CHAPTER 1

Introduction
1.1 Relevance of Surfactants as Aquatic Pollutants

Aquatic environment has been the dumping ground of several man-made pollutants. The detrimental effects of these anthropogenic compounds on aquatic flora and fauna have always been an interesting and relevant topic of research. Marine pollution research started 30 years ago with studies on radioactive wastes dumped into the sea. The first international congress on marine pollution took place in 1959. It was found that the major contaminants of the aquatic environment were petroleum hydrocarbons, heavy metals, oil, waste heat, radioactive wastes, pathogens etc. Based on their adverse effects and laws regarding their disposal these pollutants have been classified into black list and grey list compounds (Ruivo, 1972). Domestic wastes containing detergents and sewage effluents were a neglected group of pollutants as they were thought to be mild in their adverse effects.

India has a rich tradition of the use of natural products especially in the field of body adornment. This includes natural cleansing agents, herbal shampoos, pollen based powders, perfumes and the like. Ever since the evolution of Homo sapiens, next to the basic needs of food, shelter and clothing, cleanliness and sense of beauty have received priority. The traditional materials used for such purposes in earlier days gave way in course of time to many synthetic formulations.

Until 1918, soap (sodium/potassium salt of long chain fatty acids) was the main product used for cleaning. With the introduction of chemical industries, the production and use of detergents and cosmetics developed and the demand for
these products automatically increased with better standards of living. Moreover there has been an increased use of these synthetic detergents (syndets) since 1960. The syndets have an edge over soap as they are unaffected by the hardness of water and are superior to soaps in their efficacy. The manufacture of detergents in India is at present carried out by many units scattered in organised and small-scale sectors. There is no law so far pertaining to Indian Standard Specifications for the quality and quantity of the ingredients to be used in detergents and cosmetics. Ministry of Health and the Bureau of Indian Standards are now jointly working for setting up quality standards for detergents and cosmetics. Testing and analytical facilities for detergents are few and there is lack of stringent regulation or legislation (Mathur and Gupta, 1998). However, in spite of all these, the demand and the use of detergents have attained new dimensions in the fields of laundry industry, in pesticide formulations, pharmaceuticals, plastics, herbicides and many other products of day to day use.

Detergents are complex mixtures of surface-active compounds or surfactants (10-18%), builders and bleaches. Surfactants are mixtures of homologues of a material differing in chain length, degree of substitution etc. Usually the properties of these compounds are additive i.e., the total property is the sum of the properties of individual constituents. After use these are discharged as domestic waste and reach the environment via sewers and/or sewage treatment plants. Surfactant is an amphipathic molecule and may be anionic, cationic, non ionic or zwitter ionic based on the characteristic ionisable group present in it. The builder component used in earlier days was sodium tri polyphosphate (STPP). These phosphate containing detergents were found to cause accumulation of phosphate in rivers (40%) in early sixties which led to eutrophication and subsequent nitrogen imbalances (Patrick and Khalid, 1974; Salas and Martino,
Thereafter a strict regulation was imposed which reduced the permissible amounts of phosphates in detergents. It was replaced by the less toxic zeolites/silicates. The builder additive commonly used is polycarboxylate and perborate is added as the bleaching agent. The studies reveal that almost all the detergent components are toxic to the aquatic organisms, especially the surfactant and the builder. In addition, the detergents may also contain enzymes, perfumes, dyes etc. (de Oude, 1992).

Detergents were also found to have adverse effects on humans. They affect the skin by removing the stratum corneum and react with other skin proteins. They also aid in the penetration of other substances as well. Skin irritation due to detergents is not a problem where machine washing is the rule, but in India where washing is done largely by hand it is of great significance. Skin irritation potential of several anionics like sodium dodecyl sulfate and non ionics were investigated and these were found to cause allergic dermatitis (Manning et al., 1998). Occupational dermatitis was noted among workers in detergent manufacturing plant. Children are the victims of poisoning by oral consumption of household detergents. Detergents and cleaners rank third in the number of reported cases of accidental ingestion every year (Mathur and Gupta, 1998).

Extensive research has been done recently in the evaluation of the toxicity of detergents. This group has now received top priority among other aquatic pollutants because of their ever increasing use and discharge into water bodies. Also they have synergistic effect on other pollutants like oil, metals, pesticides etc. (Dennis, 1997; Panigrahi and Konar, 1990). Approximately 15 million tons of soap and synthetic surfactants were used world wide in 1987 (Berth and Jeschke, 1989). Surfactants most commonly used in commercial detergents were linear alkyl benzene sulfonate (LABS/LAS), alkyl ethoxylates (AE), alkyl phenol
ethoxylates (APE) and quaternary ammonium compounds (QAC). Alkyl benzene sulfonate (ABS) was the commercially important laundry surfactant in earlier days, but was banned as it was non biodegradable and also was highly toxic to aquatic life. It was substituted by the linear alkyl benzene sulfonate (LAS), an anionic compound. LAS is a petroleum product and is treated with oleum or sulphur trioxide gas to obtain LAS. It is then neutralised with alkali and then other ingredients like fillers are added (Wagle, 1996). LAS was the most extensively studied surfactant and several references are available regarding quantification and toxicity of the chemical to a large number of invertebrates and vertebrates (Kikuchi et al., 1986; Huber, 1989; Kimerle, 1989).

Though legislators prescribe surfactant biodegradability, they are not completely mineralised in biological waste treatment plants. Unfortunately India being a developing country has poor wastewater treatment facilities. Water pollution prevention law (revised in 1990) puts up a policy for proper treatment of domestic wastewater. Also it has been identified that the presence of detergents create significant cost increases in sewage treatment. In India, domestic sewage treatment is limited only to 15 class1 cities (out of total 142) where as full sewerage treatment is present only in 7 (out of 190) class 2 towns. 55 class 1 cities, 35 class 2 towns have partial sewerage. Also 27 class 1 cities and 12 class 2 towns have partial treatment facilities. 72 class 1 cities and 147 class 2 towns have neither sewerage nor treatment facilities. Thus in our country sewage or domestic waste pose a major cause of aquatic pollution and would undoubtedly be the major threat in years to come (Chittikkara, 1998).

Coastal areas are the most prone to pollutant effects as they receive domestic waste/sewage/industrial effluents directly and it has been reported by Mukherjee et al. (1992) that detergent inputs into rivers have reached a point of
rising concern in India. The quality of coastal waters is largely affected by sewage pollution from large human settlements and industries that dump the wastes into the sea. It has been observed that the coasts most stressed by pollution load from sewage are Maharashtra, Tamilnadu and Kerala. In Kerala the hot spot is the southern half of coastline including Cochin, Ernakulam and Trivandrum (Chitkkara, 1998).

Thus, surfactants and their metabolites form the biggest group of anthropogenic pollutants. The ability of these compounds for foam formation is a serious problem as organic contaminants and pathogenic micro organisms are concentrated in the foam and thus present an epidemiological threat. In addition foaming also reduces aeration and causes hypoxia. Moreover the quantification of surfactants in rivers, sewage, marine waters etc has been done mainly in European and U.S. water bodies (Painter, 1992; Holt et al., 1992). The local conditions of temperature, humidity, water quality etc also influence the extent of toxicity. Hence the researches done in foreign countries may not apply to the Indian scene.

Historically, LAS has received utmost attention among surfactants whereas other anionics like alkyl sulfates which are now increasingly used were neglected. Also the non ionics like alkyl ethoxylates and the cationics excluding ditallow dimethyl ammonium chloride have not been properly studied for their chronic toxic effects on aquatic species. Hence there is need for a database investigating on the toxicities of the alkyl sulfates, non ionics and the cationic, all the more so because of the increased use of the non ionics in pesticide formulations, emulsion stability and pharmaceuticals. Also alkyl phenol ethoxylates (APE) are now the most widely used at the industrial level and rank third for all types of applications with an annual volume of production of 370,000 tonnes (Raymond, 1996). Also
the estrogenic potential of alkyl phenols is said to cause a detrimental effect on male reproductive health (Jobling and Sumpter, 1993; White et al., 1994). The cationics now find applications in fabric softeners, drilling mud, antiseptics, disinfectants, eye drops etc. Though the laboratory studies have their own limitations while extrapolating the results to complex natural environmental conditions, yet they are of unquestionable benefit to provide an insight into the sub lethal chronic effects of the test compounds.

The present work is a base line attempt to investigate and assess the toxicities of three surfactants viz. anionic sodium dodecyl sulfate (SDS), non ionic Triton X-100 (TX-100) and cationic cetyl trimethyl ammonium bromide (CTAB). These compounds represent simple members of the often neglected group of aquatic pollutants i.e. the anionic alkyl sulfates, non ionics and the cationics. These compounds are widely used in plastic industry, pesticide/herbicide formulations, detergents, oil spill dispersants, molluscicides etc. The test organisms selected for the present study are the cyanobacterium *Synechocystis salina* Wislouch representing a primary producer in the marine environment and a fresh water adapted euryhaline teleost *Oreochromis mossambicus* (peters) at the consumer level of the ecological pyramid. The fish species, though not indigenous to our country, is now found ubiquitously in fresh water systems and estuaries. Also it is highly resistant to pollutants and has been suggested as an indicator of pollution in tropical region (Ueng and Ueng, 1995).

### 1.2 Scope of the Work

The present work investigates the chronic biochemical changes induced by the sub lethal concentrations of the three surfactants viz. anionic sodium dodecyl...
sulfate (SDS), non ionic Triton X-100 (TX-100) and cationic cetyl trimethyl ammonium bromide (CTAB).

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\text{SDS} - \text{H}_3\text{C}-(\text{CH}_2)_{11}-\text{OSO}_3\text{Na}^+
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\text{TX-100} - \text{H}_3\text{C}-(\text{CH}_2)_7-(\text{OCH}_2)(\text{CH}_2\text{O})_{10}\text{H}
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\text{CTAB} - \text{H}_3\text{C}-(\text{CH}_2)_{15}-\text{N}^+\text{CH}_3\text{Br}^-
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The thesis is divided into two sections.

**Section 1** discusses the effects of surfactants on the teleost fish *Oreochromis mossambicus* after an exposure to 1 ppm (1/10 of LC50) of each of the three surfactants for a period of 30 days. This corresponds to 3.468μM SDS, 0.0015μM TX-100 and 2.74μM CTAB. The parameters studied include

1. **Membrane stability**
   
The stability of the hepatic lysosomal membrane (*in vitro and in vivo*) and erythrocyte membrane (*in vitro*) was studied in presence of surfactants. The release of acid phosphatase and hemoglobin respectively were used as the criteria for assessment of membrane stability.

2. **Effect on lipid peroxidation**
   
Studies were done for evaluating the peroxidative effects of surfactants on biological membranes. The important markers in lipid peroxidation viz., catalase,
superoxide dismutase, glutathione reductase, glutathione, malondialdehyde and conjugated dienes were assayed in tissues like liver, kidney and heart.

3. **Hepatic enzymes and other biochemical parameters**

Enzymes like acid and alkaline phosphatases (ACP and ALP respectively), alanine transaminase (ALT) and aspartate transaminase (AST) were assayed to assess the impacts of the surfactants on metabolic functions of the cell. The levels of glycogen, protein and lipid were also estimated.

4. **Osmoregulation and branchial ATPases**

Fresh water fishes engage in active ion uptake to maintain ionic homeostasis. Gill Na\(^+\)-K\(^+\) ATPase and Mg\(^{2+}\) ATPase play a significant role in this respect. The activity of these enzymes as influenced by the surfactants was studied.

**Section 2** deals with the biochemical effects of surfactants on the marine cyanobacterium *Synechocystis salina* Wislouch. Here the parameters studied include

1. Growth - as determined by cell count/fluorescence measurement.
2. Estimation of Chlorophyll *a*
3. Estimation of protein
4. Estimation of carbohydrate
5. Estimation of lipid