Chapter 1: Introduction

One of the primary aims of environment quality study is to decipher the impact of anthropogenic compounds, such as organic micropollutants on ecosystem, in order to prevent its deleterious effects. As these organo-micropollutants and their geochemical fates are interrelated, it is of utmost importance to understand the control measures on the fates of these compounds through various analyses (Maskaoui et al., 2007).

Marine environment receives input of hazardous chemicals through reverine sources of harbor, direct discharge of effluents and other atmospheric depositions which leads to an elevated level of micropollutants in marine ecosystem. Organisms residing in such areas are thus exposed to these contaminants which initiate hazardous consequences.

It should be evidently noted that, in almost all cases, aquatic organisms are not exposed to any single substance but to a mixture of chemicals. Therefore, an increasing concern about the potential adversities of pollutants of various origins and types is seen as compared to studies on individual components (Gheskiere et al., 2013).

Experimental mixture studies in ecotoxicology and human toxicity demonstrate the concept of dose/concentration addition and independent actions provide good approximations of observed combination effects. Chemicals in mixture
act by the same mechanisms, whereas, a chemical independently can act through different modes, that does not influence each other.

In the present era, petroleum hydrocarbon contamination is considered a major widespread environmental problem distributed in atmosphere, terrestrial soil, marine waters and sediments. Marine sediments are of great concern due to their associated risk to public health and ecological damage to coastlines. In last few years marine environment is subjected to contamination by variety of organic pollutants from various sources that results from uncontrolled releases from industrial manufacturing, discharge from effluent treatment plants, run-off from terrestrial sources, combustion of fossil fuels, refuse burning, and oil spillages during transportation (Head and Swannell, 1999).

**Polycyclic aromatic hydrocarbons (PAHs)**

PAHs are a group of organic molecules consisting of two or more fused aromatic rings. PAHs are persistent and recalcitrant in the environment due to high thermodynamic stability of benzene moieties, and their hydrophobic nature, especially the high molecular weight PAH molecules (HMW, with four or more rings), which exhibit higher hydrophobicity and toxicity and which persist in the environment for a longer period of time (Sutherland et al., 1995).

The omnipresence of PAHs in almost all environmental matrices is one of the major ecological concerns, due to their persistence in nature. It leads to carcinogenic and mutagenic effects to both aquatic and terrestrial flora and fauna. Occurrence
and distribution of PAHs in sites distant from direct exposure can provide an understanding of the mobility of these compounds and the integrity of the system (Scott et al., 2012). Hence, it is of utmost importance to develop various strategies for its significant removal.

**Source of PAHs**

PAHs are formed due to incomplete combustion of organic compounds. Different types of combustion, such as domestic, industrial and agricultural, contribute to their emissions. Industrial and daily human activities such as coal processing, wood, crude oil and natural gas combustion for heating, vehicles, cooking and smoking, or even natural processes such as carbonization are responsible for the incomplete combustion of organic materials. Ravindra et al., (2008) reviewed that, in general, there are four major emission sources of PAHs, i.e. mobile, industrial, agricultural, and natural.

**Mobile sources**

Mobile sources include the emission from vehicles such as aircraft, shipping, railways, automobiles, off-road vehicles, and machinery. The emission of PAHs from these sources is a function of engine type, load and age, fuel type and quality (e.g. aromaticity). PAHs emissions are also associated with the extensive use of diesel, coal, gasoline, oil, and lubricant oil. Trains, aircrafts, and ships also contribute significantly to the mobile sources of PAHs.
**Industrial emissions**

Industrial emissions are the most important sources of PAHs that include primary aluminium production, coke production (e.g. as part of iron and steel production), creosote and wood preservation, waste incineration, cement manufacture, petrochemical and related industries, bitumen and asphalt industries, rubber tire manufacturing, and commercial heat/power production.

**Agricultural sources**

Agricultural sources of PAHs are open burning of biomass which is a common method for crop and forest residue disposal. Burning of agricultural waste, however, is a source of atmospheric PAHs. Agricultural sources include the stubble burning, open burning of moorland heather for regeneration purposes, and open burning of brushwood and straw. All of these activities involve the burning of organic materials under sub-optimum combustion conditions.

**Natural sources**

Natural sources include burning of forest, volcanic eruptions and decaying of organic matter. The degree of PAHs in atmosphere depends on environmental conditions like wind, temperature and humidity.

**Physical and chemical properties of PAHs**

PAHs are typically hydrophobic pollutants with high adsorption to organic matter and other particulates. Each PAH is different in terms of their physical and chemical properties such as solubility and vapor pressure. Molecular weight plays
an important part in solubility and persistence of PAHs. Low molecular weight (LMW) PAHs are more water soluble as compared to high molecular weight (HMW) PAHs, whereas HMW PAHs are more hydrophobic when compared to LMW PAHs (Juhasz and Naidu, 2000).

![Chemical structures of 16 PAHs designated by the United States Environmental Protection Agency (USEPA) as priority pollutants](image)

**Fig. 1.1** Chemical structures of 16 PAHs designated by the United States Environmental Protection Agency (USEPA) as priority pollutants

The most distinct characteristic of PAHs is their aromaticity, and the extended π-electron system resulting in chemical stability, which is why they tend to retain the π-electron conjugated ring systems in reactions. Hydrophobicity is another important property of PAHs. Because of their low solubility in aqueous
environment, PAHs tend to associate with particles and eventually sink into soil and sediments (Xiao et al., 2003).

PAHs are a class of priority pollutants with benzene rings arranged either in linear, angular and cluster arrangement (Fig. 1.1). Many PAHs have bay- and K-region (Ramesh et al., 2011), responsible for the formation of much reactive compounds such as epoxides and diols, leading to formation of very reactive species chemically and biologically (Fig. 1.2).

Phenanthrene is a simple PAH contains both – bay and K-regions at C 4-5 and C9, respectively. The bay area is a hindered area having oleinic double bonds with high electron density, which in turn makes the molecule rigidly stable (Fig. 1.2). Moreover, K region participates to yield epoxides, which according to the theory of Schmidt-Pullman, are more carcinogenic when compared to their parent molecule. LMW PAHs (2 or 3 rings) are volatile and water soluble and relatively easy to degrade than HMW PAHs (four or more rings). HMW PAHs resists biodegradation as they are strongly sorbed to particulate matter and posses high stereostability. Because of their solid state, high molecular weight and higher hydrophobicity, expressed as its log P value between 3 and 5, PAHs are very toxic to whole cells (Cerniglia, 1992). Hence, USEPA has listed 16 PAHs as prority pollutants. The properties of 16 priority PAHs are as represented in Table 1.1.
Toxic and carcinogenic effects of PAHs

It is evidently known that PAHs have catastrophic effect on humans. The toxicity of PAHs on higher life forms has continued, with many PAHs displaying acute carcinogenic, mutagenic and teratogenic properties (Bamforth and Singleton, 2005).

Benzo[a]pyrene is recognized as a priority pollutant by the USEPA as this compound is known to be one of the most potent carcinogens of all known PAHs. Because of the potential of benzo[a]pyrene to bind cellular proteins and DNA with toxic effects, it is of major health concern to humans and marine biota (Kim et al., 2013). The resulting biochemical disruption and cell damage can lead to mutations, developmental malformations, tumors, and cancer (Bach et al., 2003). Table 1.2
indicates the carcinogenic classification of PAHs on humans as determined by various organizations.

**Table 1.1** Physico-chemical properties of 16 PAHs as classified by USEPA.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>PAH</th>
<th>No. of Rings</th>
<th>M&lt;sub&gt;r&lt;/sub&gt;</th>
<th>Melting Point (°C)</th>
<th>Boiling Point (°C)</th>
<th>Water Solubility (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Vapour Pressure (Pa)</th>
<th>K&lt;sub&gt;ow&lt;/sub&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naphthalene</td>
<td>2</td>
<td>128.17</td>
<td>80.6</td>
<td>218</td>
<td>31</td>
<td>10.4</td>
<td>3.37</td>
</tr>
<tr>
<td>2</td>
<td>Acenaphthene</td>
<td>3</td>
<td>154.21</td>
<td>95</td>
<td>279</td>
<td>3.47</td>
<td>3.0x10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3.92</td>
</tr>
<tr>
<td>3</td>
<td>Acenaphthylene</td>
<td>3</td>
<td>152.20</td>
<td>93.5-94.5</td>
<td>265</td>
<td>3.93</td>
<td>8.93x10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4.07</td>
</tr>
<tr>
<td>4</td>
<td>Fluorene</td>
<td>3</td>
<td>166.22</td>
<td>116</td>
<td>295</td>
<td>0.190</td>
<td>8.0x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>4.18</td>
</tr>
<tr>
<td>5</td>
<td>Anthracene</td>
<td>3</td>
<td>178.23</td>
<td>217.5</td>
<td>340</td>
<td>0.0434</td>
<td>1.0x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>4.54</td>
</tr>
<tr>
<td>6</td>
<td>Phenanthrene</td>
<td>3</td>
<td>178.23</td>
<td>99.5</td>
<td>340</td>
<td>1.18</td>
<td>2.0x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>4.57</td>
</tr>
<tr>
<td>7</td>
<td>Fluoranthene</td>
<td>4</td>
<td>202.26</td>
<td>110.8</td>
<td>375</td>
<td>0.265</td>
<td>1.23x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>5.22</td>
</tr>
<tr>
<td>8</td>
<td>Pyrene</td>
<td>4</td>
<td>202.26</td>
<td>156</td>
<td>404</td>
<td>0.013</td>
<td>6.0x10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>5.18</td>
</tr>
<tr>
<td>9</td>
<td>Benz[a]anthracene</td>
<td>4</td>
<td>228.29</td>
<td>159.8</td>
<td>437.6</td>
<td>0.014</td>
<td>2.8x10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>5.91</td>
</tr>
<tr>
<td>10</td>
<td>Chrysene</td>
<td>4</td>
<td>228.29</td>
<td>255.8</td>
<td>448</td>
<td>0.0018</td>
<td>5.70x10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>5.86</td>
</tr>
<tr>
<td>11</td>
<td>Benzo[k]fluoranthene</td>
<td>5</td>
<td>252.31</td>
<td>215.7</td>
<td>480</td>
<td>0.00055</td>
<td>7.0x10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>6.04</td>
</tr>
<tr>
<td>12</td>
<td>Dibenzo[a,h]anthracene</td>
<td>5</td>
<td>278.35</td>
<td>266</td>
<td>524</td>
<td>0.0005</td>
<td>1.33x10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>7.16</td>
</tr>
<tr>
<td>13</td>
<td>Benzo[a]pyrene</td>
<td>5</td>
<td>252.31</td>
<td>176.5</td>
<td>495</td>
<td>0.0038</td>
<td>1.40x10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>6.25</td>
</tr>
<tr>
<td>14</td>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>6</td>
<td>276.34</td>
<td>162.5</td>
<td>536</td>
<td>0.0620</td>
<td>1.0x10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>6.58</td>
</tr>
<tr>
<td>15</td>
<td>Benzo[b]fluoranthene</td>
<td>6</td>
<td>252.31</td>
<td>167</td>
<td>357</td>
<td>0.0012</td>
<td>6.67x10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>6.57</td>
</tr>
<tr>
<td>16</td>
<td>Benzo[g,h,i]pyrene</td>
<td>6</td>
<td>276.34</td>
<td>278.3</td>
<td>500</td>
<td>0.00026</td>
<td>1.39x10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>7.10</td>
</tr>
</tbody>
</table>

The effect of PAHs on human health depends mainly on the length and route of exposure, their concentrations and toxicity. Preexisting health status and age are
also the decisive factors. Occupational exposure to high levels of PAHs mixture results in acute symptoms such as eye irritation, vomiting, nausea etc. Chronic health hazards such as kidney and liver damage, lung malfunction, skin inflammation can arise from long time exposure to PAHs (Fig. 1.3). Higher levels of PAHs can also lead to embryotoxic effect during pregnancy and have also been reported to suppress immune reactions. Moreover PAHs and their reactive derivatives can form DNA adducts causing genotoxic and carcinogenic effects on humans (Rengarajan et al., 2015; Dudhagara et al., 2016).

The carcinogenicity of PAHs on mammals is a result of metabolic formation of diol epoxides which binds to DNA and forms adduct. A variety of PAHs taken up by the human body undergo metabolic activation. The initial step in the metabolism of PAHs involves the multifunctional P-450 enzyme system forming different epoxides, which are short-lived compounds and may rearrange spontaneously to phenols or undergo hydrolysis to dihydrodiols. This dihydrodiol epoxide may interact with proteins, RNA and DNA, which in turn causes mutation and possibly cancer (Xue and Warshawsky, 2005).

**Environmental fate of PAHs**

The persistence of PAHs in the environment is determined by various factors. These factors include its chemical structure, concentration, hydrophobicity, dispersion, as well as the bioavailability of the co-existing contaminants. Besides these, environmental factors such as soil matrices, pH and temperature also control
the persistence of PAHs in the environment. The persistence of PAHs is also
influenced by the ‘age’ of the co-existing contaminants in the soil matrix. If the age of
co-existing contaminants is higher, the persistence of PAHs is longer.

Table 1.2 Carcinogenic classifications of PAHs reported by various organizations (Kim et al., 2013).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Agency</th>
<th>PAHs</th>
<th>Carcinogenic Classification</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agency for Toxic Substances and Disease</td>
<td>• Benz(a)anthracene,</td>
<td>Known animal carcinogens</td>
<td>ATSDR (1995)</td>
</tr>
<tr>
<td></td>
<td>Registry (ATSDR)</td>
<td>• Benzo(b)fluoranthene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(a)pyrene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dibenzo(a,h)anthracene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Indeno(1,2,3-c,d)pyrene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benz(a)anthracene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(a)pyrene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>International Agency for Research on Cancer</td>
<td>• Benzo(a)fluoranthene,</td>
<td>Possibly carcinogenic to</td>
<td>IARC (2010)</td>
</tr>
<tr>
<td></td>
<td>(IARC)</td>
<td>• Benzo(k)fluoranthene,</td>
<td>humans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Indeno(1,2,3-c,d)pyrene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Anthracene,</td>
<td>Not classifiable as to their</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(g,h,i)perylenen,</td>
<td>carcinogenicity to humans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(e)pyrene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Chrysene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fluoranthene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fluorene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Phenanthrene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pyrene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>U.S. Environmental Protection Agency</td>
<td>• Benz(a)anthracene,</td>
<td>Probable human carcinogens</td>
<td>USEPA (2008)</td>
</tr>
<tr>
<td></td>
<td>(EPA)</td>
<td>• Benzo(a)pyrene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(b)fluoranthene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(k)fluoranthene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Chrysene,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dibenzo(a,h)anthracene and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Indeno(1,2,3-c,d)pyrene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Acenaphthylene,</td>
<td>Not classifiable as to human</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Anthracene,</td>
<td>carcinogenicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Benzo(g,h,i)perylenen,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fluoranthene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The fate of organic contaminants in the environment is associated with both abiotic and biotic factors such as volatilization, photo-oxidation, bioaugmentation, chemical oxidation, and microbial degradation (Pathak et al., 2009).

**Fig. 1.3** Effect of PAHs on human health.

**Volatilization**

The process of conversion of a chemical substance from a liquid or solid state to a gaseous or vapor state by the application of heat, reducing pressure, or by a combination of both is called volatilization. However most of the compounds are degraded slowly and thus tend to accumulate in the environment (Urgun-Demirtas et al., 2006). PAHs compounds have different vapor pressures (Table 1.1) as a result of their solubility and structure, and this causes variable rates of volatilization. In
general, LMW PAHs have higher vapor pressures and are more soluble making them more likely to volatilize at room temperature (30-40 °C) than HMW PAHs.

**Photo-oxidation**

Photo-oxidation of PAHs is mainly determined by the concentration of oxygen, the ambient temperature and the solar radiation intensity. The PAH molecule absorb infra-red electromagnetic radiation from ultraviolet and visible light causing increase in molecular rotation or vibration that may result in fragmentation, oxidation or polymerization of the molecule. It has been found that when aromatic compounds are exposed to UV light, partially oxidized intermediates are produced which are more susceptible to degradation than their parent ones. Because of this property of aromatic compounds, photo-degradation has been recommended as an early stage strategy for biodegradation (Mueller et al., 1997).

**Chemical-oxidation**

Chemical oxidation can improve the bioavailability of PAHs by directly utilizing the oxidizing agents, and is considered as a promising technique for the remediation of PAHs-contaminated environment. Non-enzymatic or non-photochemical reactions are prominent in soil, but rarely result in the complete modification of a compound. Usually, chemical oxidation causes slight modification of PAHs that result in a compound that is chemically similar to the original compound (Alexander, 1999).
**Microbial degradation**

Microbes such as bacteria and fungi play a vital role in biodegradation of PAHs due to their metabolic capabilities, high reproduction rates, effective metabolism with unique catabolic enzymes and vast biological diversity. Microbes can attack organic compounds, such as PAHs as initial substrate into less complex metabolites, and through mineralization into inorganic minerals, H$_2$O, CO$_2$ (aerobic) or CH$_4$ (anaerobic). Biodegradation of a pollutant and its rate depends on environmental factors, number and kind of microbes, biochemistry and physiology of the molecule being degraded (Haritash and Kaushik, 2009). Thus, to devise a bioremediation system, a number of factors need to be considered. Both bacteria and fungi have been extensively studied for their ability to degrade xenobiotics including PAHs.

**Bacteria**

Bacteria are actively involved in the degradation of organic pollutants from contaminated sites. A number of bacterial species isolated from contaminated soil or sediments are known to degrade PAHs. Many of the PAH-degrading bacteria have also been reported to metabolize HMW PAHs such as fluoranthene, pyrene, chrysene, and benzo[a]anthracene. In general, *Pseudomonas*, *Sphingomonas*, *Flavobacterium*, and *Burkholderia* are the most versatile genera which are involved in PAHs degradation (Kanaly and Harayama, 2000; Haritash and Kaushik, 2009).
**Actinomycetes**

Actinomycetes are common soil bacteria best known from an environmental perspective for the breakdown of recalcitrant organic materials (Goodfellow et al., 1993). Among prokaryotes, these bacteria possess some of the largest genomes observed (up to 8 million base pairs) and a complex life cycle that can include the formation of a well-developed substrate mycelium followed by differentiation into actinospores, usually in response to nutrient limitation or unfavorable environmental conditions. Actinomycetes generally grow slowly relative to many Gram-positive bacteria and produce structurally diverse, biologically active secondary metabolites that perform important but largely undefined ecological functions (Chalis and Hopwood, 2003).

Actinomycetes possess many properties that make them good candidates for application in bioremediation of soils contaminated with organic pollutants (Balachandran et al., 2012). They possess extracellular enzymes, able to degrade an array of complex organic compounds. The exospores can combat unfavorable conditions such as draught, making them xerotolerant. In addition, filamentous growth favors colonization and better interaction with soil particles. Highly filamentous structure impacts large surface to volume ratio making them suitable for elevated remediation (Pizzul, 2009).

Another interesting feature within this group of microorganisms, especially regarding the degradation of hydrophobic compounds, is their surfactant producing
ability. In case of actinomycetes, degradation may be due to (i) the production of extracellular biosurfactants, specially glycolipids produced by *Rhodococcus* sp. (Rapp et al., 1979; Kretschmer et al., 1982; Singer and Finnerty, 1990) or the lipopeptide produced by *Arthrobacter* sp. strain MIS38 (Morikawa et al., 1993) and (ii) mycolic acid, a cellular biosurfactant responsible for adhesion of cells to hydrophobic phase in biphasic system (Neu, 1996).

Since many bacteria have been reported to have the ability to degrade PAH (Cerniglia, 1992), bioremediation is an alternative for cleaning up PAH-contaminated soils. It has been suggested that nocardioform actinomycetes (*Mycobacterium*, *Rhodococcus* and *Gordonia*) play an important role in the mineralization of these compounds in soils (Kästner et al., 1994). Members of the genus *Mycobacterium* are widely distributed in soils and are able to degrade a large number of organic compounds (Churchill et al., 1999) including PAHs as naphthalene, phenanthrene, anthracene, pyrene and fluoranthene.

**Fungi**

Several fungi are known to have the property for degradation of persistent organic pollutants (POPs). Microbial degradation of xenobiotics by lignolytic fungi has been intensively studied during the past few years. Due to irregular structure of lignin, lignolytic fungi produce extracellular enzymes with very low substrate specificity, making them suitable for degradation of different compounds (Novotný et al., 2004). The lignolytic system consists of three main enzymes as lignin
peroxidase (LiP), manganese dependent peroxidase (MnP), and phenol oxidases which can improve the degradation ability of PAHs present in environment (Hofrichter et al., 2002). The monooxygenase system of cytochrom P-450 generating epoxides may also be involved in degradation. The epoxides can be rearranged into hydroxyl derivatives or they may be hydrolyzed to vicinal dihydrodiols (Fig. 1.4).

White-rot fungi (WRF) can degrade a wide range of organopollutants and their extracellular enzymes have played a significant role in PAHs biodegradation. They grow on decaying wood, wastage of pulp and paper mill and crop waste which can be used as the nutrients. However, the growth conditions could have an effect on their degradative capability. Additionally, degradative ability can be increased by addition of surfactants which can partially improve the bioavailability of PAHs for the induction of WRF degradative system.

**Mechanism of biodegradation**

Bacteria/nocardioform actinomycetes degrade PAHs via either metabolism or co-metabolism. There is a great diversity of bacteria capable of exclusively degrading not only the LMW PAHs, such as naphthalene, acenaphthalene, phenanthrene, and anthracene but also the HMW PAHs, such as fluoranthene, pyrene, chrysene, benzo[a]anthracene and benzo[a]pyrene. Amongst them benzo[a]pyrene is considered as the most carcinogenic and toxic PAHs and is usually used as one of the model PAHs for investigation of toxicity and risk assessment (Cerniglia, 1984; Peng et al., 2008). Co-metabolism is very important for
degradation of HMW PAHs and a mixture of PAHs and HMW PAHs. In contrast, several 2, 3 and 4 ring PAHs have been known to be growth substrates for bacteria.

The bacterial aerobic degradation of PAHs is generally initiated by the action of multicomponent dioxygenases that can catalyze the incorporation of both atoms of oxygen and two electrons from NADH to form cis-dihydrodiol. These multicomponent dioxygenases usually consist of reductase, a ferredoxin and a third component consisting of two proteins, large and small iron sulfur protein (Labana et al., 2007). Subsequent dehydrogenation by dehydrogenase forms dihydroxylated intermediate, which can further be degraded through ortho or meta ring cleavage pathway which then eventually enters the TCA cycle (Fig. 1.4).

The other route of PAHs degradation is accomplished by the action of monooxygenases. Initial oxidation by monooxygenases in bacteria forms trans-dihydrodiols, this activity is slower than the dioxygenases. The cytochrome P-450 monooxygenase is a complex multi-enzyme protein of fungal origin that shares similarities to its bacterial counterparts. This enzyme is located in periplasmic space or is embedded in inner side of membrane. They can target an array of substrates as they can act upon various chemicals. One atom of molecular oxygen is incorporated into PAH by the monooxygenase to form an arene oxide (Haritash and Kaushik, 2009).
Fig. 1.4 Pathways involved in metabolism of PAHs by microorganisms.

Bioremediation of PAHs

There seems to be a general understanding that innate organisms need to be exploited for biodegradation of hazardous pollutants. Nutrient addition could aid heterotrophic population and enhance degradation regimes. This approach can be fused with already established strategies and robust technologies to eliminate the bottlenecks such as nutrient unavailability and insufficiency of microbial communities to degrade such pollutants.
A successful implementation of a remediation regime requires a consideration of the indigenous biota, nutrient availability as well as other environmental parameters necessary to achieve optimum results. A combination of technologies regulated with stringent conditions will prove to be tremendously important in reducing contaminants.

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state. Bioremediation involves the use of living organisms to degrade environmental contaminants into less toxic forms. It uses naturally occurring bacteria, fungi or plants to degrade or detoxify substances that are hazardous to human health and/or the environment (Vidali, 2001). Microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation the process is known as bioaugmentation.

Microbes are one of the most adapted organisms to various environmental extremes. Thus microbes can be isolated from almost all ecological niches. At present, microbes can be exploited for enhanced biodegradation due to their two prime virtual characterizations viz. versatility and adaptability. For effective
bioremediation strategy, microbes must act upon the pollutants and convert them to benign products such as CO$_2$ and H$_2$O. Nevertheless, various physical and chemical parameters of the habitats in which microbes thrive, can also affect the overall outcome of remediation.

Bioremediation strategies are categorized as *in-situ* and *ex-situ*. The selection of the strategy depends up on the nature and degree of saturation of specific contaminant or pollutant. Bioventing, *in-situ* biodegradation, biosparging, bioaugmentation are the sub-classes of *in-situ* bioremediation whereas landfarming, composting, biopiles, bioreactors are sub-classes of *ex-situ* bioremediation strategies (Vidali, 2001).

**Soil bioremediation**

Soil is a very unique part of the natural and agricultural aspect of the terrestrial ecosystem given its role in the growth of plants and the degradation and recycling of dead biomass. It is heterogeneous in nature and often comprises mineral and organic solids, aqueous and gaseous components. Soil bioremediation provides a way to study microbial interactions with contaminants in a controlled and reproducible way, while retaining the complexity of the matrix and being representative of the processes occurring in the field. Laboratory microcosms allow the measuring of biodegradation as well as mineralization (CO$_2$ production) rates (Arias et al., 2008) and can be used to study the effect of bioremediation treatments, including bioaugmentation (Reid et al., 2001; Cavalca et al., 2002).
**Effect of Nutrients**

PAHs degrading bacteria require nutrients as nitrogen (N), phosphate (P) and potassium (K) for the growth and degradation of PAHs. In contaminated sites, where organic carbon levels are often high due to the nature of the pollutant, available nutrients can become rapidly depleted during microbial metabolism. Under aerobic conditions, the supply of macronutrients, nitrogen and phosphorous, has been shown to have a beneficial effect on PAHs biodegradation. However, inorganic nitrogen and phosphorous additions have been shown to increase transformation rates of hydrocarbons, without apparent increase in microbial biomass (Breedveld and Sparrevik, 2000). Therefore, soil hydrocarbon degradation can be increased by the addition of supplemental nutrients, particularly nitrogen and phosphorus.

**Effect of Compost**

Composting strategies for soil bioremediation are diverse, including direct composting, compost addition, and bioaugmentation. Bacteria and fungi, the main pollutant degrading microbes in composts, have been widely considered to be the most crucial factors governing the remediation of contaminated soils. Remediation of contaminated soils by composting or compost addition mainly relies on two mechanisms: (i) adsorption by organic matter and (ii) degradation by microorganisms (Puglisi et al., 2007). The decomposition of organic pollutants in soil/compost mixture relies mostly on microbial activity. Organic amendments from
compost are an important source of nutrients, which provide more available carbon sources for indigenous microbes. In addition, organic amendments from compost also directly increase the density of microbes that are responsible for the decomposition and biotransformation of pollutants in soils (Chen et al., 2015).

**Effect of Surfactant**

Surfactant-enhanced remediation has been suggested as a promising technology for the remediation of contaminated soil. Many attempts have been made to remove PAHs from the environment; however, the success of these remediation processes, in many cases, is hindered by the existence of low number of PAHs degrading microorganisms and the low solubility and availability of hydrocarbons. Therefore, several bioremediation approaches have been designed that involves the addition of hydrocarbon degrading bacteria or the addition of chemicals such as surfactants to increase its bioavailability of PAHs (Tyagi et al., 2011; Chen et al., 2015).

Surfactants are amphiphilic in nature. They lower the interfacial tension at the oil-water interface and the surface tension of water and thus favor mass transport of hydrocarbons from the oil phase into the aqueous phase. Primarily, three mechanisms are responsible for enhancing the bioavailability of PAHs i.e. emulsification, pseudo-solubilization, and facilitated transport (Volkering et al., 1997; Paria, 2008). Emulsification results in the reduction of interphase tension between the aqueous phase and non-aqueous phase liquid (NAPL). As a result,
interphase gets expanded and promotes mass transfer of compound from NAPL to the aqueous phase.

**Fig.1.5** Uptake mechanism of substrate by micelle formation (Li and Chen, 2009).

Figure 1.5 shows the mass transfer of micelle into cell which comprises three steps. The first step is the transport of the micelles solubilized with a substrate to the vicinity of the cells or enzymes by mixing. The second step is the exchange of the filled micelles with the hemi-micellar layer of surfactant molecules formed around the cells. The formation of hemi-micelle layer around the cell or other substrates has been proposed and used successfully by many authors to describe biodegradation and dissolution of PAH (Guha and Jaffé, 1996; Guha et al., 1998). The third step is the transfer of the substrate from the hemi-micelle to the cell (Li and Chen, 2009).

Solubilization results in increased solubility of hydrophobic substrate due to their partitioning within the hydrophobic core of surfactant micelle. A micelle, unlike a liposome, is made up of monolayer with tails of the monomers oriented
towards the centre (Fig. 1.5). Hydrocarbons, present in NAPL (i.e. oil phase) are preferentially transported to the lipophilic core of micelle.

This facilitated transport favors mass transfer either by interaction of NAPL (i.e. oil) with single surfactant, by the interaction of surfactant aggregates or by interacting with oil-sorbed surfactant.

The effect of surfactant-like compounds produced by the microorganisms, when growing on aromatic hydrocarbons, solubilizes the PAH and leads to an increase in concentration in the medium. Surfactant compounds produced by *Pseudomonas aeruginosa* can increase the concentration of PAHs in the aqueous phase of the system. Increase in aqueous concentrations is generally in direct proportion to the amount of surfactant present. It could also, at times, cause inhibition of the degradation process (Cerniglia, 1997). Some of the studies have reported no effect of chemical surfactant on degradation (Avramova et al., 2008).

**Enzymes as tool for bioremediation**

The use of enzymes for environmental purpose has increased with time due to specific and peculiar properties of this class of proteins. Enzymes are versatile, efficient and specific catalysts acting behind all chemical reactions occurring in living organisms. They are capable of performing specific reactions at an elevated rate.

The enzymes play a major role in biological remediation, a process in which they are used to target, transform and remove specific pollutants. Their role may
range from natural processes such as intrinsic bioremediation, i.e. the transformation of pollutants to less toxic compounds in a contaminated environment (Gianfreda and Bollag, 2002), to manipulation of the contaminated environment by addition of specific chemical or biochemical additives or to bioaccumulation of all processes occurring in living cells and in enzymatic removal of organic pollutants (Whiteley and Lee, 2006; Demarche et al., 2012). The enzymes involved in biotransformation of PAHs by various microorganisms are listed in Table 1.3.

**Table 1.3** Enzymes involved in biotransformation of PAHs.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Substrate</th>
<th>Organisms</th>
<th>References</th>
</tr>
</thead>
</table>
| MnP     | • Anthracene  
          • Pyrene | *Bjerkandera* sp.  
          BOS55 | Eibes et al., 2006 |
| LiP     | • Anthracene  
          • Pyrene  
          • Benzo[a]pyrene | Fungi-Bacteria consortium | Hamdi et al., 2007 |
| Cytochrome P-450 | • Chrysene  
          • Fluoranthene  
          • Fluorene  
          • Pyrene | *Pseudomonas putida*,  
          *Novosphingobium* sp.  
          strain TYA-1 | Rao et al., 2014 |
| MnP     | • Benzo[a]anthracene  
          • Chrysene  
          • Benzo[b]fluoranthene | *Pleurotus ostreatus* | Baldrian et al., 2000 |
| LiP, MnP | • Anthracene  
          • Pyrene  
          • Perylene | *Phanerochaete chrysosporium* | Ding et al., 2008 |

LiP= Lignin peroxidase, MnP= Manganese peroxidase
**Microbial oxygenases**

Oxygenases belong to the oxidoreductases that utilize FAD/NADH or NADPH as co-substrate and catalyze oxidation by transferring molecule of $O_2$. Oxygenases are grouped into two categories; the monooxygenases and dioxygenases on the basis of number of oxygen atoms used for oxygenation (Fig. 1.6(A, B)). They play a key role in the metabolism of organic compounds by increasing their reactivity, water solubility and bringing about cleavage of the aromatic ring.

**Monooxygenases**

Monooxygenases are versatile biocatalysts cytochrome P-450 family of enzymes having a multicomponent system and are usually used by fungi. They are much like bacterial aromatic ring dioxygenases. Cytochrome P-450 enzymes are heme-proteins, ubiquitously distributed and capable of transforming a large range of substrates and catalyzing several chemical reactions, including the bioconversion of pollutants. These enzymes are involved in the degradation of hydrocarbons such as alkenes, PAHs, PCB and heterocyclic compounds (Karigar and Rao, 2011) (Fig. 1.6(A)). Under oxygen-rich conditions, monooxygenase catalyzes oxidative dehalogenation reactions, whereas under low oxygen levels, reductive dechlorination takes place. Oxidation of substrate can lead to dehalogenation as a result of the formation of products that undergo subsequent chemical breakdown.

Examples of reactions catalyzed by cytochrome P-450 are carbon hydroxylation, epoxidation, heteroatom oxygenation, dealkylation, aromatic
hydroxylation, reduction, and dehalogenation. This broad catalytic activity renders this enzymatic family very promising for biotechnological and environmental applications. However, there has been a limited use of these catalysts for biotechnological/environmental applications because of their high complexity and often low catalytic activity.

![Degradation of PAHs](image)

**Fig. 1.6** Degradation of PAHs by (A) monoxygenase and (B) dioxygenase enzyme systems (Karigar and Rao, 2011)

**Dioxygenases**

Figure 1.6(A, B) shows the major route for the degradation of PAHs by mono- and dioxygenase enzyme systems. Among these, degradation of PAHs by dioxygenase-dehydrogenase enzyme system is commonly used by bacteria.
Biodegradation of PAHs is catalyzed by multicomponent enzymes from microbes. A key enzyme that attacks the aromatic ring structure of PAHs under aerobic condition is the initial dioxygenase which is substrate specific (Cerniglia, 1992; Kauppi et al., 1998; Juhasz and Naidu, 2000). This initial hydroxylation step of unsubstituted PAHs is catalyzed by a dioxygenase. Since PAHs, such as phenanthrene, pyrene, benzo[a]pyrene and benzo[a]anthracene, are complex fused ring structures, bacteria metabolize PAHs at multiple sites to form isomeric cis-dihydrodiols. Monooxygenases have also been shown to be involved in oxidation to form trans-dihydrodiols. The cis-dihydrodiols undergo rearomatization by dehydrogenases to form dihydroxylated intermediates. Further, catabolism involves ring cleavage by dioxygenases to form aliphatic intermediates. Cleavage of these ortho-dihydroxylated intermediates occurs either between the two hydroxyl groups (intradiol or ortho-fission) or adjacent to one of the hydroxyl groups (extradiol or meta-fission). There are different enzymes for different ring fission substrates, each forming a different aliphatic product.

**Extracellular enzyme system**

The extracellular enzymes produced by bacteria, fungi and plants are also believed to underlie the ability of degrading PAHs and other organic pollutants (Novotný et al., 2004). These enzymes are responsible for the initial oxidation of PAHs. Some of them can oxidize certain PAHs directly, whereas others co-oxidize them indirectly during enzyme mediated lipid peroxidation. Moreover, the catalytic
action of some enzymes also has the possibility to enhance the solubility of pollutants, which results in the improvement of bioavailability.

**Lignin peroxidases (LiP)**

LiPs are heme-proteins of the secondary metabolism of fungi such as WRF. They use \( \text{H}_2\text{O}_2 \) as co-substrate and low molecular weight mediators such as veratryl alcohol (VA). They can degrade amorphous refractile molecules such as lignin and other phenolic compounds non-specifically. During the reaction, \( \text{H}_2\text{O}_2 \) is reduced to \( \text{H}_2\text{O} \) and in turn LiP gets oxidized. The LiP (oxidized) with gaining an electron from VA returns to its native reduced state, forming veratraldehyde (Fig. 1.7).

![Catalytic mechanism of LiP (Tien and Kirk, 1983)](image)

**Fig.1.7** The catalytic mechanism of LiP (Tien and Kirk, 1983).

In addition, LiP can oxidize a wide range of environmentally persistent pollutants having high ionization potential (IP) values of over 9.0 eV (Ward et al.,
2003a; Huang et al., 2003), due to the fact that LiP possesses a higher redox potential than other peroxidases and oxidases. LiP thus can act as an attractive degrader which acts non-specifically with low substrate specificity, making them useful in waste water treatments or catalyzing different chemical transformations, such as phenol, chloro- and bromo-phenols (Ward et al., 2003b) as well as PAHs. In order to achieve a high and sustainable activity, some mediators should be added into the reaction systems, such as H$_2$O$_2$, VA, tween 80, etc (Ding et al., 2008; Pizzul et al., 2009).

**Manganese peroxidases (MnP)**

MnP are non-specific heme proteins which catalyze oxidation of Mn$^{2+}$ to Mn$^{3+}$ and vice-versa, in series of reactions. Mn$^{2+}$ induces the production of MnP (Fig. 1.8). It is produced by very few species belonging to ascomycetous and basidiomycetous fungi (Hofrichter, 1998; Lopez et al., 2007). There are no reports of MnP production by bacteria or yeast (Hatakka et al., 2001). The catalytic process of MnPs is initiated by binding H$_2$O$_2$ or an organic peroxide to the native ferric enzyme and forming an iron-peroxide complex. Different from other peroxidases, MnPs oxidize Mn$^{2+}$, which is used as a preferred electron donor, into Mn$^{3+}$, and the latter one subsequently mediates the oxidation of a variety of amorphous molecules (i.e. lignin) or other phenolic as well as nonphenolic compounds, including PAHs (Hofrichter, 2002). The chelates of Mn$^{3+}$ with carboxylic acids can cause one-electron oxidations on the substrates by hydrogen abstraction to form different
radicals (Hatakka, 2001). The formative radicals are considered to be a source of peroxides which are generated through the autocatalytic reactions and can be used by MnP without the presence of external H$_2$O$_2$.

**Fig. 1.8** The catalytic mechanisms of MnPs by fungi (Hofrichter, 2002).

**Laccases**

Laccases ($p$-diphenol:dioxygen oxidoreductase) constitute a family of multicopper oxidases produced by certain bacteria, fungi, and plants, that catalyze the oxidation of a wide range organic compounds. The broad substrate specificity and non-specific catabolism to reduce an array of compounds is the unique property of these enzymes which can be exploited for biodegradation of organic compounds.

The copper centres of laccase drive electrons from a reducing substrate to molecular oxygen without releasing toxic peroxide intermediates. Laccase catalysis
is believed to comprise three major steps (Gianfreda et al., 1999) as depicted in Fig. 1.9. (i) Reduction of the mononuclear copper center: The reducing substrate (usually phenolic compounds) loses an electron to laccase (Gianfreda et al., 1999). This electron reduces the T1 copper (at the mononuclear copper center), which is positioned just below the substrate-binding site. The oxidized substrate now becomes a radical, which can either donate the second electron to the T1 copper and become a quinone or directly take part in any non-enzymatic reactions leading to either polymerization or depolymerization. The reduced T1 copper oxidizes itself by transferring the electron to the trinuclear copper cluster, (ii) internal electron transfer from the mononuclear copper to the trinuclear copper center: O₂ molecule first binds to the T2 and any one of the T3 copper atoms. This then undergoes asymmetric activation leading to the formation of four O-H bonds during the generation of two molecules of water. The oxygen-binding pocket appears to restrict the access of oxidizing agents besides molecular oxygen, which may account for the exclusivity of laccase for the oxidizing substrate, which is molecular oxygen as opposed to its low affinity for the reducing substrate (iii) reduction of molecular oxygen at the trinuclear copper centre: They can either lead to polymerization by the cross linking of monomers or depolymerization of the already existing polymers (Gianfreda et al., 1999; Claus, 2004).

Moreover, many microorganisms produce laccase isozyme which actively participates in radical depolymerization of lignin, resulting in a variety of phenolic
compounds (Gianfreda et al., 1999; Mai et al., 2000). In addition, these compounds are utilized as nutrients by microorganisms or are repolymerized to humic materials by laccase.

![Laccase catalytic cycle](image)

**Fig.1.9** Laccase catalytic cycle (D’souza-Ticlo, 2008).

**Versatile peroxidases (VPs)**

VPs are able to oxidize Mn$^{2+}$ directly and can oxidize aromatic substrates similar to that of MnP, LiP and horseradish peroxidase. It has a Mn-binding site and an open tryptophan residue, similar to veratryl alcohol oxidation by LiP. It is postulated that catalytic nature of few peroxidase is due to a hybrid molecular architecture combining different substrate binding and oxidation sites (Camarero et al., 2000; Wu, 2010).

**Degradation of fluoranthene a HMW PAHs**

The biodegradation of PAHs containing more than three aromatic rings is not well understood as is the utilization of di- and tri-cyclic aromatic hydrocarbons. This
is due to large size, high molecular weight and extreme insolubility of HMW PAHs as pyrene, fluoranthene, chrysene, and benzo[a]pyrene (Kelley et al., 1993). The study of enzymatic oxidation of PAHs, their degradative mechanisms, identification of intermediates and the elucidation of metabolic pathway has gained an emphasis in research as meager information is available about the biochemistry of pathways involved in degradation and its genetic control.

Microbes play a key role in degradation of aromatics irrespective of being in the terrestrial or aquatic ecosystem. It requires thorough knowledge and understanding to implement microbes for bioremediation of such molecules. However, successful strategies are still being hindered by the failure to remove HMW PAHs such as fluoranthene from the environment (Wilson and Jones, 1993).

**Bacteria**

Fluoranthene and pyrene degradation has been observed in *Mycobacterium* sp. strain PYR-1. *Mycobacterium* is a well-known genus capable of mineralizing high molecular weight PAHs including fluoranthene, pyrene, and benzo[a]pyrene.

Fluoranthene degradation is generally initiated by 1,2- or 7,8-dioxygenation to form cis-1,2- or cis-7,8-fluoranthene dihydrodiol, respectively. These two dihydrodiols are dehydrogenated to 1, 2- or 7,8-dihydroxyfluoranthene, respectively. 7,8-dihydroxy-fluoranthene is further transformed via meta-cleavage to 1-acenaphthenone and 3 hydroxymethyl-3H-benzo[de]-chromen-2-one through 2-hydroxyl-4-(2-oxo-2H-acenaphthylen-1-ylidene)-but-2-enoic acid, 2-
hydroxymethyl-2H-acenaphthylen-1-one, and 2-oxo-acenaphthene-1-carboxylic acid. Lee et al., (2007) suggested that there are four possible initial dioxygenations at 1,2-, 2,3-, 7,8-, and 8,9-positions of fluoranthene and four possible dimethoxy fluoranthene in *Mycobacterium* sp. JS14.

Rehmann et al., 2001 reported 2,3-dioxygenation of fluoranthene and found five metabolites, namely *cis*-2,3-fluoranthene dihydrodiol, 9-carboxymethylene-9H-fluorene-1-carboxylic acid, *cis*-1, 9a-dihydroxy-1-hydrofluorene-9-one, 8-carboxylic acid, 4-hydroxybenzochromene-6-one-7-carboxylic acid, and benzene 1,2,3-*cis*-2,3-fluoranthene dihydrodiol is catabolised by ortho-cleavage to benzene-1,2,3-tricarboxylic acid via 9-carboxymethylene-9H-fluorene-1-carboxylic acid, *cis*-1, 9a-dihydroxy-1-hydrofluorene-9-one-8-carboxylic acid, and 4-hydroxybenzochromene-6-one-7-carboxylic acid.

Bacterial catabolic pathways of fluoranthene and the corresponding metabolites are as proposed in Fig. 1.10. The biochemical pathways for the biodegradation of aromatic compounds have been well described by Kanaly and Harayama, (2000). It is understood that the initial step in the aerobic catabolism of a PAH molecule by bacteria occurs via oxidation of PAH to a dihydrodiol by a multicomponent enzyme system. These dihydroxylated intermediates may then be processed through either an ortho cleavage type of pathway or a meta cleavage type of pathway, leading to central intermediates such as protocatechuates and catechols, which are further converted to tricarboxylic acid cycle intermediates.
Metabolite designations: 1) fluoranthene; 2) cis-7,8-fluoranthene dihydrodiol; 3) 7,8-dihydroxy fluoranthene; 4) 7-methoxy-8-hydroxy-fluoranthene; 5) 2-Hydroxy-4-(2-oxo-2H-acenaphthylene-1-ylidene)-but-2-enoic acid; 6) 2-oxo-acenaphthene-1-carboxylic acid; 7) 2-methoxy-2H-acenaphthylene-1-one; 8) 1-acenaphthenone; 9) 3-methoxy-3Hbenzo[de]chromen-2-one; 10) 2 methoxy-acenaphthylene-1-ol; 11) cis-1,2-fluoranthene dihydrodiol; 12) 1,2-dihydroxy-fluoranthene; 13) 9-fluorenone-1-(carboxy-2-hydroxy-1-propanol); 14) 9-fluorenol-1-carboxypropyl-2-one; 15) 9-fluorenone-1-carboxylic acid; 16) 9-fluorenol-1-carboxylic acid; 17) 9-fluorenone; 18) 9-fluorenol; 19) cis-2,3-fluoranthene dihydrodiol; 20) 9-carboxy methylene-9H-fluorene-1-carboxylic acid; 21) cis-1,9a-dihydroxy-1-hydrofluorene-9-one-8-carboxylic acid; 22) 4-hydroxybenzochromene-6-one-7-carboxylic acid; 23) benzene-1,2,3-tricarboxylic acid.

Fig. 1.10 Proposed catabolic pathways of fluoranthene degradation by bacteria.
**Exploration of sites for the study**

Gujarat with the longest coastline of 1653 km constituting 21% of the total coastline of the country has largely remained unexplored in terms of bioavailability of organic pollutants and its remediation for the restoration of contaminated marine ecosystem. Gujarat coast, especially Bhavnagar due to extensive ship breaking and recycling activities is highly polluted. Pollutants such as PAHs, heavy metal, chlorinated compounds etc. are extensively released due to ship breaking and recycling activities at the coast. These pollutants are finally exposed to marine ecosystem which gets adsorbed and deposited in sediments as they have strong affinity towards particulates, making sediments the ultimate sinks.

Amongst all pollutants, PAHs have been successful enough to gain priority of scientists for its removal from the exposed ecosystem. Scientists are motivated to put efforts for remediation of PAHs impacted environments, as they have toxic, mutagenic and carcinogenic properties which lead to serious health consequences. However, the coastal areas are contaminated with mixture of organic pollutants rather than individual compounds. Therefore, this study has focused on the development of bioremediation strategies for restoration of heavily contaminated sites at Bhavnagar coast.

The Alang-Sosiya ship breaking and recycling yard (ASSBRY) and Kumbharwada at Bhavnagar coast, Gujarat (Fig. 1.11) has gained profound interest due to historical pollution of PAHs and deposition of heavy metals in sediments at
the coastal regions by ship breaking and recycling activities (Reddy et al., 2005; Basha et al., 2007). The possible sources of PAHs in the coastal vicinity of ASSBRY and Kumbharwada have not yet been surveyed.

**Fig. 1.11** Map of sampling sites at Bhavnagar coast.

Hence, this study has been initiated with the following objectives:

**Objectives of the study**

- Assessment of PAH prevailing at contaminated sites.
- Isolation of multiple hydrocarbon degrading actinomycetes from polluted sites and characterization of the potential isolate.
- Optimization of cultural conditions for maximum PAHs degradation using statistical analytic tools.
- Detection, purification and characterization of one of the enzymes involved in biodegradation of PAHs.
- Microcosm studies for enhanced PAHs degradation for remediation of contaminated sediments.

**Futuristic Road Map**

The present study has been focused on biodegradation of PAHs by marine actinomycetes, one of the potential mechanisms to eliminate the contaminating hydrocarbons and its derivative compounds present in the environment. The present study would thus lead to assess the potential of bioremediation in heavily contaminated marine environment. Study of soil microcosm experiments can give insight information about the degradation behaviour of PAHs by actinomycetes-Gram positive bacteria in polluted soils. This would truly lay the foundation for future *in situ* experiments for restoration of our coastline for its sustainable development.