Earth has an ocean covering approximately 71% of the planet's surface and containing 97% of the planet’s water. It influences all known life by forming part of the carbon cycle, and influencing climate and weather patterns. It is inhabited by countless species of living organisms, from tiny phytoplankton to large mammals. The biota of the oceans has huge socioeconomic value through food production, recreation, nutrient recycling, and gas regulation. A major portion of the organic carbon for Earth's petroleum reserves, especially from shallow continental shelves is provided by fossilized organic remnants of phytoplankton blooms.

Phytoplankton in the ocean play a key role as primary producers in the marine ecosystem. Also known as microalgae, more than 5000 species that have been identified form the base of marine food web especially in the shelf seas by producing organic substances. As they form the base of the food web, the evolutionary trajectories of the microalgae have also governed the evolution of organisms at higher trophic levels. Microalgal communities in the ocean comprise many different taxonomic groups; diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae), blue green algae (Cyanophyceae), green microalgae (Chlorophyceae), silicoflagellates, coccolithophores and the very small
nanoplankters, which together determine primary production and various trophic level interactions. In this respect, the quantification of phytoplankton biomass and community composition is important for understanding the structure and dynamics of marine ecosystems.

The rate at which energy is stored up by microalgae determines the basic primary productivity of the ecosystem. Coastal and estuarine environments are the most productive ecological systems on earth. The stability of the oceanic photosynthetic activity by microalgae, which contributes about 80% of the atmospheric oxygen, is dependent on the physical circulation of the upper ocean. The proliferation of phytoplankton, its intensity, and spatial and temporal distribution in the ocean is determined by the depth of the upper mixed layer and the vertical fluxes of nutrients. Most of the planktonic microalgae are distributed through the euphotic zone, which can be influenced by temperature, light intensity, nutrient limitation, freshwater influence, and tidal activity.

Microalgal growth and abundance are primarily regulated by both abiotic and biotic interactions (Armstrong, 1994). Microalgae are particularly good indicators of climate change in the marine environment and any change in the physico-chemical parameters can be indicated by the distribution of microalgae because they are free floating and can respond easily to those changes. Thus, the information obtained from phytoplankton community structure can significantly contribute to assessing levels of climate change and the subsequent effect on the structure and functions of the ecosystem.

High fertility and large phytoplankton populations in estuarine environments have been frequent due to nutrient enrichment from river water,
General Introduction

organic pollution, and by the entrainment of coastal waters in a subsurface counter-current, which transport nutrients into the estuary. The ecological health of estuaries responds to nutrient enrichment in a positive or negative way, thereby influencing microalgal distribution.

Microalgae usually have a fairly recognizable annual cycle of growth. Sometimes this synchrony is disrupted by any drastic change in the above mentioned abiotic factors and leads to the explosive growth of some species. Under favourable conditions, this leads to the enormous concentration (millions of cells per litre) and often causing discolouration of the surface of the sea. These natural phenomena are termed as Harmful Algal Blooms (HABs). An algal bloom goes through sequential developmental phases such as initiation, growth, maintenance and termination.

The International Council for the Exploration of Seas (1984) defined algal blooms as 'those which are noticeable, particularly to the general public, directly or indirectly through their effects such as visible discolouration of the water, foam production, fish or invertebrate mortality or toxicity to humans'. Consequences of blooms have been noticed from the positive and negative points of view. The positive aspect of algal blooms is that it may be beneficial to fisheries (D'Silva et al., 2012), whereas the negative impact of algal blooms includes mass mortalities of wild and farmed fish, shellfish, human illness, death of marine mammals, sea birds and alteration of marine habitats or trophic structure through shading, overgrowth or adverse effect on life-cycle of fish and other marine organisms.

Algae that can cause a variety of deleterious effects, including oxygen deficiency as mentioned above, on aquatic ecosystems are termed
“Harmful Algae”. Of the several thousand planktonic algae species, only about 300 species are known to cause water discolouration. Out of these, only around 80 species are known to produce harmful toxic effects (Hallegraeff, 2003).

HAB species can be broadly classified into two groups – the high biomass producers, and the toxin producers. The high biomass producers affect fish, shellfish, and marine mammals of shallow bays or seas by inducing physical damage by virtue of gill-clogging or oxygen depletion during decay of the dead cells. The toxin producers may cause haemolytic, hepatotoxic, osmoregulatory and other unspecified toxic effects to cause mortality in animals that ingest them. Toxin accumulation in mammals and other higher levels in food chain is possible upon consumption of such contaminated shellfish whose tissues may have accumulated the toxin after digestion. The major poisoning syndromes include Paralytic Shellfish Poisoning (PSP), Ciguatera Fish Poisoning (CFP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), Amnesic Shellfish Poisoning (ASP) and Azaspiracid Poisoning (AZP). Some species have characteristics of both groups.

Harmful algal toxins are secondary metabolites released in the dissolved state into sea water, that are primarily involved in allelopathy to outcompete other phytoplankton species for resources, like nutrients under limiting conditions (Graneli and Hansen, 2006). They are associated with processes such as nitrogen storage, nucleic acid biosynthesis, bioluminescence, bacterial endosymbiosis, and pheromones inducing sexuality during bloom decline as well as serving as grazer deterrents. These metabolites are only coincidentally toxic. Temperature, salinity and nutrient status influence the toxin content of
the cell, while its production is stimulated by the presence of grazers (Bates, 1998).

Non-toxic but prolonged algal blooms can have other impact on coastal ecosystem, such as reduction of light penetration to submerged aquatic vegetation which nourish and shelter fingerlings of commercially important fish and shellfish.

Despite being a natural event, human related phenomena like ‘anthropogenic eutrophication’ and transport of algal species by ballast water, apart from natural mechanisms of species dispersal, has led to increased reports of occurrences of harmful algal blooms over the past decades. Economic loss on account of blooms, type of resources affected, and the number of toxins and toxic species have increased dramatically throughout the world (Anderson, 1989). Anthropogenic eutrophication of the highly susceptible coastal waters occurs due to pollution on account of a dramatic increase in aquaculture activities, and ever-increasing quantities of industrial, agricultural and sewage effluents, which stimulate HABs. Consequently, more observers are involved in monitoring of HABs leading to increased awareness and advancement of monitoring systems.

The occurrence and spreading of HABs can be prevented by reducing the inflow of industrial, agricultural and sewage effluents into coastal waters. Proper monitoring for evidences of HAB events and detection of toxins in contaminated organisms can be done to mitigate its effects. Physical and chemical methods are employed to control HAB’s by directly targeting the bloom cells, though they can produce unwanted side effects on the surrounding ecosystem. Biological control of HABs through application of
agents like bacteria and viruses is gaining ground. Algal blooms usually have an ordered and structured associated bacterial community rather than a random assemblage of species from the marine bacterial community. This indicates a probable role this community plays especially in bloom termination.

The interactions between algae and bacteria are an important aspect of the microbial loop. Algae represent the primary source of organic nutrients for heterotrophic microbes in the mixed layer of the ocean and the abundance of the bacteria has shown a positive correlation with algal concentrations (Kjelleberg et al., 1993). Their physical relationship, which may be mutually beneficial, extends from intracellular to extracellular. By providing a habitat to the intracellular bacteria, the algae benefit from the nutrients synthesized by them (Seibold et al., 2001). Bacteria colonizing the surface give them access to nutrients, protection against toxins, and protection against predators (Dang and Lovell, 2000).

Bacteria adhering to algal cells are potentially important players in the dynamics of HABs and this action may be of either direct or indirect mode. In accordance with the variations in the abundance and type of organic matter during an algal bloom, qualitative and quantitative changes in the associated bacteria may occur (Doucette, 1995) that ultimately reflects in the inhibitory and/or stimulatory effect on the organisms involved. Algae and bacteria may establish commensalism which, under nutrient stress, shift to competition and finally lead to killing and lysis of algae by bacteria (Mayali and Azam, 2004). The bacterial flora associated with the HABs may change at the time of bloom declination. Approximately 30% of algicidal bacteria attack their target algal
species through direct contact, and the remaining exhibit an indirect mode of attack by dissolved lytic agents.

The dissolved lytic agents mainly include biologically active compounds, exopolymers (Decho, 1990) which increase the tendency of cells to flocculate and its sticky nature may enhance the sinking rate and degradation of decaying blooms. Serine protease produced by algicidal bacteria have shown lytic activity against the diatom *Skeletonema costatum* (Lee et al., 2000) lending support to the theory that algicidal bacteria kill their prey using extracellular proteases.

It is essential to understand how the distribution and composition of microalgae as well as dynamics of HABs in economically important shelf seas relate to the particular physico-chemical and biological properties of the water column in which they live. In view of the importance of southwest coast of India, which is considered as one of the most biologically productive areas in the world, regular monitoring of distribution and abundance of microalgae is important. The present work is concentrated on the estuarine and coastal open sea stations along the southwest coast of India. In order to get further insights into the abiotic factors governing bloom dynamics, the physico-chemical parameters that regulated three particular bloom events during this period were studied. Bearing in mind the role of bacterial fauna associated with algal blooms as a biological factor in regulating its dynamics, isolation of bacteria associated with the algal blooms, their identification, enumeration, and ability to produce extracellular enzymes have been duly incorporated into this study.
Objectives of the study:-

- To make an in-depth study on the temporal and spatial variation in the distribution of microalgae along the southwest coast of India.
- Monitoring and surveillance of algal blooms.
- To isolate and to identify bacterial strains associated with algal blooms.
- To study the potential of associated bacteria to produce extracellular enzymes and to study their probable role in algal bloom dynamics.

The thesis comprises of seven chapters. The first chapter gives a general introduction to microalgae with special reference to algal blooms and the role of associated bacteria. In the second chapter, methodology adopted for studying planktonic microalgae, algal blooms and associated bacteria is described. Third chapter mainly comprises the analysis of physico-chemical variables, such as temperature, salinity, pH, nitrate, nitrite, silicate, phosphate, dissolved oxygen and primary productivity, which influence the distribution and abundance of microalgal species. The fluctuations of these factors for a period of two years, from pre-monsoon 2009-10 through post-monsoon 2010-11, in the estuarine and coastal monitoring stations along the southwest coast of India are described in this chapter. The distribution of microalgae is described in the fourth chapter. The fifth chapter focusses on the investigations of three algal bloom events observed along the southwest coast of India during the study period. The sixth chapter projects the determination of the bloom associated bacterial community and its ability to produce extracellular enzymes. The major findings of the study are summarised in the seventh chapter.