SUMMARY AND CONCLUSIONS

The present study was mainly aimed at isolation of a bloom forming alga from Cochin estuary and to study the physical, chemical and biological parameters leading to algal blooms. Important findings of the study are summarised as follows:

- The spatial distribution of phytoplankton groups along the Cochin estuary was studied. The major groups identified belong to class Bacillariophyceae, Chlorophyceae, Dinophyceae, Cyanophyceae and Xanthophyceae. The harmful algae such as Dinophysis, Gymnodinium, Protoperidinium and Noctiluca were also observed in the preserved natural algal sample. The major groups identified belong to class Bacillariophyceae.

- Clonal cultures of various microalgae including Asterionella sp., Bacteriastrium sp., Biddulphia sp., Chaetoceros sp., Coscinodiscus sp., Gyrosigma sp., Navicula sp., Pleurosigma sp., Rhizosolenia sp., Skeletonema sp., Ceratium sp., Gymnodinium sp., Noctiluca sp., Protoperidinium sp. and Phaeocystis sp. were developed under laboratory conditions.

- In the light of increased frequency of harmful algal blooms of novel organisms all over the world, the presence of Phaeocystis sp. in Cochin estuary had been taken into special consideration and the various aspects of the Phaeocystis sp. were studied in detail.
The hydrographical conditions of the stations (Marine Sciences Jetty and Barmouth) from which the *Phaeocystis* sp. was isolated showed an average temperature of 30°C, salinity 26ppt and alkaline pH. Nutrients mainly nitrate, nitrite, phosphate and silicate in the stations were found to be higher compared to other stations indicating the signs of eutrophication in these areas.

*Phaeocystis* regularly forms blooms in the Oceans. It is believed to be entered into our ecosystem as a result of bioinvasion. The selected alga was identified as *Phaeocystis* sp. by observing the cell morphology under microscope and by studying its culture characteristics.

The alga showed a complex life cycle with three types of cells, non-motile solitary cells, motile flagellated cells and colony cells. Sequential developments of single cell to colonies were observed in the culture with the advancement of incubation period.

*Phaeocystis* sp. isolated showed close similarity to *Phaeocystis jahni* mainly in colony structure without a definite colony wall. Moreover, the cells in the colony were not evenly distributed and it appeared in a cloud form with several packets of cells.

*Phaeocystis* was found to be capable of growing over a wide range of temperature, salinity and pH showing its versatile nature. However, maximum growth of the *Phaeocystis* was obtained at salinity 30ppt, pH 8, and at temperature, 20°C and 25°C. This shows that the species could be well established in tropical waters and could form blooms.
Walne’s medium was found to be supporting maximum growth of *Phaeocystis* sp. and the growth was more influenced by the nitrate concentration than the phosphate concentration in the growth medium. High concentration of nutrients, nitrate (>1000µM), phosphate (>50µM) and iron (>5µM) in the medium could lead to blooming of this alga.

Non-motile solitary cells were observed throughout the culture period with maximum occurrence during the initial and stationary growth period. Colonies started developing in the logarithmic growth phase, when the algal growth was maximum and towards the stationary phase, the colonies got disintegrated liberating the single cells from the colonies. Number of flagellated cells also increased towards the stationary phase.

The colony formation in the *Phaeocystis* sp. was high in nutrient surplus medium compared to nutrient deficient medium. High concentrations of nitrate, phosphate, and iron were found to cause mass development and colonial growth in this alga.

Protein content of the algal cell was high during the logarithmic phase than in the stationary phase, whereas, the carbohydrate content was higher in the stationary phase compared to logarithmic phase. No significant variation was observed in the total lipid content between the logarithmic and stationary phases.

The extracellular release of proteins was higher in the logarithmic phase than in the stationary phase and the secretion of carbohydrates was more during the stationary phase.
The exudates from *Phaeocystis* sp. have an allelopathic influence on other algal species and the availability of nutrients is an important factor for the regulation of allelopathic substances and allelochemicals are only produced/released under conditions of limited growth.

The allelopathic effect of *Phaeocystis* sp. on three microalgae, *Chlorella marina*, *Chaetoceros calcitrans* and *Isochrysis galbana* was studied by using algal cell extract and culture filtrate. The effect was found to be varying from species to species. The culture filtrate of *Phaeocystis* sp. showed an inhibitory effect on the growth of *Chlorella marina* whereas, the growth of *Isochrysis galbana* was promoted by the cell extract and culture filtrate. Growth of *Chaetoceros calcitrans* was found to be unaffected by the *Phaeocystis* sp. cell extract and culture filtrate.

The *Phaeocystis* sp. was found to be capable of exerting toxicity to animal cells also. It caused 20% to 30% mortality of *Artemia salina*. The toxicity was retained only for a short duration and within 48 hrs, the *Artemia salina* larvae regained the motility and no mortality occurred further.

The alga expressed haemolytic property also. The cells growing exponentially showed higher haemolytic property than the cells in the lag phase and stationary phase. The haemolytic property was found to be dependent on cell dose and the nutrient status.

Highest haemolysis was exhibited by algal cells grown in NP non limiting medium whereas, culture filtrate from the nutrient deficient algal culture exhibited higher haemolysis than the
Summary and Conclusions

culture filtrate of nutrient surplus culture. It shows that nutrient limitation cause a physiological stress on the algal cells to secrete the toxic principle into the media.

- The algae-bacteria association was found to be very specific and four groups of bacteria were isolated from the *Phaeocystis* sp.-*Alcaligenes*, *Pseudomonas*, *Flexibacter*, and *Flavobacterium*. *Alcaligenes* was present as two different species that varied morphologically in colour and colony size. The bacterial flora associated with *Phaeocystis* sp. was found to be influencing the growth and physiological property of alga.

- The bacteria were found to be very closely associated with the alga. The culture could not be made axenic by the application of antibiotics, and when high concentrations of antibiotics was used which completely eliminated the bacterial flora, the growth of algal cells were seriously affected.

- Regular succession was observed in the bacterial community along with the change in the growth phase of alga. *Alcaligenes* I was observed in higher number in the initial growth stage and by logarithmic growth phase the dominant flora were *Alcaligenes* II and *Flexibacter*. During the stationary phase, *Pseudomonas* was the predominant form. These bacteria would be playing a significant role in the initiation and termination of algal blooms. Bacterial biomass was high towards the logarithmic growth phase of alga than the lag phase and stationary phase.

- The associated bacteria and the *Phaeocystis* sp. cells did not show any antibacterial activity towards the bacterial pathogens tested.
- Total bacterial consortium was important for the haemolytic property exhibited by *Phaeocystis* sp. *Flexibacter* and *Alcaligenes* II enhanced the haemolytic property of the alga by promoting good growth. Culture filtrates of *Pseudomonas* and *Flavobacterium* also enhanced the haemolytic activity of the alga. Among these bacteria, only *Pseudomonas* could produce haemolysis in blood agar.

In the present study, the important aspects that result in the bloom (mass growth) of *Phaeocystis* were studied under laboratory conditions. The study reveals the physiochemical conditions at which the alga blooms and the effect of nutrients on the colony formation of the alga. The role of associated bacteria in the growth of the alga was thoroughly analysed along with the allelopathic effect of *Phaeocystis* sp. on other microalgae in our ecosystem.

The study shows that algal blooms are regulated by the hydrographical parameters especially temperature, availability of nutrients such as nitrate and iron and the allelopathic property of alga that help the organism to compete over the co-occurring species. The associated bacterial flora also exerts a significant influence on the blooming of the alga by regulating bloom initiation, mass growth of the algae and termination of the bloom. However, further studies are required to ascertain the role of different bacterial species in algal blooming.