CHAPTER I

GENERAL INTRODUCTION
I.1. INTRODUCTION

Rapid industrialization and modernization of agriculture has led to the large scale manufacture and use of a variety of synthetic organic chemicals. A large number of synthetic chemicals in the form of plasticizers, pesticides, herbicides, dyes, detergents, drugs, petroleum products and industrial chemicals such as lubricants, coolants, insulators, hydraulic fluids, solvents are deliberately or accidentally released into the environment (Hutzinger and Veerkamp, 1981). Almost all the major industries emit some or other undesirable chemicals into the air, water and soil. Thus, their concentrations are increasing alarmingly in the ecosystem. Unfortunately many of these chemicals and biotransformation products are reported to be potentially toxic, mutagenic or carcinogenic to man and animals. If such chemicals are not degraded, they could accumulate in the soil and cause serious environmental pollution and ecological changes.

Since most of these toxic chemicals were absent in the biosphere prior to their synthesis by man, they may be considered as environmentally foreign chemicals. The biodegradation of environmental chemicals is influenced by a wide variety of microbial community of the biosphere. Indigenous microbial populations present in the soil and water possesses versatile mechanism to degrade vast array of synthetic organic compounds into intermediates that can enter the major metabolic pathways. Mineralization of organic chemicals is very essential to maintain the carbon cycle occurring in nature. Microorganisms being important agents for destroying synthetic chemicals in nature, much attention has
been given to the studies on microbial degradation of environmental chemicals and its possible applications in decontamination of polluted effluents and sites. Microbial metabolism of various classes of aromatic compounds and pesticides has been studied extensively and several excellent reviews in this area are available (Lee et al., 1987; Sakata et al., 1992; Gunther et al., 1995; Hwang and Maloney, 1996; Londry et al., 1999; Zablotowicz et al., 1999).

1.2. PHENOLIC COMPOUNDS AS ENVIRONMENTAL POLLUTANTS

Ground water contaminated with creosote or oil contains a mixture of phenols and aromatic hydrocarbons, because of the relatively high water solubility of these compounds (Rosenfeld and Plumb, 1991; Turney and Goerlitz, 1990). Phenolic pollution is characteristic of manufacturing (pesticides, pharmaceuticals, plastics) and raw materials (crude oil, coal) conversion processes. Phenol itself is among the compounds most frequently found in rivers, industrial effluent out falls and landfill run off waters.

Phenol is widely distributed in the biosphere, both from its extensive use as an industrial chemical and as a natural product. Sheep, for example, may excrete about 60 mg of phenol each day (Martin, 1982) produced by bacteria in the anoxic environment of the rumen from plant aromatic compounds in their diet. Phenol, cresols, chlorophenols and nitrophenols are included in the US EPA priority pollutant lists (Buckman et al., 1984). Chlorophenols are used as fungicides in cooling water, paints and construction materials, as pesticide components, as
wood preservatives, and as transformer fluids. Chlorophenols are regarded as carcinogenic substances. Large amount of chlorinated aromatic compounds are produced during the bleaching with chlorine in the paper and pulp industry (Salkinoja-Salonen et al., 1981). Generally chlorinated aromatic compounds have to be regarded as toxic to most organisms. Due to their lipophilic nature these compounds are increasingly accumulated within a food chain.

Nitrophenols are common industrial components used in the synthesis of pesticides, dyes, explosives, pharmaceuticals and other chemicals and consequently have been observed as contaminants in soil, water and atmosphere (Keith and Teillard, 1979). Nitrophenols can be build up in the soil as a result of hydrolysis of several organophosphorous insecticides such as parathion (Hanne et al., 1993).

Cresols have often been found as contaminants of ground water. One of their major sources is creosote, which is widely used in wood treatment (Ehrlich et al., 1983; Goerlitz et al., 1985). Various cresols have also been found in leachate from landfills (Solwhney and Kozloski, 1984) and as ground water contaminants from underground coal gasification activities (Stuermer et al., 1982). They are fairly soluble in water and thus migrate with the ground water, causing extensive contamination of the aquifer. Cresols are components of the total phenolic fraction of toxic matter in wastewater from hydrocarbon refining and they are of ecological concern because of their toxicity and mobility in subsurface environments (Londry et al., 1999). Commercial cresols are widely
used as solvents, disinfectants and as chemical intermediates for pharmaceuticals, fragrances, antioxidants, dyes, pesticides and resins. Cresols are also used in the production of lubricating oils, motor fuels, and rubber polymers and in the manufacture of explosives. In laboratory animals, toxic effects including damage to the respiratory and gastrointestinal tracts are associated with the strong irritant and corrosive activity of cresols. Although poisoning via inhalation is judged unlikely due to the low vapour pressure of cresols, dermal exposure causes irreversible tissue damage in experimental animals and can be fatal at high concentrations.

In humans, toxic effects and clinical signs following accidental or intentional ingestion are identified by burning of the mouth and thoat, abdominal pain and vomiting. Studies of acute poisoning in workers indicate that occupational exposure is usually the result of dermal contact, which can result in severe burns and scarring of the skin, hematological changes, kidney failure, coma and death.

p-Cresol is used in disinfectants and fumigants, in the manufacture of synthetic resins, in photographic developers and explosives. Because of its wide spread uses, p-cresol frequently occurs in a variety of aquatic environments and listed as a priority pollutant by the US. EPA (Buckman et al., 1984). Among the different isomers, p-cresol appears to be the most toxic form (Patnaik, 1992). p-Cresol is highly toxic, corrosive, causes nervous system depression and it is naturally occurring metabolic product being formed from tyrosine by bacteria
under anaerobic conditions. (D' Ari and Barker, 1985). Such conditions exist in the rumen or in the digestive tract of non-ruminants and substantial amounts of p-cresol are excreted by animals (Martin, 1982).

Phenolic compounds may be present at concentrations as high as several grams per liter in coking plant effluents, with phenol accounting for 70% and p-cresol representing 25% of the total phenols (Ganczarczyk, 1979).

I.3. PESTICIDES AS ENVIRONMENTAL POLLUTANTS

In the current era where pesticides play a vital role in ones everyday life, large quantities of various pesticides some of which are highly toxic are being used extensively for enhancing agricultural productivity by protecting crops, prevention and spoilage, eradication of pathogens and control of disease vectors. A recent study of the world health organization reveals that on an average, one person is poisoned every minute by pesticides in the developing world (Rao, 1984).

Certain environmental chemicals including pesticides termed as endocrine disruptors are known to elicit their adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure are increasingly linked to human health effects such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Crisp et al., 1998; Hurley et al., 1998; Brouwer et al., 1999).
An increasingly wider spectrum of chemicals and their combinations as formulations are being used as insecticides, fungicides, herbicides, nematocides, rodentocides etc. The major kinds of pesticides that are being currently employed include organochlorine, organophosphorous, carbamates, synthetic pyrethroids, thiocarbamates, nitrochlorophenol derivatives, metal salts and organometallic compounds. The structure and uses of some common pesticides are given in Table. 1.3 a. As a darker side of these benefits, the risks to non-targets, ecosystem productivity, biological diversity and human health itself are threatened by the short and long term effects of these pesticides. In addition to risks to beneficial species like earthworms, honeybees, fish and birds, the food chain contain high amounts of pesticide residue as evident from several surveys and criterian documents (Jaffery et al., 1992). Therefore, in any effort towards environmental impact assessment of pollution, the adverse effects of pesticides are important.

The term “pesticides” comprises of hundreds of chemicals with different structures, only general aspects are considered, the toxicology of pesticides per se is not included, since several monograph and reviews are already available. The basis of this write up is the experience gained from the books, disaster preparedness in chemical industry (Vishwanathan et al., 1986); Compilation of pesticide toxicology data hand books (Jaffery et al., 1989a,b); Toxicology atlas of India (Jaffery et al., 1990) and pesticide profiles (Kamrin, 1997). In the recent past, there has been an alarming increase in the cases of pesticides poisoning of human in the country’s agricultural belt. According to the WHO (World Health Organization) some one in the developing world is poisoned by pesticides every minute. Acute poisoning by pesticides including some fatal cases, occur relatively
<table>
<thead>
<tr>
<th>Classes</th>
<th>Examples</th>
<th>Chemical Name</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organochlorine Pesticides</td>
<td>Heptachlor, DDT, Lindane</td>
<td>1,1,1-Trichloro-2,2-bis(p-chlorophenyl)</td>
<td>Insecticide applied to malaria eradication by control of house and flea vectors of human and animal diseases. Also used for thousands of species of insect pests. Soil poison, seed treatment, toxicant for the grasshopper, borer control, insect killing shelf papers. Cockroaches, ants, termites, soil insects, grasshopper control.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gamma-1,2,3,4,5,6-hexachlorocyclohexane</td>
<td>Soil poison, seed treatment, toxicant for the grasshopper, borer control, insect killing shelf papers. Cockroaches, ants, termites, soil insects, grasshopper control.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,4,5,6,7,8-Hexachloro-3a,4,7,7a-tetrahydron-7-methanoindene</td>
<td>Soil poison, seed treatment, toxicant for the grasshopper, borer control, insect killing shelf papers. Cockroaches, ants, termites, soil insects, grasshopper control.</td>
</tr>
</tbody>
</table>

### Table 1.3a: Pesticides, their common and chemical names, structures and uses

<table>
<thead>
<tr>
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</tr>
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</tr>
<tr>
<td></td>
<td></td>
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<td>Soil poison, seed treatment, toxicant for the grasshopper, borer control, insect killing shelf papers. Cockroaches, ants, termites, soil insects, grasshopper control.</td>
</tr>
<tr>
<td>II) Organophosphorous pesticides</td>
<td>Parathion</td>
<td>o,o-Diethyl o-p-nitrophenyl phosphorothionate</td>
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<td>-------------------------------</td>
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<tr>
<td></td>
<td>Methyl parathion</td>
<td>o,o-Dimethyl o-p-nitrophenyl phosphorothionate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malathion</td>
<td>Diethyl(dimethoxythiophosphorylthio)succinate</td>
<td></td>
</tr>
</tbody>
</table>

Most widely used of this class, garlic odour high toxicity to warm blooded animals, mosquito control broad spectrum insecticides, originally used on potato beetle.

Most widely used of this class, garlic odour high toxicity to warm blooded animals, control of plant pests broad spectrum insecticides, originally used on potato beetle.

Household, home garden, vegetable and fruit insect control, control of mosquitoes, flies and lice.

**Formulae:**
- Parathion: \( \text{C}_2\text{H}_5\text{O} \) \( \text{S} \) \( \text{P} - \text{O} - \text{C}_6\text{H}_4\text{NO}_2 \) \( \text{C}_2\text{H}_5\text{O} \)
- Methyl parathion: \( \text{C}_2\text{H}_5\text{O} \) \( \text{S} \) \( \text{P} - \text{O} - \text{C}_6\text{H}_4\text{NO}_2 \) \( \text{C}_2\text{H}_5\text{O} \)
- Malathion: \( \text{C}_2\text{H}_5\text{O} \) \( \text{S} \) \( \text{P} - \text{S} - \text{CH}_2\text{COC}_2\text{H}_5 \) \( \text{C}_2\text{H}_5\text{O} \) \( \text{P} - \text{S} - \text{CH}_2\text{COC}_2\text{H}_5 \) \( \text{C}_2\text{H}_5\text{O} \)
General purpose insecticides can be useful for 100 or more crops, especially cotton, forage, fruit, vegetable, lawn and garden insecticides, low toxicity to mammals.

| Systemic insecticide, acaricide and nematicide. |
| Systemic action, seed and oil treatment. |

### Carbamates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Naphthyl N-methylcarbamate</td>
<td><img src="1-naphthyl-n-methylcarbamate.png" alt="Structure" /></td>
<td>Carbamyl</td>
</tr>
<tr>
<td>2,3-Dihydro-2,2-dimethyl-7-benzofuran-7-yl N-methylcarbamate</td>
<td><img src="2,3-dihydro-2,2-dimethyl-7-benzofuran-7-yl-n-methylcarbamate.png" alt="Structure" /></td>
<td>Carbofuran</td>
</tr>
<tr>
<td>2-Methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime</td>
<td><img src="2-methyl-2-(methylthio)propionaldehyde-o-methylcarbamoyloxime.png" alt="Structure" /></td>
<td>Aldicarb</td>
</tr>
</tbody>
</table>

### III) Carbamates

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>Carbofuran</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>Aldicarb</td>
</tr>
<tr>
<td>IV) Pyrethroid Pesticides</td>
<td>Propoxur</td>
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<tr>
<td></td>
<td>Cypermethrin</td>
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<tr>
<td></td>
<td>Fenvalerate</td>
</tr>
<tr>
<td></td>
<td>Permethrin</td>
</tr>
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<td>----------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td><img src="image" alt="Permethrin Structure" /></td>
</tr>
<tr>
<td><strong>Chemical Name</strong></td>
<td>3-Phenoxybenzyl-(1RS,cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Permethrin is a broad spectrum synthetic pyrethroid insecticide used against a variety of pests on nut, fruit, vegetable, cotton. It is used to control termite</td>
</tr>
</tbody>
</table>
often and are considered as endemic among worker who handle and spray agricultural pesticides in developing countries. Therefore the pesticides have been described as “Economic Poisons”.

The most widely used pesticides are the insecticides. Insecticides are substances, which kill insects by their chemical action. Based on their chemical nature, they are classified into three general groups (Laurent, 1973). I) The organic compounds like florides, mercurials etc. II) Natural organic compounds like nicotinoids, pyrethrins and rotenoids. III) Synthetic organic compounds like organochlorides, organophosphates, carbamates and synthetic pyrethroids.

SYNTHETIC PYRETHROIDS

Since the discovery of insecticidal property of DDT in 1943, many organochlorine, organophosphate and carbamate insecticides were developed without any great increase in the level of insecticidal activity. Subsequent search for new effective insecticides led to the discovery of synthetic pyrethroids viz cypermethrin, fenvalerate, deltamethrin etc. Earlier natural pyrethrins were used in pest control but they were too expensive and unstable, as a result they could not be used in agricultural pest control. The newly developed synthetic pyrethroids offer a class of insecticides with a wide range of ideal properties viz; quick action, high insecticidal efficiency, good persistence and amenability of synergetic action. Plant derived pesticides fall into several different broad classifications. By far largest group of such pesticides consists of pyrethrum and its related synthetic compounds pyrethroids. An American merchant, Jumtikoff in his travel, reported a powder used in insect control by the tribes of caucasus. This powder was prepared from the flower heads of chrysanthemum. There are currently over 20
pyrethroids and they constitute the single largest group of natural insects chemical control agents in the world (Ray, 1991). The general structure of the pyrethroids is given in Fig (I.3a).

![Pyrethroid Structure](image)

**Fig. I.3a. Generic pyrethroid structure.**

Pyrethroids are about equally toxic whether applied topically or injected. Substituting a cyano group at the α-carbon of 3-phenoxybenzyl ester increases the toxicity of the compound. There are many pyrethroid compounds that have been used as insecticides. Some major pyrethroids are listed in the Table. I.3b.

All pyrethroids are lipophilic compounds almost insoluble in water. Physical properties determine their rapid action in insects and also minimize translaminar action in leaves and systematic movements in plants. Therefore, pyrethroids are effective as contact insecticides and to a lesser extent as stomach poisons. Their efficiency in practice is enhanced by rain fastness, again associated with their lipophilicity, which favors retention by leaf cuticles. Pyrethroids are strongly absorbed in most soils. Therefore, ground will not receive pyrethroids except direct application or erosion of soil (Elliott et al., 1978). Synthetic pyrethroids *viz.* permethrin, cypermethrin, deltamethrin and fenvalerate are significantly more stable.
Table 1.3 b. List of Pyrethroid pesticides.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chemical Name</th>
<th>Structure</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allethrin</td>
<td>(RS)-3-alkyl-2-methyl-4-oxocyclopent-2-enyl (1RS, 3RS; 1RS, 3RS)-2,2-dimethyl-3-(2-methyl-prop-1-enyl)cyclopropane carboxylate</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Allethrin is a non-systemic insecticide that is used almost exclusively in homes and gardens for control of flies and mosquitoes. It is available as mosquito coils, mats, oil formulations and as an aerosol spray.</td>
</tr>
<tr>
<td>Bioallethrin</td>
<td>(RS)-3-allyl-2-methyl-4-oxocyclopent-2-enyl (1R, 3R)-2,2-dimethyl-3-(2-methyl-prop-1-enyl) cyclopropane carboxylate</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Bioallethrin is a potent contact, non-systemic, non-residual insecticide which produces a rapid ‘knockdown’ and is used against insects and in insecticidal colis and electric thermal vapourisers.</td>
</tr>
<tr>
<td>Insecticide</td>
<td>Chemical Structure</td>
<td>Description</td>
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<td>-------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Cyflurthrin</td>
<td>(RS)-α-cyano-4-fluoro-3-Phenoxybenzyl (1 RS, 3RS; 1RS, 3RS)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclo-propane carboxylate</td>
<td>Non-systemic insecticide. Also used in public health, storal products, domestic use and animal health.</td>
<td></td>
</tr>
<tr>
<td>Cyhalothrin</td>
<td>(RS)-α-cyano-3-phenoxybenzyl (z)-(1RS, 3RS)- (2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethyl cyclo-propane carboxylate</td>
<td>Control of animal ectoparasites. Applied as an animal dip or as a spray around animal houses.</td>
<td></td>
</tr>
<tr>
<td>Tetramethrin</td>
<td>Cyclohex-1-ene-1,2-dicarboximidomethyl (1RS, 3RS; 1RS, 3RS) - 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclo propane carboxylate</td>
<td>Normally used in combination with synergists and other insecticides for control of flies.</td>
<td></td>
</tr>
<tr>
<td>Bioresmethrin</td>
<td>5-benzyl-3-furyl methyl-(1R, 3R)-2,2-dimethyl-3-(2-methyl prop-1-enyl)-cyclopropane carboxylate</td>
<td></td>
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</tbody>
</table>

Bioresmethrin is a potent contact insecticide, effective against a wide range of household and public health insects.

<table>
<thead>
<tr>
<th>Resmethrin</th>
<th>5-benzyl-3-furyl methyl-(1RS, 3RS; 1RS, 3RS)-2,2-dimethyl-3-(2-methyl prop-1-enyl)-cyclopropane carboxylate</th>
</tr>
</thead>
</table>

It is used for fabric protection and shampoos and it is applied to horses or in horse stables. It is also used to control of flying and crawling insects.
On a wide range of surfaces even persist longer than many organophosphates and carbamates (Elliott et al., 1978). Hence, pyrethroids will accumulate to contaminate the environment and having high degree of ovicidal action will also help in knocking down the pest species at the base level (Shersiya, 1982).

The precise mode of action of synthetic pyrethroids including bioresmethrin, permethrin, cypermethrin, deltamethrin and fenvalerate probably involves an interaction with the sodium channel in the nerve membranes (Vijverberg et al., 1982). Their principal effect is to induce a continuous series of nerve impulses, referred to as repetitive activity, which completely upsets the proper functioning of the entire nervous system and eventually results in death (Wouters and VandenBercken, 1978; Vijverberg et al., 1982). Based on symptomology of pyrethroid toxicity, the poisoning is broadly classified into two types. Type I poisoning syndrome produced by the pyrethroids without cyano substituent is characterized by restlessness, incoordination, frustration and paralysis in the cockroach (Gammon et al., 1981) and whole body tremors in rat. The type II (with cyano substituent) poisoning syndrome symptoms include convulsions, intensive hyperactivity, burrowing behavior, coarse tremors, clonic seizures and salivation in rats (Gammon et al., 1982; Eldefrawi et al., 1985). However, there is evidence suggesting that type II pyrethroids can also interact with GABA receptors, inhibition of both (3,5)-t- butylbicyclopophosphorothionate (TBPS) binding at the GABA-A-receptor-lonophore complex (Lawrence and
Casida, 1983) and GABA- included chloride fluxes (Abalis et al., 1986) has been reported in studies on CNS preparation. In mammals, the symptoms of pyrethroid poisoning result from interaction of these insecticides with CNS (Ray and Cremer, 1979; Staatz et al., 1982). A single dose produces toxic signs in mammals, such as tremors, hyperexcitability, salivation, choreoathetosis and paralysis. At near-lethal dose levels, synthetic pyrethroids cause transient changes in the nervous system, such as axonal swelling and / or breaks and myelin degeneration in sciatic nerves.

**CYPERMETHRIN AND FENVALERATE**

Cypermethrin [(+/-)-α-cyano-3-phenoxybenzyl (RS)-cis,trans-3 (2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] and fenvalerate [α-cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3-methyl butyrate] are the active ingrediants of the widely applicable pesticides. Products containing cypermethrin are classified as restricted use pesticides (RUPs) by the EPA because of the cypermethrin toxicity to fish. RUPs may be purchased and used only by certified applicators. Cypermethrin is classified toxicity class II (Moderately toxic).

Cypermethrin and fenvalerate are synthetic pyrethroid pesticides used to control many pests, including moth pests of cotton, fruit and vegetable crops. They are also used for crack, crevice and spot treatment to control insect pests in stores, warehouses and green houses. They may also be used in non-food areas in schools, nursing homes and as barrier treatment insect repellent for horses. Cypermethrin and fenvalerate were moderately toxic material by dermal
absorption or ingestion (Ray, 1991; U.S. Environmental Protection Agency). Symptom of high dermal exposure includes numbness, tingling, itching, burning sensation, loss of bladder control, incoordination, seizures (Ray, 1991). They adversely affect the central nervous system and cause allergic skin reactions and eye irritant. The oral LD$_{50}$ in rats is 250mg/kg (in corn oil), 4123mg/kg (in water) for cypermethrin and for fenvalerate oral-rat LD$_{50}$: 451-3200mg/kg. Cypermethrin and fenvalerate are highly toxic to fish and aquatic invertebrates. EPA has classified cypermethrin as a possible carcinogen.

1.4. MICROBIAL DEGRADATION OF PHENOLIC COMPOUNDS

The microorganisms present in the soil and water play an important role in the biogeochemical cycles occurring in the environment by degrading or transforming a variety of aromatic compounds of natural and synthetic origin (Alexander, 1981). Bacteria and fungi are most versatile in metabolizing aromatic compounds. Among the bacteria Pseudomonas, Bacillus, Micrococcus, Rhodococcus, Alcaligenes, Nocardia, Flavobacterium, Azotobacter, Acinetobacter and Mycobacterium. And among fungi Phanerochaete, Aspergillus and Cunninghamella are the genera having maximum versatility (Fewson, 1981). Thus, the bacterial and fungal populations present in the soil and water are the chief agents for biodegradation of the environmental pollutants.

Microorganisms are most versatile in utilizing a vast array of aromatic compounds as sole source of carbon and energy for their growth. These
compounds undergo intricate degradative pathways before entering the central metabolic cycles that can yield energy or cellular constituents. These pathways are essentially aerobic. When an aromatic compound serves as the sole source of carbon and energy for the growth of an organism, it is generally completely degraded i.e., mineralized. On the other hand, a compound may not serve as the growth substrate but still may be partially or completely degraded, if some other substrate is provided for the growth of an organism. Such processes known as cooxidation or cometabolism are of great significance in the biodegradation of recalcitrant compounds such as halogenated hydrocarbons and pesticides (Alexander, 1974; Perry, 1979). Many of the synthetic organic compounds are recalcitrant to the microbial degradation because these structures do not occur naturally and therefore, microorganisms are exposed to them only recently (Hutzinger and Veerkamp, 1981). However, microorganisms regenerate quite rapidly in the environment and over a period of time develop the genetic competence to synthesize enzymes and other cellular components that are necessary for the dissimilation of environmental chemicals. Though cometabolism, mixed microbial populations can work in concert to provide necessary enzymes for the degradation of recalcitrant compounds. For example, the first step of the catabolic pathway might be catalyzed by a microorganism, which is unable to perform the second step. It thus excretes the metabolic product and another group of microbes use it as substrate. Thus, in natural environment the total gene pool of microbial population is important for the complete biodegradation of compounds.
Many recalcitrant compounds such as various halogenated, nitrated, phenols, sulphonated aromatic compounds and pesticides are of potential environmental concern as many of them are highly toxic to man and animals. Microbial degradation of aromatic compounds has a great ecological consequences (Alexander, 1981). Microbial action often results in detoxification, particularly when the compound is completely degraded i.e., mineralized. However, some instances of enhancement of toxicity of a compound due to microbial transformation, termed as "Metabolic activation" are also known e.g. transformation of secondary amines to carcinogenic N-nitrosoamines (Oliver et al., 1979).

In the following pages, the microbial degradation of phenol, cresols, chlorophenols and nitrophenols have been highlighted.

PHENOL

Several species of bacteria and fungi posses the ability to utilize phenol as the sole source of carbon and energy. The phenol was catabolized through the formation of the catechol and cleavage either by an ortho cleaving enzyme, catechol-1,2-dioxygenase, or by a meta cleaving enzyme catechol-2,3-dioxygenase, to yield the product cis,cis-muconic acid and 2-hydroxymuconic semialdehyde, respectively. Generally, bacterial species degrade phenol through the meta cleavage pathway (Bayly et al., 1966; Dagley, 1971; Bettmann and Rehm, 1984; Schmidt, 1987; O'Reilly and Crawford, 1989). *Pseudomonas putida* (Feist and Hegeman, 1969; Bayly and Wigmore, 1973; Wigmore et al., 1977;
Morsen and Rehm, 1990), *Pseudomonas aeruginosa* (Ribbons, 1970), thermophilic bacteria *Bacillus* sp. A2 (Mutzel et al., 1996) and *Bacillus stearothermophilus* (Gurujayalakshmi and Oriel, 1989) degraded phenol through *meta* cleavage pathway.

Most of the yeast species reported to degrade phenol through *ortho* cleavage pathway e.g. *Cryptococcus elinovii* H1. (Morsen and Rehm, 1990), *Trichosporon cutaneum* (Neujahr and Varga, 1970; Neujahr and Gaal, 1973) and *Candida tropicalis* (Neujahr et al., 1974). Bacteria utilizing *ortho* cleavage pathway for the degradation of phenol are *Alcaligenes* sp. A7-2 (Menke and Rehm, 1992), *Streptomyces setonii* (Antai and Crawford, 1983). *Pseudomonas cepacia* strain CMA (Stockinger et al., 1992) and *Pseudomonas stutzeri* strain SPC2 (Aneez Ahamad and Kunhi, 1996).

Phenol on an anaerobic oxidation by the dissimilatory iron reducing bacteria (GS-IS) degrade through the intermediate 4-hydroxybenzoate (Lovely and Lonergan, 1990). *Aspergillus fumigatus* degraded phenol by two routes; in one route phenol undergoes *ortho*-hydroxylation to give catechol, which is then cleaved by an intradiol mechanism leading to 3-oxoadipate. In the other route, phenol is hydroxylated in the *para* position to produce hydroquinone, which is then converted into 1,2,4-trihydroxybenzene for ring fission by *ortho* cleavage to give maleylacetate. (Jones et al., 1995). The different pathways for the bacterial and fungal degradation of phenol are illustrated in Fig. I.4a and I.4b.
Fig. 1.4a. Proposed pathway for the metabolism of phenol by *Bacillus stearothermophilus*.

Fig. 1.4b. Proposed pathway for the metabolism of phenol by fungus *Aspergillus fumigatus*.
CHLOROPHENOLS

Chlorinated phenolic compounds constitute an important class of pollutants because of their widespread use in industry (e.g., pulp and paper) and their toxicity and persistence in the environment (Haggblom, 1990). As a result, they have been the targets of a number of investigations focused on their possible biotreatment (Madsen and Aamand, 1991; Mohn and Kennedy, 1992). Microorganisms are capable of metabolizing the mono, di, tri, tetra and pentachlorophenol (Reineke and Knackmuss, 1988; Commandeur and Parsons, 1990; Chaudhry and Chapalamadagu, 1991; Khadar and Gold, 1991; Joliane et al., 1994; Ilya Utkin et al., 1995; Mannisto et al., 1999). Major pathway for the bacterial degradation of mono, di and trichlorophenols involve hydroxylation at the ortho position to chlorocatechols. 3-Chlorophenol and 4-chlorophenol were converted to 4-chlorocatechol as shown in Fig 1.4c. 4-Chlorocatechol was further converted to cis-4-carboxymethylene-but-2-en-4-diole. (Haggblom et al., 1989; Riedel et al., 1993).

The bacterial degradation of pentachlorophenol (PCP) involves initial hydrolytic dechlorination in the para position to form tetra chlorohydroquinone and its reductive dechlorination was responsible for the degradation of PCP by anaerobic Flavobacterium strain (Steiert and Crawford, 1986). This strain was also able to degrade and dechlorinate a range of di-, tri- and tetrachlorophenols (Steiert et al., 1987). Rhodococcus chlorophenolicus was also shown to degrade PCP to tetrachlorohydroquinone, which is then converted to dichlorotrihydroxybenzene by a reaction involving both hydrolytic and reductive dechlorination (Apajalahti and Salkinoja Salonen, 1987) as shown in Fig. 1.4d.
Fig. 1.4c. Proposed pathway for the degradation of chlorophenols by bacteria.

Fig. 1.4d. Bacterial degradation of pentachlorophenol by Rhodococcus chlorophenolicus.
(Apajalahti and Salkinoja Salonen, 1987, J. Bacteriol. 196, 675 - 681)
Mineralization of chlorophenols in methanogenic environments often starts with reductive dechlorination to phenol and ends with formation of methane and carbon dioxide (Gibson and Suflita, 1986; Suflita and Miller, 1985).

NITROPHENOLS

Nitroaromatic compounds are released into the biosphere almost exclusively from anthropogenic sources (Spain, 1995). They are used as insecticides, herbicides, fungicides and explosives. Recent research revealed a number of microbial systems capable of transforming or biodegrading nitroaromatic compounds (Heitkamp et al., 1990; Zablotowicz et al, 1999). Anaerobic bacteria can reduce the nitro group via nitroso and hydroxylamino intermediates to the corresponding amines (Mc Cormick et al., 1976; Oren et al., 1991; Rafii et al., 1991). Sulfate reducing bacteria, Desulfovibrio sp. reduce the nitro compounds to the corresponding amines and proposed that the amino groups are removed from the aromatic ring by a reductive deamination mechanisms (Boopathy and Kulpa, 1992; 1993; Boopathy et al., 1993). In contrast to the non-specific metabolism by fungi and anaerobes, some aerobic bacteria utilized nitroaromatic compounds as growth substrates. Bacteria able to degrade a wide range of polar and non-polar nitroaromatic compounds, Pseudomonas strain (Simpson and Evans, 1953) could convert 4-nitrophenol to hydroquinone with concomitant release of nitrite (Fig I.4e). Moraxella species degraded 4-nitrophenol in two ways. In one, 4-nitrophenol was degraded to hydroquinone by an initial oxygenase attack. The hydroquinone was further oxidized to maleylacetic acid and then reduced to β-ketoacidate. In second one, 4-nitrophenol
Fig. I.4e. Metabolism of 4-nitrophenol by *Pseudomonas* sp.
(Simpson and Evans, (1953). Biochem. J. 55, XXVI)

![Metabolism of 4-nitrophenol by Pseudomonas sp.](image)

Fig. I.4f. Biodegradation of 4-nitrophenol by *Moraxella* sp. and *Arthrobacteria* sp.
was degraded to 4-nitrocatechol via 1,2,4-benzenetriol with concomitant release of nitrite (Fig. I.4f.) (Raymond and Alexander 1971; Mitra and Vaidyanathan, 1984; Hanne et al., 1993; Jain et al., 1994). 2-Nitrophenol converted to catechol with the concomitant release of nitrite (Fig. 1.4g) (Simpson and Evans, 1953; Zeyer and Kearney, 1984).

_Bacillus_ sp. degrades 3-nitrophenol to CO₂ and NO₂ (Barik et al., 1976) _Flavobacterium_ (Raymond and Alexander, 1971) also cometabolically transform 3-nitrophenol to nitrohydroquinone (Fig. I.4h). Recently in a study of anaerobic biodegradation of several organic priority pollutants in digested municipal sludge (Boyd et al., 1983) have observed that di, tri, tetra nitrophenols were completely mineralized to CH₄ and CO₂ under anaerobic condition. Wood-rot fungus _Fusarium oxysporium_ reduce 2,4-dinitrophenol to a mixture of 2-amino 4-nitrophenol and 4-amino 2-nitrophenol (Fig. I.4i). Interestingly, the same organism could also oxidize 2-amino 4-nitrophenol to 2,4-dinitrophenol indicating the presence of a reversible nitro-reducing system (Madhosingh, 1961). _Pseudomonas_ and _Arthrobacter_ sp. (Jensen Lautrup-Larsen, 1967) and _Corynebacterium simplex_ (Gundersen and Jensen, 1956) degrades 2,4,6-trinitrophenol (picric acid) with release of nitrite ion. _Mycobacterium avium_ (Wyman et al, 1979) reduce picric acid to picramic acid (2-amino 4,6-dinitrophenol) under aerobic and anaerobic condition respectively (Fig. I.4j). _Rhodococcus erythropolis_ (Lenke and Knackmuss, 1992; Lenke et al., 1992; Reiger et al., 1994; Vorbeck et al., 1994) degrade picric acid by the initial addition of a hydride ion to the aromatically to form a hydride–Meisenheimer complex. Addition of a second hydride ion leads
Fig. 1.4g. Metabolism of 2-nitrophenol by *Pseudomonas* sp.


Fig. 1.4h. Co-metabolism transformation of 3-nitrophenol by *Flavobacterium*.


Fig. 1.4i. Transformation of 2,4-dinitrophenol by *Fusarium oxysporum*.

Fig. 1.4j. Transformation of picric acid to picramic acid by *Mycobacterium avium* and *Pseudomonas aeruginosa*.

to the eventual formation of 2,4,6-trinitrocyclohexanone, which decomposes to form 1,3,5-trinitopentane. In contrast, protonation of the hydride Meisenheimer complex leads to the enzyme-catalyzed rearomatization of the molecule and elimination of nitrite, which can be assimilated by the bacteria. The 2,4-dinitrophenol generated from picric acid is degraded by the bacteria and nitrite is eliminated.

**CRESOLS**

Microorganisms have been reported to degrade p-cresol (Mansi, 1986; Gunther et al., 1995). The degradation of p-cresol involves two pathways. In one, the ring is hydroxylated to give 4-methylcatechol as the substrate for a ring-fission dioxygenase (Dagley et al., 1960; Dagley and Gibson, 1965; Bayly et al., 1966; Chapman, 1972; Hopper and Taylor, 1975). In other, initial attack takes place on the methyl group, which is oxidized to carboxyl group, the 4-hydroxybenzoic acid formed is then ring-hydroxylated, giving protocatechuic acid, as the ring-fission substrate (Dagley and Patel, 1957; Hopper and Taylor, 1975; Hopper, 1978; Jones et al., 1993).

A novel feature of this later pathway is that the initial methylhydroxylase, in a number of bacterial species, is a flavocytochrome c that acts by dehydrogenation of the substrate to form a guinone methide. This is then hydrated to give the 4-hydroxybenzyl alcohol (Hopper, 1988). The proposed pathway for the degradation of p-cresol is shown in Fig.1.4k and Fig.1.4l. Protocatechuate was formed by two alternative routes, either by initial attack on the methyl group,
Fig. 1.4k. Proposed pathway for the metabolism of p-cresol by *Pseudomonas putida.*

Fig. 1.4l. Proposed pathway for the metabolism of p-cresol by *Aspergillus fumigatus.*
which is oxidized to carboxyl, followed by ring-hydroxylation, or by ring hydroxylation as the first step with subsequent oxidation of 4-methylcatechol to the acid. There are also some reports on the anaerobic degradation of p-cresol (Suflita et al., 1989; Haggblom et al., 1990; Rudolphi et al., 1991).

Hopper and Taylor (1975) reported that Pseudomonas putida degrade m-cresol by oxidation to 3-hydroxybenzoic acid followed by hydroxylation to gentisate, the ring fission substrate. Pseudomonas putida (Pseudomonas U) (Dagley et al., 1964; Bayly et al., 1966) metabolized the m-cresol by hydroxylation to 3-methylcatechol and subsequent meta cleavage of the ring. The proposed pathway for the bacterial degradation of m-cresol is shown in Fig. I.41. There are also several reports on anaerobic degradation of m-cresol (Smolenski and Suflita, 1987; Roberts et al., 1988; Mueller et al., 1989; Roberts et al., 1990; Ramanand and Suflita, 1991; Londry and Fedorak, 1993; Bonting, 1995).

The o-cresol generally being more resistant to biodegradation than the para and meta-isomers (Flyvbjerg et al., 1993). There are some reports on the ability of bacteria to degrade o-cresol (Ribbons, 1964; 1966; Bakker, 1977; Aneez Ahamad et al., 2001). Pseudomonas species degraded o-cresol to 4-hydroxy-2-oxo-valeric acid via 3-methylcatechol as shown in Fig.I.4o. Cometabolic transformation of o-cresol to 3-methylbenzoic acid with protease peptone as the primary substrate has recently been demonstrated by Bisaillon et al. (1991) while Kominski et al. (1990) reported the complete degradation to methane and CO₂ when o-cresol was fed as the only organic substrate to acclimated river sediment.
Fig. 1.4n. Proposed pathway for the metabolism of m-cresol by *Pseudomonas putida*.

Fig. 1.4o. Proposed pathway for the degradation of o-cresol by *Pseudomonas aeruginosa.*

1.5. BIODEGRADATION OF PESTICIDES

Pesticides used in agriculture are the classic examples of anthropogenic chemicals that enter the earth's environment in large amounts via non point sources. These compounds can adversely affect non target organisms and may be detrimental to human health, if people are exposed to residues of the molecules in food, water or agricultural products, for example pesticides such as DDT, Lindane and Dieldrin have shown to be recalcitrant. Hence, they persist in the environment for a long time and accumulate into food chains decades after their application to soil. The longer such chemicals persist in the environment, the greater the chance of their causing harm. Thus scientists have long recognized the need to study degradative processes particularly biodegradation, mineralization.

The microbial degradation of pesticides has a great effect on the environmental fate and efficiency of the compound. When a pesticide is degraded at a reasonable rate, it persists long enough to control pests but does not become a pollution problem. If a pesticide is degraded too rapidly, it may not adequately control target pests. Rapid microbial degradation of pesticides has been shown to contribute to the 'problem' or 'aggressive' soil phenomenon observed after repeated use of certain soil incorporated pesticides. Several pesticides fail to control insects after they are used continually for a number of years, resulting in economic loss from failed crops. It could result from the fact that soil microorganisms that repeatedly or continuously encounter such chemicals may develop new capabilities to degrade them. Microorganisms with newly evolved traits have been implicated in the rapid inactivation of pesticides in problem soils.
To understand the fate of pesticides in the environment, it is important to know the biochemical pathways utilized for the degradation of pesticides by the microorganisms (Somasundaram and Coats, 1990). This information allows prediction of the fate of not only the pesticides but also the metabolites generated. The biodegradation of pesticides may result in metabolites that are more toxic than the parent molecule. Some metabolites may accumulate in the environment. A number of aerobic and anaerobic microbes capable of degrading pesticides have been isolated from soil, water and other habitats. A diverse group of bacteria, including member of the genera Alcaligenes, Flavobacterium, Pseudomonas and Rhodococcus metabolize pesticides. The bacteria can utilize the pesticide as sole source of carbon or alternately cometabolize it. Microbial degradation depends not only on the presence of microbes with the appropriate degradative enzymes, but also on a wide range of environmental factors. There are several reviews on the microbial metabolism of pesticides (Roberts and Standen, 1977; Hill and Wright, 1978; Cork and Krueger, 1991; Aislabie and Jones, 1995; Borse et al., 1998). Recent reports on the biodegradation of pyrethroid pesticides have been discussed.

MICROBIAL DEGRADATION OF PYRETHROID PESTICIDES

Pyrethroid pesticides such as cypermethrin, fenvalerate, deltamethrin and permethrin have been widely used to control pests in cotton and vegetable crops and also used for repelling black flies by preventing them from taking a bread meal. They are highly toxic to fish and aquatic invertebrates. Pyrethroid pesticides interfere with the balance of sodium ions in the nerve junctions of target and non-
target organisms rendering them inactive. Pyrethroid compounds have a strong tendency to absorb to soil particles and are moderately persistent. Microorganisms capable of degrading pyrethroid pesticides play a significant role in breaking down and detoxifying pesticides in the environment. Such microorganisms have received considerable attention because of their potential use in pesticide waste detoxification as well as their effect on the fate of pyrethroid pesticides in the environment.

CYPERMETHRIN AND FENVALERATE

Cypermethrin and fenvalerate were marketed under trade name of cyperkill and sumicidine respectively. There are few reports on the ability of bacteria to degrade cypermethrin and fenvalerate (Roberts and Standen, 1977; 1981; Kaufman et al., 1981; Lee et al., 1987; Class, 1992; Sakata et al., 1992; Borse et al., 1998). Bacillus sp. (Hashemi and Mohandes, 1999) and Pseudomonas sp. (Asghar et al., 1994) have been found to utilize cypermethrin and fenvalerate as a growth substrate. The degradation of cypermethrin involves initial hydrolysis of ester linkage, yielding 3-phenoxybenzoic acid and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid. Fenvalerate is hydrolysed to yield 3-phenoxybenzoic acid and 2-(4-chlorophenyl)-3-methylbutyric acid.

The further degradation of 3-phenoxybenzoic acid was confirmed by Halden et al. (1999; 2000). According to Halden, the degradation of 3-phenoxybenzoic acid by Pseudomonas pseudoalcaligenes POB 310 (pPOB), Pseudomonas strain B13-D5 and Pseudomonas strain B13-ST1 (pPOB), involves
Fig. I.5a. Pathway for the degradation of cypermethrin by soil bacteria.

incorporation of O$_2$ by phenoxybenzoate dioxygenases in the 1,6-position of the carboxylated ring, yielding a hypothetical hemiacetal, that decomposed to form phenol and protocatechuate. Phenol was oxidized to catechol. Protocatechuate and catechol were metabolized via respective ortho and meta cleavage pathways (Fig. I.5c).

Maloney et al., (1988) reported the transformation of 3-phenoxybenzoic acid to 4-hydroxy-3-phenoxybenzoic acid by strains of Bacillus cercus, Pseudomonas fluorescens and Achromobacter sp. The different pathways for a bacterial degradation of cypermethrin and fenvalerate were illustrated in Fig. I.5 a and I.5 b.

The microbes present in the soil play a vital role in controlling pollution. Sensing and adaptation to fluctuations in environmental conditions are essential processes of these soil microbes. Hence, the current research mainly focused on the utilization of soil microbes for degradation of toxic chemicals.

I.6. MICROBIAL DEGRADATION OF BENZOIC ACID AND ITS HYDROXY DERIVATIVES

a) BENZOIC ACID

The bacterial degradation of benzoic acid occurred most often by an initial double hydroxylation followed by ring cleavage, although in a few instances monohydroxy derivatives were also postulated as intermediates. Cis-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid was shown to be an intermediate in the conversion of benzoate to catechol by Acinetobacter alcaligenes and Pseudomonas sp. (Reiner, 1971; Knackmuss and Reineke, 1973).
Fig. 1.5b. Pathway for the degradation of fenvalerate by *Bacillus cercus* (Maloney *et al.*, 1988). Appl. Environ. Microbiol. 54(11), 2874-2876
Fig. I.5c. Degradative pathway for 3-phenoxybenzoic acid by "Pseudomonas pseudoalcaligenes" POB 310.

Yamaguchi and Fujisawa (1982) purified benzoate-1,2-dioxygenase from *Pseudomonas arivilla*. The enzyme was composed of an NADH-Cytochrome c-reductase and a benzoate oxygenase, which required Fe$^{2+}$ and NADH for maximum activity. The dihydrodiol dehydrogenase catalyzing the conversion of cis-diol to catechol has been purified from *A. eutrophus* (Reiner, 1972).

In few instances, benzoate was degraded by the initial formation of salicylate or 3-hydroxybenzoate or 4-hydroxybenzoate (Ogata *et al.*, 1970; Aftring and Taylor, 1981). Most of the fungi degraded benzoate through monohydroxylation reaction to yield 4-hydroxybenzoate (Moore *et al.*, 1968; Jamaluddin *et al.*, 1970; Perrin and Towers, 1973; Yuasa *et al.*, 1975). The monohydroxy benzoic acids further metabolized through the introduction of another hydroxy group at the ortho or para position to the existing hydroxy group.

**b) 3-HYDROXYBENZOIC ACID**

The degradation of 3-hydroxybenzoic acid proceeds through hydroxylation at the 4th or 6th position yielding protocatechuic acid or gentisic acid respectively. 3-Hydroxybenzoate was hydroxylated to protocatechuic acid by *Pseudomonas testosteroni* (Michalover and Ribbons, 1973), *A. japonicus* (Milstein *et al.*, 1983) and *Asperigillus niger* (Premakumar *et al.*, 1973). Several *Pseudomonas* sp. (Groseclose and Ribbons, 1973) and *Streptomyces* (Sutherland *et al.*, 1981) converted 3- hydroxybenzoate to gentisic acid. Both these pathways were shown in several species of *Bacilli* (Crawford, 1975) and *Amycolatopsis* (Grund *et al.*, 1990). A mutant strain of *Pseudomonas testosteroni* (Engelhardt *et al.*, 1979)
accumulated 2,3-dihydroxybenzoic acid from 3-hydroxybenzoate as dead-end metabolite. Starovoitov et al., (1985) reported the catabolism of 3-hydroxybenzoate by *Pseudomonas putida* BS893, by a new pathway via 2,3-hydroxybenzoic acid and catechol. This organism was also shown to convert 3-hydroxybenzoate to gentisate and protocatechuate. 3-Hydroxybenzoate-4-hydroxylase was purified from *Pseudomonas testosteroni* (Michalover and Ribbons, 1973) and *Aspergillus niger* (Premakumar et al., 1973). 3-Hydroxybenzoate-6 hydroxylase was purified from *Pseudomonas aeruginosa* (Groseclose and Ribbons, 1973). Both the enzymes were shown to be flavoprotein requiring NADH or NADPH as an electron donor.

c) 4-HYDROXYBENZOIC ACID

Most of the bacteria and fungi appear to degrade 4-hydroxybenzoate through protocatechuate. 4-Hydroxybenzoate-3-hydroxylase was purified from four different *Pseudomonads* (Sugumaran and Vaidyanathan, 1978). Conversion of 4-hydroxybenzoate to gentisic acid was reported to occur in some *Bacillus* sp. (Buswell and Clark, 1976; Crawford, 1976). Such a reaction obviously involved the migration of carboxyl group to the 2\textsuperscript{nd} position. However, the enzyme catalyzing this hydroxylation has not been purified.

The various pathways for the degradation of benzoic acid and monohydroxybenzoic acids are shown in Fig. I.6a.
Fig. 1.6a. Microbial degradation of benzoic acid and hydroxybenzoic acids.
1.7. AROMATIC RING CLEAVAGE PATHWAYS

The oxidative cleavage of the aromatic ring catalyzed by the dioxygenases is the most critical step in the mineralization of aromatic compounds as the aliphatic compounds formed by the action of dioxygenases enter the TCA cycle by simple decarboxylation, hydrolysis and isomerization reactions. Dioxygenases cleave the aromatic ring containing two hydroxyl groups that are either ortho or para to one another. There are two distinct modes of oxidative cleavage of the aromatic rings namely ortho and meta cleavages. Cleavage of the bond between two adjacent carbon atoms that carry hydroxyl groups is known as “ortho” or “intradiol” cleavage and the pathway by which the products of such cleavage are metabolized are known as the ortho or β-ketoacid pathway. Cleavage of the bond between two carbon atoms, only one of which carries a hydroxyl group, the other carbon atom being either unsubstituted or substituted with other than a hydroxyl group. This type of cleavage is known as meta or extradiol cleavage and the pathway by which products of such cleavage are metabolized is called the meta-pathway. When the hydroxyl groups are para to one another as in gentisic acid (2,5-dihydroxybenzoic acid), oxidative cleavage is catalyzed gentisate-1,2-dioxygenase, and the subsequent pathway is the gentisate pathway. Most of the ring cleavage dioxygenases have been shown to contain nonheme iron as the sole cofactor except for the homoprotocatechuate-2,3-dioxygenase from Bacillus brevis which was shown to contain manganese II (Que et al., 1981). While most of the meta cleavage dioxygenases have been shown to contain ferrous ion (Fe^{2+}), ortho-cleavage dioxygenases contain ferric ion (Fe^{3+}).
a) RING CLEAVAGE OF CATECHOL

Bacteria can metabolize catechol by ortho or meta cleavage pathways. Some bacteria especially members of the genus *Pseudomonas* have enzymes for both pathways. Catechol dioxygenase cleaves the aromatic ring of catechol and its derivatives by the insertion of both atoms of molecular oxygen to give the corresponding muconic acid derivatives or its semialdehyde depending on the site of dioxygen insertion as shown in Fig.1.7a. Several catechol dioxygenases were obtained in crystalline form or in homogeneous state and their properties have been extensively studied (Hayaishi and Nozaki, 1969; Nozaki and Ishimura, 1974; Que, 1983). Catechol-1,2-dioxygenase also known as pyrocatechase catalyzes the intradiol ring cleavage of catechol to yield cis, cis-muconic acid. It was first isolated by Hayaishi and Hashimoto in 1950. This enzyme has been purified and characterized from *Pseudomonas arvilla* (Kojima et al., 1967), *Pseudomonas fluorescens* (Nakazawa et al., 1967), *Acinetobacter calcoaceticus* (Patel et al., 1976), and *Brevibacterium fuscum* (Nakagawa et al., 1963). Catechol-1,2-dioxygenase from *Pseudomonas arvilla* has a mol.wt. of 63,000 and contains 1g atom of ferric ion per mole of enzyme (Nozaki et al., 1982). The enzyme consists of two non-identical subunits (α-β). Catechol-1,2-dioxygenase purified from different bacterial species vary in their molecular weight, number of subunits and iron content. There are some reports of extradiol cleavage of 3-methylcatechol by catechol-1,2-dioxygenases (Hou et al., 1977).

Catechol-2,3-dioxygenase (Metapyrocatechase) catalyzes the conversion of catechol to 2-hydroxymuconic semialdehyde. It was the first dioxygenase to be
obtained in the crystalline form (Nozaki et al., 1963). The enzyme has a molecular weight of 140,000 with four identical subunits and contains 4g atoms of ferrous ion (Nakai et al., 1983).

b) RING CLEAVAGE OF PROTocatechuIC ACID

Protocatechuate-2,3-dioxygenase catalyzes the extradiol proximal cleavage of protocatechuate to 5-carboxy-2-hydroxymuconic semialdehyde. This enzyme was isolated from Bacillus circulans (Crawford, 1975) and was shown to act on methyl and ethyl esters of protocatechuate also.

Protocatechuate-3,4-dioxygenase catalyzes the intradiol cleavage of 3,4-dihydroxybenzoic acid to yield 3-carboxy-cis, cis-muconic acid. This enzyme has been studied from a number of microorganisms. Protocatechuate-3,4-dioxygenase from Pseudomonas species (Fujisawa et al., 1972; Pujar and Ribbons, 1983), Acinetobacter calcoaceticus (Hou et al., 1976), Brevibacterium fuscum (Whittaker et al., 1984; Kurane et al., 1984) were purified to homogeneity and that of Pseudomonas aeruginosa (Satyashur et al., 1980) and Brevibacterium (Whittaker et al., 1984) were crystallized. The active site of the enzyme contains catalytically essential Fe$^{+3}$.

Protocatechuate-4,5-dioxygenase catalyzed the extradiol cleavage of protocatechuate to yield 4-carboxy-2-hydroxymuconic semialdehyde. It was purified from Pseudomonas testosteroni and was shown to oxidize gallate and 3-o-methylgallate (Dagley et al., 1968; Zabinski et al., 1972). All the three modes of ring cleavage of protocatechuate are shown in Fig. 1.7 b.
Fig. I.7a. Pathways for the ring cleavage of catechol by microorganisms.

Fig. I.7b. Pathways for the ring cleavage of protocatechuic acid by microorganisms.
c) THE GENTISATE PATHWAY

Gentisic acid (2,5-dihydroxybenzoic acid) is an intermediate in the degradation of 3-hydroxybenzoate, 4-hydroxybenzoate, salicylate, anthranilic acid, m-cresol, β-naphthol (Chapman, 1972) and certain halogenated compounds.

Gentisate-1,2-dioxygenase catalyzes the conversion of gentisic acid to maleylpyruvic acid. The enzyme was purified from a Pseudomonas sp. (Lack, 1959; Sugiyama et al., 1960), Klebsiella pneumoniae M5al (Suarez et al., 1996) and it required Fe$^{2+}$ for activity. Crawford et al., (1975) isolated gentisate-1,2-dioxygenase from Morexella Osloensis in a homogenous form. It was a tetramer with identical subunits of M, 40,000. Maleylpyruvic acid was further metabolized to fumarate and pyruvate by a GSH-dependent isomerization reaction and the subsequent hydrolysis of fumarylpyruvate. Some organisms contained a GSH-independent isomerase (Hogedrin et al., 1985) and others hydrolyzed maleylpyruvate directly to maleate and pyruvate (Hopper et al., 1971). To distinguish between the GSH-independent hydrolysis and GSH-dependent isomerization of maleylpyruvate occurred in the same strain (Crawford and Frick, 1977), used N-ethylmaleimide (NEM) to inhibit the activity of GSH-dependent isomerase. All the mechanisms for the metabolism of maleylpyruvate have been shown to occur independently in different species of Bacillus (Crawford and Olson, 1979). The pathway for the degradation of gentisic acid is shown in Fig. 1.7c.
Fig. I.7c. Bacterial metabolism of gentisic acid.
AIM AND SCOPE OF THE PRESENT INVESTIGATION

The review of the literature presented in the preceding pages highlights the unity and diversity in microbial degradation of aromatic compounds and pesticides. It is apparent that a wide variety of pathways are operating for the degradation of aromatic compounds in different organisms. Oxygenases occupy a pivotal position in the utilization of these compounds.

Phenol, a corrosive aromatic compound contaminates ground water, because of the relatively high water solubility. Phenolic pollution is characteristic of manufacturing (pesticides, pharmaceuticals, plastics) and raw materials (crude oil, coal) conversion processes. p-Cresol is component of the total phenolic fraction of toxic matter in wastewater from hydrocarbon refining and they are of ecological concern because of their toxicity and mobility in subsurface environments. Phenol and p-cresol are included in the US EPA priority pollutant lists. The pathway for the degradation of phenol and p-cresol has been studied mainly in the bacteria of genus Pseudomonas. But the ability of Bacillus brevis to degrade phenol and p-cresol has not been reported.

The pyrethroid pesticides such as cypermethrin have been used extensively in agriculture and public health and are known to be toxic environmental pollutants. The pathway for the degradation of cypermethrin has been studied in soil bacteria. But the ability of Micrococcus luteus to degrade cypermethrin has not been reported. The metabolic fate of the pesticides in the environment is determined principally by the action of microorganisms.
Studies on the microbial degradation of pesticides and phenols are extremely relevant in our national context of establishing sustainable agriculture and providing clean environment.

In comparison with the wealth of information available on the biodegradation of various classes of aromatic compounds, much less is known about the biodegradation of phenol, p-cresol and cypermethrin in different organisms. Nevertheless more studies in other bacterial systems are necessary in order to evaluate the universality and evolutionary significance of the biodegradative pathways. Therefore, the present investigation was undertaken to elucidate the biodegradative pathway of phenol, p-cresol and cypermethrin by Bacillus brevis and Micrococcus luteus respectively isolated from soil by enrichment cultures. Thesis describes (i) the isolation and identification of Bacillus brevis and Micrococcus luteus capable of degrading phenol, p-cresol and cypermethrin. (ii) Elucidation of pathway for the degradation of phenol, p-cresol and cypermethrin by Bacillus brevis and Micrococcus luteus. (iii) Partial purification and characterization of 4-hydroxybenzoate-1-hydroxylase from Bacillus brevis. (iv) Degradation of p-cresol and phenol by immobilized cells.

Immobilization technique offers a great potential for their use in bioremediation process. The degradation of phenols by immobilized cells is faster compared to degradation by free cells because of viability of cells, increased cell density, cell metabolism, cell wall permeability and stability of the enzymes. The immobilization of microbial cells offers an easy to handle biocatalyst, which has been shown to be of great advantage in several industrial processes, which is of valuable application.