1. GENERAL INTRODUCTION

The coastal stretch of Tamil Nadu (Southeast coast), India is being attracted by the human population for inhabitation, urbanization, development of harbors and various industries in addition to intensive agriculture, aquaculture and fishing activities. These developments put tremendous pressure on this pristine and productive coastal environment and therefore, many sites of this coast have become most critical pollution hotspots. Hence, protection of the coastal and near shore regions from continuing pollution becomes the most essential for the effective management of coastal resources and their dependents. Proper planning for controlling and combating coastal pollution requires knowledge about the magnitude of the pollution, the major route and transport mechanisms, behaviors in the coastal ecosystem etc.

Hydrocarbons are entered into the marine environment mainly through oil tanker operation & accidents, coastal refineries, offshore oil exploration, dry (aerosols) and wet atmospheric depositions (heavy rains), river runoff, municipal/industrial discharges etc. (Tolosa et al., 1996; Lipiatou et al., 1997). Marine sediments act as temporary or long term reservoir of natural and anthropogenic organic pollutants from land based sources. The hydrocarbons are readily adsorbed onto particulate matter, and settled in bottom sediments and act as a reservoir for these hydrophobic contaminants for very long period (Budzinski et al., 1997).
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1.1. Polycyclic Aromatic Hydrocarbons (PAHs)

An important class of petroleum hydrocarbons is Polycyclic Aromatic Hydrocarbons (PAHs) and their derivatives. The PAHs are ubiquitous in the environment such as air, water, soil, sediments including living organisms and comprise more than 10,000 compounds which consist of two or more fused benzene rings in different arrangements (Blumer, 1976). In general, PAHs are released into the environment from natural and anthropogenic sources. The natural sources are oil seeps, volcanoes, forest fires, chlorophyllous and non chlorophyllous (bacteria and fungi) plants. Anthropogenic sources of PAHs include discharge from routine oil transportation, oil spill, coastal refineries, power plants based on fossil fuel consumption, biomass burning, pyrolysis of wood and internal combustion in industrial and vehicle engines (Yunker et al., 2002). For decades, this pollution has been of global concern due to significant health risk posed by these compounds because of their toxic, mutagenic and carcinogenic properties (Churchill et al., 1999; Seo et al., 2007).

The properties of PAHs are largely controlled by the organic particulate fraction of suspended and deposited sediments (Baker et al., 1986). The particle bound PAHs have a short residence time in the water column before they started settle in the bottom sediments where they may be re-suspended, degraded or subjected to long-term retention. The extent of risk to the water column depends on the degree of bioturbation, physical re-suspension and the nature of the compound etc., (Wong et al., 1995).

PAHs compounds are categorized as environmental high priority contaminants which consist of 16 PAHs such as (a) seven PAHs with low molecular weight (i. e., Naphthalene (NA), Acenaphthylene (ACY), Acenaphthene
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(ACE), Fluorene (FL), Phenanthrene (PH), and Anthracene (AN) and (b) 10 PAHs with high molecular weight (i.e., Fluoranthene (FLU), Pyrene (PY), Benz(a)anthracene (BaA), Chrysene (Chry), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Benzo(g,h,i)perylene B(ghi)P, Dibenz(a,h)anthracene (DBA), and Indeno(1,2,3-cd)pyrene (IP)). Of which, some of the PAHs such as BaA, Chry, BbF, BkF, BaP, IP, and DBA are potentially carcinogenic (US EPA, 1993). In general, PAHs are lipophilic compounds consist of 2 to 7 benzene rings; the 2 - 4 rings are classified as lower molecular weight (LMW) compounds and 5 - 7 benzene rings are classified as higher molecular weight (HMW) compounds (Neff, 1979). The LMW PAHs are more soluble in water and are acute toxic to humans and living organisms whereas HMW are highly soluble in lipid and more carcinogenic, mutagenic with more time period effects (Neff, 1979). The hydrophobic and lipophilic properties of some HMW PAHs make them relatively insoluble in water and tend to accumulate on surfaces or in non-polar substance (Neff, 1979).

The effect of PAHs is become common and persists in the aquatic ecosystem. This occurs because most of the PAHs exist with high hydrophobicity, sorbs strongly to the organic matter in sediment and are resistant to bacterial degradation in an anoxic environment (Neff, 1979). Under this environmental condition, PAHs may be re-suspended to the water column because of the disturbances and become continuing source and threaten the aquatic marine ecosystem through bioaccumulation in food chains (Neff, 1985).

1.2. Source and Fate of PAHs in aquatic environment

The PAH are derived into the environment either naturally or anthropogenically. There are mainly four types of PAHs such as (i) Diagenic, (ii)
Petrogenic, (iii) Pyrogenic and (iv) Biogenic. (i) Diagenic PAHs (perylene, retene, phenanthrane and chrysene) are derived at low temperature (< 70 °C) in a relatively rapid process (days to years). (ii) Petrogenic PAHs are derived in a slow manner with a long-term process at 100-300 °C and these are mainly from combustion of petrol and petroleum products. (iii) Pyrogenic PAHs are derived rapidly at high temperatures (> 500 °C) and from incomplete (when there is a small amount of oxygen) combustion of organic biomass produces pyrogenic PAHs. (iv) Biogenic PAHs are derived by biosynthesis of plants and animals. As a result of biosynthesis, either individual PAHs or simple mixtures are formed. Retene, C4-Phe isomer and simonellite are the examples for biogenic PAHs (Neff, 1979; Mazeas and Budzinski, 2001; Wang et al., 2010; Anyakora and Coker, 2006).

1.3. Pyrogenic and petrogenic sources of PAHs

PAHs from anthropogenic source enter the marine environment as two ways such as Pyrogenic and Petrogenic. The pyrogenic source includes pyrolytic processes such as combustion of fossil fuel, urban and industrial activities, natural fire and biomass burning that produce high molecular weight and less or non-alkylated PAHs. Combusted PAHs tend to attach into soot particles, move in far distances and get deposited on soil in terrestrial and sediments in sea bottom. Some of pyrogenic products of PAHs such as fine particles from charcoals are also washed out from the place of production to the marine environment (Neff, 1985). The petrogenic sources of PAHs are mainly from the release of crude oil and petroleum products such as lubricating oil, diesel fuel, gasoline, asphalt and kerosene which enters in to the environment via oil spill, tanker accident, routine tanker operation such as ballast water discharge and discharge from vehicle workshops (NAS, 1975).
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Accumulation and bioaccumulation of PAHs in the marine environment and organisms are inversely correlated to the potential and ability of hydrocarbons to metabolize them either chemically or biologically (Sakari et al., 2011, 2010a, b and 2009). Investigation of the sources for hydrocarbon pollution is an important task to execute remedial measures properly. In Southeast Asia, intensive studies on specific compound analysis have been started in the Straits of Malacca (Zakaria et al., 1999, 2000, 2001, 2002 and 2006) and Gulf of Thailand etc., (Boonyatumanoond et al., 2006 and 2007).

1.4. Identification of source of PAHs compounds through ratio analysis

In order to identify the sources of hydrocarbon pollution in the environment, there are several techniques such as use of isomer pair ratios (Yunker et al., 2002), individual compound ratios (Baumard et al., 1998a,b,c; Zakaria et al., 2000) and biomarkers (Zakaria et al., 2002; Wang and Fingas, 2005). Recently, some molecular ratios of specific hydrocarbons were developed to distinguish the origin of PAHs from various sources. Of which, Phenanthrene/anthracene (Ph/An) and fluoranthene/pyrene (Fl/Py) ratios were widely used (Steinhauer and Boehm, 1992; Budzinski et al., 1997; Baumard et al., 1998 a,b,c, 1999). The ratio of Fl/Py (fluoranthene/pyrene) has been widely used to identify fuel sources, showing values < 1.4 for coal combustion (Lee et al., 1977) and < 1.0 for wood (Lee et al., 1977; Knight et al., 1983). In sediments, value for this ratio was about 1.3-1.7 for remote sites and < 1.0 near to the urban centers (Gschwend and Hites, 1981; Helfrich and Armstrong, 1986). Similarly, phenanthrene/anthracene ratio also applies as an indicator for measuring the remoteness (>15) or vicinity (<10) of PAHs sources to urban areas (Zhang et al., 1993).
High temperature processes such as combustion of organic matters generates PAHs characterized by low ratio of Ph/An (<10), whereas slow maturation during catagenesis, reach to higher values (Ph/An <15). Same trend observed in ratio of flouranthene/pyrene (Fl/Py), where values greater than 1 come from pyrogenic sources and less than 1 is the indication of petroleum input. Further, Flu over Flu plus Pyr ratio [Flu/(Flu+Pyr)] that is generally greater than 0.5 in grass, wood or coal combustion, and the petroleum boundary ratio appears closer to 0.40 than 0.50, whereas the Flu/(Flu+Pyr) ratios between 0.40 and 0.50 are more characteristic of liquid fossil fuel combustion (Yunker et al., 2002). The above mentioned values were shown to be relatively less reliable in different geographical locations due to the various combustion material sources (Budzinski et al., 1997).

1.5. Bacterial degradation of PAH

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants, which cause a public concern due to their potency as carcinogens and mutagens. Originated mainly from anthropogenic activities, PAHs are now distributed in natural waters worldwide (Baumard et al., 1999; Rogers, 2002). In the aquatic environment, PAHs tend to sorb onto the particle phase due to their high hydrophobicity and solid-water distribution ratios (Wang et al., 1988a, b; Johnsen et al., 2005). The distribution of PAHs in the aquatic environment is significantly affected by aquatic particulates which act as aggregates of numerous complicated organic materials (Tang et al., 2000). Biodegradation is one of the most key processes for PAHs to disappear from the environment. Therefore, it is critically important to study the influence of particles on degradation of PAHs in natural water systems. Bacteria are commonly considered to degrade organic substrates
only when they are dissolved in water (Subba Rao and Alexander, 1982; Weissenfels et al., 1992; Johnsen et al., 2005)

PAHs in the environment have definite behaviors which are controlled by several processes. Processes which can control the transport and degradation of PAHs in sediment include: 1) partition of the compounds between aqueous (pore-water) and particulate phase, 2) microbial degradation, 3) uptake, metabolisms and depuration of PAHs by benthic organisms, 4) photooxidation (surface sediment), chemical oxidation and 5) biosynthesis (Sakari et al., 2011).

Recently, several bacterial species which are capable of degrading a variety of PAHs have been isolated. It is now well established that microbial degradation of PAH occurs primarily in the aerobic zone (Bauer and Capone, 1985) with highest rates occurring with low molecular weight homologues (Lee et al., 1977; Gardner et al., 1979; Readman et al., 1982). The ability of bacteria to degrade PAHs is usually determined by (i) ability of bacteria to transport the PAH into the cell (dependent largely on molecular size and water solubility), (ii) the PAH being a suitable substrate for the available enzymes and iii) the PAH being an inducer for the appropriate transport system or degradative enzymes (Juhasz and Naidu, 2000). Bacterial degradation predominantly occurs via aerobic oxygenase mediated pathways; although an alternative pathway of degradation involving utilization of nitrate as the alternative electron acceptor has been reported (Eriksson et al., 2003). In the latter process, oxidized forms of nitrogen are reduced and organic carbon from soil material or contaminants is oxidized (Pothuluri and Cerniglia, 1994). The anaerobic degradation has been suggested as occurring in a similar manner to aerobic degradation, namely modification into intermediates followed by
ring cleavage and the creation of metabolites for cell function (Holliger and Zehnder, 1996).

The present study is undertaken to understand the status and magnitude of Polycyclic Aromatic Hydrocarbons (PAHs) in selected spots of Tamil Nadu coast and attempted for their degradation is to identify suitable microbes for the biodegradation of high molecular PAH (Benzo(a)pyrene). The microorganisms which have the potential and ability to survive in the contaminated environment are useful for a successful biodegradation research. In view of this, the present study aims to study the degradation of PAHs by bacterial cultures isolated from PAHs contaminated sediments and for better bioremediation applications in future.