estuary becomes tidal. The observations of Iyengar and Venkataraman[2] on abundance of *C. meneghiniana* Kütz. in the Coum estuary are relevant: *C. meneghiniana* Kütz. becomes abundant during two periods of the year when heavy rain decreases the salinity of waters lending support to our laboratory studies.

In our opinion, the ability of *Cyclotella meneghiniana* Kütz. to grow in estuaries involves efficient maintenance of a small inoculum throughout the year in various places in the river and the sea and

Table 1 *Percentage increase in average cell diameter of C. meneghiniana Kütz. in Reimann medium amended with different NaCl concentrations*

Inoculum	NaCl concentration in g/l R	1	2	5	10
From Reimann	0	71	124	166	78
From Reimann + 25 g/l NaCl	7	11	47	85	11