REVIEW OF LITERATURE

The concern over deterioration of water quality has attracted wider attention from all spheres of mankind. Since long, concern over this has stimulated studies on limnology of water bodies like lakes, rivers and oceans and efforts have been made to correlate distribution of the algae with the amounts of different physical and chemical factors (Jayangoudar, 1964; Olga and Owens, 1976; Rai, 1978; Noyelles and Brien, 1978; Moore, 1979). However, rarely any attempt has been made to establish the relationship between coliform bacterial presence, their distribution and periodicity with that of physical and chemical factors.

Bacteria has been widely used as a tool to determine whether water has been polluted by pathogenic, faecal intestinal flora. In sanitary microbiology, some bacterial group of organisms have been universally accepted as indicator of water pollution (A.P.H.A., 1975; Geldreich and Clark, 1972; Grabow, Prozesky and Smith, 1974; W.H.O., 1971). It is also because, their presence in water is always associated with faecal pollution (Geldreich and Clark, 1972; Bagley and Seidler, 1977). The access of faecal pollution to water may add a variety of intestinal pathogens at anytime, and at one time or another, pathogenic organisms
would be present in water degraded by a variety of pollutional discharges from warm-blooded animals. The most common pathogens include strains of *Salmonella*, *Shigella*, *Leptospira*, enteropathogenic *Escherichia coli*, *Pasteurella*, *Vibrio*, *Mycobacterium*, human enteric viruses, cysts of *Entamoeba histolytica* and hookworm larvae (Geldreich, 1972). However, although modern bacteriological methods have made it possible to detect these pathogenic bacteria in sewage and sewage effluents, it is not practicable to isolate them as routine procedure from samples of drinking water because laboratory methods of isolation and identification remain too cumbersome for routine use.

When pathogenic organisms are present in faeces or sewage, they are always greatly outnumbered by the normal excremental organisms and these normal organisms are easier to detect in water. These are coliform group of organisms. If these organisms are not found in the water, it can in general, be inferred that disease producing organisms are also absent and the use of normal excremental organisms as an indicator of faecal pollution in itself introduces a margin of safety. Yet, the total coliform count should not be regarded as specific indicator of faecal pollution and faecal coliform count should also be used to support it, as faecal coliforms are reported to be reliable indicators of faecal pollution (Clark and Kabler, 1964; Dufour, 1977).
Since a wide range of pathogenic organisms are associated with transmission of disease by water, (Geldreich, 1972) recently, interest has been focussed particularly on organisms which are indicative of faecal contamination (Dutka, 1973; Feachem, 1975). Of these faecal indicators, it is faecal coliforms which have received the most attention, (Feachem, Khan and Rosbergen, 1977) and have become most widely accepted as parameters of detecting faecal pollution.

The coliforms include *Escherichia coli*, *E. freundii* and *Aerobacter aerogenes*. These are Gram-negative, non-spore forming rods, capable for fermenting lactose with the production of acid and gas at 37°C in less than 48 hours. However, many species, like *Aerobacter aerogenes* and others belonging to coliform group, are non faecal in origin and are widely distributed in nature. But *E. coli* is of proven faecal origin and its presence should be considered as a sure indication of faecal pollution, calling for immediate action. For the purpose of hygienic analysis of water, *E. coli* is regarded as a gram negative, non spore forming rod which is capable of fermenting lactose with the production of acid and gas, both at 37°C and 44°C in less than 48 hours, which produces indole in peptone water containing tryptophan, and which is capable of utilizing sodium citrate as its sole source of carbon (W.H.O., 1970).
Although, since long *E. coli* was considered as non-pathogenic, and used only as an indicator of faecal pollution, the status of *E. coli* as a pathogen has been recognized in recent years, (Sack, 1976). Certain strains were regarded as causing enteritis in only young children. It has now been suggested that some strains may also have a role in adult diarrhoea (Hobbs, Rowe, Kendall, Turnbull and Ghosh, 1976). Food products of animal origin have been found to contain enterotoxin producing *E. coli* that are similar serological type of those that cause human disease. Various serotypes of *E. coli* frequently cause gastroenteritis characterized by a profuse watery diarrhoea with little mucus and no blood, nausea, prostration and dehydration with a general absence of fever (A.P.H.A., 1970). Serious diarrhoea among children under 5 years, particularly of the newborn is frequently a result of the etiologic agents, enteropathogenic *E. coli* (A.P.H.A., 1970). Adults may also succumb to a diarrhoea caused by these organisms. In addition most urinary infections of adults have been reported to be caused by pathogenic *E. coli* (Turck and Peterdorf, 1969).

There is an increasing awareness of enteropathogenic *E. coli* serotypes, as a cause of gastroenteritis occurring in adults, in addition to being a prime cause of infant
diarrhoea. Most of the reported instances of water borne enteropathogenic *E. coli* infections have been related to consumption of contaminated drinking water (Geldreich, 1972). Enteropathogenic *E. coli* are present in streams and lakes polluted with warm-blooded animal faeces (Geldreich, 1967). Once separated from the intestinal tract, survival of any *E. coli*, pathogenic or nonpathogenic, is influenced by a host of environmental factors including pH (Rogers and Wilson, 1966) metal ion toxicity (Malaney, Sheets and Quillin, 1959), addition of bacterial nutrients (Kittrell and Furfari, 1963), water temperature (Hanes, Rohlich and Sarles, 1966), sunlight exposure (Gameson and Saxon, 1967) intermittent stream riffles (Kittrell and Furfari, 1963), bacterial adsorption with sedimentation (Weiss, 1951) and predation (Varon and Shilo, 1969).

When *E. coli* is found in fresh water or estuarine environment, its occurrence indicates a recent introduction of faecal contamination. Storm water can be a major source of intermittent pollution to designated recreational reaches of streams and lakes. In rural community, storm water runoff transports the faecal contamination from livestock pastures, poultry and pig feedling pens, cattle feed lots, and to a lesser magnitude, the faecal contributions from
During storm periods, adjacent stream water quality is dramatically degraded by addition of runoff from feedlots, which is characterized by high B.O.D. values and high faecal content (Geldreich, 1972).

Quite, apart from the question of their being indication of faecal pollution, organisms of coliform group as a whole are foreign to water and must at least be regarded as indication of pollution in its widest sense. The search for faecal streptococci of which *Streptococcus faecalis* may well be of value in confirming the faecal nature of pollution in doubtful cases. Faecal streptococci regularly occur in faeces, in varying numbers, which are usually considerably smaller than those of *E. coli*. In water they probably die and disappear at approximately the same rate as *E. coli* and usually more rapid than other members of the coliform group. Therefore, the finding of faecal streptococci is an important confirmatory evidence for the faecal nature of pollution.

Anaerobic spore forming organisms of which the most characteristics is *Clostridium perfringens*, are also regularly present in faeces, though generally in much smaller numbers than *E. coli*. The spores are capable of surviving in water for a longer time than other coliforms and usually resist
chlorination at doses normally used in water works. The presence of spores of \textit{C. perfringens} in natural waters suggests faecal contamination and their presence in the absence of organisms of the coliform group, suggests that contamination occurred at some remote time.

In India, Health and Sewage Disposal Department routinely carry out periodical check of coliform organisms by multiple tube method or membrane filtration method. As far as this survey goes, only few systematic studies on coliform bacteria have been carried out in Indian Lake Water (Varma, 1977). No attempt has been made to correlate its presence with the physico-chemical or biological factors (Bagde, Khan and Varma, 1981). Some systematic studies carried out in water bodies like, streams, lakes, rivers, wells as well as oceans have been discussed below.

Aboo, Sastry and Alex (1968), made a survey of the quality of well waters in the city area of Bhopal, M.P. and found a definite seasonal variation in number of coliform and enterococci present in the well waters. Smaller numbers were present in winter. Maximum bacterial densities occurred in rainy season. They attributed this to the effect of rains in bringing polluted water into wells. They also stress the need for further work to establish whether there is any definite relationship between change in water temperature and bacterial counts.
Sastry, Aboo and Khare (1970) noted that in Upper lake, Bhopal, the seasonal variations in coliform and enterococci were well marked. The bacterial density varied from sampling point to sampling point because of seasonal variation and pollutional load. The counts were low during winter and post monsoon months. The density gradually increased in summer and monsoon and a high coliform index was found to be accompanied in most of the cases by a high MPN of enterococci.

Panicker, Wagle and Rao (1966) studied the coliform spectra and their seasonal variations in raw water sources from Nagpur City, Maharashtra (India). They analysed a total of 363 water samples from three water supply sources, viz. Kanhan river, Gorewara and Ambazari lakes over a period of three years and found that coli-I was present at high percentage in water samples. Next highest in frequency of occurrence was aerogenes I. It was seen that there was a tendency for coli-I to be in association with aerogenes-I more than any other member of the coliform group. However, they noticed no seasonal variation as far as the presence of particular coliform was concerned. A few water samples from the lake were noted to be coliform free in winter, once, but this was not consistent every year.

Rao, Parhad, Rao and Subbarao (1968) studied the coliform spectra of faeces and sewage samples collected
from different parts and reported that *E. coli* type I was in combination with other coliform organisms at 80.7 and 69.9% and indicated that it would be appropriate to consider the coliform group as a whole, as indication of faecal pollution rather than *E. coli* type I alone.

Thapliyal, Ahluwalia, Sethi and Negi (1970) analysed samples, collected from 18 different sources in the Tarai region, bacteriologically and found significant positive correlations between total coliform counts and enterococcus counts. With increase in coliform counts there was corresponding increase in the enterococcus counts and vice-versa. Correlation coefficient value between the coliform and enterococcus counts was 0.56. They also assessed bacteriological quality of the same Tarai waters and noted that most sources revealed counts higher than that allowed by permissive limits (Thapliyal, Ahluwalia, Sethi and Negi, 1972). They found that the MPN/100 ml coliforms was higher than that of enterococcus in 74 samples, whereas in 14 samples the reverse was the true, while 12 samples gave consistent counts. It was observed that enterococcal count could be of greater value for assessing the sanitary quality of water as compared to that of coliform.

William and Frans (1972) made a hydrobiological study of the polluted river Lieve (Ghent, Belgium) and noted that
the number of bacteria varied with the time of collection and the sampling place. The total number decreased from summer towards winter, at all places, and the highest bacterial numbers were found at the most polluted places.

Seenayya (1973) studied certain fresh water ponds in Hyderabad, India and found that bacteria were present in the surface waters all through the year but attained maxima in summer in two ponds (Shatum and Hydrilla ponds) when temperature was 36-39°C. Whereas in Golkonda pond their largest number was recorded towards the end of winter, when the temperature fluctuated between 28-31°C. During monsoon, bacteria multiplied well but they never exceeded the number attained during summer.

Saxena, Chakrabarty, Khan, Chattopadhya and Chandra (1966) carried pollution monitoring studies considering physical, chemical and bacteriological parameters of river Ganges near Kanpur during different seasons for a year and found that coliform were high at almost every station during summer season and attributed this to the less available dilution.

O.R.SAN.CO. (1971) studied total coliform and faecal coliform ratio for evaluation bacterial quality of raw water of Ohio river and stated that ratio of total coliform and faecal coliform varied from 0.04 to 0.80 with average figure of 0.14.
Feachem (1974) carried assessments of faecal coliforms and faecal streptococci in streams in New Guinea Highlands and stated that the water there constituted a health hazard. Reporting detailed data of faecal coliform and faecal contaminations in the streams he noted that waters investigated were grossly contaminated with faecal material.

Agarwal, Gaur, Sen and Marwah (1976) assessed bacteriological quality of Ganges river water at Varanasi, India, and noted that at bathing ghats mean MPN index/100 ml was $9.219 \times 10^3$ and the mean value of a faecal coliforms was $4.598 \times 10^3$. The mean increased manifolds at the sewage out falls. Pathogenic bacteria, *Vibrio-chlorae*, *Salmonella* and *Shigella* were isolated both from ghats and sewage out falls. The mean MPN index and faecal coliforms were statistically highest during summer and lowest in winter.

Mancini (1978) studied numerical estimates of coliform mortality rates under various conditions and found that solar radiation had a significant influence on coliform mortality rates.

Qureshi and Dutka (1979) studied microbiological quality of urban storm water runoff in Southern Ontario, Canada and found that microbial densities were similar to those found in dilute raw waste-waters and therefore represent
a potential health hazard. The recovery of pathogenic bacteria (Pseudomonas aeruginosa and Salmonella) further substantiated the existence of health hazards. There appeared to be little relationship between the duration, intensity and amount of rainfall and the occurrence of peak microbial population. As a result, no typical pattern of time-related distribution of indicator and pathogenic bacteria could be established in this investigation. They also suggested that initial flushing has minimal effect on the microbial quality of an individual storm event and indicated the seriousness of urban storm water runoffs as a major factor in nonpoint source pollution of receiving waters.

Gracey (1978) analysed polluted water in Jakarta, Indonesia and provided evidence, supporting the view that environmental pollution, especially of water, is a major contributor to childhood diarrhoeal diseases in crowded, tropical, urban environments.

Meyer-Reil, Bolter, Liebezeit and Schramm (1979) studied short term variation in microbiological and chemical (PO₄, SiO₂, NH₃, NO₃, NO₂ as well as dissolved organic carbon and particulate organic carbon and nitrogen) parameters and established inverse relationship between microbiological and organic chemical parameters (dissolved and particulate organic carbon, total free amino acids). The study also indicated that microorganisms were responsible for the variations in
organic matter. The strong variations in the number of colony forming units, and concentration of dissolved inorganic nutrients obviously reflect water body changes. There was no indication of a distinct pattern common to variations in microbiological and/or chemical parameters. However, for two stations at which an uniform, individual water body was sampled at short intervals, distinct patterns of fluctuations or rhythms of microbiological and chemical parameters could be observed. The patterns were different obviously reflecting different ecological situations.

Paerl, Payne, Mackenzie, Kellar and Downes (1979) carried microbiological, biochemical and chemical analysis of samples from nine lakes and stated that they were unproductive and were also with substantial numbers of bacterial cells. In the heavily stained lakes, bacterial biomass accounted approximately 80-90% of microbial mass which indicated the presence of abundant non-pigmented micro-organisms. Apparently, these heavily stained lakes allowed bacterial growth while restricting algal growth. They also concluded that phosphate was most limiting in these lakes.

Brooks and Cech (1979) evaluated well water supplied in rural East Texas focussing on nitrates, their extent, causes and sources in rural well water. They also tried to
obtain information about the source of nitrates in these wells by microbiological tests using faecal coliforms and faecal streptococci as indicators of faecal contamination and found that the wells with the highest nitrate levels had bacterial ratio of 1 or higher, suggesting that domestic pollutions were most likely the primary source of nitrates.

Petrilli, Renzi, Orlando and DeFlora (1980) assessed microbiological quality of coastal waters in the Tyrrhenian sea including Tuscany littoral in the district of Livorno (Leghorn, Italy), three isles (Elba, Capraia and Gorgona) and the terminal tracts of two rivers (Calambrone and Cecina) to know the bacteriological and virological pollution of the monitored areas, as well as the correlation between different bacteriological parameters (total coliforms, E. coli and faecal streptococci) and between E. coli and animal viruses. They found a positive correlation between total coliforms and E. coli (r = 0.826), total coliforms and faecal streptococci (r = 0.793) and E. coli and faecal streptococci (r = 0.929).

The life of micro-organisms in aquatic environment is affected by a variety of physical and chemical factors. The life process of all micro-organisms is affected by the temperature of water. Bacteria are able to survive in wide
ranges of temperature, but the range in which they grow and carry on their activities generally falls between 0 and 90°C. The mesophilic organisms were found to have a minimum temperature of 5-20°C, optimum, 18 to 45°C and maximum of 30 to 50°C (Salle, 1974). The growth of micro-organisms is also affected by hydrogen ion concentration (pH) of the water body. Most organisms could grow only within a pH range of 4 to 9 (Thimann, 1964). The optimum pH for most aquatic microbes was noted to be between 6.5 to 8.5 which corresponded to the pH range of the larger bodies of water (Thimann, 1964).

Living micro-organisms require oxygen to maintain their metabolic processes. Under aerobic conditions micro-organisms stabilized decomposable carbonaceous matter as well as oxidized inorganic nitrogen compounds (Morrissette and Mavinic, 1978). The current measure of pollutional strength is the B.O.D. (Biological Oxygen Demand). It measures the amount of oxygen required by microorganisms for their activities. It is an appropriate index to assess the pollution load of some water bodies (Rai and Kumar, 1977).

Different inorganic substances like nitrogen, phosphorus and sulphur compounds also affected the life of micro-organisms in water, which represented the limiting factor for microbial life (Staples, 1973; Schindler and
Nighswander, 1970; Schindler, Armstrong, Holmgren and Brunskill, 1971; and Likens, 1972). However, the inorganic requirements of bacteria are not well understood (Salle, 1974). In oligotrophic lakes, ammonia, nitrate, nitrite, orthophosphate and sulphate could hardly be demonstrated because as soon as they were released, they were immediately bound again by microbes. Nitrogen appeared to be the major nutrient, limiting primary production of biomass in certain fresh waters (Likens, 1972). Sulphur is an universal constituent of living cells. Cowie (1950) found that the sulfate was readily passed through cells of *E. coli* and that the uptake of the element was directly proportional to cellular growth. Iron is also an important growth requirement for bacteria. Winder and O'Hara (1962) reported that *M. smegmatis* required the element for full growth. Many investigations have reported the necessity of iron for other bacteria. Shanker and Bard (1952) reported the necessity of Ca, Mg, Fe, Na, K for the growth of *Clostridium perfringens*. Similar results were reported by Webb (1948), MacLeod (1951) and Rochford and Mandle (1953) on the same and other organisms.

Antimicrobial drugs and chemicals have been used since long to control microbial growth and by now there is
extensive literature concerning the toxicity of chemicals and inhibitors that can be used to control microbial growth under variety of conditions. In these studies effectiveness of different inhibitors to kill or inhibit growth of pure cultures of micro-organisms have been considered (Stutzenberger, and Bennett, 1965). However, studies pertaining to biochemical changes induced by inhibitors in the population of micro-organisms and particular reference to bacteria are scarce. It is observed that the heavy metal concentration which will kill or inhibit the growth of organisms is much dependent both on the metal and on the organisms and one metal may be more toxic than other to an organism and less toxic at high concentration to another organism. The susceptibility of different species to heavy metals can vary enormously (Bryan, 1971). According to Bryan (1971) Hg, Ag and Cu were the most toxic metals followed by Cd, Zn and Pb and then by Cr, Ni and Co. However, he stated that this was not the rigid order of toxicity and was different in different species. In order of decreasing toxicity towards, Brevibacterium, Alcaligenes, and Pseudomonas. Lester, Perry and Dadd (1979) reported toxicity of Cu>Cd>Pb>Cr. Towards E. coli Ni>Co>Cd>Zn>Mn have been established by Abelson and Aldous (1950). Laborey and Lavollay (1967) noted Cu>Cr towards A. aerogenes and Zn>Cd towards Aspergillus. Lamb and
Tollefson (1973) reported $\text{Cu} > \text{CrO}_4 > \text{Cr}$ whereas Huckelkian and Gellman (1955) found $\text{Ni} > \text{Cu} > \text{Cr} > \text{Cd} > \text{Co} > \text{CrO}_4$. However, these anomalies may be explained by variations in experiment and operating conditions (Lester, Perry and Dadd, 1979). Some systematic studies pertaining to effects of various inhibitors on microorganisms have been described below:

Schade (1949) has investigated the effect of Co on the growth of 21 different organisms. In all cases cobalt was toxic. The amount required to stop growth was around 10 to 100 ppm in broth and about 0.1 to 1.0 ppm in synthetic medium.

Abelson and Aldous (1950) studied the influence of magnesium on the toxicity of Ni, Co, Cd, Zn and Mn on *E. coli*. The elements in order of decreasing toxicity for *E. coli* were found to be $\text{Ni} > \text{Co} > \text{Cd} > \text{Zn} > \text{Mn}$. The toxicity of these cations was markedly lowered in presence of much magnesium. If magnesium was not present in the medium, these elements were toxic at very low levels. The toxicity of Ni and Co was similarly reduced by Mg in the case of three other organisms tested, *Aerobacter aerogenes*, *Torulopsis utilis* and *Aspergillus niger*. They noted that the toxicity of these ions at low concentration of magnesium was a consequence of their interference in the normal metabolic role of magnesium.
In all four organisms investigated higher levels of Mg diminished the nickel and cobalt bound by the cell.

Several mechanisms of resistance of heavy metal toxicity in bacteria have been elucidated. While studying the specificity of the manganese requirement of Lactobacillus arabinosus, MacLeod and Snell (1950) noted that zinc toxicity could be overcome by increasing the concentration of manganese. They explained that zinc inhibited the growth of this organism by interfering with the formation of metabolically essential metalloproteins and that manganese counteracted this inhibition by the formation of active complexes with the same proteins.

Weed and Longfellow (1954) studied morphological and biochemical changes induced by copper in a population of E. coli and found that if traces of copper were present in liquid cultures of E. coli, subsequent platings of this suspension contain a variant form whose colonial, morphological and chemical composition differ from the untreated parent strain. The morphological variant showed small colony size and the rate of oxygen uptake of the copper treated organisms was slower than that of normal E. coli.

Harold and Ziporin (1958) reported cessation of cell division and marked reduction of viable count in E. coli on treatment with nitrogen and sulfur mustard. These compounds
induced a concentration dependant transient inhibition of DNA. Growth (increase in turbidity) and RNA synthesis continued, while DNA synthesis was blocked and deoxyribose accumulated in the acid soluble fraction of *E. coli*.

Doudney (1959) studied the effect of chloramphenicol on nucleic acid synthesis during the cellular reproduction of *E. coli*. His results suggested that protein synthesis during certain periods of cellular reproduction cycle was a requisite to subsequent nucleic acid formation. Chloramphenicol blocked DNA synthesis when added just prior to cellular division whereas the addition of chloramphenicol during short period of time subsequent to cellular division blocked RNA synthesis. It is suggested that the appearance of new proteins at specific times related to cellular division were requisite to subsequent nucleic acid synthesis. While Gale and Folkes (1953) and Wisseman, Smadel, Hann and Hopps. (1954) have demonstrated that chloramphenicol inhibited protein synthesis but permitted the synthesis of nucleic acid in log phase cultures of bacteria.

Bennett and Bauerle (1960) studied sensitivities of mixed population of bacteria to inhibitors. The study made was concerning the effects of mixed population of *Pseudomonas aeruginosa* and *Desulfovibrio desulfuricans* on the sensitivities
of the individual species to three groups of inhibitors (phenols, lactate, acetate). They found that the presence of *Pseudomonas aeruginosa* produced in some cases an increase in resistance of *D. desulfuricans* to substituted phenols, a consistent increase in resistance of *D. desulfuricans* to nitroparaffins and had no marked effect on their sensitivity to mercurials. The presence of sulphate reducing bacteria produced a significant increase in resistance of *Pseudomonas* to some of the nitroparaffins, consistent increase in resistance of *Pseudomonas* to mercurials, and a consistent increase in sensitivity of the *Pseudomonas* to phenols.

Stutzenberger and Bennett (1965) studied sensitivity of mixed population of *Staphylococcus aureus* and *E. coli* to mercurials and found that *Staphylococcus* had a higher resistance to merbromin and mercuric chloride in presence of *E. coli*. The protective effect of the gram negative organism on *S. aureus* was due to the production of extracellular glutathione and hydrogen sulphide and to an unequal distribution of the inhibitor between the two species. However, *S. aureus* did not significantly influence the resistance of *E. coli* to mercurials. Also Stutzenberger and Bennett (1963) reported that *E. coli* could protect *S. aureus* against numerous antibiotics and phenols in mixed cultures.
Smith (1967) studied fifty five clinical isolates and laboratory stocks of *E. coli* and *Salmonella* for resistance to each of ten metals. Eleven clinical isolates carrying R factors were resistant to Hg and in each case the resistance was mediated by a previously undefined R factor gene. Fourteen strains, 12 infected with R factors, were resistant to Co and Ni, but their resistances were mediated by R factor in only two strains and a separate R factor gene mediated the resistances to Ni and Co. All strains tested were equally resistant to Al, Cd, Cr, Cu, Pb and Ag.

Loveless and Painter (1968) studied the influence of metal ion concentration and pH value on the growth of a *Nitrosomonas* strain isolated from activated sludge and noted that copper, sodium, calcium and magnesium stimulated growth of pure cultures. EDTA improved growth in the basal medium and abolished the toxic effects of added copper. It was however, inhibitory at low calcium concentration.

Blundell and Wild (1969a,b) studied the action of six different metal salts, Co, Ni, Zn, Cd, Mg and Cu and they were found to be inhibitory for the growth of *E. coli* MRE 600. The treated cultures showed a greater decrease in the RNA synthesis than protein synthesis in presence of all the salts except CoCl₂. Cobalt caused a high synthesis and accumulation
of RNA. But protein synthesis was comparatively lower than RNA synthesis.

Komura, Izaki and Takahashi (1970) studied the resistance of cell free extracts of drug resistant \textit{E. coli} to inorganic mercury and reported that three multiple drug resistant strains of \textit{E. coli} isolated in Japan were HgCl$_2$ resistant too. It was also shown that the resistance to HgCl$_2$ could be transferred from a drug resistant strain of \textit{E. coli} to sensitive strains of both \textit{E. coli} and \textit{A. aerogenes} as in case of the resistance to drugs such as chloramphenicol and tetracycline. Also, Komura and Izaki (1971) noted that mercury poisoning in a strain of \textit{E. coli} was dependent on the reduction of the mercury salt to elemental mercury which was lost from the medium as vapour. The resistant strains could grow in presence of 0.02 mM HgCl$_2$, whereas a sensitive strain failed to grow in the presence of 0.01 mM HgCl$_2$. During cultivation in the presence of 203 HgCl$_2$, glucose and NaCl in phosphate buffer, the cells of resistant strain vapourized radioactive mercury, while the cells of sensitive strain showed no such activity.

Summers and Silver (1972) reported that a strain of \textit{E. coli} carrying genes determining mercury resistance on naturally occurring resistance transfer factor (TRF) converts 95% of $10^{-5}$M Hg$^{2+}$ (chloride) to metallic mercury at a rate of
4 to 5 moles of Hg$^{2+}$ per minute per $10^8$ cells. The metallic mercury was rapidly eliminated from the culture medium as mercury vapour. The volatilizing activity was temperature dependant and Ag$^+$ and Au$^+$ were markedly inhibitory for Hg volatilization.

The biochemical effects of lead were described by Vallee and Ulmer (1972), while Den Doorem de Jong (1971) showed with the agar diffusion technique that $10^{-4}$M of lead nitrate inhibited growth of *Azotobacter* strains. Tornabe and Edwards (1972, 1973) found that the cells of *Micrococcus leuteus* and *Azotobacter* species bound lead bromide mainly to the cell walls and membranes and not cytoplasmic fractions. The lead bound in the bacterial cell membrane interacted with the lipid fraction of the membrane.

Kondo, Ishikawa and Nakahara (1974) studied resistance of *Staphylococcus aureus* mediated by the penicillinase (PC-ase) plasmid to divalent metal ions of Hg and Cd and found to be controlled by different mechanisms. The Hg resistance of the PC-ase plasmid carrying organism was based upon a process of changing the ion incorporated in the cell into somewhat innocuous form. This process was independent of temperature and seemed to be controlled by an inducible enzyme. The killing effect of Hg salts was not influenced by
the co-existence of other di- or monovalent ions such as MgCl₂, CaCl₂, MnCl₂ and NaCl. Whereas Cd ion resistance was mediated by some protective mechanism to retain the ion outside the cell. Sensitive organisms not carrying the PC-ase plasmid incorporated Cd ions into the cells, whereas PC-ase plasmid carrying organisms do not. The incorporation of this ion was temperature dependent and does not take place at 4°C. When incubated with this ion at 4°C, sensitive as well as PC-resistant organisms also showed resistance. The addition of CaCl₂ could eliminate the killing effect of CdCl₂ with a dose effective response.

Mitra, Gray, Chin, and Bernstein, (1975) reported that E. coli has the ability to accommodate growth inhibiting concentration of Cd. The cells which proliferate in the presence of Cd accumulate the ion to a very high concentration. In accommodated cell, 56% of the Cd was associated with the cell wall, 13% in the membranes and 37% in the cytoplasm. While in unaccommodated cells the figures were 21, 75 and 23% respectively. It appeared that accommodation of E. coli to the presence of Cd involves exclusion of the ion from the cell and reversal of damage caused by prior exposure to ion.

Carter and Dean (1975) studied effect of gentamycin on Aerobacter aerogenes NCIB 418 and noted that a resistance of
trained strains to gradually increasing concentration of gentamycin in a minimal liquid medium, was continuously graded to the concentration at which training was carried out.

Pickett and Dean (1976) studied cadmium and zinc sensitivity and tolerance in *Aerobacter aerogenes*. The resistance of strains of *Aerobacter aerogenes* trained to Cd and Zn in liquid medium was graded to training concentration. Training of Cd increased the sensitivity of Zn but training to Zn reduced the sensitivity to Cd. The trained organisms, particularly those trained to Cd grown slowly in medium containing the metals and the growth rates after 20 and 200 subcultures were not significantly different. The survival of untrained organisms on Cd agar decreased progressively as the Cd concentration was increased, but a threshold concentration of Zn was necessary before any decrease set in.

Cenci and Morozzi (1977) studied the effect of Cd and Cd(CN)$_4$ ions on the growth and enzymatic activity of mixed microflora from activated sludges. Both ions tested, inhibited the growth of micro-organisms significantly and the inhibitory effect of Cd was greater than that of Cd(CN)$_4$ at the same concentrations. There was also inhibition of maximum uptake rate of glucose, but in this case there were no statistically significant differences between inhibitory
effect of the corresponding mass of molar concentrations of Cd and Cd(CN)$_4$.

Guha and Mookerjee (1978) studied effect of cobalt chloride on *E. coli* and stated that cobalt chloride was inhibiting cell free protein synthesis directed by natural mRNA. When cells of *E. coli* were treated with cobalt chloride (300 µM), an imbalance developed in protein and RNA content of the cell. The protein synthesis got selectively inhibited with concomitant RNA accumulation. Also, Guha and Mookerjee (1978) found that NiCl$_2$ at 50 µM concentration inhibited growth of *E. coli*. The synthesis of RNA, DNA and protein was directly affected.

Babich (1978) stated that 10 mM concentration of Zn decreased the survival of *E. coli*, enhanced the survival of *Bacillus cereus*, but did not significantly affect the survival of *Pseudomonas aeruginosa*, *Nocardia*, *Corallina* and coliphages and fungi. The toxicity of zinc to the fungi, bacteria and coliphages was unaffected, lessened or increased by the addition of high concentration of NaCl. The increased toxicity of zinc in the presence of high concentration of NaCl was not a result of a synergistic interaction between Zn and elevated osmotic pressures but of the formation of complex anionic ZnCl$_2$ species that exerted greater toxicities than did cationic Zn. Conversely the decrease in zinc toxicity with increasing concentration of NaCl probably reflected the decrease in the
levels of Zn. due to formation of ZnCl₂ species, which was less inhibitory to these microbes than Zn. *A. niger* tolerated higher concentrations of zinc in the presence of NaCl at 37°C than at 25°C.

Lester, Perry and Dadd (1979) studied the influence of Cd, Cr, Cu and Pb as shock doses at different concentrations upto 50 mg/l and found that the response of the population vary for each metal. It appeared that concentration of lead as low as 5 mg/l caused modification in the individual population sizes. They also stated heavy metal toxicity in decreasing order towards, *Brevibacterium, Alcaligenes, Pseudomonas* and unidentified gram negative rod as Cu>Cd>Pb>Cr.

Pickett and Dean (1979) studied the action of Cd and Zn on *Bacillus subtilis* sub sp *niger* and on *Pseudomonas* sp. and compared results with *Klebsiella aerogenes* (Pickett and Dean, 1976). They pointed out that in liquid medium the lag and mean generation time of *Bacillus subtilis* increased with increasing Cd and Zn concentration whereas only the total biomass of *Pseudomonas* sp. was affected. Nevertheless, the responses of both species indicated a specific action at low concentrations and more general toxic action at high concentration.
Much work has been done to investigate lead toxicity on man, animals and plants, but little is known about the effect of lead on bacteria (Tan, 1980). Also, relatively few studies have been made on the interaction of chromium with micro-organisms.