Chapter I:
Introduction, Review of literature and Objectives
It is an established fact that air, water and soil are among vital things for the life on earth (Rose, 1998). During the long period of development, human beings along with other living organisms enjoyed the benefit of clean water, soil and air, but industrial revolution of the past few centuries, gradually caused the atmosphere to become severely polluted. Now a days, environmental pollution has reached to such an alarming level that it is endangering the health of human beings and survival of other living organisms. It is in this context that, many countries of the world in general and industrial countries in particular have taken certain fundamental measures for the prevention of environment pollution (Sadatipour et al., 2004).

Pollution implies the introduction of contaminants into a natural environment that causes instability, disorder, harm or discomfort to the ecosystem i.e. physical systems or living organisms (Hughes, 2005). Pollution can take the form of chemical substances or energy, such as noise, heat, or light. It is often categorized as point source or nonpoint source pollution. Environmental pollution implies any alteration in the surroundings but is restricted in use especially to mean any deterioration in the physical, chemical or biological quality of the environment (Gleick, 2001). Pollutants, the elements of pollution, can be xenobiotics or naturally occurring or energies; are released into the environment by the action of man and occur in concentrations higher than “natural levels”. All types of pollution directly or indirectly affect human health. The pollutants fall under the broad classification of xenobiotic compounds.

Many studies have shown that air, water, soil and food are frequently contaminated with mutagens and carcinogens, which can reach humans and increase environmental carcinogenic hazards. For this reason, monitoring of genotoxic compounds in the environment has become an important objective of public health, with the intention of avoiding or minimizing direct and indirect human exposure to these toxic substances (Feretti et al., 2007).

1. **Forms of pollution**

The major forms of pollution are:

a. Air pollution

b. Soil pollution
c. Water pollution

1.1. Air pollution

Air pollution is caused by the release of gaseous chemicals and particulates into the atmosphere. Common gaseous air pollutants include carbon monoxide, sulfur dioxide, chlorofluorocarbons (CFCs) and nitrogen oxides produced by industry and motor vehicles. Air pollution comes from both natural and man made sources, though globally man made pollutants from combustion, construction, mining, agriculture and warfare are increasingly significant in the air pollution equation. In fact, motor vehicle emissions are one of the leading causes of air pollution (Boubel, 1994). China, United States, Russia, Mexico, and Japan are the world leaders in air pollution emissions. Principal stationary pollution sources include chemical plants, coal-fired power plants, oil refineries, petrochemical plants, nuclear waste disposal plants, incinerators, large livestock farms (dairy cows, pigs, poultry, etc.), PVC factories, metals production factories, plastics factories, and other heavy industry (Nevers, 2000). Air pollution from agriculture sources comes from contemporary practices which include clearing, felling and burning of natural vegetation as well as spraying of pesticides and herbicides.

1.2. Soil pollution

Soil pollution is caused by the entry of the man made chemicals and/or other alterations in the natural soil. This type of contamination typically arises from the rupture of underground storage tanks, application of pesticides, percolation of contaminated surface water to subsurface strata, oil and fuel dumping, leaching of wastes from landfills or direct discharge of industrial wastes to the soil. The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, and heavy metals. In fact soil pollution is correlated with the degree of industrialization and intensities of chemical usage. The concern over soil contamination stems primarily from health risks from direct contact with the contaminated soil, vapors from the contaminants, and from secondary contamination of water supplies within and underlying the soil (USEPA (a), 1989).
Contaminated or polluted soil directly affects human health through direct contact with soil or via inhalation of soil contaminants which have vaporized; potentially greater threats are posed by the infiltration of soil contamination into groundwater aquifers used for human consumption, sometimes in areas apparently far removed from any apparent source of contamination (Magrini et al., 2008). Health consequences from exposure to soil contamination vary greatly depending on pollutant type, pathway of attack and vulnerability of the exposed population. Chronic exposure to chromium, lead and other metals, petroleum, solvents, and many pesticide and herbicide formulations can be carcinogenic, can cause congenital disorders, or can cause other chronic health conditions. Industrial or man-made concentrations of naturally-occurring substances, such as nitrate and ammonia associated with livestock manure from agricultural operations, have also been identified as health hazards in soil and groundwater (Xiao et al., 2006).

### 1.3. Water pollution

Water pollution is referred to as the contamination of water bodies (e.g. lakes, rivers, oceans and groundwater) and occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Water pollution affects plants and organisms living in these bodies of water; and, in almost all cases the effect is damaging not only to individual species and populations, but also to the natural biological communities. Water pollution is a major global problem. It has been suggested that it is the leading worldwide cause of deaths and diseases (Pink and Daniel, 2006; West and Larry, 2006), and that it accounts for the deaths of more than 14,000 people daily (West and Larry, 2006). In addition to the acute problem of water pollution in developing countries, industrialized countries continue to struggle with this pollution problem as well (USEPA, 2007).

The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens, and physical or sensory changes such as elevated temperature and discoloration. While many of the chemicals and substances that are regulated may be naturally occurring (calcium, sodium, iron, manganese, etc.), the concentration is often the key in determining what is a natural component of water and what is a contaminant. Petroleum hydrocarbons, fuel, lubricants, fuel combustion
byproducts, and chemicals derived from stormwater runoff containing high concentrations of naturally-occurring substances, can also have negative impacts on aquatic flora and fauna (Burton and Robert, 2001). Pathogens can also produce waterborne diseases in either human or animal hosts (Imoisi et al., 2012). Alteration of water's physical chemistry includes acidity (change in pH), electrical conductivity, temperature, and eutrophication. Eutrophication is an increase in the concentration of chemical nutrients in an ecosystem to an extent that increases the primary productivity of the ecosystem. Depending on the degree of eutrophication, subsequent negative environmental effects such as anoxia (oxygen depletion) and severe reductions in water quality may occur, affecting fish and other animal populations (Imoisi et al., 2012).

There has been an increasing awareness among the scientists in the past few decades and they have been attempting to find a solution to this perennial problem of water pollution. Several studies have been carried out in the past three decades with a view to find out the effects of industrial effluents on the growth of economically important plants and soil characteristics. The possibility of making use of effluents and domestic wastes for agricultural purposes, after appropriate treatment and desired dilution, has also been explored by several workers. The diluted effluents of the factories, industries and domestic wastes are subjected to such experiments (Pragasam and Dixit, 2006).

Water contaminants pose a high potential risk for the health of human and animal populations; for this reason their toxic effects should be urgently established (Novelli et al., 1998). Organic compounds, pesticides, oils, solvents, and heavily used industrial products reach streams and rivers via run off from unregulated waste disposal (Wegman, 1992). The rate of urbanization has also undergone a rapid increase, including an increase in waste water discharge, so that exposure of humans to water pollutants is rarely limited to a single chemical or organic residue (Heindel et al., 1995).

1.3.1. Categories of water pollution

Surface water and groundwater have often been studied and managed as separate resources, although they are interrelated (USGS, 1998). Sources of surface water
pollution are generally grouped into two categories based on their origin: namely point source and non-point source.

1.3.2. Groundwater vs surface water pollution

Interactions between groundwater and surface water are complex (USGS, 1998). By its very nature, groundwater aquifers are susceptible to contamination from sources that may not directly affect surface water bodies, and the distinction of point vs. non-point source may be irrelevant. A spill of a chemical contaminant on soil, located away from a surface water body, may not necessarily create point source or non-point source pollution, but nonetheless may contaminate the underground aquifer.

1.3.3. Water pollution: National scenario

During the past few decades Indian industries have registered a quantum jump, which has contributed to high economic growth but simultaneously it has also given rise to severe environmental pollution. Consequently, ambient air and water quality got seriously affected which is far lower in comparison to the international standards. The problem is worse in the case of water pollution. It has been found that one-third of the total water pollution comes in the form of effluent discharge, solid wastes and other hazardous wastes. The surface water is the main source of industries for waste disposal. Almost all rivers are polluted in most of the stretches by one industry or the other. Although all industries function under the strict guidelines of the Central pollution control board (CPCB) but still the environmental situation is far from satisfactory. Different norms and guidelines are given for all the industries depending upon their pollution potentials. In India there is sufficient evidence available related with the mismanagement of industrial wastes (Mishra, 2008). Consequently, at the end of each time period the pollution problem becomes of menacing concern. So far no clear-cut estimations have been made to determine the overall effects of the industrial pollution, especially industrial water pollution. In very few instances the problem has been identified partially. However, many studies have listed heavy metals and pesticides as the major water pollutants in India (Malik and Ahmad, 1995; Rehana et al., 1995; Datta, 1999; Singh et al., 2005a; 2005b; Fatima and Ahmad, 2005; 2006; Tabrez and Ahmad, 2010).
1.3.4. Global picture of water pollution

Water pollution is also a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases (Pink and Daniel, 2006; West and Larry, 2006) and accounts for the deaths of more than 14,000 people daily (West and Larry, 2006; Solanki et al., 2011). In addition to the acute problems of water pollution in developing countries, industrialized countries also continue to struggle with these problems.

2. Oil refineries as a major source of water pollution

Pollution of the aquatic environment occurs from multitude sources including from oil refineries. Oil refinery effluents contain various chemicals at different concentrations including ammonia, sulphides, phenols, heavy metals and hydrocarbons. The exact composition cannot, however, be generalised as it depends on the refinery and which units are in operation at any specific time. It is, therefore, difficult to predict what effects the effluent may have on the environment (Wake, 2005).

Petroleum refineries generate a substantial amount of waste water through many operations during the refineries of crude oil. When large quantities of contaminated effluent is discharged in soil and aquatic sources, it slowly deteriorates the quality of both environmental compartments. As pollutants, petroleum hydrocarbons are moderately bioaccessible substances. The different oil pollutants are introduced into the soil and water through the irrigation of agricultural fields or leakages (Solanki et al., 2011).

Petroleum refining involves the transformation of crude oil into final useful products such as gasoline, gas oil, kerosene and jet fuel, and petrochemical feed stocks. The refined products are produced after a series of separation and treatment processes (Al Zarooni and Elshorbagy, 2006). After initial crude desalting and fractionation, several treatment and conversion processes are employed to reach the final blending stocks. Examples of conversion processes include thermal and catalytic cracking, steam and catalytic reforming, isomerization, alkylation and lube oil units. Treatment processes on the other hand include naphtha and gas oil desulfurization, sour water strippers and catalyst regeneration units. Petroleum refining uses relatively
large quantities of water, especially for cooling systems, desalting water, stripping steam, and water used for flushing during maintenance and shut down. In addition, surface water runoff and sanitary waste waters are accounted in the waste water system. The quantity of waste water generated and their characteristics depend on the process configuration, as a general rule, approximately 3.5–5 m³ of waste water is generated per ton of crude oil processed when cooling water is recycled (Dold, 1989; Al Zarooni and Elshorbagy, 2006; Ponce-Ortega et al., 2011).

2.1. Oil refineries as a source of pollution in India with special reference to Mathura refinery

Oily sludge and chemical sludge are the major sludges generated from the processes and effluent treatment plants of refineries engaged in crude oil refining operation. Refineries in India are estimated to generate about 28,220 tons of sludge per annum. Pollutants like phenols, heavy metals etc are present in the refinery sludge and they are considered as hazardous wastes (Bhattacharyya and Shekdar, 2003).

The Indian Oil Corporation Ltd. set up the Mathura Refinery as the sixth Indian oil refinery to meet the growing demand of petroleum products in North and North-Western regions of the country, including the national capital region and its adjoining areas. Petroleum refineries generate a substantial amount of waste water through many operations during the refineries of crude oil. Many investigations have been carried out to analyze the physico-chemical parameters of this refinery waste effluent. Most of them found a high level of original crude oil stock as well as metallic (Zn, Cr, Va, Ni, Pb,Cu) and non-metallic constituents. High levels of phenols, nitrogen, total grease and oil, total hydrocarbons, fluoride, chloride, calcium have also been reported in this refinery waste effluent (Solanki et al., 2011).

Chaudhary et al. (2011) analyzed and compared the activities of different enzymes in soil samples of native contaminated sites of a Mathura refinery and adjoining agricultural land. They collected the soil samples from the nearby area of Mathura refinery, India, and biological health parameters (dehydrogenase, aryl esterase, aryl sulphatase, alkaline phosphatase, acid phosphatase, lipase, laccase and catalase activities) were estimated in the soil samples. Among all the samples, sewage sludge impregnated soil showed the maximum activity of the enzymes, microbial
biomass carbon and most probable number of polycyclic aromatic hydrocarbon (PAHs) degraders in soils spiked with three- to four-ring PAHs at 50 ppm.

3. Major toxic pollutants of refinery waste water

According to Brown and Donelly (1984), petroleum refinery waste waters may contain a wide range of organic and metallic pollutants such as oil and greases, phenols, sulfides, ammonia, nitrogen compounds, heavy metals and polycyclic aromatic hydrocarbons. Because petroleum refining is a water-intensive practice, the petroleum industry uses and discharges large volumes of waste waters into surface waters. Although most of the contaminants are treated and recovered in the refinery before they enter the final effluent, a significant amount of toxic substances and compounds can enter the waste water (Avci et al., 2005).

Refinery effluents tend to have fewer of the lighter hydrocarbons than crude oil but more polycyclic aromatics which tend to be more toxic and more persistent in the environment (Wake, 2005). Polycyclic aromatic hydrocarbons (PAHs) are the most prominent among the genotoxic and carcinogenic agents present in polluted sites (Conney, 1982).

PAHs are chemicals of concern in many waste site investigations that are undertaken pursuant to the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the Resource Conservation and Recovery Act (RCRA), and state hazardous waste programs. The USEPA (1993) has identified seven PAHs as “probable human (B2) carcinogens”: benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, and indeno(1,2,3-c,d)pyrene (USEPA, 1993).

Besides the presence of PAHs, phenols are also a major component of refinery waste waters. The phenols are produced in the cracking process and special gasoline washeries (Al Zarooni and Elshorbagy, 2006). Phenols and its vapors are corrosive to the eyes, the skin, and the respiratory tract. Repeated or prolonged skin contact with phenols may cause dermatitis, or even second and third-degree burns due to phenols caustic and defatting properties (Lin et al., 2006). Inhalation of phenols vapor may cause lung edema. The substance may cause harmful effects on the central nervous
system and heart, resulting in dysrhythmia, seizures, and coma (Warner and Harper, 1986). Long-term or repeated exposure of the substance may have harmful effects on the liver and kidneys (World Health Organization/International Labour Organization, 2006). Besides its hydrophobic effects, another mechanism for the toxicity of phenols may be the formation of phenoxy radicals (Corwin, 2006).

The refinery effluents consist of compounds from original crude oil stock, metallic (Zn, Cr, Va, Ni, Pb, Cu) and non-metallic constituents (Solanki et al., 2011). Heavy metals may be categorized generally as toxic or very toxic to aquatic animals and to many plant species, though large interspecific differences in susceptibility occur even within closely-related groups of organisms (Hellawell, 1986).

4. Toxicity with particular reference to genotoxicity vis-à-vis surface waters

Among the various types of toxicities, organ toxicity like hepato/ cardio/ neuro/ nephro-toxicity is limited to animal kingdom, phytotoxicity restricts itself to plant kingdom and other toxicities though covering the different trophic levels but limit themselves to the exposed organisms only. However, it is the genotoxicity which encompasses the whole range of biota and all living organisms. Moreover, such harmful effects extend up to the future generation of living organisms.

Genotoxicity refers to the harmful effects of an agent on the genetic material (Pratt and Barron, 2003). It has been found to be directly associated with the neoplastic transformation (i.e. cancer) in humans and animals (Zeiger, 2001). Any measure directed at prevention of this as yet incurable disease would be highly desirable. Thus genotoxicity testing not only addresses the problem of toxicity at the very root level since targeting the genetic material is just like targeting the king of the country or the heart in the body, it also provides a clue to the cancer-causing potential of an agent at the individual level as well as that at incidence of genetic diseases in the exposed population. A wide variety of genotoxicity tests have been developed for biomonitoring purposes. These include use of micronuclei counts (Spies et al., 1990), DNA adducts (Varanasi et al., 1987), strand breakage (Stamato and Denko, 1990), his+ reversion (Kummarow et al., 2003; Umbuzeiro et al., 2004) etc. A combination of these assays provides a powerful method for assessing the short and long term genotoxicity.
5. Genotoxicity testing methods for surface water: An overview

5.1. *Salmonella* mutagenicity test

Among the microbial bioassays, *Salmonella* mutagenicity test has been the most widely used for detecting mutagenicity/genotoxicity in surface waters. The different responses of the *Salmonella typhimurium* strains can provide information on the classes of mutagens present in the water samples. This test has been proposed by the USEPA for clean water compliance monitoring (USEPA (b), 1989).

Rehana et al. (1995) used five different *Salmonella typhimurium* strains to compare the mutagenic activity of water samples from four sites of Ganga River, India. Samples always showed extreme mutagenic activity for TA98 and TA100 strains, both with and without S9 fraction. They also found a similar pattern in the responsiveness of the tester strains for a mixture of pesticides suggesting that the mutagenicity of the water extracts might be attributed to the pesticides used in the upstream region. Fatima and Ahmad (2006) compared genotoxic potential of waste water samples from two different stations namely Aligarh and Ghaziabad. Both the test water samples were found to be highly mutagenic by this test and the best sensitivity was recorded in case of TA102 and TA98 strains. An extremely high mutagenic potential of the water samples from a river in Brazil was suggested by Vargas et al. (1993) employing TA98 strain and S9 fraction. Numerous other studies have also employed the Salmonella mutagenicity test for the evaluation of water pollution (Kummrow et al., 2003; Umbuzeiro et al., 2004; Kutlu et al., 2007; Gana et al., 2008).

5.2. SOS chromotest/umu-test

Although the Salmonella microsome test has been widely used for the detection of mutagenicity in environmental samples, a variety of other assays also exist for investigating complex environmental mixtures. The SOS-chromotest and the umu test were developed as alternatives to the Ames test by Quillardet et al. (1982) and Oda et al. (1985) respectively. These are widely used for the routine monitoring of water samples as the results are available in a single day with minimal advance preparation. The microplate version of the SOS chromotest and umu test was
developed as a rapid and sensitive screening tool for the detection of genotoxicants in surface waters (Langevin et al., 1992; White et al., 1996). The application of a fluorometric umu-test system has been developed in order to increase the sensitivity of the test for the detection of genotoxic compounds in surface water (Reifferscheid and Zipperle, 2000).

5.3. *Escherichia coli* lacZ reversion mutagenicity assay

The *Escherichia coli* lacZ reversion mutation assay was introduced by Cupples and Miller (1988). The lacZ assay uses a set of *E. coli* lacZ strains. Each strain carries a lacZ allele which codes for an inactive β-galactosidase protein. The use of lactose as a carbon source by *E. coli* requires the activity of β-galactosidase which catalyzes the hydrolysis of lactose to glucose and galactose. Therefore, reversion to lacZ results in a colony which can grow on lactose minimal medium. The lacZ alleles used in the Cupples and Miller (1988) system were rationally designed so that only a single DNA sequence change generates a selectable mutant from each allele. This means that the lacZ assay can be used directly to test the mutational specificity of a particular mutagen without need for DNA sequencing. This test has been used in the detection of mutagenicity of effluent from dye industry (Chung et al., 1998; 2000).

5.4. Single cell gel electrophoresis/ comet assay

In recent years the comet assay has gained broad attention, because the test is relatively easy to handle and can be applied with cells from different organisms and tissues. The alkaline version of the comet assay has been developed by Singh et al. (1988). Several studies have employed the comet assay for assessing the level of DNA strand breakage in cells from aquatic organisms treated with surface water samples *in vivo* and *in vitro* (Klobucar et al., 2003; Russo et al., 2004; Woo et al., 2006). Advantages of the test are the option to choose a broad range of test organisms and tissues, the use of even non-proliferating cells, and that results can be obtained within one day. On the other hand there are still no standard test protocols and a certain degree of handling skills is a necessary prerequisite to routinely perform the test (Angerer et al., 2007).
5.5. *Saccharomyces cerevisiae* gene mutation assay

The test performance of the gene mutation assay with unicellular yeast (*Saccharomyces cerevisiae*) is more comparable with the bacterial assays than with other eukaryotic tests. The test principle is the detection of forward or reverse mutations (Zimmermann, 1984).

5.6. Chromosome aberration assay

Chromosome aberrations include structural aberrations such as fragments or intercalations and numerical aberrations. Cytogenetic effects of the sample can be studied either in whole animals or in cells grown in culture. Generally the cell culture is exposed to the test substance and then treated with a metaphase-arresting substance. Following suitable staining the metaphase cells are analysed microscopically for the presence of chromosomal abnormalities (Fucic et al., 2007).

5.7. Micronucleus induction assay

The micronucleus assay is a widely used cytogenetic assay for the assessment of *in vivo* or *in vitro* chromosomal damage. There are several reports on micronucleus induction in aquatic organisms, plants and cultured cells treated with surface water (Campana et al., 2001; Dixon et al., 2002). Micronucleus formation along with the sister chromatid exchanges and chromosome aberration assays is considered as a clastogenic endpoint. In principle flow cytometric measurement of micronuclei is possible (Kohlpoth et al., 1999; Sanchez et al., 2000) but the costs of equipment are high.

5.8. Other genotoxicity assessment methods

Sister chromatid exchange (SCE) assay, UDS assay, DNA adduct formation and the Tradescantia stamen hair mutation assay have also been widely used for the detection of aquatic pollution (Duan et al., 1999; Grummt, 2000; Bakare et al., 2003). Fluorescence-based screening assay for DNA damage has been recently introduced as a screening tool to check genotoxic potential of industrial chemicals.
5.9. The *Allium cepa* test: a sensitive tool in environmental monitoring

The *Allium cepa* test was introduced in 1938 by Levan. Since then, it has been widely used for environmental monitoring. This test has been widely used for the toxicity assessment of heavy metals, pesticides and industrial waste waters (Chauhan et al., 1986; Fiskesjö, 1988; Grover and Kaur, 1999; Siddiqui and Ahmad, 2003; Fatima and Ahmad, 2006). Kovalchuk et al. (1998) used this assay to evaluate the genotoxic potential of waste water. Our group (Siddiqui and Ahmad, 2003; Fatima and Ahmad, 2006; Tabrez and Ahmad, 2010) have also used this test for the genotoxicity assessment of waste water from Aligarh and other region. The *Allium cepa* test has also been used by other investigators for detecting the genotoxicity of surface waters (Monarca et al., 2003; Leme and Morales, 2009) and the clastogenicity of atrazine (Bolle et al., 2004).

The *Allium cepa* anaphase-telophase chromosomal aberration test has been used to study the mutagenic effects of N-methyl-N-nitrosourea, maleic hydrazide, sodium azide and ethyl methane sulphonate (Rank and Nielsen, 1997). Both versions of the *Allium cepa* test viz. root length inhibition assay as well as genotoxicity assay are cost-effective, easy to perform and sensitive enough to respond to low concentrations of toxicants (Kailasam and Rogers, 2007).

6. Hazard assessment: Toxicity bioassays

The widespread release of natural and synthetic chemicals into the environment, singly or as complex domestic and industrial effluents, has necessitated the development of rapid and cost effective toxicity tests to protect humans and other biota (Sherry et al., 1997; Dalzell et al., 2002). Thus both the short-term and long-term bioassays exist utilising all trophic levels including microorganisms, invertebrates, higher plants and animals. The general purpose of toxicity testing is to establish the potential impact of chemicals on biota in the environment. The information gained can then be used to manage the treatment or release of chemicals. No single toxicity test can determine the effect of toxicants on all biota because of differences in response by organisms at different trophic levels (Kaiser, 1993). With respect to biological testing, it is known that organisms within the same (Codina et al., 1993) and in different (Ribo, 1997; Shoji et al., 2000) trophic levels respond
differently to a range of toxicants, either as single or complex mixtures, and hence there is a need to develop toxicity bioassays using a battery of susceptible organisms.

7. Relevance of biomarkers in environmental toxicology

Limitations in understanding the relationship between occupational and environmental exposures and diseases present ample opportunities for using biological markers to fill the gaps in knowledge. The most compelling reason for using biomarkers is that they can give information on the biological effects of pollutants rather than a mere quantification of their environmental levels. Moreover, biomarkers may provide insight into the potential mechanisms of the harmful effects of contaminants (Altenburger et al., 2003). The goal of biomarker research and application is to prevent disease by reducing exposures to hazardous agents through the early identification of exposure and response (Silbergeld and Davis, 1994). Potential utility of biomarkers for monitoring of environmental quality has received increasing attention during the last few years (Zhang et al., 2004). Identification of early and sensitive biomarkers of exposure allows the development of strategies to prevent cell damage that results in persistent or irreversible injury (Brooks, 2001).

7.1. Biomarker concept

Typically, biomarkers are defined as quantitative measures of changes in the biological system that respond to either (or both) exposure and/or doses of xenobiotic substances that lead to biological effects. Although not explicitly contained in most definitions, the use of the term ‘biomarkers’ or ‘biomarker response’ is often restricted to cellular, biochemical or molecular or physiological change that are measured in cells, body fluids, tissues or organs within an organism and are indicative of xenobiotic exposure and/or effect (Oikari and Jimenez, 1992; Lam and Gray, 2003).

Changes that occur at the organismic, population and assemblage levels are usually referred to as ‘bioindicators’. One possible reason for limiting the term ‘biomarkers’ to sub-organismic changes is that one of the functions of biomarkers is supposedly to provide early warning signals of biological effects and that it is generally believed that sub-organismic (molecular, biochemical and physiological)
responses tend to precede those at the organismic or higher levels (Jimenez and Stegeman, 1990; Lam and Gray, 2003). Biomarkers can also be used to test the effectiveness of environmental controls. The utility of a biomarker is expected to influence by the degree to which it is validated (Schulte, 1995; Molitoris et al., 2008).

According to the National Research Council of Canada (NRC, 1985), WHO (1993) and Martin-Diaz et al. (2004) biomarkers can be subdivided into three classes:

I. Biomarkers of exposure, which cover the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism.

II. Biomarkers of effect, which include measurable biochemical, physiological or other changes within tissues of body fluids of an organism that can be recognized as associated with an established or possible health hazard.

III. Biomarkers of susceptibility, which include the inherent or acquired ability of an organism to respond to the challenge of exposure to specific xenobiotic substances including genetic factors.

At a practical and operational level, there are four desirable characteristics of the biomarker assay: sensitivity, specificity, simplicity and stability. The assay should be sensitive enough to detect early stage of the toxicity while specificity is desirable because it can provide evidence of the harmful effect of a particular type of pollutant. Simplicity is desirable to make an assay available to non-experts in a cost-effective way. Stability is also important in the sense that unstable and short-lived responses are difficult to measure and interpret in field studies (Walker, 1998; Vander Oost et al., 2003). Use of biomarker is regarded as ethically acceptable. Good biomarkers are sensitive indices of both pollutant bioavailability and early biological responses.

The selection of the appropriate markers for the study of the effect of contaminants is frequently a controversial issue especially when information on the mechanism of action of the contaminant is insufficient (Lauwerys et al., 1995). In reality, no biomarker assay exists that has all of the aforementioned attributes and it is unlikely that there ever will be such a biomarker. This limitation can be overcome by using a combination of biomarkers (Bocquen et al., 1997). By screening multiple
biomarker responses, important information will be obtained about organism’s toxicant exposure and stress. A pollutant stress situation normally triggers a cascade of biological responses, each of which may serve as a biomarker at least theoretically (Besten and Munawar, 2005).

7.2. Biomarkers vis-à-vis xenobiotic biotransformation

Xenobiotics are usually biotransformed in the liver according to the simplified mechanism, which can be subdivided into phases I, II and III. Phase I is a non-synthetic alteration (oxidation, reduction or hydrolysis) of the original foreign molecule, which can then be conjugated in phase II and catabolized in phase III (Vander Oost et al., 2003). The enzymes of phase III (e.g. peptidases, hydrolases and β-lyase) catalyze the catabolism of conjugated metabolites to form easily excretable products.

7.2.1. Phase I of biotransformation

Phase I is the predominant biotransformation pathway. It generally involves the addition or exposure of functional groups on the xenobiotic, e.g. by oxidation or hydrolysis (Goepfar et al., 1995). The most extensively examined system, from the point of view of biomarkers, is the mixed function oxidase system (MFO) which involves oxidation by a variety of isozymes of cytochrome P450 (Lewis, 2001). Since the MFO system is sensitive to certain environmental pollutants, its activity may serve as a biological monitor for exposure to certain classes of xenobiotic chemicals (Bucheli and Fent, 1995; Vander Oost et al., 2003).

Cytochrome P450 was discovered as a pigment that in a complex with CO could absorb light at 450 nm (Schenkman and jansson, 1998). Its inactive form has an absorption maximum of 420 nm. Cytochromes P450, comprising a large and still expanding family of heme proteins, are membrane-bound proteins which predominantly are located in the endoplasmic reticulum of liver of animals (Jia et al., 2009). Its activity depends on the presence of NADPH-cytochrome P450 reductase and phospholipid membrane function. All these components constitute a monooxygenase system (Kvanickova, 1995).
7.2.2. Biochemistry of cytochrome P450

In a simplified form, cytochrome P450 function is shown by the following equation:

\[
\text{NADPH} + \text{H}^+ + \text{RH} + \text{O}_2 \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{NADP}^+
\]

This is a monooxygenase reaction in which one molecule becomes more planar by the insertion of an oxygen atom.

For the detection of pollution in aquatic environments, the CYP1A1 family members have so far been proved to be the most sensitive indicators (Machala et al., 2000; Schelnk and DiGiulio, 2002). They respond to water contamination at levels too low to be detected by other laboratory methods.

7.2.3. Phase II enzymes

Enzymes active in Phase II are located in the cytoplasm and endoplasmic reticulum and promote conjugations of Phase I products with endogenous ligands such as glutathione (Talalay, 2000) to inactivate the xenobiotics by making it more water-soluble (polar), which is important for elimination and excretion. The function of Phase II enzymes can be defined by the following properties (Talalay, 2000):

- Regulation by mechanisms that are very similar and may involve common promoter elements (e.g. Antioxidant Responsive Element, ARE).
- Catalysis of a wide variety of reactions that serve to protect cells against toxicities of electrophiles and reactive oxygen species by converting them to less toxic products.
- The induction of Phase II enzymes is effective and sufficient to accomplish cellular protection against toxic and neoplastic effects of electrophiles and reactive oxygen species (ROS).

Numerous endogenous sources of oxyradical production exist, but of more immediate interest with respect to environmental biomarkers is the ability of a number of structurally diverse compounds to enhance intracellular oxyradical production through the process of redox oxidant-mediated effects with a potential suitability as biomarkers. These include either adaptive responses, such as increased activities of
antioxidant enzymes and concentrations of non-enzymatic compounds, or manifestations of oxidant-mediated toxicity in terms of oxidations of proteins, lipids and nucleic acids, as well as perturbed tissue redox status (Filho, 1996; Howcroft et al., 2009). Defense systems that tend to inhibit oxyradical formation include the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR). SOD, CAT, GST and GPx are critically important in the detoxification of radicals to nonreactive molecules. Numerous low-molecular-weight antioxidants, such as GSH, \( \beta \)-carotene (vitamin B), ascorbate (vitamin C), \( \alpha \)-tocopherol (vitamin E) and ubiquinol 10 have also been found to play a vital role (Torres et al., 1993; Griffiths et al., 2002).

### 7.2.3.1. Glutathione-S-transferases (GST)

The family of glutathione-S-transferase (GST) enzymes is of physiological importance because its members provide protection against electrophilic xenobiotics, such as heavy metals, pesticides, carcinogens, etc. by conjugating them to glutathione (GSH) (Mannervik and Danielson, 1988). GSTs are also essential components of the cellular antioxidant defence system, since they catalyze the conjugation of GSH to several dangerous compounds produced by lipid peroxidation (Arrigo, 1999; Rahaman et al., 1999).

As regards with CYP1A, the mechanism of induction for most GSTs in mammals is regulated via the Ah-receptor (George, 1994; Kirby and Ottea, 1995). An additional form of GST induction which functions independently of the Ah-receptor has been elucidated. It requires metabolism of the compound before transcriptional activation of the respective subunit gene takes place (Rushmore and Pickett, 1990). Due to the role that GSTs play in conjugating reactive epoxide species and other electrophiles, induction of these enzymes must be considered to be beneficial, although metabolic activation of halogenated xenobiotics by GST is also well recognized (Armstrong, 1990; Commandeur et al., 1995; Castel et al., 2005).
7.2.3.2. Superoxide dismutase (SOD)

The superoxide dismutase comprises a family of multi functional enzymes with broadly overlapping substrate specificities, which play important roles in detoxification of superoxide radical (Pereira et al., 1998). Similar to other antioxidant enzymes, SOD is known to be influenced by a large number of toxic compounds (Novelli et al., 1997).

SOD is considered as the first line of defense against oxygen toxicity and the central regulators of ROS levels by catalyzing the decomposition of superoxide, the first but most abundant ROS, into hydrogen peroxide and water. SOD can be activated to scavenge excessive superoxide in the presence of moderate oxidative stress with compensation (Landis and Tower, 2005).

7.2.3.3. Catalase (CAT)

Catalases are hematin-containing enzymes that facilitate the removal of hydrogen peroxide (H$_2$O$_2$), which is metabolized to molecular oxygen (O$_2$) and water. Unlike some peroxidases that can reduce various lipid peroxides as well as H$_2$O$_2$, CATs can only reduce H$_2$O$_2$ (Filho, 1996; Menone et al., 2008). It was demonstrated that peroxisome-proliferating compounds (a class of non-genotoxic carcinogens) induce both the activities of H$_2$O$_2$-generating fatty acid oxidases and CAT in rodents (Reddy and Lalwani, 1983; Halliwell and Gutteridge, 1999). Since CATs are localized in the peroxisomes of most cells and are involved in fatty acid metabolism, changes in activities may often be difficult to interpret (Stegeman et al., 1992; Vander Oost et al., 2003). Therefore, CAT activities in erythrocytes may be a more appropriate marker for oxidant exposures in vertebrates.

7.2.3.4. Glutathione peroxidase (GPX)

Peroxidases are enzymes that reduce a variety of peroxides. While CAT employs one molecule of H$_2$O$_2$ as donor in the reduction of another H$_2$O$_2$ molecule, peroxidases employ other reductants. GPX catalyzes the metabolism of H2O2 to water, involving a concomitant oxidation of reduced GSH to its oxidized form (GSSG). GPX is considered to play an especially important role in protecting membranes from damage
due to lipid peroxidation (LPO). This observation led to the view that the major detoxification function of GPx is the termination of radical chain propagation by quick reduction to yield further radicals (Lauterburg et al., 1983). The use of peroxidases in plants may be used to detect early oxidant responses, but the use of GPx received relatively little attention as a biomarker in animals (Stegeman et al., 1992). The GPX/glutathione system is considered to be a major defense in low-level oxidative stress (Wassmann et al., 2004).

7.2.3.5. Ascorbate oxidase (AO)

This enzyme belongs to the family of oxidoreductases, specifically those acting on diphenols and related substances as donor with oxygen as acceptor. The systematic name of this enzyme class is L-ascorbate:oxygen oxidoreductase (Boyer et al.,). AO participates in ascorbate metabolism. Cell wall ascorbate and cell wall-localized ascorbate oxidase (AO) have been implicated in control of growth. High AO activity is associated with rapidly expanding cells and a model which links wall ascorbate and ascorbate oxidase to cell wall extensibility has been presented (Smirnoff, 1996).

7.2.3.6. Glutathione reductase (GR)

Glutathione reductase perhaps is not involved in antioxidant defense in the same way as described for the aforementioned enzymes. However, GR merits attention because of its importance in maintaining GSH/GSSG homeostasis under oxidative stress conditions (Winston and DiGiulio, 1991). GR catalyzes the transformation of the oxidized disulfide forms of glutathione (GSSG) to the reduced form (GSH), with the concomitant oxidation of NADPH to NADP⁺.

7.2.3.7. Monodehydroascorbate reductase (MDHAR)

Monodehydroascorbate reductase belongs to the family of oxidoreductases. In plants, the MDHAR is an enzymatic component of the glutathione-ascorbate cycle that is one of the major antioxidant systems of plant cells for the protection against the damages produced by ROS. This is found in several cell compartments, such as chloroplasts, cytosol, mitochondria, glyoxysomes, and leaf peroxisomes (Leterrier et al., 2005). MDHAR could account for the regeneration of ascorbate from monodehydroascorbate
(MDA) produced by ascorbate peroxidase (APX) activity. In the absence of MDHAR, MDA disproportionated to ascorbate (ASC) and dehydroascorbate (DHA). The DHA is reduced to ASC by dehydroascorbate reductase (DHAR) in chloroplasts (Chen and Gallie, 2006).

7.2.3.8. Dehydroascorbate reductase (DHAR)

Dehydroascorbate reductase is an enzyme that is critical for maintenance of an appropriate level of ascorbate in plant cells (Kato et al., 1997). It is responsible for regenerating ASC from an oxidized state and regulates the cellular ASC redox state which in turn affects cell responsiveness and tolerance to environmental ROS (Chen and Gallie, 2006).

7.2.3.9. Reduced and oxidized glutathione (GSH and GSSG)

Reduced GSH, a tripeptide consisting of glutamine, cysteine and glycine, can be conjugated in the initial step of mercapturic acid formation (Commandeur et al., 1995; Mutlib et al., 2000). Among its functions are two contrasting roles in detoxifications, as a key conjugate of electrophilic intermediates, principally via GST activities in phase II metabolism, and as an important antioxidant (Stegeman et al., 1992; Commandeur et al., 1995).

Perhaps the most obvious direct effect of certain pollutants is a decrease in thiol status, i.e. the ratio of reduced to oxidized glutathione (GSH: GSSG) (Stegeman et al., 1992; Otto and Moon, 1995). Alternatively, normal GSH: GSSG ratios can be maintained due to increased activities of GR or increased GSH synthesis. In mammals, GSH synthesis is considered to be tightly regulated via feedback inhibition by GSH on a rate-limiting synthetic enzyme (Suh et al., 2004). In the healthy cell, GSH: GSSG ratios are typically very high i.e usually greater than 10 (Stegeman et al., 1992; Harwood et al., 2009). There are strong indications that the tissue thiol status modulates Ah receptor inducible CYP1A gene expression and catalytic activity, indicating a ‘cross-talk’ between the GSH and cytochrome P450 systems (Vander Oost et al., 2003).
7.3. Non-enzymatic biomarkers

7.3.1. Ascorbic acid (ASC)

Ascorbate is a major metabolite in plants. It is an antioxidant and in association with other components of the antioxidant system protects plants against oxidative damage resulting from aerobic metabolism, photosynthesis and a range of pollutants (Smirnoff, 1996).

7.3.2. Tocopherol

Tocopherol (Toc) is a lipid soluble antioxidant found in all plant parts and is a potential scavenger of ROS and lipid radicals (Kruk et al., 2005). α-Tocopherol is the major vitamin E found in leaf chloroplasts, where it is located in the chloroplast envelope, thylakoid membranes and plastoglobuli and plays both the antioxidant and non-antioxidant functions (Bosch, 2005). Tocopherol prevents the chain propagation step in lipid autoxidation and this makes it an effective free radical trap (Serbinova and Packer, 1994). In addition, Toc acts as scavenger of oxygen radicals, especially that of \( \mathrm{O}_2^- \) (Fryer, 1992).

7.3.3. Carotenoid

Carotenoids (Car) are the pigments that are found in plants and microorganisms. There are over 600 carotenoids occurring in nature. These lipid soluble antioxidants, play a multitude of functions in plant metabolism including oxidative stress. Carotenoids trap light energy and transfer it to chlorophyll molecules in reaction centers. They also protect the photosynthetic apparatus by quenching a triplet sensitizer, singlet oxygen and other harmful free radicals which are naturally formed during photosynthesis (Ahmad et al., 2009).

7.3.4. Plant phenolics

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants (Randhir et al., 2004). These compounds owing to one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in
There have also been a number of reports suggesting that dietary phenolics exhibit prooxidant and cytotoxic properties under certain conditions (Summers and Felton, 1994; Yamanaka et al., 1997; Sugihara et al., 1999). The antioxidant/prooxidant activity of phytophenolics can depend on such factors as metal-reducing potential, chelating behavior, pH, and solubility characteristics (Decker, 1997).

### 7.4. Oxidative stress parameters

Many environmental contaminants (or their metabolites) have been shown to exert toxic effects related to oxidative stress (Winston and DiGiulio, 1991). Oxygen toxicity is defined as injurious effects due to cytotoxic reactive oxygen species (ROS), also referred to as reactive oxygen intermediates (ROIs), oxygen free radicals or oxyradicals (DiGiulio et al., 1989). Reduction products of molecular oxygen are of particular interest, which may react with critical cellular macromolecules, possibly leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and ultimately cell death (Winston and DiGiulio, 1991). The activities of the antioxidant enzymes, which defend the organisms against ROS, are critically important in the detoxification of radicals to non-reactive molecules.

#### 7.5.1. Ethoxyresorufin-O-deethylase (EROD)/ CYP1A1

EROD activity describes the rate of the CYP1A mediated deethylation of the substrate, 7-ethoxyresorufin (7-ER) to form the product, resorufin. The catalytic activity towards this substrate is an index of amount of the enzyme present and is measured as the concentration of resorufin produced per mg protein per min (mol/mg/min) (Kennedy and Jones, 1994). Because metabolism is generally highest in hepatic tissue, the assay is typically conducted using liver. For this reason, EROD is often termed an “early warning system” (Payne et al., 1987). EROD is a highly sensitive indicator of contaminant uptake in fish, providing evidence of receptor-mediated induction of cytochrome P450-dependant monooxygenases (the CYP1A subfamily specifically) by xenobiotic chemicals. The most useful aspect of CYP1A for biomonitoring purposes is the enzyme’s tendency to increase in concentration upon chemical exposure. Induction of CYP1A is mediated through the binding of xenobiotics to a cytosolic aryl hydrocarbon receptor (AhR). Induction of EROD is an extremely sensitive indicator of environmental alterations and is usually one of the
first detectable, quantifiable responses to exposure (Stegeman, 1992). Receptor
binding is followed by a series of molecular events leading to the expression of
several genes (including CYP1A) known as the “Ah-gene battery” (Nebert et al.,
1993).

![Structural formulae of 7-ethoxyresorufin and resorufin. Deethylation of the
substrate is mediated by CYP1A (7-ethoxyresorufin-O-deethylase) to yield
the fluorescent product resorufin.]

**Fig. 1:** Structural formulae of 7-ethoxyresorufin and resorufin. Deethylation of the
substrate is mediated by CYP1A (7-ethoxyresorufin-O-deethylase) to yield
the fluorescent product resorufin.

7.5.2. Pentoxyresorufin-O-deethylase (PROD)

Pentoxyresorufin-O-deethylase (PROD) is a major cytochrome P450 enzyme which is
inducible by phenobarbital in both liver and small intestine. It is active in the
metabolism of some compounds like pentoxyresorufin, testosterone.

7.5.3. Ethoxycoumarin-O-deethylase (ECOD)

7-Ethoxycoumarin-O-deethylase (ECOD) is a drug-metabolizing enzyme found in the
hepatic, placental and intestinal microsomes that metabolizes 7-alkoxycoumarin to 7-
hydroxycoumarin. 7-ethoxycoumarin-O-deethylation has been used widely as a
marker activity for assessing substrate specificities of cytochrome P450 (P450) in
liver microsomes of mammals, and extensive studies have shown that in rats and mice
the major catalysts are P450 1A1, 1A2, and 2B enzymes. In contrast to findings in
experimental animal models, P450 2E1 has been reported to be a principal enzyme
involved in 7-ethoxycoumarin-O-deethylation in human livers (Yamazaki et al.,
1996).
8. Limitations of biomarkers

Biomarker responses are powerful because they integrate a wide array of environmental, toxicological and ecological factors that control and modulate exposure to, as well as effects of, environmental contaminants. However, these factors may also complicate interpretation of the significance of the biomarker responses in ways that may not always be anticipated (McCarthy, 1990). Many non-pollution-related variables may have an additional impact on the various enzyme systems, and may thus interfere with biomarker responses when experimental conditions are not thoroughly analyzed or controlled. Examples of such ‘confounding’ or ‘modifying’ factors are the organisms’ health, condition, sex, age, nutritional status, metabolic activity, reproductive and developmental status, and population density, as well as factors like season, ambient temperature, heterogeneity of the environmental pollution, etc. Unfortunately, most available toxicity data rarely quantify the potency that confounding factors are likely to exhibit in natural environments (De Kruijf, 1991). Moreover, estimates of confounding factor interactions are scarce, as evidenced by the extensive use of uncertainty factors in risk assessment to address unknowns (Power and McCarty, 1997).

9. Fate of the refinery effluent

The fate of oil refinery effluent, once it is discharged into the environment depends on the conditions and hydrodynamics of the receiving water. The effluent is inevitably diluted within the receiving water but to what extent depends on the size of the recipient and where the outfall is located, whether it is intertidal or subtidal. Grahl-Nielsen (1987) dyed the discharge water from an offshore operation and found that the discharge was unevenly distributed in the recipient waters. Most studies on the fate of refinery wastes just consider the hydrocarbons within the effluent. The volatile compounds are lost from the water column through weathering (Cranthorne et al., 1989). The remaining compounds undergo sedimentation and biodegradation. Knap and Williams (1982) found that the most important removal mechanism was sedimentation and that in Southampton Water, 70% of the hydrocarbons were found in the sediments after 1 h. Compounds with high water solubility such as aromatics were absorbed slower than non-polar compounds like aliphatics. In Southampton Water biodegradation occurred rapidly, hydrocarbon concentrations were reduced by
70% after 40 days, much faster than in other areas. The increased speed of biodegradation was attributed to the substantial population of oil degraders in the area that had accumulated over the 50 years of chronic discharge. Most of the hydrocarbons that are degraded are lower molecular weight aliphatic fractions. This means that over time hydrocarbon concentrations do decrease but due to the constant effluent discharge they are always being replenished. Therefore, if the discharges were to cease or the hydrocarbon concentration within effluents were to be reduced then there is the potential for the hydrocarbon concentrations to decrease to lower levels within the sediment. Le Dreau et al. (1997) observed three zones of contamination of the sediment around a petroleum refinery in the Gulf of Fos (South France). Firstly, a highly contaminated zone near the refinery (50 g kg\(^{-1}\) sediment dry weight), followed by a less contaminated zone in the deep creek (3 g kg\(^{-1}\) sediment dry weight), with a final slightly contaminated zone in the open sea (0.1 g kg\(^{-1}\) sediment dry weight). Other studies have also shown that the area of high contamination is often localised to the vicinity of the outfall and decreases with distance (Knap et al., 1982; Armannsson et al., 1985; Moore et al., 1987; Talsi, 1987).

9.1. Toxicity of refinery waste water in plant system

In soils polluted with organic chemicals (generally entering into the soil through petroleum derived waste), plants may experience a combined stress from nutritional deficiency and chemical toxicity. Reactive oxygen species (ROS) are generated as by-products of normal metabolism in different subcellular compartments. Furthermore, the imposition of biotic or abiotic stress may give rise to an excessive concentration of ROS. Plants use ROS as second messenger in many signal transduction cascades and thus ROS accumulation is crucial to plant development and defence. For these reasons, the plant antioxidant defence network is important in controlling the life-time of the ROS signals and in preventing uncontrolled oxidation (Gomez et al., 2004; Romero-Puertas et al., 2007; Meyer, 2008). The enhanced activities of MDHAR, GR and SOD were noticed by Marti et al. (2009) in the leaves of alfalfa plants exposed to refinery waste sludge. Different *Allium* species are used to evaluate environmental pollution, *Allium cepa* being the most often used due to the knowledge of its cell cycle duration and its reaction in the presence of many known mutagenic agents (Evseeva et al, 2003). The refinery effluent inhibited the growth of the alga *Selarolstrom*
apricorntum and the duckweed Lemna gibba. It also reduced the germination in Lactuca seed by 15% (Wake, 2005).

Numerous reports have catalogued MN formation, enhanced oxidative stress and chromosomal aberration in plants exposed to refinery waste contaminated water (Hoshina and Marin-Morales, 2009). According to Spencer et al. (2007), such exposures significantly increase the number of micronuclei in plant and animal system. Mitteregger et al. (2007) demonstrated toxicity in Allium cepa after exposure to environmental samples of water and sediment containing phenolic compounds. A significant increase in the MN formation was observed by Cavusoglu et al. (2010) in Vicia faba L. seeds exposed to petroleum waste water and also suggested that heavy metals in petroleum waste water had cytotoxic activity induced MN formation in Vicia faba L. These observations are also in agreement with cytotoxicity data reported by other authors so far. In most of the studies, results indicated that the test substances as heavy metals ions can produce chromosomal or spindle damage and mitotic apparatus damage leading to formation of MN (Inceer et al., 2003).

9.2. Toxicity of refinery waste water in animal system

Petroleum hydrocarbons, salt water, and caustic chemicals have the potential of altering rumen flora and enzymatic processes as well as damaging the ruminal and gastrointestinal epithelium (Edward, 1989). The toxicity of petroleum hydrocarbons appears to be related more closely to the volatility and viscosity of the product than to other factors. The more volatile straight chain and aromatic petroleum hydrocarbons have a greater potential for aspiration pneumonia and may produce an anesthetic-like action if absorbed systemically (Ohe et al., 2004). The more volatile petroleum hydrocarbons also are more irritating to skin and mucous membranes and appear to be more damaging to rumen flora (Edward, 1989; Sciencecorps Lexington, 2010).

Genotoxic effects of petroleum refineries are well documented. Petroleum refinery runoff sludge induces mutations in Salmonella and chromosomal damage in A. nidulans (Brown and Donelly, 1984), MN formation in newt Pleurodeles waltl (Fernandez and Haridon, 1994), DNA damage, chromosomal aberration, p53 protein accumulation and apoptotic cell death in Chinese Hamster ovary cells (Krishnamurthi et al., 2003).
Vanzella et al. (2007) evaluated the genotoxicity and mutagenicity of the diesel water soluble fraction (DWSF) collected from Brazilian rivers contaminated by petroleum waste, on the neotropical fish *Prochilodus lineatus* under acute (6, 24 and 96 h) and subchronic (15 days) exposures, using the comet (SCGE) and micronucleus assays. Comet scores and micronuclei frequency in experimental fish exposed to DWSF were significantly higher than the respective negative control groups (fish exposed to clean water for the same period).

9.3. Effect on human health

People's health could be adversely affected by oils either when inhaling or touching oil products, or when eating contaminated sea food. Concentrations of petroleum contaminants in fish and crab tissue, as well as contamination of shellfish, could pose a significant potential for adverse human health effects. The presence of heavy metals in water bodies contaminated by refinery effluent can cause severe damage to human beings.

Oil refineries emit about 100 chemicals everyday. These include metals like lead, very small dust particles called PM10, many gases like sulphur dioxide (SO₂), nitrogen oxide (NO₂), carbon dioxide, carbon monoxide, methane, dioxins, hydrogen fluoride, chlorine, benzene and others. Many of these are harmful to humans, and can cause skin irritations, nausea, eye problems, headaches, birth defects, leukemia, and cancers. Young children and the elderly are the worst affected. A study conducted in Durban showed that school children at a school situated next to a refinery suffered between 30% - 40% more respiratory problems than children living more than 10 km away (Naidoo, 2010).

10. Objectives of the study

In view of the literature presented in the preceding pages as well as the surveys conducted by Ashok et al. (1995), Hayat et al. (2002), Chaudhary et al. (2011) and Solanki et al. (2011) that reported various ailments probably associated with refinery toxicants exposure to living system, an attempt was made (i) to estimate the genotoxicity of Mathura refinery waste water and (ii) to identify certain biomarkers of
waste water contamination in the water bodies. The detailed objectives of the study are listed below:

1. Chemical analysis of the Mathura refinery waste water (MRWW) was proposed to carry out to identify the major toxicants in the test water samples.
2. Since *Allium cepa* test is a simple, sensitive and cost effective indicator of toxicity, it was thought that its efficacy would be greatly increased if biomarker studies were carried out in the same onion bulbs employed for the MRWW toxicity bioassay.
3. Various enzymes of the detoxification machinery like GST, GR, SOD, CAT, APX, GPx and MDHAR have been shown to get modulated in response to pollutant exposure (Panda, 2003; Fatima and Ahmad, 2005; Metwally and Fouad, 2008; Sinha et al., 2009). It was proposed to carry out the biomarker studies on the various components of detoxification machinery of *Allium cepa* system in response to Mathura refinery waste exposure.
4. EROD, PROD and ECOD, the representative markers of the phase I detoxification enzymatic machinery have also been suggested as valid biomarkers of pollution (Fernandes et al., 2002; Ferrat et al., 2003; Lee et al., 2008). It seemed also reasonable to study the potential of these enzymes of *Allium cepa* system as the candidate biomarkers of refinery waste pollution.
5. The chromosomal aberration and micronucleus assays are the rapid and sensitive tests of genotoxicity and routinely employed for testing the mutagenicity/genotoxicity of water samples. The formation of different forms of chromosome abnormalities and micronuclei in *Allium cepa* caused by the test water sample, if any, can also serve as a biomarker of genotoxicants present in water samples, which needs proper scientific investigation.
6. In view of the increasing popularity of comet assay, it seemed worthwhile to investigate the efficacy of comet assay for detecting the DNA damage in human peripheral lymphocytes exposed *in vitro* to different concentrations of the Mathura refinery waste water.
7. The toxicity of water pollutants has been reported to be mediated by reactive oxygen species in many cases (DiGiuiilo et al., 1995; Livingstone, 1998; Hassoun et al., 2004; Wenhua, 2005). The generation of ROS can provide an indication about the presence of specific pollutants and can thus be utilized as
a biomarker. The involvement of ROS in general and individual species in particular in the toxicity/genotoxicity of the test water samples was also proposed to be investigated.