Chapter I: Review of literature
The riverine system in India is severely polluted and at several locations it has reached alarming levels. The Yamuna River originates from the Yamunotree glacier near Bandar punch (30°58' N, 78°27' E) in the Mussorie range of lower Himalayas. The problem of pollution of the Yamuna water is due to the discharge of industrial effluents and domestic waste into the river, in addition to other sources of pollution such as disposal of dead bodies, cattle bathing and washing of clothes etc. (Trivedy, 1988).

Water borne diseases have been a major health problem throughout history during this century. However, improved quality of drinking water have decreased the incidence of outbreaks of water borne diseases, although they are still a problem especially in developing countries. Controlling outbreaks of water borne diseases has been attained by decreasing the direct input of wastewater to natural waters, improving wastewater treatment, protecting raw water resources and monitoring hygienic quality of water (Craun, 1990).

Fecal indicator bacteria are enumerated in waters in order to evaluate hygienic quality because in practice all the pathogenic agents that are potentially present can not be enumerated. If indicator bacteria are present, there is probability that pathogenic organisms (viruses, bacteria and protozoa) excreted in feces are also present and that the water can transmit waterborne infectious diseases. Indicator bacteria have traditionally been monitored in water pollution control to help limit the spread of these diseases. However, indicator bacteria are inadequate for assessing the risk of viral and protozoans (Cabelli, 1978).

Soil is an environment rich in its diversity of bacterial species; it has been estimated that half of the known bacterial genera contain species which can be considered as soil bacteria (Clark, 1967). Different types of bacteria including symbiotic and free living nitrogen fixers, nitrifiers, members of Pseudomonadaceae, Enterobacteriaceae. Alcaligenes and Cyanobacteria have been reported to be present in metal polluted soils by different workers (Diels et al., 1988; Malik and Jaiswal, 2000; Athar and Ahmad, 2002; Malik and Ahmad, 2002). Elevated levels of heavy metals can affect the qualitative as well as quantitative structure of microbial communities. Several studies have found that metals influence microorganisms by adversely affecting their growth, morphology, and biochemical activities, resulting in decreased biomass and diversity (Barkay et al., 1985; Baath, 1989; Reber 1992; Malik and Ahmad, 2002; Rajapaksha et al., 2004). Soil microorganisms play a vital role in various biochemical cycles in the soil ecosystem.
Therefore, any change in the type or quantity of soil microorganisms may disrupt the natural soil ecosystem, which in turn may influence soil fertility (Juwarkar et al., 1988).

**Escherichia coli**

The genus is named after Theoder Escherich who was first to describe the colon bacillus under the name *Bacterium coli commune* (1885). Based on minor differences in biochemical characteristics, colon bacilli were described under various names but in view of mutability of the biochemical properties of this group, they have all been included in one species. *E. coli* is Gram negative, straight rods measuring 1-3 \( \mu \text{m} \) x 0.4-0.7\( \mu \text{m} \) arranged singly or in pairs. It is motile by peritrichous flagella, though some strains may be non-motile. Capsules and fimbriae are found in some strains. Spores are not formed (Alcamo, 1997).

It is a facultative anaerobe capable of fermentative and respiratory metabolism. Under anaerobic growth condition there is an absolute requirement for fermentable carbohydrates. The temperature range is 10-40°C but the optimum temperature is 37°C. Good growth occurs on ordinary media. Colonies are large, thick, grayish, white or colorless, moist, smooth, opaque or partially translucent. This description applies to the smooth (S) form seen on fresh isolation, which is easily emulsifiable in saline. The rough (R) forms give rise to colonies with an irregular dull surface and are often auto-agglutinable in saline. The S-R variation occurs as a result of repeated subcultures and is associated with the loss of surface antigens and usually of virulence. Some strains may occur in mucoid form (Talaro and Talaro, 1993).

Glucose, lactose, mannitol, maltose and many others sugars are fermented with the production of acid and gas. The four biochemical tests widely employed in the classification of enterobacteria are indole, methyl red (MR), Voges Proskauer (VP) and citrate utilization tests, generally referred to by the mnemonic ‘IMViC’. *E. coli* is indole and methyl red positive and Voges Proskauer and citrate utilization negative. Gelatin is not liquefied, \( \text{H}_2\text{S} \) is not formed, urea is not split and growth doesn’t occur in KCN medium (Ananthnarayan and Paniker, 1994).

**Pseudomonas spp.**

*Pseudomonas* strains are absolute aerobes typically polar flagellated, Gram negative rods usually less than 1 \( \mu \text{m} \) in diameter and not more than 4-5 \( \mu \text{m} \) in length,
although some strains (fluorescent plant pathogens, *P. putida*) may have cells which are considerably longer. The flagella are usually polar but in some instances sub-polar attachment is more common (Stanier *et al.*, 1987). *Pseudomonas* strains are very common in natural habitats particularly soil, water, spoiled food and diseased plants from which both fluorescent and non-fluorescent *Pseudomonas* could be isolated. The capacity of *Pseudomonas* for growth in very simple media and their widespread occurrence made them appear as prime participants in the process of mineralization of organic matter in nature (Soakchi and Ornston, 1986). Pili or fimbriae can be observed in the cells of many species to which a number of different functions have been attributed: cell to cell contact (Heumann, 1962), phage adsorption (Bradley, 1972), attachment to cell surfaces (Buchanan and Pearce, 1979) and twitching motility (Bradley, 1980).

Properties of *Pseudomonas* colonies such as shape, color, edge and surface ornamentation in some instances give important clues for identification. *P. aeruginosa* strains normally have flat, creamy colonies which have tendency to spread over agar surface. Strains with mucoid colonies can be isolated from respiratory infection associated with cystic fibrosis. These strains produce alginate, a polysaccharide composed of D-mannuronic acid and L-glucuronic acid (Evans and Linker, 1973). *P. aeruginosa* was the only species of the genus known to have this capacity, but Govan *et al.* (1981) reported the same property in carbenicillin-resistant strains of the related species, *P. fluorescens*, *P. putida* and *P. mendocina* but not in many other species.

Pigment production is a useful property for species determination. Pigments may be of various chemical types. This may be soluble in water and freely diffusible into the culture medium or they may remain associated with cell. Best known of the soluble pigments are the fluorescent pigments. These pigments are strong iron chelators and allow growth in the medium having low iron content. Important soluble pigment is pyocyanin, a phenazine derivative characteristic of *P. aeruginosa*. Several carotenoids are characteristic of some groups (for instance *P. mendocina*) (Palleroni, 1978).

Physiological characteristics that have been frequently used to determine *Pseudomonas* species include the oxidase reaction, growth factor requirements, nitrate reduction, denitrification, hydrolysis of starch, gelatin, poly-β-hydroxybutyrate, lecithin and arginine dihydrolase reaction.
Azotobacter chroococcum

*Azotobacter chroococcum* had been isolated by Beijerinck in 1901. Generally there are seven species of the genus *Azotobacter* differentiated on the basis of cell shape, pigments, and motility. *A. chroococcum* is the species most frequently occurring in different soils but rarely exceeds $10^{4.5}$ CFU/g of soil (Subbarao, 1995). Due to its multiple physiological attributes of broad-spectrum utility, the use of the *Azotobacter* is recommended for various crops. *Azotobacter chroococcum*, mainly occurring in neutral or alkaline soil. *Azotobacter vinelandii* and *A. beijerinckii*, originally isolated from North American soil. Cell size, flagellation, pigmentation and production of extracellular slime are considered as diagnostic features of these bacteria in distinguishing species. It has been observed that *A. chroococcum* displayed three fold effect as follows (Verma and Paul, 2000): (a) Nutritional: Helps in saving nitrogenous fertilizer (10-20 Kg N/ha) and phosphate solubilization. (b) Stimulatory: Secretes growth-promoting substances (indole acetic acid, gibberellic acid etc.) which helps in better seed emergence and expanded root system. (c) Therapeutic: Suppresses the growth of saprophytic and pathogenic microorganism near the root system and reduces the damage to crop by plant diseases.

The bacteria belonging to the genus *Azotobacter* are Gram negative, some strains are motile by peritrichously located flagella, pleomorphism is common and variety of cell shapes and sizes have been observed. Cells are ovoid or even yeast like in appearance, mesophillic (optimum growth temperature 30°C) and obligate aerobe. They are catalase positive and grow best with nitrogen free or simple forms of combined nitrogen, capable of fixing atmospheric nitrogen asymbiotically, widely distributed in soil. There is only one species of *Azotobacter* i.e. *A. paspalum* (identified by Dobereiner, 1966), which was specifically associated with *Paspalum notatum*, a grass which is classed as a case of associative symbiosis (Verma and Paul, 2000).

Environmental pollution

India is a huge country with more than one billion population and only second to China in terms of man power. Since independence, an all round and unprecedented growth has taken place in several areas such as industry, agriculture, power generation, oils and minerals exploration, telecommunications, shipping and aviation, science and technology etc. (Srinivas, 1999; Sundaravadivel and Vigneswaran, 2003). The rapid industrialization, fast urbanization and over population consequently resulted in the
tremendous release of xenobiotic compounds into the environment and thus the environmental pollution has become a serious problem in India. Large quantity of various chemicals, some of which are highly toxic are used routinely by the industries and consumers either for material comfort or for enhanced agricultural productivity (Viswanathan, 1985).

One of the problems of extensive production of chemicals is associated with the release of even increasing number of various toxic chemicals in the environment and their exposure to living community. At present most of the industries in India are operating without any treatment plants for their liquid wastes or gaseous emissions. Industrial, commercial and domestic wastes are disposed off indiscriminately into the drains or on the lands. Such a callous disposal of waste material which may contain toxic and radioactive chemicals or disease producing microorganisms may lead to serious health problems (Ray and Gupta, 1986; Rawat et al., 2003; Singh et al., 2004).

**Heavy metal pollution**

Heavy metals are among the most common environmental pollutants, and their occurrence in water and biota indicate the presence of natural or anthropogenic sources (Forstner and Wittman, 1979). The main natural sources of metals in water are weathering of minerals. Industrial effluents, non-point pollution sources, as well as atmospheric precipitation (Solomons and Forstner, 1984) can also be sources of increased concentrations of heavy metals. Air masses supplying acidic pollutants also carry significant amounts of certain metals which contaminate the terrestrial and in particular, aquatic environments (Klavins et al., 2000).

**Heavy metals pollution in aquatic environment**

There are various sources of domestic and industrial effluents leading to heavy metal enrichment of water, sediments, vegetations and fish in rivers. Knowledge of the distribution of heavy metals in water, sediments, plants and fish play a key role in detecting sources of heavy metal pollution in aquatic system (Forstner and Wittmann, 1981; Moore and Ramamorthy, 1984).

Ajmal et al. (1985) have reported the high concentration of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in water and sediments of the Yamuna River due to sewage and industrial effluents. Singh et al. (1997) determined the concentration of
various heavy metals (Cr, Cu, Co, Fe, Mn, Ni, Pb, Zn and Cd) in sediments of the Gomti River (India). Alam and Ahmad (1999) also reported the presence of metals (Zn, Ni, Co, Pb and Li) in the Yamuna River water at different sampling stations in the capital of India (Delhi).

Kaushik et al. (2001) investigated the heavy metal pollution of the Yamuna River in the industrially developing state of Haryana and Delhi, selecting 16 stations covering the upstream and downstream stations for major industrial complex of the state. The concentration of Fe, Ni and Co were found to be in the range of 0.07-3.84, 0.02-0.25 and 0.05-0.41 mg/l respectively which exceeded the maximum permissible limits prescribed for drinking by WHO (WHO, 1984).

Barghigiani et al. (2001) reported the results of a monitoring program of six rivers (Serchio, Cecina, Cascina, Era, Elsa and Pavone) in Italy. In this area, agricultural, industrial and urbanization pressures affect water quality in terms of suitability to the survival of aquatic organisms. They monitored water for several physico-chemical parameters (Temperature, DO, suspended solids, total ammonia, nitrites, Cu, Cd, Cr, Ni and Pb) in order to assess the anthropogenic impact on the environmental conditions of these watercourses and their suitability for fish life. Concerning the metals, inter element relationship (between Cr and Ni, Pb and Cr, Ni and Pb and Cu, Cu and Pb) were found which allow some remarks on the origins of the anthropogenic impacts.

Various environmental problems due to heavy metal pollution in India have been reported (Chandra, 1980). Chatterjee and Banerjee (1999) determined the levels of lead and other 19 elements in the residential area of greater Calcutta (India), where 50,000 people reside in the vicinity of a lead factory and reported the presence of high concentration of these metals in the area.

Detailed studies have been carried out in and around Jeedimetla industrial area in Andhra Pradesh (India) to determine the extent of heavy metal contamination in soil and water by Govil et al. (2001) and observed that soil as well as water in the area has a high contamination of Pb, Ni, Cu, Mn, Zn, As, Sr, Cr, Cd and Ba. The level of these elements were found to be far above the normal distribution of these elements in nature.

Most of the foodstuffs like fresh vegetables, cereals and fruits contain the elevated levels of several heavy metals. Crops grown in polluted soil (from industrial contamination and from use of sewage sludge as fertilizer) or irrigated with polluted water may increase concentration of heavy metals. Some seafood also contained higher
levels of many trace elements especially the elevated level of lead in the air of rural areas have been documented. It is known to be present in milk and dairy products as well as in wine (WHO, 1984).

The mutagenic effect of metals on bacteria has been known since early 1950s (Demerec and Hanson, 1951). Some metals have been detected as genotoxins in a suspension assay using Bacillus subtilis (Nishioka, 1975; Kanematsu et al., 1980) and E. coli Lac I systems (Zakour and Glickman, 1984), Lambda phage induction (Rossman et al., 1984, Vargas et al., 2001) as well as the fluctuation assays (Arlauskas et al., 1985) have also confirmed the genotoxic effect of certain metal ions.

It is known that heavy metal contamination lessens soil respiration (Hattori. 1992), microbial biomass (Brooks and McGrath, 1984), soil enzyme activities (Mathur and Sanderson, 1980) and microbial numbers (Malik and Ahmad, 2002). Codina et al. (1995) evaluated the heavy metal genotoxicity using different microbial tests: the Ames Salmonella tests, the E. coli WP2 tests, the mutation test detecting mutagenicity, and the SOS assay with E. coli detecting enzyme induction. All the metals tested (Cd, Cr, Cu, Hg, Ni and Zn) were detected as genotoxins by the mutatox and the SOS test. The Ames Salmonella test and E. coli WP2 assays only detected chromium as genotoxic causing mutagenicity. Pagano and Zeiger (1992) reported that cobalt chloride (Co\(^{2+}\)), ferrous sulfate (Fe\(^{2+}\)), manganese sulfate (Mn\(^{2+}\)), cadmium chloride (Cd\(^{2+}\)) and zinc chloride (Zn\(^{2+}\)) could be reportedly detected as mutagens in Salmonella strain TA97a when preincubation exposure were made in sterile distilled deionized water or in hepes buffer in NaCl/ KCl rather than standard sodium phosphate buffer. The insensitivity of bacteria in metal mutagenesis has generally been attributed to the lack of bioavailability or uptake into the cells. Insoluble metals which could be expected to be non-mutagenic and in some cases non-toxic in bacterial systems, have been shown to cause toxicity in mammalian cells after phagocytosis (Rossmann et al., 1987). Wong (1988) has also reported the mutagenicity and carcinogenicity of several heavy metals that are commonly found in polluted areas using the Ames Salmonella mutagenicity test.

**Heavy metals pollution in soil**

The soil is a long-term sink for the group of potentially toxic elements often referred to as heavy metals like zinc, copper, nickel, lead, chromium and cadmium. Whilst these elements display a range of properties in agricultural soil including
differences in mobility and bioavailability, leaching losses and plant uptake are usually relatively small compared to the total quantities entering the soil from different diffuse and agricultural sources. As a consequence these potentially toxic elements slowly accumulate in the soil profile over long periods of time. This could have long-term implication for the quality of agricultural soils, including phytotoxicity at high concentrations, the maintenance of soil microbial processes and the transfer of zootoxic elements to the human diet from increased crop uptake or soil ingestion by the grazing livestock (Nicholson et al., 2003).

Soil serves many vital functions in our society, particularly for food production. It is thus of extreme importance to protect this resource and ensure its sustainability. Deteriorating environmental conditions and increasing reliance on agrochemicals have led to the growing concern over the potential accumulation of heavy metals and other contaminants in agricultural soils (Kabata-Pendas, 1995; Wang and Tao, 1998; Manz et al., 1999; Barman et al., 2000; Sanghi and Sasi, 2001; Wong et al., 2002, Nicholson et al., 2003).

Owing to rapid economic development, heavy metal contamination of agricultural soils has become increasingly serious in India (Dar, 1997; Farooq et al., 1999; Barman et al., 2000; Sanghi and Sasi, 2001; Roychowdhury et al., 2002; Singh et al., 2004). As one of the most significant sources of soil pollution, wastewater from industries and domestic sources introduces a huge amount of organic and inorganic contaminants including heavy metals, apart from nutrients such as nitrogen and phosphorus into agricultural land. This is a particularly acute problem in developing countries, where scarcity of water necessitates wastewater reuse for a variety of different purposes, most importantly crop agriculture (Wang and Tao, 1998; Lombardi and Gracia, 1999; Barman et al., 2000; Wong et al., 2002; Singh et al., 2004). Nicholson et al. (2003) found that the heavy metal input in agricultural soils of England and Wales were mainly due to atmospheric deposition, sewage sludge, livestock, manures, inorganic fertilizers and lime, agrochemicals, irrigation water, industrial by-product and wastes. They also reported high concentration of heavy metals in agricultural soils irrigated with wastewater.

**Pesticide pollution**

The worldwide consumption of pesticides is about two millions tones per year, of which 24% is consumed in the USA alone, 45% in Europe and 25% in rest of the world.
India’s share is just 3.75%. The main use of pesticides in India is in agriculture and public health sector to combat the various pests and diseases that affect man. To achieve this goal, the production of basic pesticides commenced in 1952 with the manufacture of benzene hexachloride (BHC), followed by DDT. India is presently the second largest manufacturer of basic pesticides in Asia. It ranks 12th globally. Despite the fact that the consumption of pesticides in India is still very low, there has been a wide spread contamination of food commodities with pesticides residues (Gupta, 2004).

The most important pollutants among the toxicants in India are organochlorine (OC) and organophosphorus (OP) pesticides. The later type is tending to replace the former type due to its fast degradation in the environment (Jiries et al., 2002; Golfinopoulos et al., 2003). In India, alarming levels of pesticides have been reported in air, water, soil as well as in foods and biological materials (Viswanathan, 1985; Agarwal et al., 1986; Nair and Pillai, 1992; Hans et al., 1999; Gupta, 2004). OC insecticides have been extensively used in India since 1954 in agriculture as well as in public health sector (Gupta, 1986). The use of OC which are banned or restricted in most developed countries are still being used in this country. The major factors responsible for detrimental effects on the environment from use of these chemicals are that they are very persistent, extremely toxic to fish, used more as preventive measures rather than cure in controlling insects and some of them were found to biomagnify in organisms (Ahmad et al., 1996). Their accumulation in low concentration in the body fat of mammals may pose health problems in the long run (Hung and Thiemann, 2002).

**Pesticide pollution in aquatic environment**

Most of the pesticides enter into the aquatic environment through intentional applications, aerial drifts and run off and then become rapidly distributed through the action of wind and water. Some pesticides are directly applied to water to control the aquatic weeds, algae, unwanted invertebrates and noxious insects (Nimmo, 1985). Agricultural run off from fields and grazing lands is considered to be the major route of pesticide movement into water. It has been observed that industrial waste from pesticide manufacturing plant is the second largest source of pesticide in aquatic environment. Another source of pesticide that is poorly understood and is not well studied is the hazardous waste disposal (Rodrigues et al., 1998).
Agarwal et al. (1986) evaluated the DDT residues in water and sediments from the four different sites of the Yamuna River at Delhi (India) from 1976-1978. They reported that all the samples contained DDT residues in varying concentrations. The concentration of T-DDT residues ranged from 0.04-3.42 $\mu$g/l in water and 0.007-5.6 mg/kg in sediments. They also found that the total DDT concentration was comparatively higher at downstream Wazirabad site where the mixing of river water with the discharge from Najafgarh drain which also carried effluents of a DDT factory along with other factories.

Pico et al. (1994) monitored the levels of the pesticides in natural waters of the Valencia community (Spain). They collected water from the rivers, lakes and irrigation canals. They found high levels of OC, OP and carbamate pesticides. Tan and Vijayaletchumy (1994) carried out a study to measure the extent of environmental contamination of the OC pesticides in river water from peninsular Malaysia. They collected water samples from twenty five major rivers. Highest concentration were found in Sg. Selangon river (T-BHC- 280 ng/l, T-endosulfan 310 ng/l, heptochlor 100 ng/l, T-DDT 110 ng/l and dieldrin 47 ng/l).

Nayak et al. (1995) tested 34 water samples for OC pesticide residues from the middle stream of the Ganges River, India and observed that total HCH were found in 30 samples ranging from 0.105-99.517 $\mu$g/l. Rehana et al. (1995) also analyzed the water samples for the presence of some OC and OP from the Ganges River, India (between Kachla and Kannaulj, UP), and HPLC analysis of these samples showed the presence of some pesticides like DDT, BHC, DDD, aldrin, dieldrin, dimethoate and methyl parathion.

Abbassy et al. (1999) studied the seasonal occurrence of pesticide residues and other organic contaminants (polychlorinated biphenyls) in water at the estuaries of Rosetta and Damiatta branches of the river Nile from Summer 1995 to Summer 1997. The results indicated that OC compounds including lindane, $p, p'$-DDE, $p, p'$-DDD, $p, p'$-DDT, aroclor 1254 and aroclor 1260 were present in all the water samples at concentration levels ranging between 0.286-0.352, 0.035-0.067, 0.019-0.033, 0.024-0.031, 0.390-0.70 and 0.166-0.330 $\mu$g/l, respectively.

Hung and Thiemann (2002) investigated the surface water samples in Hanoi and its surroundings for fifteen insecticides, which were banned in Vietnam from 1990 to 1998. Their investigation was focused on an area of approximately 30 by 20 km. A total of 30 water samples from river, lakes, irrigation canals and wells were analyzed and they
found that the concentration of the banned pesticides were highest in the rivers and then in the irrigation canals, followed by the lakes and wells. The mean concentration of ί-HCH (a, β, γ, and δ HCH) and T-DDT (2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT) in the river were 17.2 and 43.7 ng/l in dry season (November 1998) and 29.3 and 56.1 ng/l in the rainy season (August 1999), respectively.

Turgut (2003) conducted a study from 2000 to 2002 to determine the residues of OC pesticides and metals in surface water of Kucuk Menderes River in Turkey. His results showed that Kucuk Menderes River was polluted with OC pesticide despite the existence of bans over a long time. The T-DDT (DDT, DDD, and DDE) were detected in most water samples. The highest concentration among OC pesticides was heptachlor epoxide (281 ng/l).

**Pesticide pollution in soil**

Most potential contaminants are necessary for agricultural production but become hazardous when they occur in excess in soil. Much soil contamination is the result of human activity, including the entry of industrial wastes into soil through atmospheric deposition or application of agro-chemicals and domestic waste to the land. These organic (industrially derived compounds such as agricultural pesticides which are intentionally applied to the soil) contaminants reduce the soil quality for agricultural production. Soils thus play an important role for the global flux for pesticides in the environment (Sanghi and Sasi, 2001).

The fate of a pesticide applied to soil depends largely on its persistence and solubility properties. Once applied to cropland, pesticides may either be taken up by plants (Nair *et al.*, 1993) or ingested by animals, insects, worms, or microorganisms in the soil, or may move downward in the soil (Sujatha and Chacko, 1992) and either adhere to it or dissolve in water or may vaporize (Maguire, 1992) and enter the atmosphere or may breakdown via microbial and chemical pathways into other, less toxic compounds or may be leached out (Li and Migita, 1992) of the root zone by rain or irrigation water. Chemical, biological and physical forces play an important role in the fate of pesticides in soil. Pesticides remain or persist in soil for a limited time, which may vary from days to years depending on the type of pesticide, soil moisture, organic matter, temperature and pH. Persistence may differ considerably because of varying environmental conditions and application rates (Sanghi and Sasi, 2001).
In spite of the ban to pesticides like DDT, developing countries like India still use these insecticides due to cost benefit efficacy and broad spectrum toxicity. In agricultural country like India, DDT and HCH contribute to more than 70% of the pesticide consumption (Sanghi and Sasi, 2001). In a typical study conducted by Geography Department (1990) Aligarh Muslim University (India) revealed that in a selected area (between Narora and Kannauj) of the Ganges plain, the OC pesticides used for crop production was about 0.46 Kg/hectare. It has been documented that 40% of the pollution in the Ganges River was due to sewage discharge and 13% owing to chemical waste released from factories (Ahmad et al., 1996).

Kashyap et al. (1994) selected two locations in Gujrat state (India) on the basis of the use of insecticides in agriculture and vector control programs and analyzed the locations for T-DDT and T-BHC residues and they observed that residue levels were significantly higher in that location where the insecticides were used both in agriculture and vector control programs as compared to another location where insecticides were used in agriculture.

Pesticides can affect the human body directly through water, air and food of agricultural origin as well as many other biological processes. Not only the environment (air, water and soil) has been shown to have a toxic level of pesticides but the animal kingdom as well as the plants have also been contaminated by their hazardous level (Viswananathan, 1985). The pesticides toxicity in water depends mainly on their chemical stability and solubility in water as well as the quality of water and other physico-chemical parameters (Nimmo, 1985). It has been reported that OC pesticides build up in the environment since they are not at all biodegradable, or degraded very slowly (Lal and Saxena, 1982; Nawab et al., 2003).

The toxicity studies of pesticides have been conducted on both the bacterial system (Ghosh et al., 1997; Ruiz and Marzin, 1997) as well as on animals (Tandon and Dubey, 1983) and humans (Misra et al., 1985). Methyl parathion has been reported to induce reverse mutation in Salmonella with or without S9 (Waters et al., 1980). Tremolada et al. (2004) also reported the toxicity of pesticides to rainbow trouts, daphnia and algae. Several pesticides and their degradation products are reported to be highly mutagenic in Ames tester strains and also induced unrepairable DNA damage in E. coli (Kamal and Ralph, 1986; Antony et al., 1989; Mehrotra et al., 1990, Rehana et al., 1995, 1996). Some pesticides have also been found to be tumorogenic (Ray and Prasad, 1987).
According to Kurrinj (1984), among the 400 pesticide preparations studied for mutagenic activity half of them were found to be mutagenic in one or more test systems.

**Heavy metal resistance in bacteria**

The introduction of heavy metals in various forms in the environment can produce considerable modifications of the microbial communities and their activities. 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. However, at low concentration some metals are essential for microorganisms since they provide vital co-factors for metallo-proteins and enzymes (Doelman et al., 1994; Guzzo et al., 1994). The pollution of the environment with heavy metals has led to the appearance of heavy-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 (Ivanov et al., 1999).

Bacterial plasmids harbor housekeeping genes (governing their replication, segregation and copy number control) and also genes that provide the host cell with ancillary phenotypic functions, those that are not needed by the cell under growth conditions. These extra functions include the synthesis of bacteriocins, the ability to mediate cell-to-cell conjugation, resistance to antibiotics, resistance to toxic heavy metals, and a range of other activities, including some (lactose fermentation and hydrogen uptake) that might be considered central cellular metabolism. Heavy metal resistance have been frequently found on plasmids of Gram negative and Gram positive eubacteria (Silver and Misra, 1988; Silver and Walderhaug, 1992, Unaldi et al., 2003; Verma et al., 2004).

The known mechanisms of bacterial heavy metal resistance are basically four (Silver, 1992):

i) Keeping the toxic ion out of the cell by altering a membrane transport system involved in initial cellular accumulation.
ii) Intracellular or extracellular sequestration by specific mineral-ion binding components (analogous to the metallothioneins of eukaryotes and phytochelatins of plants, but generally at the level of cell wall in bacteria).

iii) Highly specific cation or anion efflux systems encoded by resistance genes. This is the most commonly found mechanism of plasmid controlled bacterial metal ion resistance.

iv) Detoxification of the toxic cation or anion by enzymatically converting it from a more toxic to a less toxic form.

Prokaryotes (bacteria, actinomycetes) are more sensitive than eukaryotes (fungi) to heavy metal pollution. Doelman and Haanstra (1979) showed that 75% of the bacteria isolated from Pb polluted soils (184-1177 mg/kg of Pb) were able to grow on a medium containing 30 of Pb µg/ml, whereas only 5% of bacteria from reference soils (12-41 mg/kg of Pb) were able to grow on the same medium. These data clearly show the difference in microbial resistance between relatively unpolluted and polluted soils.

Diels and Mergeay (1990) found a correlation between heavy metal content in soils contaminated with industrial wastewater and metal resistant bacterial strains. Diels et al. (1988) isolated Alcaligenes eutrophus strains from severely polluted sites and found both as multiple heavy metal resistant and chloroaromatic compound degrading. Dong et al. (1998) found nec-nre nickel resistance in Comamonas, Sphingobacterium heparinum, Flavobacteria, and even Gram positive bacteria. Anisimova et al. (1993) studied metal resistance in 112 strains of Gram negative bacteria from sewage and soil samples in the vicinity of industrial area. All the isolates were examined for the plasmid DNA and the percentage of plasmid bearing bacterial strains was found to be 50%. Rasmussen and Sorensen (1998) isolated bacteria from mercury contaminated site inside the harbour of Copenhagen (Denmark) and an unpolluted control site of Kogebuge and these bacteria were compared with respect to diversity indices of antibiotic and metal resistance pattern and abundance of plasmids in the resistant bacterial isolates. They also found that the incidence of plasmid was higher at polluted site than at the unpolluted zone.

**Mercury resistance**

Mercuric ions and organomercurial compounds, which have strong affinities for the thiol groups in proteins, are toxic to cells. Mercury compounds are leached from natural sources (weathering of rocks and soil). Major sources of environmental
contamination of mercury from human activities are from burning coal and petroleum products. Mercury compounds are used in industry as catalysts. Phenylmercury, merthiolate and mercuriochrome have been used as household and hospital disinfectants. Mercury resistance is widely distributed among different bacterial genera and species (Misra, 1992).

Mercury resistance determinants are frequently found on plasmids (Robinson and Tuovinen, 1984; Trevors et al., 1985, Silver and Misra, 1988, Rasool et al., 1995: Bruins et al., 2003). In some cases mercury resistance determinants have been found to be chromosomally determined (Nies, 1999). Gene conferring resistance to mercury compounds are clustered in an operon in most naturally occurring mercury resistance system. Mercuric reductase (MerA), one of the protein encoded by the mercuric reductase (mer) operon catalyses a unique reaction in which mercuric ion $\text{Hg}^{2+}$ are reduced to mercury metal $\text{Hg}^0$ using NADPH as a source of reducing power (Schottel, 1978; Fox and Walsh, 1982; Misra, 1992; Osborne et al., 1997).

To prevent toxic effects of $\text{Hg}^{2+}$ on periplasmic proteins in Gram negative bacteria, $\text{Hg}^{2+}$ is transported into the cell via specific uptake systems. In Gram negative bacteria, it is bound by the periplasmic $\text{Hg}^{2+}$ binding protein MerP as the first step of detoxification (Qian et al., 1998). MerP probably delivers the toxic cation to the mercury transporter MerT for transport into the cytoplasm (Hobman and Brown, 1996). Alternatively, or in addition to MerTP, another uptake route exists which involves the MerC protein (Hamlett et al., 1992; Sahlman et al., 1997).

Organomercurials, which are more toxic than $\text{Hg}^{2+}$ may also be detoxified if the mer resistance determinant encodes a MerB organomercurial lyase in addition to the other Mer proteins (Silver, 1996; Silver and Phung, 1996). After cleavage by MerB, the resulting $\text{Hg}^{2+}$ is reduced by MerA. The high toxicity of organomercurials and other methylated and alkylated heavy metal compounds makes it very unlikely that these kinds of chemical modification of heavy metals are metal resistance mechanism. Methylation has been observed for arsenic, mercury, tin, lead, selenium and tellurium (Fatoki, 1997).

Chromium resistance

The presence of high concentrations of chromate in the environment inhibits most microorganisms (Aislabie and Loutit, 1984). Chromate also promotes the selection of resistant variants (Summers et al., 1978; Luli et al., 1983; Wong and Trevors, 1988).
Bacterial resistance to chromate can be due to chromosomal mutations, usually affecting sulfate transport (Ohta et al., 1971), or plasmid borne (Silver and Misra, 1988; Verma et al., 2004). It appears that chromosomal and plasmid determinants function by different mechanisms, as resistance to chromate are additive in cells possessing both determinants.

Plasmid determined bacterial resistance to chromate has been found in *Streptococcus* (Efstathiou and McKay, 1977), *Pseudomonas* (Summers and Jacoby, 1978; Bopp et al., 1983; Cervantes et al., 1986; Cervantes and Ohtake, 1988; Sultan and Hasnain, 2003), and *Alcaligenes* (Nies and Silver, 1989a; Peitzsch et al., 1998). Chromate resistance results from decreased chromate accumulation by the resistant cells (Cervantes and Ohtake, 1988; Nies and Silver, 1989; Iyer et al., 2004).

Chromate resistance is probably based on an interaction of chromate reduction and chromate efflux (Nies, 1999) and a broad variety of bacteria able to reduce chromate have also been found (Cervantes and Silver, 1992). Chromate resistance was then mainly thought to be based on chromate efflux; however, data for *Ralstonia* sp. (formerly *Alcaligenes*) CH34 suggest that both processes, efflux and reduction are involved (Peitzsch et al., 1998).

Additional bacterial system exists that reduce more toxic Cr$^{6+}$ to a less toxic Cr$^{3+}$ (Silver, 1992). A chromate resistant strain of *Pseudomonas mendocina* MCM108, capable of reducing hexavalent chromium was found to harbor a single plasmid (Dhalkephalkar et al., 1996). Plasmid mediated chromate resistance and chromate reduction are independent process as chromate sensitive and chromate resistant *P. fluorescens* are both equally able to reduce chromate (Bopp and Ehrlich, 1988).

**Lead resistance**

Lead has been used in large amounts for 2500 years, as fuel additives, although the toxicity of lead for animals and man has been well known for a long time (Hong et al., 1994). Lead tolerant bacteria have been isolated (Trajanovska et al., 1997; Malik and Ahmad, 2002; Malik et al., 2002) and precipitation of lead phosphate within the cells of these bacteria has been reported (Levinson et al., 1996; Levinson and Mahler, 1998). *Ralstonia* sp. CH34 has been shown that resistance to lead is mediated by a P-type ATPase. Moreover, the Cad A P-type ATPase is also able to transport Pb$^{2+}$ (Rensing et al., 1998). Thus, lead resistance may also be based predominantly on metal ion efflux (Nies, 1999).
Copper resistance

The mechanism of copper resistance is not yet clearly understood though the copper resistance conferred by plasmid pPT23D has been elucidated at the molecular level (Jain, 1990). Copper resistance has also been reported to be chromosome encoded (Vargas et al., 1995a). Although the copper resistance determinants were shown to be homologous in E. coli and Pseudomonas species, the phenotype of the two copper resistant bacteria is different, while E. coli remains colorless, resistant Pseudomonas strains turn blue on high copper containing media because copper is accumulated in the periplasm and outer membrane (Cooksey, 1993; 1994). The periplasmic CopA protein shows conservation of several predicted copper binding sites. In addition the CopC and CopD proteins seem to catalyze copper uptake into the cytoplasm. Related copper resistance determinants were found in several Pseudomonas strains (Lin and Olson, 1995; Vargas et al., 1995a) and Xanthomonas campestris (Lee et al., 1994). Copper resistance is associated with antibiotic resistance plasmid in E. coli (Ishihara et al., 1978) and P. syringae (Bender and Cooksey, 1987).

Cadmium, cobalt, zinc and nickel resistance

The four heavy metals Zn, Co, Cd and Ni are used for a variety of applications. Metallic Zn is used for alloys like brass, galvanized iron and for fabrication of batteries. Cd is used for electroplating, for batteries and for TV tubes. Co and Ni are chemically related to iron and both are used for the production of steel and for electroplating. Cobalt and nickel salts have been used for centuries for the production of blue and green pigments, respectively. These examples illustrate the extensive use of these four heavy metals by humans which consequently leads to contamination of soil and fresh water habitats with these metals. In addition to anthropogenic contamination, heavy metal ions may leak from naturally occurring minerals into soil or fresh water habitats. In both cases it is not generally one cation that is present in toxic concentrations, but usually a major cation plus some accompanying ions e.g., Zn\(^{2+}\) plus Cd\(^{2+}\), Ni\(^{2+}\) plus Co\(^{2+}\) and CrO\(_4^{2-}\). As a response to this challenge, multiple-metal ion resistant bacteria evolved which contain a variety of plasmid encoded metal resistance determinants e.g. Staphylococcus aureus (Novick and Roth, 1968; Xiong and Jayaswal, 1998) and Alcaligenes eutrophus strain CH34 (Mergeay et al., 1985; Schmidt and Schelegel, 1994) in addition to the cze and cnr determinant. Alcaligenes eutrophus CH34 harbors three mer determinants (resistance to
Hg$^{2+}$; Dressler et al., 1991), chr (resistance to chromate; Nies and Silver, 1989b) and cop (resistance to Cu$^{2+}$) (Brown et al., 1992). When copper resistant Alcaligenes strains were isolated from wastewater these strains contained czc, cnr, chr, and mer resistance determinants although the corresponding metal ions were not used for selection (Dressler et al., 1991). Moreover, Alcaligenes eutrophus CH34 like organisms were easily isolated from metal contaminated habitats in Germany, Belgium and Zaire (Schmidt and Schlegel, 1994; Kaur et al., 1990; Diels and Mergeay, 1990). Thus the presence of a variety of metal resistant determinants in one bacterium seems to occur frequently in nature.

The Alcaligenes eutrophus strain CH34 was isolated from a zinc decantation tank and contains two large plasmids designated pMOL28 (163 kb) and pMOL30 (238 kb). These plasmids harbor a variety of metal resistance determinants (Mergeay et al., 1985). A. eutrophus KT02 was isolated from the wastewater treatment plant of Gottingen (Timotius and Schlegel, 1987); it harbors the following three plasmids: plasmid pGOE1 (250 kb), which determines cadmium and zinc resistance, plasmid pGOE2 (210 kb), which encodes nickel and cobalt resistance and plasmid pGOE3 (170 kb), for which so far no function is known (Schmidt et al., 1991). Alcaligenes xylosoxidans 31A was isolated from the metal working industry in Holzminden, Germany and it harbors two large plasmids, pTOM8 (340 kb) and pTOM9 (200 kb), both of which determine resistance to nickel, cobalt, zinc, cadmium and copper ions (Schmidt and Schlegal, 1989). Alcaligenes denitrificans, isolated from the wastewater treatment plant in Dransfeld, Germany (Timotius and Schlegal, 1987) and Klebsiella oxytoca CCUG 15788 isolated from the metal working industry in Goteborg, Sweden (Mattsby-Baltzer et al., 1989) are the strains which have been shown to carry nickel resistance genes on the chromosomes (Kaur et al., 1990; Stoppel et al., 1995).

**Antibiotic resistance in bacteria**

Expanded application of antibiotics has caused an increase in the incidence of resistance to these antimicrobial compounds, even within bacterial species that are not directly subject to antibiotic control. Numerous genes conferring resistance to antibiotics are presently circulating in bacterial populations, and such factors were not as prevalent prior to the selective pressures produced by the increased use of antibiotics (Hughes et al., 1983; Davies, 1994). Therefore, it is not surprising that contemporary strains of Gram negative bacteria frequently display resistance to high levels of commercially
administered antibiotics, and such cases have been documented repeatedly with these and other bacteria (Guillemot, 1999).

A number of common antibiotic resistance mechanisms were described for bacteria by Davies and Smith (1978), which may be listed as follows:

i) Alteration of the target site in the cell that reduces or eliminates the binding of the drug to the target site.

ii) Blocking the transport of the antibiotics into the cell—regardless of whether or not specific or active mechanisms of drug transport are involved, because a change in the transport system can reduce the penetration of the drug into the cell.

iii) Detoxification or inactivation of the antibiotic.

iv) Providing the cell with a replacement for the metabolic step that is inhibited by the antimicrobial agent. e.g. a by-pass mechanism.

v) Increasing the level of the enzyme inhibited by the drug, so that the drug is saturated, or titrated out.

vi) Production of a metabolite that can antagonize the inhibitory effect of the inhibitor.

vii) Decreasing the cell's metabolic requirements for the pathway or reaction inhibited by the drug.

The presence of antibiotic resistant bacteria in freshwater sources throughout the world has been documented (Kelch and Lee, 1978; Niemi et al., 1983; Ogan and Nwiika, 1993; McArthur and Tuckfield, 2000, Ash et al., 2002; Lin et al., 2004). Most surveys of antibiotic resistance in bacteria isolated from natural waters have been restricted to organisms that are indicators of fecal pollution (Niemi et al., 1983; Malik et al., 1995; Niemi et al., 1997; Sabry et al., 1997). Bacterial species of soil, water and sewage may have resistance to natural antibiotics or may acquire these characters from other bacteria through genetic exchange (Linton, 1986; Amyes and Gemmell, 1992; Malik et al., 1995; Malik and Jaiswal, 2000).

Several water-borne disease outbreaks occur due to the presence of drug resistant enteric pathogens which occur due to the failure of patients to respond to treatment with antibiotics (Gangarosa et al., 1972; Baine et al., 1977; Malik et al., 1995). E. coli with transmissible resistance to some broad spectrum antibiotics is considered to be potentially very dangerous because its resistance might be transmitted to some other pathogenic bacterial strains, thus rendering the treatment of infectious diseases very difficult (Malik
et al., 1995). The selective processes leading to the emergence and maintenance of bacteria resistant to antibiotics are mainly brought about by the incorrect or abusive utilization of these drugs (Anderson, 1968; Silva and Hoffer, 1993; Fluit et al., 2000).

Microorganisms resistant to both antibiotics and metals have been isolated frequently from environments and has led to the suggestion, that the combined expression of resistance to antibiotics and of resistance to heavy metal may be due to gene determinants presenting loci in the same plasmid (Smith, 1967; Aiking et al., 1984; Silva and Hoffer, 1993; Unaldi et al., 2003).

Conjugation

Bacterial gene transfer has been shown to occur in diverse environments including bulk soil (Top et al., 1990; Klingmuller, 1991), the rhizosphere (Knudsen et al., 1988; Lilley et al., 1996), the phylloplane (Normander et al., 1998), water and epilithon (Trevors et al., 1987; Hill et al., 1992). The transfer of plasmid conferring resistance to heavy metals has also been demonstrated in metal contaminated sites such as those near smelters (Top et al., 1994). Many experiments have used metal resistance genes as markers, especially Hg resistance, to select the transconjugants because they are relatively stable and easy to work (Mergeay et al., 1990). These studies and other transfer studies showed that the frequency of plasmid transfer and the propagation of the transconjugant formed are strongly affected by genetic and ecological factors (Verma et al., 2002).

Over thirty years of studies have established that conjugative transfer of plasmid-encoded resistance to drugs and heavy metals can take place at high frequency between various organisms under laboratory conditions. The detected transfer frequency in soil, in aquatic environments, and in the urogenital and respiratory tracts of healthy animals and man has generally been low. However, the conversion of bacteria from susceptible to resistant to antibiotics has been observed during antimicrobial therapy. This has formed a challenge for the antibacterial treatment of pathogenic bacteria and called for the evaluation of the extent of conjugative transfer in various environments (Viljanen and Boratynsski, 1991).

The process is usually encoded by conjugative plasmids which have been isolated from a diverse range of Gram-negative bacteria and include members of more than 20 incompatibility groups (Bradley, 1980). All these plasmids encode the production of sex
pili (Ippe-Ihler, 1989). These pili play an essential role at least in the cellular interactions that precede the conjugative transfer of DNA (Ippe-Ihler, 1989).

R plasmids are composed of two genetically and physically distinguishable components: i) a RTF factor that harbors the gene for self transmissibility (tra) and autonomous replication (rep) ii) a resistant determinant (R-determinant) that harbor majority of the resistance genes. The plasmid transfer is possible both within and outside the boundaries of the genus (Bale et al., 1988, Rani and Mahadevan, 1992; Silva and Hoffer, 1993; Sikander and Shahida, 1994; Davison, 1999).

Curing

The elimination of plasmid DNA from plasmid carrying host strains, or curing has been described for several species of mesophilic bacteria. Techniques for this purpose involve growth of the strains at elevated temperature (May et al., 1964; Saha et al., 2000; Unaldi et al., 2003) or in the presence of sodium dodecyl sulfate (Ruiz-Barba et al., 1991; Saha et al., 2000; Bruins et al., 2003), intercalating drugs (Bouanchaud et al., 1968) inhibitors of DNA replication (McHugh and Swartz, 1977; Taylor and Levine, 1979) or transcription (Johnston and Richmond, 1970) and formation of protoplasts (Novick et al., 1980). Curing is of practical significance both in chemotherapy of drug resistant bacteria and in bacterial genetics. Genetic determinants of antibiotic resistance (Shahid et al., 2003), oil degradation (Chakrabarty, 1976), serum resistance (Reynard and Beck, 1976), haemolysin, colicin and enterotoxin biosynthesis (Goebel et al., 1974; Smith, 1974; Gyles et al., 1974), and heavy metal resistance (Bruins et al., 2003) are located extrachromosomally on plasmids. Curing of a plasmid DNA by mutagens depends on (i) surface structure of bacterial cells which may prevent the mutagen to enter the cell, and (ii) nature of the plasmid DNA (Singh and Yadava, 1988).

Genotoxicity of water

An aquatic environment such as river, is a depository of different types of anthropogenic discharges and a wide range of human activities e.g. domestic, agricultural and industrial activities are potential sources. Several studies have revealed that the river water in many countries has been contaminated by genotoxic compounds (Kusamran et al., 1994; Filipic et al., 1995; Filipic, 1995; Rehana et al., 1995; 1996; Duan et al., 1999; Vargas et al., 2001; Avishai et al., 2004; Umbuzeiro et al., 2004).
Environmental mutagens may be a major risk factor for human health. Of particular concern are hazardous industrial wastes and effluents. Their chemical complexity precludes a detailed chemical analysis of individual genotoxic components. Therefore short-term bioassays are often used for screening the potential genotoxic risk of complex environmental mixtures (Cerna et al., 1996). The identification of specific chemical substances with genotoxic activity in drinking water, in untreated waters or even in industrial effluent is quite difficult because few compounds are present at high concentration (Malik and Ahmad, 1995; Vargas et al., 1995b). Many times genotoxic activity cannot be attributed to specific compounds in mixture but rather to a set of properties and chemical interactions of the sample as a whole (McGeorge et al., 1983).

Mutagenicity evaluations of surface water provide an indication of potential hazard in the absence of priority knowledge about the identification or physical/chemical properties of the putative toxicants. The Salmonella mutagenicity assay in particular has been widely used to detect mutagenic activity in complex environmental mixtures such as surface waters, especially river waters (Ohe et al., 2004).

The presence of genotoxins in source water have been determined both by direct detection of cytogenetic effect in aquatic species (Prein et al., 1978; Van der Gaag et al., 1983; Rajaguru et al., 2003) and other test organisms (Klekowski and Levin, 1979; Ma et al., 1985; Steinert et al., 1998) and by demonstration of genotoxic activity in organic and inorganic concentrates of these waters (Van Kreijl et al., 1980; Kool et al., 1981; Rehana et al., 1995; 1996; Kummrow et al., 2003). Mutagenic activity have been detected in organic and inorganic extracts of industrial wastewater from a variety of industries (Johnston et al., 1982; McGeorge et al., 1985; Malik and Ahmad, 1995; Vargas et al., 1993; Lemos et al., 1994; Alzuet et al., 1996; Umbuzeiro et al., 2004). Similarly concentrates of municipal wastewater frequently contains detectable amount of genotoxic agents when the ratio of industrial and domestic waste input is high (Meier and Bishop, 1985; Meier et al., 1987; Magdaleno et al., 2001).

Previous work had suggested that the compound responsible for mutagenicity of river water were primarily of industrial origin (Meier and Bishop, 1985). Significant mutagenic activity has been detected in a variety of wastewater effluents and sludges (Pancarbo et al., 1987; Malik and Ahmad, 1995). A coke plant waste was tested for mutagenicity in the standard Salmonella assay by Andon et al. (1986). The sample was extremely potent in conventional Salmonella assay especially in TA98 with metabolic
activity. Sanchez et al. (1988) collected 10 wastewater effluents from metallurgical industries operating in an industrial complex in Brazil. Organic extracts of the samples were tested for acute toxicity and mutagenicity in short term microbial assays. Of the 10 metallurgical wastes tested, a large percentage (60%) was mutagenic either in *Salmonella* or *E. coli* fluctuation test. Mutagenic metallurgical effluents have also been reported by McGeorge et al. (1985).

Maruoka et al. (1986) concentrated the organic fractions of the river water in Japan by XAD adsorption followed by elution with ethyl ether. *Salmonella typhimurium* tester strains TA1538 and TA98 were used for detecting mutagenic activity. Mutagenic fractions separated by a liquid-liquid fractionation and two consecutive runs with thin layer chromatography were analyzed using high performance liquid chromatography. Their results indicated that at least six different frame shift mutagens all requiring metabolic activation were present in the river water.

Ziaee and Rastgar-Jazii (1996) tested water extracts prepared from river and stored rain water and found that these extracts induced high mutagenicity in *Salmonella typhimurium* TA98 strain, while TA100 strain did not show any response. Spring and canal water extracts caused a significant increase in the TA98 his revertants which was not observed in TA100. Among the 40 samples of deep and shallow water concentrates, only one third of the later induced significant mutagenicity in the TA98 strain. Among all the prepared extracts, only those belonging to river and stored rainwater caused remarkable induction of DNA breaks in V79 fibroblasts. They further stated that as an important part of human diet water and its constituents and quality, may seriously influence the health status of the exposed population. The presence of genotoxic pollutants in this vital constituent of diet could induce certain genetic disorders such as cancer.

Otsu et al. (1998) evaluated the mutagenicity of the river water in Korea using blue rayon method. Their results showed very high mutagenicity of the river water flowing through an industrial district, suggesting serious pollution of river water in such districts. The source of the pollution appears to be waste fluids from the surrounding factories. Though its direct effects on human health are unclear, long-term exposure to the pollutants via fishes and drinking water may have toxic effects on human health. The river water into which waste fluid of daily living and agriculture flows was not
mutagenic. On the other hand, the river water into which industrial waste fluid flows was highly mutagenic in TA98 with S9 mix.

Park *et al.* (2000) extracted the organic contents of drinking tap water from Seoul, Taejon and Suwon (Korea) with XAD-2 resin column and organic solvents. Four doses of the extracts equivalent to 4, 2, 1 and 0.5 L water were tested for mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 in the presence and absence of S9 mix. The organic extracts of water from all these cities were mutagenic in TA98 in the absence of S9 mix and in TA100 with and without S9 mix. They also tested three doses of the extract (equivalent to 22, 11 and 3.7 L water) in bone marrow micronucleus test using BDF1 mice and reported a significant increase of micronucleus frequency at the highest dose.

Since 1979, the environmental agency of Sao Paulo State in Brazil, CETCEB, has been using the *Salmonella* mutagenicity assay to assess the quality of natural waters. They compiled the data obtained during the last 20 years from more than a thousand of samples. Potencies up to 30,000 reverants/l were obtained in 137 positive samples (Umbuzeiro *et al.*, 2001).

Kummrow *et al.* (2003) extracted the water sample from river under the influence of an azo dye-processing plant discharge using XAD-4 and blue rayon. The organic extracts were tested for mutagenicity with the *Salmonella* assay using TA98 and TA100 strains and they found that the efficiency of the blue rayon to recover the mutagenicity of 2-aminoantracine was similar to XAD resin. This was also observed for the recovery of PAHs extracted by the XAD-resin and blue rayon.

**Genotoxicity of soil**

Thousands of chemicals are released and find their way into the environment i.e. air, land, ground and surface water, by industrial activity etc. Numerous genotoxic compounds have been detected in both the particulate and gas phases of outdoor air particularly in densely populated urban regions (Cohen, 2000). Combustion of fossil fuels for power generation and transportation in industrial facilities, power plants and motor vehicles are 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).
Most of the atmospheric compounds eventually descend to the ground, and therefore the ground surface may be contaminated with these genotoxic compounds. It was reported that some industries, e.g. pulp and paper mills, steel foundries and organic chemical manufacturing facilities discharge waste of noteworthy genotoxic potency (Houk, 1992).

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 ground water 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 (Watanabe and Hirayama, 2001).

Although there are large number of genotoxicity assays, a relatively small number have been used to examine soil genotoxicity, and most of these used the Salmonella mutation assay (Smith, 1982; Knize et al., 1987; DeMarini et al., 1992; Watanabe et al., 1998; Goto et al., 2000; Wesp et al., 2000; Edenharder et al., 2000, Ehrlichmann et al., 2000; Watanabe et al., 2000; Garcia and Donnelly, 2002; Courty et al., 2004). Other DNA damage assays, chromosome assays and so forth using rat lung DNA (Randerath et al., 1994), bacteria (DeMarini et al., 1992; McDaniels et al., 1993; Ehrlichmann et al., 2000), cultured cells (Wesp et al., 2000), mice (Wesp et al., 2000), and plants (Wang, 1999; Gichner and Veleminsky; 1999; Cotelle et al., 1999).

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 biphenyls (Donnelly et al., 1988; DeMarini et al., 1992; Cotelle et al., 1999), pesticides (Ruiz and Marzin, 1997), polycyclic aromatic hydrocarbons (Ehrlichmann et al., 2000), heavy metals (Wang, 1999; Ehrlichmann et al., 2000) munition wastes (Ehrlichmann et al., 2000), wood preserving wastes (McDaniels et al., 1993; Randerath et al., 1994) etc. Donnelly et al. (1988) evaluated the genotoxicity of soil samples collected from the vicinity of a PCB disposal area using Salmonella mutation assay. They reported that sequential extracts of the soil samples with methylene chloride and methanol were mutagenic towards TA98 in the presence of the mammalian metabolic activation system (S9 mix). Ehrlichmann et al. (2000) evaluated genotoxicity of
concentrated and non concentrated aqueous soil extracts from various soil samples using three bacterial assays: the umu test with *Salmonella typhimurium* TA1535/pSK1002, the NM2009 test with *Salmonella typhimurium* NM2009 and SOS chromotest with *E. coli* PQ37. They reported that the concentrated and non concentrated aqueous extracts from the samples contaminated with nitroaromatic compounds exhibited an extremely high genotoxic potential in all of the genotoxicity tests.

Agricultural soil was also reported to be mutagenic in the *Salmonella* mutation assay both in the presence and absence of S9 mix (Smith, 1982; Goggleman and Spitzauer, 1982; Brown *et al*., 1985; Edenharder *et al*., 2000). Goggleman and Spitzauer (1982) examined n-hexane/aceton extracts of soil from several agricultural fields on which crops such as asparagus, rye, oat, pasture and meadow grew. and showed that all soil samples were mutagenic towards *S. typhimurium* TA98 and TA100 with some differences in potency. Brown *et al*., (1985) demonstrated that dichloromethane extracts of three types of agricultural soil exerted mutagenicity in eukaryotic test using *Aspergillus nidulans* as well as in *Salmonella* assay, and suggested that the activity was related to past agricultural practices, including biocide application, fertilization and cultivation.

Soil samples from roadsides and some points where there is no apparent industrial or agricultural pollution source have also been reported to be positive in the *Salmonella* mutation assay (Tamakawa *et al*., 1985; Knize *et al*., 1987; Aboul-Enein *et al*., 1989; Arashidani *et al*., 1992; Nishimura *et al*., 1992; Watanabe *et al*., 2000; Wesp *et al*., 2000) and plant assays (Gichner and Veleminsky, 1999).