CHAPTER-VII : GENERAL DISCUSSION
General Discussion

Water has become a precious commodity worldwide, and its quality has become a major concern because of rampant environmental pollution. Water quality has not only to be monitored for high load of salts and metals but also for the presence of toxic and even mutagenic and carcinogenic material. Normally, many dangerous chemicals are present in very low concentrations in water bodies, but they can be concentrated again in the food chain. Agents which are acutely toxic are readily detected because the immediate effects allow rapid identification of the source of toxicity, but the mutation and cancer are not the immediate processes they are only detected many years after exposure.

Therefore, the screening of environmental chemicals for potential mutagenic and carcinogenic activity in the human population continues to be a high priority activity all over the world. The screening has continued and new assay systems designed to detect mutagenic/carcinogenic activity in various environmental samples. Much of the new research is concerned with the evaluation of complex mixtures as they occur as air pollutants or pollutants of fresh and sea water.

Two analytical methods have been used to concentrate the water samples i.e. Amberlite XAD-4/8 absorption technique and the other one is liquid-liquid extraction. XAD-concentrates contain all the organic constituents and
liquid-liquid extracts contain only organochlorine and organophosphorus pesticides (Wilcox and Williamson, 1986, Singh et al., 1987). Our results also support this finding (Chapter III). HPLC system was used to resolve the pesticides present in water samples, whereas the identification and quantification of heavy metal was done on atomic absorption spectrophotometer. Our results of HPLC system indicated that the water samples contained the high level of certain commonly used pesticides like DDT, BHC, Lindane, Aldrin, Endrin, dieldrin, endosulfon, Dimethoate, Methylparathione and 2,4-D in all the four test samples (Table 2, Figs. 2-5, Chapter III). Some major peaks remained unidentified, because they did not match to the known pesticides. All these pesticides are commonly used by cultivaters for agricultural purpose in the test stretch as well as in the upstream region (Narora).

The total consumption of different varieties of pesticides and insecticides was 378.54 Kilograms over an area of 822.27 hectares as reported in Integrated study of Ganga Ecosystem between Narora to Kannauj (1990). It is significant that the environmentally more damaging and persistant organochlorines are the dominant group of pesticides and insecticides used. This may be because of their longer persistence and more specific nature than other alternative chemicals and are safer for formers to apply because of less short term toxicity to humans (Anon, 1984).
The pesticidal load increased as we moved towards the down stream from Narora to Kannauj. The concentration of 10 heavy metals Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn at all the four sampling stations are shown in Table 4, Chapter III. Most of the heavy metals except Hg, Fe, Pb, Cd and Cr are within the permissible limits. Iron is found 5 times greater than the permissible limit at all the monitoring stations. The concentration of heavy metals in local sediments and river water has an effect on crop production as well as on the health of inhabitants. The changes in the course of the river means loss of agricultural land for one village and may gain for another village. It has been observed that minor changes in the concentration of elements dissolved in the soil and mercury in the river and manganese can cause several plant diseases, effect on agricultural crop and fish population, and it has rendered fish harmful for human consumption (ISGEBN to K, 1990).

The water samples, from the four sampling stations have been shown to possess a significant amount of mutagenic activity by means of Ames testing (Chapter IV). Our results indicated a higher degree of mutagenicity of all the test samples with AT — GC frameshift and base pair mutants, TA98, TA97a and TA100 except for Narora water sample suggesting that the test samples preferentially act upon GC base pairs to bring about frameshift and substitution mutations (Table 1, 2; Figs. 2-4 & 6-10; Chapter IV). It is also noteworthy that, even in the absence
of Sg microsomal fraction, all the test samples responded significantly but the addition of microsomal fraction further enhanced the mutagenic activity of some test samples (Table 1; Figs. 1-4B; Chapter IV) suggesting that the metabolic products of river water are even more mutagenic.

It is interesting to point out that the mutagenicity of Ganga water concentrate (Prepared from XAD resin) further enhanced in the presence of Sg fraction as compared to those samples obtained from Liquid-Liquid extraction method. In the presence of Sg fraction these extracts slightly reduced the mutagenic activity (Table 2, Figs. 5-8(B); Chapter IV) suggesting that these extracts contained only those pesticides whose metabolic products are not more mutagenic. The mixture of those pesticide which were detected by HPLC in the test water samples (Chapter III) also displayed the same pattern providing support to our contention that mutagenic activity displayed by the sample is mainly due to the presence of pesticides. Since we had carried out the experiment taking only the identified pesticides and the quantities into consideration too. It was found that the mixture of pesticides was less mutagenic compared to the liquid-liquid extracted water samples (Chapter IV). This finding obviously suggests that certain unidentified pesticides or other substances were also contributing to the mutagenic potential of water. Moreover, the water samples concentrated from XAD4/8 ion exchange columns showed
remarkably higher mutagenic activity as compared to those samples extracted from liquid-liquid extraction method. This is not surprising because the XAD-concentrates would obviously contain a broad range of organic pollutants including the PAHs, PCBs, pesticides etc. This is interesting finding but identification of such a broad range of organics would require great efforts and thus we left this aspect for future studies.

As mutagenicity at all the stations is of the same magnitude, it is believed that the entire reach is contaminated with almost equal amounts of mutagenic compounds. Since the river coliforms are in contact with pesticides and other mutagens for longer periods, even low concentration of these compounds in river water may be detrimental. Bioaccumulation and bioconcentration will also be a crucial factor (ISGEBN to K, 1990). The Ames testing of bile fluid of Ganga water fish provides support to this idea (Fig 9; Chapter IV).

The genotoxic activity of water samples was further confirmed by the E.coli DNA-repair defective mutants. In many cases, the reversion tests has proven to be superior for compounds requiring metabolic activation (DeFlora et al., 1984), while DNA repair tests are superior for direct acting agents. It is also believed that the Ames tester strains carrying the pKM101 plasmid enhance the error-prone repair process (Levin et al., 1982b; Little et al., 1989).
The *Salmonella* strains lack error-prone repair (Walker, 1984) due to the absence of a functional UmuD gene (Herrera *et al.*, 1988). The error-prone repair in *Salmonella* is regained in the presence of pKM101 plasmid which contains analogues of UmuCD genes making the error-prone repair functional. Our results obtained with *E. coli* and *Salmonella* system suggest that the test samples bring about the DNA damage and thus the treated cells initiate the SOS-repair with the concomitant induction of mutation. The induction of SOS-response by the test samples in our case was supported by the high sensitivity of recA, polA and lexA mutants of *E. coli* towards the test samples (Figs. 1-4; Chapter V). The role of recA<sup>+</sup>, lexA<sup>+</sup> and polA<sup>+</sup> genes are well documented in the error-prone repair in *E. coli* damage induced by various agents (Srivastava, 1978; Walker, 1985; Musarrat and Ahmad, 1988).

The test samples seem to have initiated the SOS-response with the concomitant induction of GC-AT frameshift and base-pair substitution mutation.

With regard to the damaging effects of various sample treatment, it seems that XAD-concentrate of Kannauj sample was more damaging than that of other stations, suggesting that the damaging effect is slowly increased from Narora to Kannauj. These results are in conformity with the Ames testing studies which displayed similar trend that is the mutagenicity of water samples increased from upstream to
downstream in the test stretch. (Table 1 and 2; Chapter IV). The recA and polA mutants were always found to be most sensitive followed by lexA mutant in all the sampling stations except Narora. Narora water samples exhibited relatively higher sensitivity to the lexA mutant than other mutants. Such type of response was also observed with XAD-concentrated drinking water (Bourbigot et al., 1986). However, the contributory factor in that case was suggested to be chlorine rather than pesticides or heavy metals.

The XAD-concentrated water sample from Kannauj was employed for other biochemical studies. It caused a destabilization in the secondary structure of DNA with the formation of single strand breaks, and also induced reciprocal exchanges between sister chromatids (Chapter VI). The results of SCE analysis indicated that the water sample is clastogenic at all the dose levels. It is also observed that the frequency of sister chromatid exchanges significantly increased at higher doses (Table 1; Chapter III). Similar findings have also been reported by Athanasiou and Kyrtopoulos (1983). These workers found that water samples induced the SCEs and chromosomal aberrations in CHO cells. For the majority of chemical mutagens which are S-dependent agents the sister chromatid exchange (SCE) has proved to be even more sensitive than the induction of aberration. The SCEs, like chromosome aberrations induced by
S-dependent agents, are formed by unrepaired lesions that are present when the cell passes through S phase and the chromosomes replicate. The SCE has been used as a test system to tell whether or not a chemical is potentially dangerous and to determine which of the metabolites of a premutagenic and precarcinogenic agents might be the likely ones to interact with DNA and cause its effect (Wolff, 1984; Renata et al., 1989).

The results of in vitro studies also indicated that the test sample causes a destabilization of secondary structure of DNA with the formation of ssbs. This is evident by the increased level of single strandedness in duplex DNA as observed by hydroxyapatite chromatography (Fig. 1; Chapter VI) and increased susceptibility to S1 nuclease (Table 2; Chapter VI). In addition to this fact alkaline unwinding assay also suggested the existence of single strand breaks in the treated DNA (Table 4; Chapter VI). The strand break formation was also found to be dose dependent. The increasing dose of XAD-concentrate seems to exert a sort of destabilization in the secondary structure of DNA resulting in the denaturation of DNA. An enhanced rate of hydrolysis of DNA with S1 nuclease in the presence of water sample also supports the same idea. This can also be explained in view of the modification of bases (Fig. 2; Chapter VI) which might disrupt the hydrogen bonding between complementary bases and thus locally denatured regions can
Fig. 1 - Proposed mechanism of water sample-induced DNA damage.
be formed. Our results also indicated that the water sample treated DNA became susceptible to $S_1$ nuclease hydrolysis but the DNA degradation could also be brought about by water sample alone (Table 3; Chapter VI).

In view of the present findings, we conclude that the Ganga River contain high levels of several water borne organic and inorganic pollutants which are genotoxic and mutagenic. On the basis of these results we propose the following scheme (Fig.1) for the water sample-induced mutagenesis as well as its interaction with DNA. In vivo damage leads to the initiation of SOS response and induction of mutagenesis, whereas in vitro damage leads to the modification of bases, and formation of single strand breaks and DNA degradation. It also induces genomic alterations during the cell cycle in human lymphocyte. The presence of significantly high levels of pesticides and heavy metals in Ganga River largely seems to be contributing to the genotoxic and mutagenic potential of its water.

This study calls for particular concern over the increasing level of pollution with particular reference to genotoxic pollutants in the most important and largest river of India. We would like to especially emphasis here that the Ganga water must not be taken for drinking without proper cleaning and purification in view of its remarkable mutagenic, clastogenic and genotoxic effects. Moreover, bioaccumulation of genotoxic substance are slowly rendering
the revertine organisms like fish into a forbidden eatables. Effective steps to prevent pollution in our biggest water reservoir are, therefore, immediately needed.