Introduction
If examined at molecular level, the basic process of life occurs in much the same way with all living organisms. This is particularly apparent if we consider the animals and plants on the earth’s crust, including freshwater. All higher animals have analogous respiratory pigments and all higher plants use the same chlorophyll in photosynthesis. This leads to the same sugars and amino acids from all plants and the same proteins from all animals being formed. This is called primary metabolism and primary metabolites are the components that are involved in these processes. However, it is also true that some plants can be safely eaten while others are poisonous and the poison differs from species to species. Such toxic compounds stem from processes that start with primary metabolites. Because of this and as a consequence of the unfortunate choice of the term primary metabolism, these processes and the compounds resulting from them have been called secondary metabolism and secondary metabolites respectively. Despite their names, secondary metabolites are by no means of secondary importance to the life of the organism. What is true is that secondary metabolites are in general restricted to certain organisms where they fulfil special roles, thus contributing to the uniqueness of these organisms as well as being essential to their survival.

To avoid difficulties the alternate term ‘Natural Products’ was invented for the secondary metabolites. However, secondary
metabolites are not the only ones to have a natural origin; primary metabolites have a natural origin as well.

The ocean provides a huge resource bank to the discovery of novel compounds (Cragg et al., 1997). Many compounds, which are diverse, novel and bioactive, have been isolated from marine organisms. The natural compounds with existing drug-like properties (Harvey, 1999) have the potential to become new therapeutic agents for various diseases.

Besides considering how man has used marine natural products to his advantage, we also have to examine in what way marine natural products are involved in regulating marine life and thus contribute to the sharp differentiation between the marine and terrestrial ecosystems. These ecological aspects are much less understood than nutritional and pharmacological ones.

Secondary metabolites have different roles to play within and outside the organisms. Thus, they may provide defense against potential predators not only to marine invertebrates as in molluscs but also form part of the plant defense system with the production of antifeedants and phytoalexins (Dixon, 2001). They may also act as antibacterial, antifouling as exemplified by the alga belonging to family Caulerpaceae and Udotaceae (Paul & Fenical, 1987). Marine metabolites like asterosaponins from the starfish and holothurins from
sea cucumber are known to inhibit fertilization of sea urchin eggs (Ruggieri & Nigrelli, 1960; Faulkner, 1984, 1986). These are the type of molecules a chemist is interested as they are expected to be of biomedical importance.

In order to evaluate the biomedical potential of any plant or animal one must consider both the chemical ecology of the organism and its evolutionary history. It is interesting to note that the majority of marine natural products currently in clinical trials or under preclinical evaluation are produced by invertebrates such as sponges, tunicates, molluscs or bryozoans. The wealth of bioactive metabolites isolated from these soft-bodied, sessile or slow-moving invertebrates that usually lack morphological defense structures such as spines or a protective shell is no coincidence but reflects the ecological importance of these constituents for the respective invertebrates. It has been repeatedly shown that chemical defense through accumulation of toxic or distasteful natural products is an effective strategy to fight off potential predators (e.g. fishes) or to force back neighbours competing for space (Proksch & Ebel, 1998; Proksch 1999; Mc Clintock & Baker, 2001).

Recent studies (Faulkner, 1994) have shown that unique secondary metabolites are produced not only by the organisms such as sponges, soft corals, algae, ascidians, bryozoans and molluscs (Michl et al.,
1993, Attaway & Zaborsky, 1993) but also by microorganisms associated with these invertebrates (Davidson, 1995).

Most, if not all, marine invertebrates harbour microorganisms that include bacteria, cyanobacteria and fungi within their tissues where they reside in the extra and intra cellular space (Vacelet & Donadey, 1977; Wilkinson, 1992). In some cases these associated microorganisms may constitute up to 40% of the biomass as in Mediterranean sponge *Aplysina aerophoba* (Vacelet, 1975; Friedrich *et al.*, 1999).

On the other hand many invertebrates are filter feeders and consume microorganisms from the inhaled seawater by phagocytosis. The relationships of marine invertebrates and marine microorganisms that may serve as food or that live either permanently or temporarily inside of marine macro organisms are highly complex and far from being understood (Wilkinson, 1992; Steinert *et al.*, 2000). It is believed that microorganisms might perhaps also be involved in the biosynthesis of natural products that are recovered for example from sponges. Okadaic acid first isolated from the sponges of the genus *Halichondria* such as *H. okadai* or *H. melanodocia* with habitats in two oceans was subsequently recognized as the metabolite of a marine microalgae, the dinoflagellate, *Prorocentrum lima* (Shimizu, 2000). Some marine bio-products originally isolated from macro organisms, such as sponges, have been subsequently discovered to be localized in
microbial associates (Bewley et al., 1996). If these symbiotic microorganisms can be isolated and cultured, optimization of production in marine microbial bioreactors may lead to an industrially feasible supply option. If the source of the compound is the macro organism itself, development of in vitro production methods could provide bulk supply of the compound (El Sayed, 2000).

Development in these fields can be understood from the recent reviews wherein marine microorganisms have been referred to as a new biomedical resource (Fenical & Jenson, 1993). The same authors have commented on both the chemical and ecological perspectives of pursuing marine bacteria as a source of new secondary metabolites (Jenson & Fenical, 1994; Fenical, 1993). A significant number of marine bio-products with pharmaceutical potential have been identified from heterotrophic marine microorganisms isolated from coastal sediments (Fenical, 1993; Davidson, 1995; Kobayashi and Ishibashi, 1993).

Fungi are well represented within the marine environment (Kohlmeyer & Kohlmeyer, 1979), especially in association with wood; nevertheless, marine fungi have received very little attention from natural products chemists. As interests have turned to marine microorganisms, the fungi have begun to be recognized as a likely source of potentially useful natural products (Davidson, 1995).
All of the fungi studied have been found to be associated with solid substrates. The shell of the crab Chinoecetes opilio was the source of a fungus of Phoma sp. producing diterpenoids phomactins A, B, B1, B2 and D (Sugano et al., 1991, 1994). The surfaces of marine algae are a good nutrient-rich source of microorganisms. For example, the surface of Enteromorpha intestinalis was the source of a strain of Penicillium sp. that produces the novel polycyclic alkaloids communescins A and B (Numata, 1993). Although these two molecules differ only in the structure of the acyl group, they exhibit roughly a 10-fold variance in their cytotoxicity against P388 lymphocytic leukemia (Davidson, 1995). Two classes of metabolites have been recently isolated from unidentified fungi obtained from the marine sponge Jaspis johnstoni, known as the source of the cyclic peptide jaspamide (also termed jasplakinolide) (Zabriskie et al., 1986; Crews et al., 1986).

Marine fungi are adapted to a very distinct set of environmental pressures and there is increasing evidence that these adaptations include the production of unique secondary metabolites (Jensen, 2002).

The development of seawater-based isolation and fermentation techniques have recently enabled the discovery of exciting marine
fungal metabolites such as the neomangicols, structurally unprecedented halogenated sesterterpenes that possess cytotoxic and antibacterial properties (Renner, 1998).

Clearly, marine fungi represent a frontier resource for the discovery of structurally unique secondary metabolites with anticipated biomedical potential (David et al., 2003).

Filamentous fungi are good candidates for industrial production of heterologous proteins (van den Hondel et al., 1991), organic acids (Kubicek & Rohr, 1986), and pigments (Hajjaj et al., 1997; Theobald et al., 1993). They are ubiquitous inhabitants of soil. Many of their metabolites are contaminants of feed and foodstuff. Mycotoxins are toxic metabolites produced by fungi, which when contacted by skin or ingested or inhaled can cause very serious diseases in human beings (Hsieh, 1987). They are capable of antibiotic activity, which represents a mechanism of challenge with other microorganisms. Among toxic fungi, a very important genus is Aspergillus. In particular, the species A. flavus, A. fumigatus and A. parasiticus are producers of very important toxic metabolites called aflatoxins. Aflatoxin B1 (AFB1) is by far the most toxic aflatoxins (Coulombe, 1993). It is potent carcinogen (Robens & Richard, 1992) and induces renal or hepatic lesions in many animal species, including human beings (Mollenhauer et al., 1989; Newberne & Butler, 1969).
The sea also has been a dumping ground for thousands of years, offloading rubbish, sewage and more recently industrial waste. Marine pollution frequently originates on land, entering the sea via rivers and pipelines. This means that coastal waters are much more polluted than the open seas, especially estuaries and harbours being badly affected. Additional pollution is actually created at sea by activities such as dredging, drilling for oil and minerals and shipping. By the latter half of the 1990s, it has become clear that oceans, routinely treated as limitless sources and sinks for human consumption and waste, were changing in response to intense fishing, pollution and climate change (NRC, 1995).

Most of the coastal areas of the world have been reported to be damaged from pollution, significantly affecting commercial coastal and marine fisheries. Therefore, control of aquatic pollution has been identified as an immediate need for sustained management and conservation of the existing fisheries and aquatic resources (Islam & Tanaka, 2004).

Many synthetic organic chemicals (e.g. organochlorines, organophosphates, polycyclic aromatic hydrocarbons and organometals) are of growing environmental concern, because of their toxicity and high persistence in the environment and in biological systems. Furthermore, the high lipophilicity of many of these
xenobiotics greatly enhances their bioconcentration/ biomagnification, thereby posing potential health hazards on predators at higher trophic levels (including human beings). Nowadays, persistent xenobiotic compounds have been found in every part of the ocean: from Arctic to Antarctic and from intertidal to abyssal (Islam & Tanaka, 2004).

Most xenobiotic compounds occur only at very low concentrations in the environment, and their threats to marine life and public health are still not well understood. However, sub-lethal effects of these compounds over long-term exposure may cause significant damage to marine population, particularly considering that some of these compounds may impair reproduction functions of animals while others may be carcinogenic, mutagenic or teratogenic (Islam & Tanaka, 2004). The biomarker approach is suggested as a suitable strategy for studying sublethal effects of pollutants, providing an early indication of possible adverse effects in the organisms (Hugget et al., 1992).

The work reported in this thesis deals mainly with (1) fungal metabolites of industrial importance, (2) fish biliary metabolites that are used as biomarkers of polycyclic aromatic hydrocarbon (PAH) pollution and bioremediation of PAHs (3) anti-protozoal and antimicrobial activity of some synthetic analogs of benzoxazoline-2-one a constituent of mangrove Acanthus ilicifolius.
It has been divided into three chapters:

Chapter 1: is a presentation of the study carried out on the secondary metabolites produced by marine fungus *Aspergillus sulphureus* isolated from marine grass environment. It has been sub divided into the following sections.

Section I: reviews the literature on the genus *Aspergillus*.

Section II: describes the isolation and structural elucidation of secondary metabolites produced by *Aspergillus sulphureus* using NMR and ESI-MS techniques. These include:

(i) Kojic acid

(ii) Major lipids with special reference to triglycerides, sterols and phthalates.

Section III: deals with the attempts made to enhance the production of Kojic acid, an industrially important compound by using alginate elicitor and different synthetic and commercial culture media.

Section IV: In this section, the isolation and structural elucidation of acetyl cyclopiazonic acid a novel antifungal metabolite that was obtained from the culture medium of *Aspergillus sulphureus* during the
course of investigations on the enhancement of kojic acid production
has been dealt with.

Chapter 2: studies the effect of Polycyclic Aromatic Hydrocarbon
(PAH) pollutants on the estuarine fish Oreochromis mossambicus in
the presence of nitrite. Bioremediation of these pollutants using
biosurfactants (saponins) has also been evaluated. It has been divided
into the following sections.

Section I: gives a broad description of polycyclic aromatic
hydrocarbons in the marine environment.

Section II: The effect of nitrite on the metabolism and impact of
phenanthrene in the estuarine fish, Oreochromis mossambicus has
been discussed in this section. The metabolites and DNA adducts
formed in the above study have been characterised by ESI-MS.

Section III: The studies made on the emulsifying and surfactant
properties of saponins with respect to bioavailability and bioelimination
of PAH has been reported in this section. The novel saponin was
isolated from a mangrove plant Lumnitzera racemosa and identified on
the basis of spectral data.

Chapter 3: Miscellaneous studies
In this chapter antimicrobial and leishmanicidal activities of Benzoxazoline-2-one and its derivatives have been reported.
REFERENCES:


Chapter 1

Study of Secondary Metabolites of Marine Fungus Aspergillus sulphureus