Chapter I:

Review of literature
The rapid increase in populations worldwide has resulted in the need for greater fuel demand and development of industrial chemicals, fertilizers, pesticides and pharmaceuticals to sustain and improve quality of life (Chakrabarty et al., 1988). Although many of these chemicals are utilized or destroyed, a high percentage is released into the air, water and soil, representing a potential environmental hazard (Alexander, 1995; Anwar et al., 2009). Consequently, earth’s natural resources are not only being depleted, but are also becoming polluted and unfit for human use. The use of pesticides has benefitted modern society by improving the quantity and quality of the world’s production while keeping the cost of that food supply reasonable. Unsurprisingly, pesticide use has become an integral part of modern agricultural systems. Because of continuous pest problems, their usage possibly cannot be discontinued in the near future. Extensive and improper use of these chemicals had already caused considerable environmental pollution and leads to greater health risk to plants, animals and human population which have been reviewed from time to time by several workers (Ajaz et al., 2005; Bhagobaty et al., 2007; Chowdhary et al., 2008; Murugesan et al., 2010). Unfortunately, it is not possible within a short time to replace all the industrial processes generating polluting waste streams with clean alternatives. Therefore, treatment both at source and after release, whether accidental or not, must be considered as alternatives in many cases (Betts, 1991). Current legislation and recent waste management strategies have placed significant emphasis on waste minimization, recycling and remediation rather than disposal (Colleran, 1997; Barbagallo et al., 2001; Baczynski and Pleissner, 2010). The persistence of organo-xenobiotics in the environment is a matter of significant public, scientific and regulatory concern because of the potential toxicity, mutagenicity, carcinogenicity and ability to bioconcentrate up the trophic ladder. These concerns continue to drive the need for the development and application of remediation techniques (Colleran, 1997).

Chemical pesticides have contributed greatly to the increase of yields in agriculture by controlling pests and diseases and also towards checking the insect-borne diseases (malaria, dengue, encephalitis, filariasis, etc.) in the human health sector (Abhilash and Singh, 2009). Owing to their efficiency; these compounds were considered a boon to the fields of agriculture and medical entomology. Organochlorine insecticides are more toxic to insects and less toxic to non-target organisms, but these chemicals can damage a wide variety of beneficial as well as
harmful organisms due to their persistence in the environment (Nawab et al., 2003). Therefore, organochlorine insecticides have important ecological effects in addition to those usually intended. Among these, the interaction of pesticides with microorganisms is important, since microorganisms are involved in many basic ecological processes, such as biogeochemical cycles, decomposition processes, energy transfer through trophic levels, and numerous microbe-microbe, microbe-plant, and microbe-animal interactions (Prakash et al., 2004; Lal et al., 2007).

Millions of tons of pesticides are applied annually; however, less than 5% of these products are estimated to reach the target organism, with the remainder being deposited on the soil and non-target organisms, as well as moving into the atmosphere and water (Pimental and Levitan, 1986). Microbes and plants are among the most important biological agents that remove and degrade waste materials to enable their recycling in the environment. Soil microflora, mainly bacteria, fungi, algae and protozoa make a valuable contribution in making the soil fertile through their primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrients content of soil. Pesticides that disrupt the activities of the soil microorganisms could be expected to affect the nutritional quality of soils and would therefore have serious ecological consequences (Handa et al., 1999). The fate of the pesticides in the soil environment in respect of pest control efficacy; non-target organism exposure and offsite mobility has become a matter of environmental concern (Hafez and Thiemann, 2003) potentially because of the adverse effects of pesticidal chemicals on soil microorganisms (Araújo et al., 2003) which may affect soil fertility (Schuster and Schröder, 1990). Pesticide should be toxic only to the target organism, be biodegradable and undesirable residues should not affect non-target surfaces. However, the use of pesticide has been minimized or terminated in technologically advanced countries due to their persistence in nature, susceptibility to biomagnifications, and toxicity to higher animals. But the ever-increasing world population and poor health conditions especially in developing countries, may outweigh the disadvantages caused by the extensive use of these insecticides (Handa et al., 1999; Prakash et al., 2004; Lal et al., 2007).

**Pesticide pollution**

Pesticide consumption in India has increased from 2353 MT in 1955 to 40,672 MT in 2005 for technical grade chemical pesticides. In March, 2005, 186 technical grade pesticides were registered in the country for use under section 9 (3) of
Insecticides Act, 1968. (Directorate of Plant Protection and Quarantine, Govt. of India). Indian pesticide industry has achieved the status of second largest basic pesticide manufacturer in Asia after Japan. Interestingly, India’s consumption of pesticides per hectare is low (0.5 kg/ha) when compared with world averages like those of Korea (6.60 kg/ha) and Japan (12.0 kg/ha). According to the pesticide industry statistics, India spends only $3/ha on pesticides compared to $24/ha spent by the Philippines, $255/ha by South Korea and $633/ha by Japan (TERI, 2000). However, the contamination of food products in the country is alarming. About 20% of Indian food products contain pesticide residues above tolerance level compared to only 2% globally (TERI, 2000). This is primarily due to their non-judicious use in certain areas/states, lack of awareness and inadequate information dissemination amongst the farming community.

Chattopadhyay studied “Insecticide and pesticide pollution of food stuffs and their toxic effect on man” (Chattopadhyay, 1998). He reported more than 40 pesticides in use in agriculture in Panjab, India. Experimental samples were prepared by spraying various vegetables like cabbage, cauliflower, brinjal, etc., with pesticides like quinalphos, malathion, methyl carbaryl, phosphamidon, dimethoate, dichlorovos and phorate and exposed to various environmental conditions to determine their degradation time. He observed that persistence of the insecticides was temperature dependent, whereas residues of malathion and quinalphos were observed even after one week when the temperature ranged between 15°C and 22°C, phosphamidon, dimethoate and dichlorovos were detected only upto 4/5 days, when temperature range was 30.8°C to 42.6°C. He recommended at least 10 days gap between last application of the pesticide on crop and its harvest.

Chahal et al. (1999) investigated the levels of insecticide residues in vegetables. They collected 197 samples of various vegetables from farmer fields when these were ready for transportation to the market. Their results indicated that 45 out of 65 samples of Brinjal were contaminated with different insecticides and 24 of these contained residues above their maximum residue limits (MRLs). In case of cabbage, 25 samples were found to be contaminated with monocrotophos, methyl parathion, quinalphos, chloropyriphos, cypermethrin, fenvalerate, deltametrin and endosulfan. Out of these, 19 samples contained insecticides above MRL values. Seven out of 17 okra samples were contaminated with methyl parathion, quinalphos, monocrotophos, triazophos, chloropyriphos and fenvalerate, though none exceeded their respective
MRLs. Three out of five samples of potato contained residues of dichlorovos above its MRLs of 0.5 mg/kg. Residues of phosphamidon in one and quinalphos in two samples of tomato out of 25 samples were found to exceed their respective MRLs. All the samples were also analysed for the presence of DDT and HCH/BHC residues; however, their residues were below the detection limit of 0.001 mg/kg. The study revealed that 70% of vegetables were contaminated with different insecticides and about 27% of samples contained residues above their respective MRLs.

Environmental pollution of natural waters by pesticide residues is of great concern. Pesticides are one class of compounds that, despite their benefits, may produce a wide range of toxic side effects that pose a potential hazard to the environment (Kolpin et al., 1998; Golfinopoulos et al., 2003; Zhao et al., 2007). Hans et al. (1999) tested soil samples from dry bed of the River Ganges at Kanpur (India) and reported pesticides hexachlorocyclohexane (HCH), dichlorodiphenyl trichloroethane (DDT) their isomers and metabolites in all the samples. Mean levels of 109.35, 136.76 and 145.93 µg HCH/kg and 6.64, 49.3 and 46.70 µg DDT/kg were obtained in the rural upstream, city and downstream industrial areas, respectively. Prakash et al. (2004) evaluated 45 soil samples of surface and subsurface soils from agricultural sites of Delhi, Haryana, Haridwar, Uttar Pradesh and also around the pesticide industry, Chinhat industrial area, Lucknow, (India) for the presence of residues of HCH isomers. They found residues of β-HCH (2.5 µg/kg to 463 mg/kg of soil) in 39 of the 45 soil samples, γ-HCH (0.08 µg/kg to 43.00 mg/kg) in the remaining soil samples, whereas α-HCH (0.04 µg/kg to 98.00 mg/kg of soil) and δ-HCH (0.07 µg/kg to 458.00 mg/kg of soil) were detected less frequently. Aleem and Malik (2005) found the pesticides BHC, DDE, and DDT as 25, 1.6, and 8.8 ng/l, respectively, in the Yamuna water at Delhi. The River is highly polluted with domestic as well as industrial waste at Delhi. Pandit et al. (2002) reported persistent organochlorine pesticides in marine waters at Mumbai. They found HCH, DDE, and DDT as 5.42, 0.87, and 12.45 ng/l, respectively. All the sources showing pesticide pollution may have the possibility by wastewater contamination. Ansari and Malik (2009a) evaluated industrial wastewater samples of Ghaizabad City, India and found that the concentration of pesticides (organochlorines) BHC, DDE, DDT, Dieldrin, Aldrin, and Endosulfan were of 27.06, 15.82, 18.41, 3.20, 0.93, and 1.82 ng/l, respectively and organophosphorus; Dimethoate, Malathion, Methylparathion, and Chlorpyriphos were 0.99, 1.75, 1.92, and 0.98 ng/l, respectively.
**Beneficial effects of pesticides**

Pesticides are a mixture of substances applied for the preventing, destroying, repelling or mitigating any pest. Pesticides have had a key role in improving productivity to such an extent that India, a farmer country of famine has quadrupled grain production since 1951 (Jha and Chand, 1999) and now not only feeds itself but exports produce (100 m tonnes in 2003 - Indian export stats). Similarly outputs and productivity have increased dramatically in most countries, for example, wheat yields in the United Kingdom rose from 2.5 t/ha in 1948 to 7.5 t./ha in 1997 (Austin, 1998). Corn yields in the USA went from 30 bushels per acre to over a hundred per acre over the period from 1920 to 1980. Increases in productivity have been due to several factors including use of fertiliser, better varieties and use of machinery. However pesticides have been an integral part of the process by reducing losses from the weeds, diseases and pests that can markedly reduce the amount of harvestable produce.

Webster *et al.* (1999) stated that "considerable economic losses" would be suffered without pesticide use and quantified the yield increases and 50% increase in gross margin that result from pesticide use in British wheat production. Schmitz (2003) explored the potential effect on European crop productivity of possible EU legislation to reduce pesticide usage. He concluded that the evidence for the benefits of a reduction strategy were unconvincing and an imposed cut of 75% would reduce productivity to catastrophically low levels, that is, by between one third and two thirds of the original production levels.

Insecticides are used to control insect pests that feed on crops or carry plant diseases and thus prevent huge losses. Fungi are one of several causative agents of diseases of crops that can reduce yield and quality of food crops (Agrios, 2002) and they sometimes produce toxic compounds making the produce unfit to eat. Fungicides now routinely protect many crops throughout the world from various diseases like late blight, powdery and downy mildew, leaf spot, scab, canker, rust, Botrytis grey mould. Weed competition is the major constraint that limits yield in many crops. Herbicides, i.e., chemical substances used to kill or control unwanted vegetation, are the most widely used type of pesticide and comprise around 50% of all crop protection chemicals used throughout the world, compared with insecticides and fungicides that are around 17% each. Timely weed control was found to be essential for better crop establishment, tillering, vigorous growth, higher fertilizer efficiency and increased
yield. There would be estimated US $13.3 billion loss in farm income in the US without timely application of herbicides (Anon, 2003). Yancy (2005) put the figure higher for benefits of herbicide use at $21 billion annually, against a cost of $6.6 billion for the product and application, thereby reducing losses to weeds by 23% and reducing loss of farm income valued at $8 billion. Chemical weed control reduced soil erosion by 400% (40 tonnes/ha) and provided an average production increase of 16% due to increased soil moisture and reduced root damage compared with tilled systems.

**Hazards of pesticides**

Pesticides have resulted in serious health implications to man and his environment. The high risk groups exposed to pesticides include the production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of health hazards may be higher because the processes involved are not risk free. In industrial settings, the workers are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents and inert carrier (Handa et al., 1999; Das et al., 2003).

Studies suggest that pesticides may be related to various diseases, including cancers, as well as having neurological, mental and reproductive effects. Exposure to the pesticides can also lead to immune system disorders in humans. Children may be more susceptible to the effects of pesticides due to increased exposure via food and breast milk, underdeveloped detoxification pathways, and longer life expectancy in which to develop diseases with long latency periods (Cohen, 2007).

Most of the pesticides used are organic molecules with hydrophobic properties resulting in rapid sorption of the pesticide molecules to the soil particles that can be washed into the water. After the pesticides reach the aquatic ecosystem in dissolved form, they associate with organic matter in suspension or sediment. Sorbed pesticide molecules tend to be less degradable than their dissolved forms; since they are less accessible to the degrading action of microorganisms, UV-light and dissolved oxidative chemicals (Ying and Williams, 2000).

**Impact on soil microorganisms and plants**

In modern agriculture practices, application of insecticides which belong to diverse chemical groups has become a common practice to fight against insect pests for the treatment of soil and seed. The application of such insecticides causes their accumulation in soils and affects directly or indirectly the soil enzyme activities and physiological activities of non-target soil microbiota leading thereby to the losses in
fertility of soils (Martijin et al., 1993). For the fertility and plant growth, soil microorganisms are an important and diverse community that catalyses many processes (Yedla and Dikshit, 2001). There are many processes that are important for cycling of the nutrients from the soil and fertilizers and transfer of nutrients directly to the crops by microbes Therefore, these microbial soil communities are greatly influenced by various factors including the agrochemicals. In modern agricultural trends, agronomists recommend pesticides in order to augment the productivity of various crops. Zahran (1999) and Srinivas et al. (2008) reported that continuous and abundant use of synthetic pesticides has become a major threat to beneficial soil microbes and in turn affects the sustainability of agricultural crops. It is globally, a greater concern that, how to minimize or reduce the effects of pesticides so that the consequential impact of these chemicals on the microorganisms involved in nutrient cycling, vis-a-vis the productivity of crops could be saved. Rajagopal et al. (1984) have reported both innocuous and inhibitory effects of certain pesticides on soil bacteria depending on the concentrations used.

**Heavy metal pollution**

Rapid industrialization and urbanization have resulted in elevated emissions of toxic heavy metals and radionuclides to the biosphere. Inorganic toxicants may occur as cations of metals such as mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), and uranium (U). Toxic inorganics may also include alkylated or aromatized forms of metal ions, such as methylmercury and phenylmercury. The increasing quantities of toxic metals emitted into the biosphere pose potential hazards to ecosystems and influence the metabolism of living organisms (Gazso et al., 2001; Ansari and Malik, 2007).

Heavy metals pose a significant threat to the environment and public health because of their toxicity and their accumulation in soil and food chains (Ceribasi and Yetis, 2001; Chen et al., 2009; Gurel et al., 2010). Metal pollution of the biosphere by toxic metals has accelerated dramatically since the industrial revolution (McIveen and Negusanti, 1994). Industrialized countries are increasingly concerned regarding the occurrence of toxic metals in the environment. The most effective policy to minimize their release from industrial or agricultural sources is the adoption of low waste-generating technologies coupled with effective effluent purification processes (Fourest et al., 1994; Sag et al., 2000).
In recent years, public awareness has increased regarding the long-term effects of wastewater containing toxic elements. Numerous industrial processes generate aqueous effluents contaminated with heavy metals. Metal concentrations must be reduced to meet ever increasing legislative standards and recovered where feasible. According to the World Health Organization, the metals of most immediate concern are Hg, Cr, Cd, Pb, Ni, aluminum (Al), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), and zinc (Zn) (Allen and Brown, 1995; WHO, 2002).

Many researchers have reported heavy metal pollution in soil, especially in agricultural lands in different parts of the world (Fabiani et al., 2009; Sun et al., 2009; Yang et al., 2009). Fossil fuel combustion, mineral mining and processing, and generation of industrial effluents and sludges, biocides and preservatives release a variety of toxic metal species into aquatic and terrestrial ecosystems, and this can have significant effects on biota (Wainwright et al., 1997; Pokrovsky et al., 2008; Fabiani et al., 2009). Metal-rich habitats also occur due to natural localized ores and mineral deposits, and weathering of rocks, minerals, soil, and sediments are a vast reservoir of metals. Restoration of metal-polluted habitats requires a functional microbial community for plant community establishment, soil development, and biogeochemical cycling.

Heavy metals are nondegradable and exist in number of inorganic and organic forms. Some heavy metals such as Fe, Cu, and Zn are essential trace elements but others, such as Cd and Pb, have no beneficial biological function and are toxic even in very small amounts. Cadmium, Pb, and Hg are regarded as the most toxic of the heavy metals. Another elemental toxicant, arsenic (As), is sometimes regarded as a heavy metal, although strictly speaking, it is a metalloid. Contamination of soil and water by heavy metals has significant relevance because metals cannot be degraded like most organic pollutants and they accumulate in terrestrial, aquatic and marine food chains (Smejkalova et al., 2003; Ortega-Larrocea et al., 2007). Metals such as Cd, Cr, Pb, Hg, As, copper (Cu), zinc (Zn), and nickel (Ni) are continuously being added to soils through agricultural activities such as long-term application of urban sewage sludge, and industrial activities such as waste disposal, waste incineration, and through vehicle exhausts. These sources cause the accumulation of metals and metalloids in soils and pose threats to food safety and public health due to soil-to-plant transfer of metals.
Metals cause detrimental effects on both aquatic and terrestrial ecosystems and human health due to their mobilities and solubilities which determine their speciation (Kabata-Pendias, 1992; Del Val et al., 1999). In some cases, soil may be contaminated to such an extent that it may be classified as a hazardous waste (Berti and Jacob, 1996). Soil contamination with heavy metal mixtures is receiving increasing attention from the public as well as governmental agencies, particularly in developing countries (Yanez et al., 2002; Khan, 2005).

**Pesticide Tolerance in Bacteria**

A process of developing tolerance to pesticides is a complex physiological and genetic phenomenon, on the basis of which a temporary and permanent tolerance can be distinguished (Chowdhary et al., 2008). Microorganisms that developed tolerance to xenobiotics (pesticides), are frequently capable of biodegrading them (Bellinaso et al., 2003; Anwar et al., 2009) and are able to remediate soil (Juhasz et al., 2002; Baczynski and Pleissner, 2010).

Elimination of halogens from halogenated xenobiotic molecules is a key step in their degradation; because the carbon-halogen bond is relatively stable (Fetzner and Lingens, 1994; Anwar et al., 2009). Reports have shown that bacteria, mainly *Pseudomonas* isolated from aerobic soils could degrade not only the γ-isomer (Senoo and Wada, 1989) but also the α- and β-isomers of HCH under aerobic conditions (Sahu et al., 1990; Nawab et al., 2003; Lal et al., 2007). The rapid disappearance of γ-benzenehexachloride from soil and aquatic environments has been attributed to its susceptibility to degradation by microorganisms (Johri et al., 2000; Baczynski and Pleissner, 2010). Evidence from soil degradation studies indicates that cleavage and mineralization of the heterocyclic ring of organophosphates occur in soil due to the activities of microorganisms (Racke and Coats, 1990; Gupta et al., 1994). Tolerance of pesticides namely endosulfan, carbofuran and malathion were observed in *Pseudomonas*, *Azotobacter* and *Rhizobium* strains isolated from soil (Shafiani and Malik, 2003). Soil bacteria degrading chlorpyriphos include *Flavobacterium* sp. ATCC 27551, *Pseudomonas diminuta* strain GM and *Pseudomonas putida* (Rani and Lalitha-Kumari, 1994). Chlorpyriphos has been shown to be degraded co-metabolically in liquid medium by bacteria (Mallick et al., 1999; Horne et al., 2002; Bhagobaty and Malik, 2008). Enhanced degradation of chlorpyriphos by *Enterobacter* strain B-14 was reported by Singh et al. (2004). Yang et al. (2005) isolated
Alcaligenes faecalis DSP3, capable of degrading chlorpyriphos and 3,5,6-trichloro-2-pyridinol (TCP).

**Metal Resistance in Bacteria**

The pollution of the environment with heavy metals has led to the appearance of metal resistant microorganisms in the soil and water of industrial regions. In many cases, resistance to heavy metals is determined by plasmids, which can be used for the creation of novel strains with a high detoxifying activity against heavy metals. The investigation of such strains has greatly contributed to our knowledge of the structure and function of the determinants and mechanism of metal resistance (Pal *et al*., 2005; He *et al*., 2010). Heavy metals generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions, or modifying the active conformation of biological molecules. Copper, chromium, cadmium and nickel are known to be the most commonly heavy metals used and the more widespread contaminants of the environment (Singh *et al*., 2010a, 2010b). However, at low concentration some metals are essential for microorganisms since they provide vital co-factors for metallo-proteins and enzymes (Ganguli and Tripathi, 2002; Liu *et al*., 2006; Abou-Shanab *et al*., 2007; Begum *et al*., 2008) but high levels of them may cause extreme toxicity to living organisms due to inhibition of metabolic reactions.

Heavy metal resistance have been frequently found on plasmids of gram negative and gram positive eubacteria (Rochelle *et al*., 1989). The metal resistance is often associated with resistance to single or multiple drugs, phenolic compounds and pesticides (Lokeshwari and Chandrappa, 2006; Ansari *et al*., 2008; Begum *et al*., 2008). The known mechanisms of bacterial heavy metal resistance are (Silver, 1992):

1. Keeping the toxic ion out of the cell by altering a membrane transport system involved in initial cellular accumulation.
2. Intracellular or extracellular sequestration by specific mineral-ion binding components (analogous to the metallo-thioneins of eukaryotes and the phytochelatins of plants but generally at the level of the cell wall in bacteria).
3. Highly specific cation or anion efflux systems encoded by resistance genes (analogous to multiple resistance of animal tumor cells). This is the most commonly found mechanism of plasmid controlled bacterial metal ion-resistance.
4. Detoxification of the toxic cation or anion by enzymatically converting from a more toxic to a less toxic form. This last surprising mechanism does indeed occur, as best known for detoxification of inorganic and organomercurials. It may also be used for oxidation of As (III) and reduction of Cr (VI) to less toxic forms, but these known microbial processes have not been associated with plasmids (Liu et al., 2006).

The concentration of metal pollutants in the environment is low excepting in specific area which are polluted by various hospital and industrial wastes. However, unpolluted environments may also harbor metal resistant organisms that readily adapt to high concentration of metals. The incidence of plasmid bearing strain is more in polluted site than in the unpolluted region (Hada and Sizemore, 1981; Malik et al., 2002).

**Antibiotic resistance in bacteria**

Antibiotic resistance is a public health concern of great urgency because of growing inefficacy of antimicrobial agents to treat infectious diseases. This is mainly caused by the propagation of antibiotic resistance genes due to the overuse of antimicrobials in humans and intensive use of antibiotics in animals and agriculture (Mollen et al., 2001; Rysz and Alvarez, 2004). Several studies have reported the presence of antibiotic resistant bacteria outside the hospitals setting worldwide, namely in food, sewage, waters for recreational activities, soil, air, animals, healthy human faeces and among others (Kümmnerer, 2004; Kumar and Schweizer, 2005).

The presence of antibiotic resistance bacteria in water sources throughout the world has also been well documented. Bacteria are able to adapt rapidly to new environmental conditions such as the presence of antimicrobial molecules and as a consequences, resistance increases with the antimicrobial use (Lewinson and Bibi, 2003; Auerbach et al., 2007; Böckelmann et al., 2009). Many of the microorganisms presented multiple antibiotic resistance (Ansari et al., 2008; Baquero et al., 2008; Binh et al., 2008).

Proliferation of resistance genes can take place by vertical transfer (multiplication of cells harboring a resistance gene), and by three recognized mechanisms of prokaryotic gene transfer (transformation, conjugation, and transduction). Horizontal transfer by conjugation, or conjugation like processes, seems to be of particular importance under environmental conditions, because resistance genes are often found on plasmids that are either transferable or
mobilizable (van Elsas et al., 1990; Sørensen et al., 1996). Conjugative plasmids have been identified in bacterial populations inhabiting, for instance, the phytosphere of sugar beet, the rhizosphere of wheat, contaminated soils, River epilithon, marine sediments, marine air water interfaces, marine water, marine biofilm communities, sewage and activated sludge (Davison, 1999; Dröge et al., 1999; Ansari et al., 2008; Malik et al., 2008). Because of the fact that soil can continuously be enriched with antibiotic resistant genes, it is important to investigate the role ecosystems play in the transmission of antibiotic resistant genes. Enteric bacteria from the intestinal flora of humans and animals are spread to the soil through wastewaters and the use of manure as fertiliser (Haack and Andrews, 2000; Smalla et al., 2000; Binh et al., 2008). Furthermore, the natural production of antibiotics in soil by the micro-biota can be another potential source of selection for antibiotic resistance genes within the soil environment (van Elsas, 1992; Bahl et al., 2009).

**Bacterial Plasmids**

Plasmid is an extrachromosomal part of the DNA circular duplex molecule naturally present in some bacterial species (Amabile-Cuevas and Chicurel, 1992) ranging in size from a few to more than one thousand kilobases (kb) and can represent a large proportion of the whole bacterial genome. Screenings from different environments have revealed that the plasmid incidence in the agricultural soil, wastewater, sediments, piggery manure and wastewater treatment plant was 18, 18, 32, 34, 50 % respectively (Götz et al., 1996; Malik et al., 2002; Smalla et al., 2006; Oregaard and Sorensen, 2007; Binh et al., 2008). These accessory chromosomes can replicate independently of the host chromosome. In contrast with the host genome, they are dispensable under certain conditions. A bacterial cell may have no plasmids at all or it may house as many as 20 copies of plasmids.

Our knowledge of prevalence and diversity of bacterial plasmids has been very limited, while, well characterized plasmids are mostly belonging from clinical bacteria (Smalla et al., 2000; Grohmann et al., 2003; Smalla et al., 2006). For the characterization of plasmid, sequencing the plasmid or its part has become a straightforward approach. Recently, Ramirez-Díaz et al. (2011) determined the complete nucleotide sequence of conjugative plasmid pUM505 containing virulence and heavy metal resistance genes which was isolated from a clinical strain of *Pseudomonas aeruginosa*. The plasmid had a length of 123,322 bp and contained 138 complete coding regions, including 46% open reading frames encoding hypothetical proteins.
Plasmids isolated from environmental bacteria are often very large ranging in size up to 500 kilobases (kb), tend to be transferred by conjugation and are able to replicate in a number of different bacterial hosts (Grohmann et al., 2003; Binh et al., 2008). The traits precise by plasmids include toxic heavy metal resistance, antibiotic resistance, degradation of xenobiotic compounds, symbiotic and virulence determinants, bacteriocin production, resistance to radiation and increased mutation incidence (Snyder and Campness, 1997; Thomas, 2000; Sota et al., 2006). Therefore, by transferring genetic material, plasmids play a major role in enhancing the genetic diversity and adaptation of bacteria.

**Narrow and Broad-host-range plasmids**

The host range of conjugative plasmids has been divided into narrow host range plasmids and broad-host-range plasmids, afterwards these plasmids were habitually called promiscuous plasmids. The original definition of BHR plasmids was used to describe plasmids isolated in species belonging to the *Enterobacteriaceae* that, not only transferred to members of this family, but also to *Pseudomonas* species and *vice versa* (Datta and Hedges, 1971). Since then, many of the isolated plasmids from Gram negative bacteria have been shown to transfer and replicate among members of γ subdivisions of *Proteobacteria*. Consequently, a more stringent definition of BHR plasmids was proposed, which refers to those plasmids transferring and maintaining in bacteria from different phylogentic subgroups (Top et al., 1998). Some plasmids are able to replicate in a limited number of bacterial species they have a narrow host range. Examples are ColEl, pBR322, pUC18 plasmids which are limited to *E. coli* and some closely related species. Other plasmids are able to replicate in a wide range of bacterial species; they have a broad-host-range. The study of the difference between broad-host-range and narrow host range plasmids revealed that there was low number of restriction sites in BHR plasmids than narrow host range plasmids. Hence, owing to this characteristic, BHR plasmids easily overcome the restriction barrier of the host cells (De Gelder et al., 2008; Bahl et al., 2009). It may be possible to eventually track the plasmids history and predict from which organism the different genes or mobile genetic elements originate or have passed through. Subsequently, a more stringent definition of BHR plasmids was anticipated, which refers to those plasmids transferring and maintaining in bacteria from different phylogentic subgroups (Top et al., 1998; De Boever et al., 2007).
Since there are several examples of plasmid transfer to recipient strains where the plasmid cannot replicate, it is possible that the key difference between BHR plasmids and NHR plasmids is the replication systems (Krishnpillai, 1988). Although some plasmids control by themselves the frequency of initiation of their replication, most plasmids rely extensively on the host replication machinery (Espinosa et al., 2000). This implies an appropriate communication between plasmid and host-specific factors. The narrow host range plasmids of Gram negative bacteria (ColE1, F, P1, R6K, pSC101, and R1) have solved this host/plasmid interplay with only a limited number of highly related hosts (Espinosa et al., 2000). The BHR plasmids such as the IncP-1 (RP4) and IncQ (RSF1010) incompatible groups have designed different strategies allowing them to be propagated in most Gram negative hosts and at least in some Gram positive bacteria. In Gram positive bacteria, many rolling circle and some theta type replicating plasmids exhibit a BHR, being able to maintain themselves in several unrelated Gram positive and negative bacteria (Espinosa et al., 2000).

IncP-1 incompatibility group plasmids of broad-host-range spread in most of the gram negative bacteria (Espinosa et al., 2000; Ansari et al., 2008). The IncP-1 plasmids are one of the most promiscuous groups of plasmids which are capable for both self transfer and maintenance within most of the members of the tested Gram negative bacteria (Smith and Thomas, 1987; De Gelder et al., 2008). The IncP-1 plasmid was proficient to mobilize transfer of DNA among bacteria and Saccharomyces cerevisae (Heinemann and Sprague, 1989) while, IncP plasmids are not maintained in species other than Proteobacteria, unless modified to a shuttle vector.

**Plasmid classification by incompatibility**

Plasmids have been classified according to their physical and phenotypic characteristics or by incompatibility testing, replicon typing and complete plasmid sequencing (Smalla et al., 2000). At present, plasmids are mainly classified according to their universal inheritable property, the incompatibility (Inc), which indicates relatedness in their replication system and partitioning (Datta and Hedges, 1972; Novick, 1987), and hinders plasmid coexistence with an “incompatible” group in a cell, but not coexistence of plasmids from different Inc groups.

Plasmid classification was developed on the basis of incompatibility group in the early 1970s by Datta and Hedges and has been used to classify many plasmids from Gram positive and Gram negative bacteria (Datta and Hedges, 1979; Couturier et
al., 1988, Table 1). Incompatibility testing is usually accomplished by investigation of the ability of an incoming unknown plasmid, introduced by conjugation or a reciprocal transformation (Tietze, 1998), to establish in a cell already containing another known plasmid. If the resident plasmid is eliminated in the progeny selected for incoming plasmid markers, the incoming plasmid is assigned to the same Inc group (Datta and Hedges, 1971).

Table 1. Classification of incompatibility group plasmids.

<table>
<thead>
<tr>
<th>Incompatibility group</th>
<th>Plasmids</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>F, R386</td>
</tr>
<tr>
<td>FII</td>
<td>R1</td>
</tr>
<tr>
<td>FIII</td>
<td>Col B-K99, Col B-K166</td>
</tr>
<tr>
<td>FIV</td>
<td>R124</td>
</tr>
<tr>
<td>I</td>
<td>R62, R64, R483 (at least 5 subgroups)</td>
</tr>
<tr>
<td>J</td>
<td>R391</td>
</tr>
<tr>
<td>N</td>
<td>R46, pULB2432</td>
</tr>
<tr>
<td>O</td>
<td>R724</td>
</tr>
<tr>
<td>P</td>
<td>RP4</td>
</tr>
<tr>
<td>Q</td>
<td>RSF1010, pJE723</td>
</tr>
<tr>
<td>T</td>
<td>R401</td>
</tr>
<tr>
<td>U</td>
<td>pRSA1</td>
</tr>
<tr>
<td>W</td>
<td>R388</td>
</tr>
</tbody>
</table>

PCR amplification of specific plasmid regions (oriT for IncW and IncP; oriV for IncQ etc.) (Göttz et al., 1996) is also frequently used as a classification method among isolated plasmids, or for monitoring their distribution in the environment (Göttz et al., 1996; Smalla et al., 2000, 2006; Binh et al., 2008; Malik et al., 2008). For instance, the presence and diversity of IncP-1 plasmids in a wastewater treatment plant was studied by PCR amplification of the trfA gene, located in the replicon region of IncP-1 (RP4) plasmids (Bahl et al., 2009). PCR amplification of the trfA gene was also used by Oregaard and Sorensen (2007) for classification of pKris-1 (IncP-1) plasmid. PCR-based detection of plasmids belonging to IncP, IncN, IncW and IncQ plasmid groups (Göttz et al., 1996) is extended to cover most plasmid incompatibility groups circulating among the Enterobacteriaceae, as the specific primers recognizing FIA, FIC, HI1, HI2, I1-Ig, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA replicons have been created (Carattoli et al., 2005).
Plasmids classified in *E. coli* and in *Pseudomonas* as IncP are a particularly well studied group of plasmids because they are very promiscuous (they can replicate in many different hosts) and have been found not only in clinical contexts, but also in soil and aquatic environments. IncP-1 plasmids carry a variety of phenotypic markers including antibiotic resistance and toxic heavy metal ion resistance (Zatyka and Thomas, 1998; Ansari et al., 2008; Bahl et al., 2009). IncP-1 plasmids have been divided into two subgroups, IncP-1α and IncP-1β, which appear to represent major evolutionary branches of the IncP-1 group (Smith and Thomas, 1987; Binh et al., 2008). This grouping was primary based on the patterns obtained by Southern blotting *Haell* digests of 10 IncP plasmids with a probe carrying the *oriT* region of RP4 (Yakobson and Guiney, 1983). The IncP-1β plasmid R751 genome was also sequenced, and compared to the sequence of the RP4. The comparison between the two sequences confirmed the conservation of the IncP-1 backbone genes for replication, conjugative transfer and stable inheritance functions between the two branches of this family. The typical IncP-1 plasmid backbone is interrupted by 1 or 2 MGEs (Smith and Thomas, 1987).

**Conjugation**

The first published research on conjugal exchange of circular DNA elements by a sex-resembling process among intestinal *E. coli* was reported by Joshua Lederberg and Edward Tatum at Yale University (USA) (Tatum and Lederberg, 1947). Conjugative plasmids are considered to be auto transmissible (or self-transmissible), provided that they contain a self-sufficient conjugative transfer system, and historically the conjugation has been studied most thoroughly through analysis of antibiotic resistance plasmids of Gram negative bacteria. Self-transmissible conjugal plasmids carry the genes necessary for transfer initiation at origin of transfer (*oriT*) and mating pair apparatus formation (Zatyka and Thomas, 1998; Grohmann et al., 2003; Binh et al., 2008). However, a large group of plasmids frequently lack genes for the mating pair apparatus, but might be co-transferred (non self-transmissible but mobilizable) via a mating apparatus provided by a self-transmissible plasmid, as they also contain their own *oriT* and genes for conjugal DNA processing. Conjugation systems were emphasized (on one hand) because of the prevalence and importance of antibiotic resistance plasmids, and on the other because of the ease in the manipulation of *E. coli* (Norman et al., 2010). Historically, the majority of molecular and biochemical understanding of the conjugative processes was derived from the
examination of F plasmid conjugation system in *Escherichia coli* (Manchak *et al.*, 2002), today recognized as the F-like conjugation system (IncF I, II, III and IV group). Other conjugative plasmids that have been studied in detail are RP4, R388 and Ti-pasmids (Pansegrau and Lanka, 1996; Zatyka and Thomas, 1998; Grohmann *et al.*, 2003; Norman *et al.*, 2010). Due to the high genetic homology among the IncP-1 plasmids, it must be assumed that the transfer of other plasmids from this group is very similar to the transfer of RP4. Although these conjugative plasmids differ significantly in the organization of transfer genes, pilus type, etc., still share certain similarities to IncF plasmids (Llosa *et al.*, 2002).

**Horizontal gene transfer in agricultural soils**

The exogenous isolation of mobile genetic element was applied to capture MGEs from soil and phytosphere microbial communities (Smalla and Sobecky, 2002). Antibiotic resistance or mercury resistance was often used as selective markers to exogenously isolate conjugative plasmids from phytosphere of different crops (e.g. Lilley and Bailey, 1997; Malik *et al.*, 2008) and from mercury-polluted soils (Smalla *et al.*, 2006) in gram-negative plasmid recipients. Biodegradative genes encoded on MGEs were captured from soils treated with 2, 4-D, but not from untreated controls (Top *et al.*, 1998). Two different cultivated-independent approaches were used to isolate naphthalene-catabolic genes from oil-contaminated soil in Japan (Ono *et al.*, 2007). One approach was the construction of broad-host-range cosmide metagenomic library. The other involved exogenous plasmid isolation. The cosmide clone was obtained that carried naphthalene-catabolic pathway operon for conversion of naphthalene to salicylate. The operon was similar to the corresponding operon on the IncP-9 naphthalene-catabolic plasmid pDTG1. Using the exogenous approach the microbial soil community was mated with a *Pseudomonas putida* recipient. Transconjugants had acquired either a 200- or 80- kb plasmid containing all the naphthalene-catabolic genes for the complete degradation of naphthalene. Both plasmids belong to the IncP-9 incompatibility group, and the naphthalene catabolic genes are highly similar to those of other IncP-9 plasmids, namely, pDTG1 and pSLX928-6 (Ono *et al.*, 2007).

Miyazaki and coworkers determined the nucleotide sequence of exogenously isolated plasmid pLB1 involved in γ-hexachlorocyclohexane degradation (Miyazaki *et al.*, 2006). pLB1 was isolated from hexachlorocyclohexane-contaminated soil and transferred from *Sphingobium japonicum* to other alpha-proteobacterial strains by
conjugative transfer. Thus, pLBl may contribute to the dissemination of genes for γ-hexachlorocyclohexane degradation in agricultural soils (Miyazaki et al., 2006).

Conjugative plasmids encoding multiple antibiotic resistance were captured from manure used for soil fertilization (Smalla et al., 2000; Heuer et al., 2002, 2008; van Overbeck et al., 2002; Heuer and Smalla, 2007; Binh et al., 2007, 2008). van Elsas et al. (1998) isolated mobilizing plasmids from the rhizosphere of wheat plants by using microbial communities detached from the rhizosphere as donors in triparenatal matings. Plasmid pIPO2 was isolated in Ralstonia eutropha on the basis of its mobilizing capacity. Replicon typing and plasmid sequencing showed that this 45-kb cryptic plasmid was not related to any of the known broad-host-range plasmids except pSB102 (Schneiker et al., 2001). Sequencing of plant associated bacteria has revealed that many phytopathogenic and symbiotic bacteria harbor plasmids (Vivian et al., 2001; Zhao et al., 2005; Sundin, 2007; Crossman et al., 2008; Li et al., 2008a), pathogenicity or symbiosis (Arnold et al., 2003; Nakatsukasa et al., 2008), or integrons (Gustin et al., 1986; Szezepanowski et al., 2004; Gillings et al., 2005).

Genotoxicity of wastewater

Many studies have shown the presence of different mutagenic and carcinogenic compounds in wastewater, and epidemiological studies have highlighted some cancer hazard in populations using treated drinking water (Golfinopoulos et al., 2003; Jolibois and Guerbet, 2004; Preeti et al., 2009; Şik et al., 2009; Gartiser et al., 2010). These genotoxic substances are derived from industrial, agricultural and urban pollution, and also from the disinfection treatment used for drinking water production, particularly when water is obtained from surface sources and then chlorinated (Stumm, 1992; Kataoka et al., 2000; Umbuzeiro et al., 2004; Lah et al., 2005). Chlorination has long been used as a simple, effective and economic method for disinfection of water for drinking water purposes, but genotoxic products can be formed by the interaction of chlorine and organic components, such as fulvic and humic acids, which are naturally present in raw waters. The detection and chemical identification of the genotoxic compounds present in drinking water is considered an essential step to predict the effects of the consumption of this complex mixture on human health (Oguri et al., 1998; Jung-Hwan et al., 2008; Gartiser et al., 2010).

The Salmonella assay can be a useful tool in the quantification of the genotoxic activity of those complex mixtures and the different responses of the several Salmonella strains can help in the identification of the classes of genotoxicants
present in the wastewater samples (Fatima and Ahmad, 2006; Preeti et al., 2009; Gartiser et al., 2010). The water samples must be concentrated, and proper extraction of the mutagenic compounds is required for both the chemical analysis and Salmonella test. Different strategies can be used to extract organic genotoxicants from water samples and the Amberlite XAD resins have been the most applied method (Chenon et al., 2003; Bull et al., 2006; Jung-Hwan et al., 2008; Siddique et al., 2010). The XAD resins allow the recovery of a wide range of chemicals and are very efficient to extract all the polar and the non polar chemicals potentially effective in toxicity and genotoxicity assays (Siddique and Ahmad, 2003; Chen and White, 2004; Aleem and Malik, 2005; Ansari and Malik, 2009a; Siddique et al., 2010). The blue rayon hanging method has also been used to concentrate mutagenic polycyclic compounds from freshwater and seawater (Kira et al., 1995; Kataoka et al., 2000). This method was employed to concentrate a new class of mutagens in River waters, the 2-phenylbenzotriazoles (PBTAs). These compounds are generated by cyclization of the azo bond of some dyes in a reductive environment after chlorination (Nukaya et al., 1997). Ohe et al. (2003) using the same hanging technique, analyzed Rivers from the US and Canada, and demonstrated positive results for the Salmonella assay, although with lower potencies than the ones observed in Japan. A study by Jolibois and Guerbet (2004), in which the genotoxicity of different wastewaters from Rouen area were evaluated with the SOS chromotest (on Escherichia coli PQ37) and the Salmonella fluctuation test on Salmonella typhimurium strains TA98, TA100 and TA102 with or without metabolic activation and they reported that genotoxic risk was present in these wastewaters.

Rehana et al. (1995) used five different Salmonella tester strains to compare the mutagenic activity of water samples from four sites of the Ganga River, India, using the XAD resin extraction method and the liquid-liquid extraction method. Their samples showed extreme mutagenic activity for TA98 and TA100, both with and without S9 fraction. The maximum activity for each strain was >10,000 revertants per liter. Siddiqui and Ahmad (2003) and Aleem and Malik (2003a) reported that TA98 (classified as extreme) was remarkably more responsive strain with XAD concentrated water extracts (River Yamuna, India) as compared to TA100 (classified as high), both with and without S9 fraction. It was also reported that XAD concentrated water extracts were more mutagenic than liquid-liquid concentrated extracts. In their findings, water samples collected during the summer exhibited
higher mutagenic activity compared with other seasons, and water samples also contained oxidative (TA102) mutagens. This extreme mutagenic contamination of the River water is likely to be derived from a combination of domestic, municipal and industrial effluents noted at the sampling site.

Vargas et al. (1993, 1995) observed extremely potent activity for TA98 without metabolic activation among 100 non-concentrated samples collected from Carí River, Brazil, and an area under the influence of a petrochemical industrial complex. The potency ranking was extreme for both TA98 and TA100, with and without metabolic activation. The highest value was more than 200,000 revertants per liter for TA98 in the absence of S9 fraction compared with 7000 revertants per liter in the presence of S9 fraction. The maximum value for TA100 was more than 50,000 revertants per liter and more than 10,000 revertants per liter in the absence of S9 fraction and in the presence of S9 fraction, respectively. It was also reported that these non-concentrated samples lost their activity upon liquid–liquid extraction using dichloromethane. In turn, they suggested that volatile compounds were responsible for the mutagenicity that was lost in the liquid–liquid extraction.

In a surface water quality monitoring program, Umbuzeiro et al. (2004) analyzed for 20 years in Sao Paulo State in Brazil demonstrated that 14% of 1007 surface water samples showed positive mutagenic activity. Among the positive samples, a total of 81 samples were analyzed using a dose-response manner. From those 81 samples, 9% were ranked as extreme for either TA98 or TA100 (5400–30,000 revertants per liter). In addition, the result showed that direct-acting mutagens induced frameshift mutations and S9 activated mutagens induced base substitution mutations. Their possible pollution sources were petrochemical industrial, oil spill and untreated domestic sludge. In a study of the Rio Tercero River (Cordova, Argentina) by Alzuet et al. (1996), the presence of S9 activated mutagens capable of causing base substitution and frameshift mutations was observed (without S9). Maximum activity was observed in TA98 with S9 fraction (8,550,000 revertants per liter), although moderate activity was found in TA100 with S9 fraction (1000 revertants per liter). The region is a heavily industrialized and holds the main oil refinery in the country, several petrochemical industries, a rolling steel mill and a sulfuric acid plant. Previous studies have demonstrated the presence of polycyclic aromatic hydrocarbons in airborne particulate matters, surface waters and sediments collected in this study area (Catoggio et al., 1989; Colombo et al., 1989; Delistraty and Yokel, 2007).
In the early 1990s, Stahl (1991), De Flora et al. (1991) and Houk (1992) reviewed the genotoxic and/or carcinogenic hazards of natural waters, the marine environment, and industrial wastes and effluents, and demonstrated that genotoxic organic compounds can enter surface waters from a wide range of industrial and municipal sources. They also stressed the importance of bioassays to detect mutagenicity/genotoxicity arising from the ubiquity of genotoxic compounds in the environment and the necessity of the identification of the sources of contaminants. White and Rasmussen (1998) noted that volumetric emissions from municipal wastewater treatment plants in large urban centers often exceed $10^9$ per day. As a result, genotoxic loadings from municipal wastewater treatment facilities are often far greater than those of industrial facilities and there is a strong relationship between a measure of human activity (i.e., population) and surface water genotoxicity. Several researchers have reported that conventional wastewater purification processes do not effectively remove many chemical contaminants (Meier and Bishop, 1985; Matsui, 1988; Claxton et al., 1998; Delistraty and Yokel, 2007). Other studies show a sharp rise in the mutagenicity/genotoxicity of water samples collected at sites downstream from wastewater treatment plants (Sakamoto and Hayatsu, 1990; Ohe et al., 1999, 2004). Consequently, the increasing use of contaminated surface waters and an increase in the magnitude of the contamination pose a serious problem for the health and welfare of humans and indigenous aquatic biota. Thus, appropriate bioassays have been needed for evaluation of surface waters on potential hazard to human and the water environment (Ohe et al., 2004).

**Genotoxicity of soil**

Thousands of chemicals are released and find their way into the environment, i.e. air, land, groundwater and surface water, by industrial activity, agricultural practices, domestic activity etc. Numerous genotoxic compounds have been detected in both the particulate and gas phases of outdoor air, particularly in densely populated urban regions (White and Claxton, 2004). It was reported that some industries, e.g. pulp and paper mills, steel foundries and organic chemical manufacturing facilities, discharge wastes of noteworthy genotoxic potency (Houk, 1992). When improperly handled and disposed of, these industrial wastes and effluents also contaminate the soil with their genotoxic compounds. An abundance of chemicals are applied to agricultural land as fertilizers, pesticides and herbicides. Soil microflora may also
convert nongenotoxic compounds to genotoxic derivatives (Asita and Makhalemele, 2008).

Combustion of fossil fuels for power generation or transportation in industrial facilities, power plants and motor vehicles is thought to be a major source of these genotoxic compounds. In addition to the genotoxic compounds released directly into the environment by combustion process, some of these compounds are thought to be formed from primary combustion products via chemical and photochemical reaction in the outdoor environment (Natusch, 1978; Guo et al., 2008). Most of these atmospheric compounds eventually descend to the ground, and therefore the ground surface may be contaminated with these genotoxic compounds.

Genotoxic compounds in soil may have an effect on human health in an exposed population through pathways such as inhalation of dust which contains these compounds, ingestion of plants that uptake the compounds from soil, and leaching of the compounds from soil to groundwater and surface water used as drinking water. Because of the complex chemical nature of soil, standard chemical analyses are limited in their ability to characterize the chemical composition of genotoxicants in soil to assess its potential genotoxicity. Bioassays, however, provide a means of assessing the toxicity of a complex mixture like soil without prior knowledge about its chemical composition. There are several reports on the genotoxicity of soil contaminated with chemicals originating from industrial sources. The contaminants of these soil samples varied widely, e.g. polychlorinated biphenyl (PCBs) (Donnelly et al., 1988; DeMarini et al., 1992; Cotelle et al., 1999), polycyclic aromatic hydrocarbons (PAHs) (Ehrlichmann et al., 2000), heavy metals (Cotelle et al., 1999; Wang et al., 1999; Ehrlichmann et al., 2000), pesticides (Vijayraghvan and Nagarajan, 1994), solvents, (Cotelle et al., 1999) munitions wastes, wood preserving wastes (Randerath et al., 1994; DeMarini et al., 1998).

The Salmonella mutagenicity test is undoubtedly the most popular bioassay used in environmental mutagenesis research, particularly for the analysis of complex mixtures such as organic extracts of soil, air, and water (DeMarini et al., 1992, 1994; Claxton et al., 1997; Claxton and George, 2002; Aleem and Malik, 2003b; Ansari and Malik 2009b). The standard version of the assay, known as the plate incorporation assay, is a reverse mutation test that quantifies the frequency of reversion from histidine auxotroph to wild-type following 48 to 72 h incubation with the test substances (Ames et al., 1975). Several tester strains of Salmonella are available,
carrying a variety of his mutations. The most popular tester strains, TA98 and TA100, carry the hisD3052 and hisG46 alleles, respectively. These strains have been extensively employed for the detection of environmental mutagens including PAHs (Moller and Alfheim, 1980; Sugimura and Takayama, 1983; Sakai et al., 1985), nitroarenes (Sugimura and Takayama, 1983; Tokiwa and Ohnishi, 1986), aromatic amines (e.g., N containing heterocyclics) (DeMarini et al., 1995; Wakabayashi et al., 1995; Ohe et al., 1997; 2004), S-containing heterocyclics (Pelroy et al., 1983; Marvin et al., 2000), and phenylbenzotriazoles (Oguri et al., 1998). Although some of these compounds have noteworthy mutagenic activity on both TA100 and TA98 (e.g., PAHs) (Sakai et al., 1985), several are known to have more potent frameshift activity (e.g., aromatic amines, phenybenzotriazoles, nitroarenes) (Sugimura and Takayama, 1983; Wakabayashi et al., 1995; Ohe et al., 1997; Oguri et al., 1998). The majority of Salmonella mutagenicity data on soil extracts employed the standard plate incorporation version of the assay with Salmonella strains TA98 and/or TA100 (Aleem and Malik, 2003b; Ansari and Malik, 2009b; Asya et al., 2009). Some researchers examining soil extracts have referred to potency expressed as net revertants per g dry soil as weighted activity (Donnelly et al., 1993, 1995; Garcia et al., 2002, Aleem and Malik, 2003b; Ansari and Malik, 2009b).

Ehrlichmann et al. (2000) evaluated genotoxicity of concentrated and nonconcentrated aqueous soil extracts from various soil samples using bacterial assays: the umu test with Salmonella typhimurium TA1535/pSK1002, the NM2009 test with S. typhimurium NM2009 and the SOS Chromotest with Escherichia coli FQ37. The soil samples included sandy samples contaminated with mineral oil hydrocarbons, soil contaminated with explosives, e.g. 2,4,6-trinitrotoluene and other nitroaromatic compounds, a sandy soil sample contaminated with heavy metals, and soil taken from a coal mine and coking plant. Each sample was extracted with distilled water and less hydrophilic compounds in the aqueous extracts were concentrated with PAD-1 resin. The concentrated and nonconcentrated aqueous extracts from the samples contaminated with nitroaromatic compounds exhibited an extremely high genotoxic potential in all of the genotoxicity tests.

Much of the soil mutagenicity literature is concerned with the identification of hazardous sites that may pose a mutagenic hazard to humans and indigenous fauna (Donnelly et al., 1993, 1995; Hughes et al., 1998), and the predominance of particular Salmonella strain activation combinations in the literature reflects their utility in this
regard. This assertion is supported by numerous studies of organic extracts from soils contaminated with a wide range of pollutants (e.g., wood preserving wastes, petrochemical wastes, sewage sludge) that yielded little or no response on the base-substitution strain TA100 (e.g., (Davol et al., 1989; Donnelly et al., 1991; DeMarini et al., 1992; Wesp et al., 2000). Moreover, many of these studies have noted the presence of known frameshift mutagens such as nitroarenes and N-containing heterocyclics in soil extracts that show strong TA98 activity (Aprill et al., 1990; Wang et al., 1990; Barbee et al., 1992; Wesp et al., 2000). However, it should be noted that some studies, such as those that examined extracts of soils contaminated with munitions wastes (e.g., di- and trinitrotoluenes) and dinitropyrenes (e.g., 1,3-, 1,6-, and 1,8-dinitropyrene), also showed strong responses in Salmonella TA100 (Watanabe et al., 2000, 2008).

Pollution of the agricultural areas as a result of used agrochemicals turn into a global problem for contemporary mankind. The genotoxic compounds in soil can affect human health in various ways. Sustainable agriculture provides protection of the environment and requires a conversion period, during which no agrochemicals are used. Some agrochemicals can persist in the soil for several years, contaminating crops that are supposed to be chemical free (Courty et al., 2008).