CHAPTER-I

Introduction,

Review of Literature

and Objectives
Environmental pollution implies any alteration in the surroundings but it is restricted in use especially to mean any deterioration in the physical, chemical and biological quality of the environment. All types of pollution, directly or indirectly affect human health. The pollutants fall under the broad category of xenobiotic compounds and are released into the environment by the action of man and occur in concentrations higher than “natural levels”.

Pollutants are generally classified under the two heads viz.

1. Biodegradable
2. Non-biodegradable

Biodegradable pollutants consist of sewage effluents and organic matter that are readily decomposed under normal circumstances. Non-biodegradable substances are those which are not degraded by microorganisms e.g. heavy metals, plastics and xenobiotics such as pesticides, detergents and other substances like polythene. Fast urbanization and industrialization have resulted in the tremendous release of xenobiotic compounds into the environment. Large quantities of highly toxic chemicals emitted by industries are generally used in India for enhanced agricultural productivity (Vishwanathan, 1985).

Most of the organic pollutants originate from five major industrial categories: petroleum refining, organic chemical and synthetic industries, steel mining and coal conversion, textile processing, and pulp and paper milling (Rawlings and Bamfield, 1979; Nye, 2000). However, industries alone are not totally responsible for exposure of the chemicals to environment, consumers too share a part. Utilization of gasolene, aerosol sprays, pesticides and fertilizers lead to release of pollutants by the consumers directly into the environment.
Effluent from waste water treatment plants is another cause of xenobiotic pollution. Accidental spillage, illegal dumping, poorly chosen landfalls and uncontrolled hazardous waste sites are other routes through which the environment is contaminated. In fact, inadequate disposal techniques have been cited as the main cause of contamination of biota as well as soil surface and ground matters which could lead to the emergence of disease producing microorganisms and ultimately result in serious health problems (Gupta 1989; Malik and Ahmad, 1995; Tibbetts, 2000).

Among the various types of environmental pollution, water pollution is an age old problem but it has gained an alarming dimensions lately because of the problems of population increase, sewage disposal, industrial waste, radioactive waste, etc. These factors have contributed so much to the pollution of water resources that about 70-80% rivers and streams all over the world carry polluted waters (Tibbetts, 2000). In the absence of adequate potable water supply, a significant proportion of the population in underdeveloped countries still depends on natural resources such as wells, rivers and ponds. In India the same body of natural water is used for irrigation, washing of clothes and utensils, sewage disposal and bathing of animals and man, as well as for drinking and cooking without any purification or sterilization, making the risks of water pollution more serious (Vishwanathan, 1985; SOI:R, 2001).

**Heavy metals in the environment and their toxicity**

Metal pollution is a widespread problem, in fact, in industrially developed countries it is normal to find elevated levels of metal ions in the environment. In addition, it has been estimated that approximately 37% of sites in the US
contaminated with organic pollutants such as pesticides, are additionally polluted with metals (Riley et al., 1992). Despite this, biological treatment or bioremediation of contaminated sites has largely focused on the removal of organic compounds and only recently attention has been directed towards the treatment of metal contaminated wastes (Brierley, 1990; Summers, 1992; Srivastava, 2000). Due to their toxic nature, the presence of metals in organic contaminated sites often complicates and limits the bioremediation process. Such metals include the highly toxic cations of mercury and lead, but many other metals and metalloids are also of concern, including arsenic, beryllium, boron, cadmium, chromium, copper, nickel, manganese, selenium, silver, tin and zinc.

Metal pollution arises when human activity either disrupts the normal biogeochemical cycles or concentrates metals (Roane et al., 1996). Examples of such activities include mining and ore refinement, nuclear processing and industrial manufacture of a variety of products including batteries, metal alloys, electrical components, paints, preservatives and insecticides (Suzuki et al., 1992; Saxena et al., 2001). Much of the research on metal bioavailability has been done in soil systems because understanding the fate of metals in soil and sediments is crucial to determining metal effects on biota, metal leaching to ground waters, and metal transfer up to the food chain (Roane et al., 1996). Soil usually exhibits higher concentrations of metals than water because metals are more likely to accumulate in soil and are composed of minerals.

Elevated metal concentrations in the environment have wide ranging impacts on animals, plants and microbial species. For example, human exposure to a variety of metals causes disorders and symptoms like hypophosphatemia, heart diseases
and liver damage, cancer, neurological and CNS disorders etc. (Trivedi and Gundap, 1992; ATSDR, 2001). Exposure to metals is the cause of most morphological and mutational changes observed in plants (Brooks, 1983; Manios et al., 2002). These include shortening of roots, leaf scorch, chlorosis, nutrient deficiency and increased vulnerability to insect attack (Canney et al., 1979). Likewise, microbial growth is often slowed or inhibited completely in the presence of excessive amounts of metals (Baath, 1989; Bojic et al., 2002). Toxic metals exert their toxicity in a number of ways including the displacement of essential metals from their normal binding sites on biological molecules (e.g. cadmium compete with zinc), inhibition of enzymatic functioning and disruption of nucleic acid structure (Freedman, 1995).

Some metals are essential components of microbial cells for example, sodium and potassium regulate gradients across the cell membrane, and copper, iron and manganese are required for activity of key metalloenzymes in photosynthesis and electron transport. However, certain metals can also be extremely toxic to microorganisms and thus affecting microbial growth, morphology and biochemical activities as a result of specific interactions with cellular components (Beveridge and Doyle, 1989; Freedman, 1995). Perhaps the most toxic metals are the nonessential metals such as cadmium, lead and mercury.

Nickel compounds are widely used in modern industries (Bennett, 1984). Several industrial processes, like nickel refining, electroplating, production of long lasting nickel-cadmium batteries, combustion of fossil fuels and the incineration of nickel-containing solid waste are responsible for the production of nickel containing aerosols in the workplace and in the surrounding
environments. Because of the widespread use of these compounds, workers in these facilities are at risk of occupational exposure. Moreover, the release of nickel into the environment represents a potential for non occupational exposure (Bennett, 1984; Oller, 2002).

Inhalation is the main route for human exposure to nickel compounds, and epidemiological studies have demonstrated a correlation between the incidence of respiratory (lung and nasal) cancer and worksite exposure to nickel (Doll et al., 1970; Langard, 1994). Tumors have also been induced in several animal models after inhalation, ingestion or injection of various nickel compounds (Sunderman, 1981; Sunderman, 1989).

Chromates are widely discharged into the environment and thus high concentrations of chromium are found in both marine and freshwater sediments and in soil associated with industrial discharges such as steel production, wood preservation, and leather tanning (McLean and Beveridge, 2001; Viti et al., 2003). Chromium occurs in oxidation states from +2 to +6 with +3 and +6 being the most important biologically (Sultan and Hasnain, 2003). Furthermore, experimental and epidemiological lines of evidence exist for the carcinogenicity of some chromium compounds (Sittig, 1985) and mutagenicity in bacteria (Losi et al., 1994). Owing to its mutagenic and carcinogenic behaviour hexavalent Cr is about 100-fold more toxic than trivalent form (Cervantes, 1991).

Cadmium ranks as a major anthropogenic pollutant especially released from the industrial effluents (Cunningham and Lundie, 1993). Its transfer in the soil-plant system may lead to cadmium accumulation in roots, stems and leaves (Mench et al., 1989), especially in the edible parts of crops (Hooda and Alloway,
1995) and most particularly in market garden products (Carlton-Smith and Davis, 1983). As a consequence, the crop production may become unfit for animal and human consumption (Decloitre, 1998).

The toxic effects of cadmium on microorganisms are well documented (Babich and Stotzky, 1977; Vymazal, 1987; Stohs and Bagchi, 1995) and derive from several mechanisms. Disruption of protein function can occur through binding of cadmium to sulfhydryl groups (Cunningham and Lundie, 1993). In addition cadmium competes with several divalent ions such as Ca^{2+}, Zn^{2+} and Mn^{2+} for metal binding sites in biological systems (Hughes and Poole, 1989). Binding of cadmium to nucleotides leads to single strand breaks in cellular DNA (Mitra et al., 1979; Saplakoglu and Iscan, 1998). Cadmium toxicity can result in prolonged lag phase, decreased growth rate, lower cell density, even death of bacteria and algae at levels below 1 ppm (Aiking et al., 1982; Les and Walker, 1984).

Lead, the most common metal found at superfund sites, was less studied compared with other metals (Enger and Smith, 1992). There are reports of high lead accumulations in surface soil horizons due to a large capacity for lead immobilization, and its accumulation in relation to the soil organic fraction, soil pH and redox (Colbourn and Thorton, 1978; Nederlof and Van Riemsdijk, 1995). Despite the apparent immobility of lead, organic soils or sediments do not retain approximately 30% of the total lead ecosystem input (Johnson et al., 1995). Consequently, the soil becomes a major source of lead exposure for microbial communities.
Lead is an environmental nephrotoxicant and probable human carcinogen, it is also reported that lead acetate cause chromosomal aberrations in human cells (Hayashi, 1983; Waalkes et al., 2004). Lead poisoning of children is common and leads to retardation and semi-permanent brain damage (Jarup, 2003).

Copper is readily available as Cu(I) or Cu(II) in inorganic salts and organic complexes. About 30-50% of the oral intake is absorbed mainly from the duodenal mucosa through a copper binding proteins. Copper is an essential trace element required for several bacterial enzymes, particularly oxidation-reduction enzymes (Cass and Hili, 1980) and proteins involved in electron transport, redox reactions and others (Lontie, 1984). However, at higher concentrations, it is highly toxic to microbial cells exerting an inhibitory effect on the bacterial growth, and has also been incorporated as a key component of agricultural bactericides (Lim and Cooksey, 1993; Cervantes and Gutierrez - Corona, 1994).

Yang et al. (2002) reported that copper pollution in soils is widespread, and its accumulation in crop products could pose a risk on human health. Moreover, Zietz et al. (2003) have found that copper in drinking water has been associated with Non Indian Childhood Cirrhosis (NICC), a form of early childhood liver cirrhosis.

**Pesticides in the environment and their toxicity**

Pesticides are one of the vital components of our modern agriculture practices. Some of these chemicals also play an equally important role in the eradication of human and domestic animal pests. Adoption of modern agricultural practices of highly intensive nature to feed the ever increasing population of
the world resulted in the widespread pollution of synthetic pesticides in the environment. Thus the presence of these compounds is ubiquitous, often contaminating surface and ground waters as they migrate from their point of application (Thomas et al., 2001). The movement of pesticides from one place to another has been thoroughly studied (Kreuger et al., 1999; Liess et al., 1999) and is dependent on factors such as soil-type, drainage, physical and chemical properties of the pesticides and weather (Brown and Hollis, 1996). In nut shell the indiscriminate use of these pesticidal agents has posed a great threat to man and his environment in several ways such as harming non-target organisms, causing ecological imbalance, destroying useful plants, entering into food chain and causing toxicity to both man and animals. Moreover, the rivers, streams and ponds have also become highly polluted with these harmful agents (Kumar and Mukerji, 1996; Phillips and Bode, 2004).

The toxicity of pesticides to aquatic microorganisms has been exhaustively reviewed by De Lorenzo et al. (2001). Microorganisms are important inhabitants of aquatic ecosystems, where they fulfil critical roles in primary productivity, nutrient cycling and decomposition. Microorganisms of the aquatic environment are exposed directly to the pesticides because of the direct and indirect input of the pesticides. Though certain pesticides are known to elicit a variety of chronic and acute toxicity effects in microorganisms, some of them still have the ability to accumulate, detoxify or metabolize pesticides to some extent (De Lorenzo et al., 2001). It is supposed that detrimental effects of pesticides on microbial species may have subsequent impacts on to higher trophic levels (Ahlgren et al., 1990; De Lorenzo et al., 2001). To understand the mechanisms of pesticides action on our environment, a summary of their action on target
organisms has been presented in Table 1. Besides these specialized actions, pesticides have been shown to cause various kinds of organ toxicities such as cardiotoxicity, neurotoxicity and ocular toxicity as a result of short term or chronic exposure to common pesticides such as DDT, endosulfan and HCH (Ray et al., 1992). A number of types of cancer have also been attributed to the exposure of pesticides (Jaga and Duvvi, 2001; Tisch et al., 2001). Toxicity of selected pesticides and their major metabolites have been compiled in Table 2.

The phenoxyacetate herbicides which include 2,4-dichlorophenoxyacetic acid (2,4-D) 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) have been used extensively for the control of weeds since their introduction in the mid 1940's. Among them 2,4-Dichlorophenoxyacetic acid (2,4-D) is being used most extensively as hormone herbicides, resulting in the pollution of soil and natural water (Schneider, 1979; Radjendirane et al., 1991). Once their accumulation touches enormity, these compounds become very hazardous to life (Hanify et al., 1981).

Lindane, γ-isomer of BHC (benzene hexachloride), due to its widespread use, is a common pollutant worldwide (Phillips et al., 2001). It has been used since 1940s for the control of disease vectors and agricultural pests (Rogers, 1996). Because of its carcinogenity, chronic toxicity to many aquatic organisms and tendency to biomagnify, BHC has been placed among the priority pollutants and it was banned in 1976 (Watts, 1998). However, the use of BHC in developing and even in some technologically advanced countries still continues and they are still manufactured to export (Watts, 1998).

After the ban or restriction on various chlorinated hydrocarbon insecticides,
Table 1: Summary of pesticide mechanisms of action on target organisms

<table>
<thead>
<tr>
<th>Pesticide Class</th>
<th>Groups included</th>
<th>General Toxic effect</th>
<th>Specific site of action</th>
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<tbody>
<tr>
<td>Organophosphate</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carbamates</td>
<td></td>
<td>Nervous system inhibition</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>Organochlorines</td>
<td></td>
<td>Nervous system inhibition</td>
<td>GABA receptor</td>
</tr>
<tr>
<td>Cyclodienes</td>
<td></td>
<td>Photosynthesis inhibition</td>
<td>Hill reaction of electron transport</td>
</tr>
<tr>
<td>Herbicides</td>
<td></td>
<td>Photosynthesis inhibition (light reaction)</td>
<td>Reducing side of photosystem I</td>
</tr>
<tr>
<td>Ureas, cyclic ureas, triazines, acylanilides, phenylcarbamates triazinones</td>
<td></td>
<td></td>
<td>Carotene accumulation</td>
</tr>
<tr>
<td>Bipyridiniums</td>
<td></td>
<td></td>
<td>Fatty acid synthesis</td>
</tr>
<tr>
<td>Pyridazinones</td>
<td></td>
<td></td>
<td>Microtubule formation</td>
</tr>
<tr>
<td>Chloroacetamide</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dinitroanilines, phosphoric amides, chlorthalidimethyl propyzamide, cholchicine, terbutol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophenols</td>
<td></td>
<td>Multiple inhibiting actions</td>
<td>Phosphorylation, protein synthesis, lipid biosynthesis Mitochondrial ATPase</td>
</tr>
<tr>
<td>Tributyl tins, tralkyl tins</td>
<td></td>
<td>Respiratory system inhibition</td>
<td></td>
</tr>
</tbody>
</table>

[Adopted from De Lorenzo et al. (2001)]
Table 2: Toxicity of selected pesticides and their major metabolites

<table>
<thead>
<tr>
<th>Pesticide factor</th>
<th>Major metabolite</th>
<th>Toxicity (mg/kg)*</th>
<th>Detoxification factor (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphates</td>
<td>p-nitrophenol</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td></td>
<td>350</td>
<td>122</td>
</tr>
<tr>
<td>Azinphos-methyl</td>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>anthranilic acid</td>
<td>4620</td>
<td>824</td>
</tr>
<tr>
<td>Carbamates</td>
<td></td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Carbaryl</td>
<td>1-naphthol</td>
<td>2590</td>
<td>7</td>
</tr>
<tr>
<td>Phenylureas</td>
<td></td>
<td>1480</td>
<td></td>
</tr>
<tr>
<td>Monuron</td>
<td>4-chloroaniline</td>
<td>300</td>
<td>1/8</td>
</tr>
<tr>
<td>Acylanilides</td>
<td></td>
<td>1800</td>
<td></td>
</tr>
<tr>
<td>Alachlor</td>
<td>2,6-diethylaniline</td>
<td>2690</td>
<td>2</td>
</tr>
<tr>
<td>Phenoxyacetates</td>
<td>2,4-dichlorophenol</td>
<td>370</td>
<td></td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
<td>580</td>
<td>2</td>
</tr>
</tbody>
</table>

* Toxicity reported as LD$_{50}$ oral, rat, mg/kg; data from Christensen (1976)
carbamates gained prominence in many developed countries. They are widely used throughout the world (Davies and Lee, 1987). Carbamates are potent inhibitors of cholinesterase and hence they are highly toxic to humans (Kuhr and Dorough, 1976).

**Phenolic compounds in the environment and their toxicity**

Phenolic compounds produced from coal gasification, chemical and petrochemical industries and oil refineries are among the most ubiquitous pollutants in industrial effluents (Swoboda -Colberg, 1995; Vijayaraghavan et al., 1995; Borja et al., 1996). Phenol is a hazardous substance and widely distributed environmental pollutant. It has been in use for disinfection for many years (Kirk and Othmer, 1978; Leonard et al., 1999).

The toxic action of phenol is always associated with the loss of the integrity of cytoplasmic membrane that results in disruption of energy transduction, disturbance of membrane barrier and related functions, and subsequent cell death (Keweloh et al., 1990; Heipieper et al., 1992). In general, several mechanisms for decreasing membrane fluidity due to various environmental stress factors have been proposed in bacterial system such as *Pseudomonas putida* (Heipieper et al., 1992), *Escherichia coli* (Keweloh et al., 1991) and *Vibrio* species (Okuyama et al., 1991). These include an increased degree of saturation of fatty acids, conversion of *cis*-unsaturated fatty acid to the *trans* isomer, and alteration of the polar head groups of phospholipids.

**Heavy metals, pesticides and phenolics pollution in India: An overview**

Various environmental problems due to heavy metal pollution in India have been reported (Chandra, 1980; Tyagi et al., 1999). The global environmental
system sponsored by WHO and UNEP reported the higher levels of various metals i.e. Fe, Mn, Cr, Cd, Hg, Pb, As and Cu in drinking water of three Indian cities i.e. Bombay, Delhi and Calcutta as compared to WHO permitted levels (GEMS, 1988).

According to a study conducted by Ministry of Environment and Forests, Govt. of India, it is reported that 17 categories of industries were responsible for pollution of surface water in India during the last couple of years (SOER, 2001). They are Aluminium, Copper, Iron and steel, Zinc, Caustic, Cement, Dyes, Fertilizers, Pesticides, Pharmaceutical, Refinery, Textiles, Petrochemical, Paper and pulp, Sugar, Distillery and Leather Industries.

On the other hand, the CPCB, has also been monitoring water quality of national aquatic resources. The monitoring data obtained by CPCB revealed that organic and bacterial contamination were the critical pollution factors in the Indian water resources. This was mainly due to discharge of untreated waste waters from the urban agglomeration (SOER, 2001).

Application of sewage sludge on agricultural, arable fields for enhancing productivity has been a common practice in India for many years and has adverse long term effects on soil microorganisms. During the last few decades the toxicity of heavy metals to plants and microorganisms has drawn the attention of many environmental scientists because of the tendency of uptake of toxic metals and metalloids like Cd, Cu, Co, Ag, Ni, Pb, Zn, Fe etc. by food crops and plants (Bhowals et al., 1987; Athar and Ahmad, 2002). A number of small and large scale industries in Aligarh are reported to spill large amount of heavy metals into the sewage in the form of industrial wastes (Ajmal et al., 1983; Malik and Ahmad, 1995). Tyagi et al. (1999, 2000) reported that the
concentration of heavy metals in industrial areas in India is much higher than the permissible limit of World Health Organization. They have also reported that these metals in the ground water have caused various diseases in human beings and also disturb the metabolic functions.

The most important pollutants among the environmental toxicants in India are the organochlorine and organophosphorus pesticides because of their massive use for the agricultural purposes. A high proportion of pesticides used in India is applied on crops and only a small quantity is used for soil and seed treatment. Soil contamination, however, has become a serious problem with the persistence of organochlorine and some organophosphorus pesticides (Sahai and Chauhan, 1977; Rehana et al., 1995; Nawab et al., 2003). Lindane (γ HCH) was banned in 1976 but still India, China, Japan, Pakistan, Brazil, some African countries, Canada and most European countries used lindane. India, China and Japan were the most polluted countries by lindane, and India was the highest contaminated country in 1990 (Li, 1999).

**Bioremediation/detoxification of pollutants**

The use of microorganisms in removing or detoxifying the pollutants, mainly the contaminants of soil, water or sediment which may otherwise threaten public health, is known as bioremediation (Gupta and Mukerji, 2001).

Bioremediation has been used as a strategy of using microorganisms for complete transformation of organic pesticides to harmless end products such as CO₂ and H₂O. Similarly, microorganisms can transform inorganic pollutants, not necessarily completely, but to compounds with decreased solubility, mobility and toxicity (Kamaluddeen et al., 2003).
Heavy metals, pesticides and phenolics contamination due to natural and anthropogenic sources is a global environmental concern. Release of heavy metals without proper treatment poses a significant threat to public health because of its persistence, biomagnification and accumulation in food chain. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and ecofriendly nature (Rajendran et al., 2003).

Microbial interactions with heavy metals and their applications in detoxification/bioremediation have been exhaustively reviewed (Ehrlich, 1997; Chen et al., 2002). The microorganisms have the capacity to remove, immobilize or detoxify metals and radionuclides through various mechanisms (Ji and Silver, 1995; Srivastava et al., 1998). The detoxification of metals by microbes has been attributed to a number of processes which include oxidation-reduction, complexation, methylation and reactions involving biosurfactants (bioemulsifiers) and sidrophores, as discussed and summarized by Francis (1990). In response to metal toxicity, many microorganisms have developed unique mechanisms to resist and detoxify harmful metals. These mechanisms of resistance may be intracellular or extracellular and may be specific to a particular metal, or a general mechanism operating for a variety of metals (Fig. 1).

The application of individual microbes and microbial communities in the large scale treatment of domestic and industrial wastes for the removal of heavy metals is well documented. In this context Markandey et al. (2002) have reviewed the efficiency of metal removal with particular reference to Cr and Pb bioremediation.
Fig. 1. Metal resistance mechanisms developed by various microorganisms
[Adopted from Roane et al. (1996)]
The role of microbes in the removal of toxic organic pollutants from natural environment involves the process of biodegradation to a large extent. This process has also been referred to as bioremediation, bioreclamation and biorestoration. Nature plays an important role in modulating the microbial activity which has been shown to be greatly influenced by environmental factors. The purpose of bioremediation, therefore, is to minimize these environmental pollutants (Timmis et al., 1994). It is to be emphasized here that any biodegradation operation to be undertaken must compete economically and functionally with other types of physical and chemical processes available in the removal of pollutants. For soils, these methods include thermal treatment (incineration) and solvent or detergent washing technique etc. (Amend and Lederman, 1992). Bioremediation, however, provides a reliable and a low cost alternative.

The degradation of pesticides by microorganisms and the mechanisms involved in this process have been extensively reviewed (Kumar and Mukerji, 1996; Kumar et al., 1996). Various reports on the chemistry, degradation and mode of action of phenoxyacetate herbicides are also available in literature (Loos, 1975; Sandmann et al., 1988; Mai et al., 2001).

Most of the organic chemicals/pollutants are subject to enzymatic attack by living organisms which may lead to the transformation of these compounds, although the end products may differ drastically (Alexander, 1994).

Microbial degradation of phenolic compounds has also been documented lately, albeit, only at low concentration. Bacteria, algae, fungi and yeast have all been reported to degrade phenolic compounds. Among them are the Pseudomonads (Kotturi et al., 1991; Hinteregger et al., 1992), Bacillus sp.
(Gurujeyalakshimi and Oriel, 1989), *Alcaligenes* sp. (Hughes et al., 1984), *Streptomyces* sp. (Antai and Crawford, 1983), *Trichosporon* sp. (Neujahr and Varga, 1970), *Candida* sp. (Neujahr et al., 1974), *Ochromonas* sp. (Semple and Cain, 1996), and the most extensively studied are the *Pseudomonas* species (Yap et al., 1999).

Enzymatic approach in the removal of toxic chemicals from water bodies has generated a lot of attraction lately. The peroxidase from horse radish has been found to efficiently remove phenol and aromatic amines from aqueous solution (Klibanov et al., 1983) and to decolorize from phenolic industrial effluents (Davis and Burns, 1990). Phenols and aromatic amines are intermediary products in the degradation pathway of pesticides and other aromatic compounds, and these intermediates can be polymerized by phenoloxidases (Berry and Boyd, 1985). The enzyme catalyzed polymerization can be considered a detoxification reaction, which could be applied to terrestrial and aquatic environments.

**Immobilization of bacterial cells for the bioremediation/detoxification of water pollutants**

Immobilized cells are defined as cells that are entrapped within or associated with an insoluble matrix. Mattiason (1983) discussed six general methods of immobilization viz. covalent coupling, adsorption, biospecific affinity, entrapment in a three dimensional polymer network, confinement in a liquid-liquid emulsion, and entrapment within a semipermeable membrane. Under many conditions, immobilized cells have advantages over either free cells or immobilized enzymes. Catalytic stability can be greater for immobilized cells
and some immobilized microorganisms tolerate higher concentration of toxic compounds than do their non-immobilized counterparts (Westmeier and Rehm, 1985; Ignatov et al., 2002).

Immobilization provides an increased resistance of substrates and products to diffusion through immobilization matrices. Although cell immobilization is commonly used in processes for microbial production of specialty chemicals (Wood and Calton, 1984), few studies have addressed the use of immobilized cells to degrade toxic compounds too (Westmeier and Rehm, 1985; Cho et al., 2000; Prieto et al., 2002).

In biological waste water treatment, immobilization of microorganisms has gained a great deal of attraction. Adsorption of organisms on activated carbon is one way which has the advantage that microbial resistance to compounds like phenol is increased because the pollutant itself is bound and thus easily removed from the water phase (Ehrhardt and Rehm, 1985).

The entrapment of cells in alginate is another promising method for microbial degradation of toxic substances (Westmeier and Rehm, 1985). These biocatalysts are also favourable for investigation of the physiology of immobilized microorganisms. The immobilization method is not toxic to the cells, and for the dissolution of gel particles, and thus the liberation of the immobilized cells, is easy and rapid (Vorlop and Klein, 1983).

The degradation of phenol by Pseudomonas putida, immobilized in calcium alginate, was examined by Bettmann and Rehm (1984). They found better degradation rates in immobilized cells than in free cells. Moreover, the immobilized bacteria could be exposed to higher phenol concentration without
loss of cell viability. The reasons for these phenomena however, were unknown (Keweloh et al., 1989). Asthana et al. (1995) reported that Ca-alginate immobilization method was very effective in nickel biosorption.

Mariano et al. (1999) have demonstrated the ability of immobilized bacterial strains in the biotransformation of aromatic compounds by barium alginate beads. On the other hand, bacterial cells immobilized by calcium alginate beads were found to be non-responsive to the toxic effects of phenol (Keweloh et al., 1989). The degradation of 3-chloroaniline by calcium alginate immobilized cells of *Pseudomonas acidovorans* CA28 was described by Ferschl et al. (1991). Immobilized *Pseudomonas putida* US2 was also found to be beneficial in the biodegradation of 2-chloroethanol (Overmeyer and Rehm, 1995). A comparative analysis of cadmium biosorption on free and immobilized cells in alginate beads was carried out by Lebeau et al. (2002). Phillip et al. (2000) on the other hand discussed the suitability of several commonly available, inexpensive matrices for immobilization of *Pseudomonas aeruginosa* cells. Several workers demonstrated a better remediation of heavy metals by the immobilized organisms as compared to free cells (Sag et al., 1995; Tsekova and Ilieva, 2001).

**Role of various living organisms in the degradation/detoxification of major Indian water pollutants**

A brief outline of various organisms used for detoxification of the major toxicants of Indian water bodies i.e. heavy metals, pesticides and phenolics are presented below.
1) **Algae and fungi:** Among the green algae, the genus *Chlorella* has been identified as a potential candidate for metal biosorption (Ting et al., 1989; Aksu and Kutsal, 1991). Gale (1986) reported effective removal of Pb by blooms of *Chlorella, Cladophora, Rhizoclonium, Hydrodictyon, Spirogyra* and *Oscillatoria*. Tsuji et al. (2002) have recently demonstrated that the synthesis of phytochelatins in a marine alga, *Dunaliella tertiolecta*, is strongly induced by Zn. Pretreatment of the cells with Zn enhanced the tolerance towards toxic heavy metals such as Cd, Hg, Cu and Pb. A marine diatom *Skeletonema costatum* was reported to degrade 2,4-dichlorophenol (Yang et al., 2002). Scarano and Morelli (2002) characterized cadmium and lead phytochelatin complexes formed in a marine microalga in response to metal exposure. Brown alga *Fucus serratus* was found to accumulate arsenate readily and transformed it into several arsenic compounds depending on the exposure concentration (Geiszirger et al., 2001). Several research groups have recently studied the possibility of reducing the toxicity of many aromatic compounds (e.g. pesticides, phenols, disinfectants etc) of polluted environment using various fungi (Valli and Gold, 1991; Kissi et al., 2001; Fountoulakis et al., 2002).

2. **Plants:** Biodegradation of organic xenobiotics by plants were reviewed by Zaalishvili et al. (2000). They further described the detoxification pathways operating in plants and their role in remediation of the biosphere. Moreover, Cobbett and Goldsbrough (2002) reviewed the roles of phytochelatins and metallothionein in heavy metal detoxification and homeostasis. It is an established fact that plants are capable of undertaking detoxification processes in air, soil and water (Arthur and Coats, 1998; Salt et al., 1998; Memon et al., 2001).
3. **Bacteria:** Role of bacteria in the biodegradation and detoxification of the toxicants is well documented (Lai et al., 1995; Johri et al., 1996). Matsumura et al. (1976) first reported aerobic degradation of HCH, a persistent pesticide, by a *Pseudomonas* strain. Later on, its degradation by a *Pseudomonas paucimobilis* was reported by Wada and coworkers (1989). The role of *Pseudomonas* species in the biodegradation of γ-HCH is also well established (Imai et al., 1989; Nawab et al., 2003).

The bacteria involved in the metabolism of 2,4-D and its other derivatives have been extensively studied by several investigators (Oh and Tuovinen 1991a; Roane et al., 2001; Cho et al., 2002).

Biodegradations of carbamate pesticides by different bacteria were also demonstrated by several workers (Larkin and Day, 1986; Hayatsu et al., 1999; Hanumanthanaik et al., 2001).

Detoxification of Cd by *Arthrobacter*, *Bacillus* or *Pseudomonas* spp. has been reported by Roane and Pepper (2000). Cadmium resistance with the probable ability of detoxification in *Pseudomonas putida* was also reported by Lee et al. (2001). Moreover, Wang et al. (1997) have reported the use of *Pseudomonas aeruginosa* for the removal of cadmium.

Detoxification of Cr by means of microbes has been demonstrated by several investigators (Shen and Wang, 1993; McLean and Beveridge. 2001; Megharaj et al., 2003; Viti et al., 2003).

Saxena and coworkers have recently isolated a *Pseudomonas putida* strain from copper mines which is capable of precipitating copper and thus an attractive candidate for bioremediation of copper (Saxena and Srivastava, 1998; Saxena et
The general heavy metal sorption mechanisms operating in various microbes are shown in Fig. 2.

Detoxification of phenols by *Comamonas testosteroni* strain has been reported by Yap et al. (1999). Moreover, *Pseudomonas putida* has also been demonstrated to degrade phenols (Allsop et al., 1993). Detoxification of the phenolics like pentachlorophenol by *Pseudomonas* sp. Bu34 has also been reported by Lee et al. (1998). Catechol degradation was clearly demonstrated by Rider et al. (1998) using a *Pseudomonas putida* strain. Moreover, O'Reilly and Crawford (1989) earlier reported the degradation of p-cresol by an immobilized *Pseudomonas* sp.

**Applications of *Pseudomonas fluorescens* strains in detoxification of water pollutants**

*Pseudomonas fluorescens* is a non pathogenic soil bacterium, it produces greenish fluorescent pigment. Various *Pseudomonas fluorescens* strains have been reported to have potential in detoxifying certain organic and inorganic water pollutants. This soil bacterium was found to be especially useful in combating heavy metal pollution (Bopp and Ehrlich, 1988; Joshi-Tope and Francis, 1995). Several investigators have also studied the mechanism of heavy metal and pesticide bioremediation in *Pseudomonas fluorescens* (Zablotwicz et al., 2001; Hamel and Appanna, 2003).

Multiple metal tolerance in *Pseudomonas fluorescens* and its biotechnological significance have been the subject of study for Appanna and coworkers (Appanna et al., 1995; Appanna and Hamel, 1996; Appanna et al., 1996). Moreover, the role of phosphate in the culture medium of *Pseudomonas fluorescens* ATCC13525 was also ascertained on the extracellular and
Fig. 2. Microbes mediated heavy metal sorption mechanisms
[Adopted from Rajendran et al. (2003)]
intracellular accumulation of lead (Al-Aoukaty et al., 1991). Lopez et al. (2000) demonstrated the effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39. Shah and Thakur (2003) carried out the enzymatic dehalogenation of pentachlorophenol by *Pseudomonas fluorescens* of the microbial community from tannery effluent. *P. fluorescens* was also found to utilize pentachlorophenol as a carbon source (Shah and Thakur, 2003). Utilization of petroleum hydrocarbons by *P. fluorescens* isolated from a petroleum contaminated soil was further reported by Barathi and Vasudevan (2001). Wedemeyer (1966) was also able to demonstrate the uptake of 2,4-D by some *Pseudomonas fluorescens* strains.

**Objectives of the present study**

In view of the literature survey conducted on the water pollutants and their bioremedial strategies employed by the earlier workers, we laid down the following objectives for the bioremediation of major water pollutants in India:

1. Isolation of the naturally occurring *Pseudomonas fluorescens* strain from the highly polluted soil.

2. Characterization of the tolerant isolate in terms of the multiplicity as well as the potency of the resistance markers.

3. Role of plasmid in the tolerance, bioremediation and detoxification of the major toxicants of Indian waters.

4. Suitability of the test isolate in terms of the efficiency of bioremediation and detoxification of the test toxicants.

5. Suitability of the immobilized cell system for the purification of contaminated water.