REVIEW OF LITERATURE
The interaction of microbes with heavy metal ions has been studied for decades from different considerations and for diverse applications. Initially, heavy metal ions were highly valued as antimicrobial agents. Compounds of silver, mercury and arsenic were among the first chemotherapeutic agents. The use of silver in opthalmic neonatal gonorrhoea, mercuric compounds as diuretic and antisyphilitic agents (Jeffries, 1982) and organo arsenic compounds for treatment of spirochetal infections (Foye, 1977) is well known. However, due to toxicity of certain metal ions to human and animal systems, their use as chemotherapeutic agents has faded away in the wake of newer and much safer drugs. In recent years, the interest in metal ions has come about from an entirely different angle. The discovery that certain metal ions (e.g. methyl mercury) accumulate into the food web of man (D'Itri, 1972) and the resultant toxicity in man due to consumption of such foods, played an important catalytic role in the vigorous effort to study the ways and means to combat this situation. The role of microorganisms in this accumulation and in fact enhancement of the toxicity of certain metal ions is now well established (Summers and Silver, 1978).

Though the medicinal use of mercurial compounds,
owing to their toxicity, has since been by and large abandoned, the use of silver compounds in topical chemotherapy and as a sanitizing agent is finding a new impetus because of several reasons. Reports about pollution with silver are not available in literature and the cases of poisoning are very rare. However, the usage of silver in photographic material might be a cause for concern (Summers et al., 1978), if efforts are not made to recover silver from the effluents.

The disinfection of drinking water, using silver salts has been achieved by several processes, e.g. by percolation of water through sand coated with silver or by electrolytic methods making use of silver electrodes (Sykes, 1965).

Applications in medicine:

Several compounds of silver including soluble silver salts and the colloidal preparations of silver are used for antiseptic purposes. It has been observed that soluble silver salts like silver nitrate and citrate are extremely irritant and colloidal silver preparations like silver proteinates are more useful for clinical purposes.

Silver compounds have proved particularly useful for topical burn wound antisepsis, because of the minimal adsorption of these potent antimicrobial agents, which are also of low toxicity. It has been observed that the colonization of burn wounds by gram negative bacteria is
the principal cause of high mortality that accompanies extensive burn injury. None of the antibiotics, whether used systemically or topically have significantly reduced the mortality in burn patients. Also, the prolonged use of antibiotics leads to dangerous systemic toxicity and to the emergence of superinfection by resistant organisms. Among the various alternatives for local treatment of burns, silver therapy appears to be most promising. However, hypotonic solution of silver nitrate, which release silver ions rapidly, were found to cause biologically dangerous deficits of sodium chloride and required frequent applications to achieve clinically effective concentrations of silver in local wounds (Fox, 1968; Moncrief, 1974). Fox in 1968 synthesized a new silver compound — Silver sulfadiazine (SSU), which is a white fluffy water-insoluble complex and it does not readily precipitate chlorides. Now approved by Food and Drug Administration (U.S.A.), Silvadene — a 1 per cent silver sulfadiazine cream effectively controls and eradicates infections in burn wounds and the superior efficacy of SSU has been reported by workers all over the world (Fox, et al., 1969; Lowbury et al., 1971; Mc Dougall, 1972; Dickinson, 1973; Fox, 1973; MacMillan, 1973). Deitch et al. (1973) reported the effectiveness of a silver-nylon fabric (which consists of a nylon substrate coated with metallic silver) as a wide spectrum antimicrobial agent and they have suggested that it might be useful for delivering
sufficient levels of silver (to eradicate local soft tissue infections) continuously. Also because of the electrical conductivity of this fabric, it might be possible to generate and maintain microbicidal concentrations of silver ions almost indefinitely by the passage of a weak electrical current.

The subsequent introduction of zinc sulfadiazine represented a further improvement in the topical therapy, by the adjuvant provision of zinc (a normal body constituent), essential for the healing of wounds, once infection is under control (Prasad, 1966; Nielsen and Jemec, 1968; Fox et al., 1976). The modification of silvadene by incorporating cerium nitrate into it has been found to strikingly enhance its topical antiseptic effect without increasing the toxicity (Fox et al., 1977). This is particularly useful, when wound size increases to 50-60% of the body surface area and SSU alone is inconsistent in preventing or suppressing the sepsis by both gram negative and gram positive bacteria. The possibility of use of other metal ions has also been explored by Fox et al., (1979). They compared the inhibitory properties, solubility and toxicity of various metal sulfonamides (Al$^{3+}$, Cu$^{2+}$, Co$^{2+}$, Fe$^{3+}$, Ag$^{+}$, Zn$^{2+}$, Ce$^{3+}$). Cobalt alone had MIC figures comparable to Ag$^{+}$, Zn$^{2+}$ and Ce$^{3+}$. LD50 of SSU, CuSU and ZnSU/CoSU for mice were 140 mg, 250 mg and 400 mg per kg. body weight respectively.

Mode of Action of Heavy metals:

The antimicrobial activity or toxicity of metal
ions to higher forms of life can be related to the inhibition of one or more processes in a cell. Generally, because of the multiplicity of binding sites, the nature of metal ion toxicity is so generalized that the specific effects are often masked or overlooked. Some information, nevertheless is available about the potential targets in a bacterial cell and these are—

a) binding to -SH group containing proteins and enzymes,
b) binding to deoxyribonucleic acid,
c) binding to structural components like cell wall or cell membrane.

The inhibitory effect of mercury, cadmium and silver is probably due to the action of these ions on conformation of enzymes causing non-competitive type of inhibition. The silver ion can combine with the thiol groups in the cysteine residues of the enzyme proteins to form mercaptides (Madsen, 1963, Dixon & Webb, 1964). The mercaptide inhibits the enzyme by altering the conformation at the active site. This can have a wide range effect on the bacterial system. It is likely that the sulfhydral groups with different reactivities are present in the dehydrogenase region of the respiratory chain (Eastbrook et al., 1968; Kim & Bragg, 1971) as well as the transport carriers for various sugars and amino acids (Reeves et al., 1972; Kaback & Hong, 1973). The reports about susceptibility of these systems to metal ions
are available in literature (Bragg & Hou, 1968; Azocar & Munoz, 1978). Zinc ions are known to inhibit the succinate oxidase system in *E. coli* respiratory particles at the level of succinate dehydrogenase (Kasahara and Anraku, 1972) and thiol reagents like pCMB, Hg$^{2+}$ and Ag$^+$ inhibit the transport of succinate in membrane vesicles of *E. coli* (Raymon et al., 1972). The interaction of silver ion with respiratory chain of *E. coli* has also been reported (Bragg and Rainnie, 1974). The site most sensitive to silver ions was located between cytochrome b and cytochrome a$_2$ and the second level of inhibition was in the NADH and succinate dehydrogenase regions of the respiratory chain. The inhibition was alleviated to a great extent by the addition of glutathione. The prevention or reversal of the inhibitory action of heavy metal ions by thiol containing compounds has been reported by other groups also (Chambers et al., 1962; Berntheim, 1971).

Synthesis of macromolecules might be particularly sensitive to poisoning by metal ions, either directly due to binding to polynucleotides (Dale and Ward, 1975) or due to release of degradative RNAase activity (Beppu and Arima, 1969). The mechanism of action of SSU has also been investigated (Modak & Fox, 1973; Fox and Modak, 1974) and it has been established that silver alone is responsible for the bactericidal action of SSU and this results from the binding of silver to the deoxyribonucleic acid in bacteria. The sedimentation
coefficient of DNA isolated from SSU inhibited bacteria was found to be higher than that of normal DNA (Wysor and Zollinhofer, 1972). The binding of zinc ions to DNA has also been reported by Fox et al. (1976) and Eichhorn et al. (1974).

Cell membrane has also been identified by some workers as the primary site of action of metal ions in certain microorganisms. An extensive loss of $K^+$ from yeast treated with $Hg^{2+}$ was reported by Jeffries (1982). The leakage of potassium ions from cells is probably due to a breakdown in the permeability of the membranes. (De Filippis, 1979). Other evidence to support the cellular membrane being an important site of $Hg^{2+}$ action. Low levels of $Hg^{2+}$ ions can cause lysis of Anabaena inequalis (Stratton et al., 1979) and E. coli (Schaechter and Santomassino, 1962) and the swelling in Pseudomonas aeruginosa (Berntheim, 1971). Similarly, other heavy metal ions like copper, zinc and cadmium also affect permeability of plasma membranes, causing leakage of electrolytes (Rothstein (Passow and , 1961; O'Kelly, 1974 and Stratton and Corke (1979). Carr and Rosencraz (1972) reported that the bactericidal action of silver sulfadiazine is due to binding of this compound with cell membrane.

Transport of metal ions into bacteria:

Passive and active mechanisms have been proposed to account for the uptake of heavy metals by bacterial and
algal species (Davies, 1973; Fujita & Hashizume, 1975). The former mechanism does not require the involvement of any metabolic process or energy and is called adsorption (Sakaguchi et al., 1979). However, it appears that the amounts of heavy metals taken up by passive mechanisms are quite low as compared to those taken up by energy-dependent processes (Bucheder & Brodas, 1974; Gadd & Griffiths, 1978). It has been shown that uptake of zinc (Bucheder & Broda, 1974) and cobalt (Nelson & Kennedy, 1971) into E. coli cells is inhibited by depletion of glucose or addition of the metabolic inhibitors.

The transport of various metal ions into bacterial cells is mediated by different carrier systems and it appears that most of the metal ions are taken into cells via the already existing cation uptake systems e.g. the uptake of cadmium into S. aureus cells occurs by the energy dependent manganese transport systems (Weiss et al., 1978; Tynecka et al., 1981). Similarly the uptake of cobalt and arsenate is by the magnesium transport (Nelson & Kennedy, 1971) and the phosphate transport systems (Willsky and Maladmy, 1980) respectively. However, the uptake of Hg$^{2+}$ seems to occur by a Hg$^{2+}$ specific transport system (Nakahara et al., 1979) though it has not been fully characterized. Zinc may be transported by a highly specific transport system (Bucheder & Broda, 1974; Failla, 1977) or via the basic Mg$^{2+}$ transport system (Jasper & Silver, 1977).
Development of resistance:

The antibiotic resistance has been most thoroughly studied and several reviews are available in literature (Benveniste and Davis, 1973; Lacey, 1975; Davies and Smith, 1978; Foster, 1983). Study of epidemiology of drug resistant bacteria indicates that the origin, selection, spread and prevalence of antibiotic resistance in microorganisms has resulted from indiscriminate usage of the antibiotic (Nakahara et al., 1977). The antibiotic resistance genetically has been attributed to the presence of plasmids (Broda, 1979; Falkow, 1975; Hardy, 1981). Also, resistance genes are often incorporated into discrete genetic units called transposons (Kleckner, 1981), which have the capacity to transpose from one DNA molecule to another. This has an important role to play in the dissemination of resistance genes and hence in the evolutionary process.

The plasmid determined drug resistance could be a consequence of one of the following processes -

1. Blocking the transport of the drug into the cell (Franklin, 1973);
2. Detoxification of the drug (Richmond and Sykes, 1973; Sykes & Mathew, 1976);
3. Alteration of the target site (Lai & Weisblum, 1971);
4. Bypass of the metabolic step that is inhibited by the drug (Skold, 1976).
Heavy metal ion resistance in a number of bacteria is seen in isolates from various sources. However, the resistance to heavy metals, like antibiotics, has arisen in response to the selective pressures in the environment. Mercury and organomercurial resistant bacteria were first isolated from mercury contaminated soil in Japan (Tanaka et al., 1983). They have since been isolated from sediments of the New York Bight, heavily polluted with mercury from industrial wastes (Timoney et al., 1978). Similarly, silver resistant bacteria have been isolated from sewage from an industrial plant that reprocesses used photographic film (Summers et al., 1978). Metal resistant enteric bacteria, Staphylococci and Pseudomonas spp. have also been isolated from clinical setting (Nakahara et al., 1977; Weiss et al., 1977; Hendry and Stewart, 1979). Recently it has been noted that reduction in the use of mercurial compounds as diuretics and disinfectants has resulted in a 66% decrease in frequency of mercury resistant S. aureus isolates from hospitals in Tokyo (Porter et al., 1982).

Metal resistance is determined by plasmids, which in many instances also encode resistance to other heavy metal ions and antibiotics (Smith, 1967; Novick and Roth, 1968; Kondo et al., 1974; McHugh et al., 1975; Nakahara et al., 1977). The evolution of multiple resistance metal ion and antibiotic strains might be a result of sequential acquisition of individual transposons to form composite transposable units.
Resistance to Mercury:

Resistance to mercurial compounds is a common plasmid determined property of both gram-positive and gram-negative bacteria (Schottel et al., 1974; Clark et al., 1977; Weiss et al., 1977; Weiss et al., 1978), which, in majority of the cases is determined by enzymatic reduction of the mercuric ion to elemental mercury, Hg⁰, which is much less toxic and evaporates from the medium because of its high vapor pressure (Summers and Silver, 1972; Summers and Silver, 1978). Mercury resistance is expressed only after exposure to sub toxic levels of Hg^{2+} (Summer and Silver, 1978), hence the volatilizing activity in Hg⁰ strains is inducible (Schottel, 1978).

The enzyme that catalyses the reduction is the FAD containing intracellular mercuric reductase (Schottel, 1978). It is specified by plasmid R 100 and transposon Tn 501, and is composed of sub units of about 63,000 molecular weight (Fox & Walsh, 1982). Strains, which detoxify organomercurials contain another cytoplasmic enzyme - organomercurial lyase which cleaves the C-Hg bond (Tezukad & Tonomura, 1978) to release Hg^{2+}, which is volatilized by the reductase. Since, the reductase enzyme is intracellular, it appears that the transport of Hg^{2+} to the inside of the cell is essential. A Hg^{2+} specific transport system under the control of a plasmid has been proposed by Nakahara et al. (1979) and Foster et al. (1979).
The plasmid specifying Hg\(^{2+}\) resistance, known as the 'mer' plasmid has been genetically analysed. Three genes have been recognised and mapped - the regulatory gene (mer R), the transport function gene (mer T), and the reductase gene (mer A). An operon structure has been proposed to explain the co-ordinate induction of mer T and mer A (Foster et al., 1979).

Several other mechanisms of resistance to Hg\(^{2+}\) ions are also known to occur in bacteria, though, they are less common. These mechanisms include the biological methylation of mercury by aquatic microorganisms (Jensen and Jernelov, 1969; Pan-Hou, 1982); detoxification of methyl mercury and conversion into HgS with the help of H\(_2\)S (Pan-Hou, 1981) and decreased accumulation of mercury (Pan-Hou et al., 1981). Enzymatic volatilization of mercury could not be observed in these cases.

**Resistance to Cadmium:**

High level resistance to cadmium in *S. aureus* has been associated with decreased accumulation of the ion into bacterial cell (Chopra, 1970, 1975; Weiss et al., 1978). The primary avenue of entry of Cd\(^{2+}\) into *S. aureus* cells is the energy dependent manganese transport system and it has been shown that Cd\(^{2+}\) inhibits the uptake of Mn\(^{2+}\) in susceptible cells, but is without effect on Mn\(^{2+}\) transport in resistant
S. aureus cells (Weiss et al., 1978). It is now clear that Cd\(^{2+}\) resistance is caused by a plasmid encoded efflux system (Tynecka et al., 1981). The resistant strains expel cadmium via a Cd\(^{2+}/2H^+\) antiport system so that one Cd\(^{2+}\) ion is exchanged for two protons.

The resistance is governed primarily by two genes on the plasmid. Cad A gene has been mapped (Smith & Novick, 1972 and causes approx. 100-fold increase in Cd\(^{2+}\) resistance (Novick & Roth, 1968). Some plasmids have an additional gene, Cad B, which maps quite distantly from Cad A locus and it provides only a smaller increase in Cd\(^{2+}\) resistance (Novick & Bouanchaud, 1971).

In contrast to the above mechanism, active accumulation of cadmium from the growth medium by a strain of Pseudomonas putida has been reported (Higham and Sadler, 1984). The resistance mechanism involves a series of cysteine rich cadmium-proteins that are induced during different growth phases. The cysteine rich proteins contain 4-7 gram atoms of cadmium per mole of the protein.

Furthermore, there is a report by Mitra et al. (1975) of accommodation to high cadmium levels in E. coli, which involves compartmentalization of Cd\(^{2+}\) in the cells.

Resistance to Arsenate:

Plasmid determined resistance to arsenate is
present in *S. aureus* and enteric bacteria (Novick and Roth, 1968; Hedges and Baumberg, 1973). Resistance to arsenate is inducible and is linked to arsenite and antimony resistance, each ion being capable of acting as an inducer.

The resistance to arsenate is specified by an efflux system (Mobley and Rosen, 1982), which is specific for arsenate. The efflux system is driven by hydrolysis of ATP.

**Resistance to Silver:**

Plasmid mediated resistance to silver ions has been observed in clinical isolates (Mc Hugh *et al.*, 1975; Hendry & Stewart, 1979), where silver salts have been used to treat burn wounds. Resistance to silver ions is expressed constitutively (Silver, 1981) and is a function which is located at the cell surface. It has been suggested (Silver, 1981; Silver *et al.*, 1982; Silver & Misra, 1984) that resistance to silver is dependent on the presence of halide ions, so that AgCl₂ precipitates are found and the resistant cells can't extract silver from the insoluble precipitates.

Studies conducted by Belly and Kidd (1982) showed that Ag⁺ bacteria convert silver salts to elemental silver and it is stored in the cells as harmless Ag-protein complexes.

**Emergence of resistance:**

Resistant organisms have appeared quickly after
the widespread usage of toxic substances. The earliest reported case appears to be the resistance of *Bacillus subtilis* to the antiseptic mercuric chloride (Kossiakoff, 1887). In the early days of the antibiotic era, there was a major controversy regarding the basis of resistance - whether the resistance had a genetic (mutational) or physiological (adaptive) basis? However, the mutation and selection theory of resistance is largely accepted now. This has its basis in the Luria-Delbruck fluctuation test (Demerec, 1948; Cavalli, 1952) and it implies that under the selective pressures of the new or old drugs, the variants possessing the genetic advantage would be selected from the normal susceptible populations.

Numerous antibiotic resistance mechanisms have evolved in microorganisms, however, what is more crucial to the effectiveness of resistance mechanism is the multiplicity of the transfer systems (viruses, plasmids, transposons etc.) that have evolved in addition to resistance. Transference of certain kinds of genes to lysogenic viruses and plasmids allows microorganisms to respond to environmental challenges by amplifying and deamplifying genes that confer resistance. Gene transfer by plasmids allows most organisms to be free of the burden of carrying the plasmid so that plasmids are stored in a minimum portion of the population and regained when needed.
There appears to have been little de novo evolution giving rise to new gene functions forced by the use of antimicrobial agents by man. The common microbial responses to even a new type of growth inhibitor have been the amplification, derepression and mobilisation of genes from other sources (Koch, 1981). Amplification can very easily be selected in the laboratory by prolonged exponential growth in liquid medium containing a high but sub-inhibitory concentration of the drug (Scholff and Puhler, 1979; Wiebauer et al., 1981). However, de novo evolution of resistance genes must have taken place at some stage during the evolution of life on earth and these must have evolved in response to toxic agents such as newly evolved antibiotics. The primitive organism must have had no resistance genes and no mechanism for gene transfer as well (Koch, 1981) and the emergence of resistance could only have taken place by point mutations, deletions and insertions.