Imperial Journal of Interdisciplinary Research (IJIR)
Vol-3, Issue-9, 2017
ISSN: 2454-1362, http://www.onlinejournal.in

Genotoxicity Assessment of Surface Water with Special Reference to Oil Refinery Effluent: A Review

Amit Kumar Gupta* & Masood Ahmad
Department of Biochemistry
F/O Life Sciences, Aligarh Muslim University, Aligarh

Abstract : *Contamination of the water bodies occurs from different sources including oil refineries. Oil refinery effluent contains different chemicals at different concentrations including ammonia, sulphides, phenol, hydrocarbons and heavy metals. Monitoring the impact of pollutants on aquatic life forms is challenging due to the differential sensitivities of organisms to a given pollutant, and the inability to assess the long-term effects of persistent pollutants on the ecosystem as they are bio-accumulated at higher trophic levels. A number of tests and system have been developed to investigate the toxicity of refinery waste effluent and other surface water. Many studies indicate that the single cell gel electrophoresis (comet assay), micronucleus, chromosomal aberration and microbial assays are sensitive enough to monitor genotoxic responses of indigenous aquatic organisms to environmental pollution. This article reviews the toxicity in general and genotoxicity as major caused by the exposure of refiner waste effluent to the living system. Mutagenicity/genotoxicity assays should be performed to assess the presence of genotoxicants in the waste water, in addition to the chemical analysis. This review high-lightens the sensitive and reliable tools to measure the mutagenic and genotoxic activities in aquatic environment.*

Introduction

Surface waters, such as rivers, lakes and seas, receive large quantities of waste water from industrial, agricultural, and domestic sources, including municipal sewage treatment plants. These surface waters, which contain many unknown compounds, are used as a source of drinking water, as well as for agricultural, recreational and religious activities around the world. Consequently, water pollution can be a serious public health and aquatic ecosystem problem. The US EPA's Toxic Release Inventory (TRI) for 2001 reported that more than 100,000 metric tonnes of chemicals are released into surface waters and approximately 762,000 metric tonnes of chemicals are emitted into the atmosphere annually by industrial use. This study show that large quantities of toxic materials are routinely released directly or indirectly (via airborne emission) into aquatic systems after industrial usage (Wake 2005).

The condition and health of the aquatic environment is constantly being monitored so that the effects of pollution can be better understood and its impact reduced. Pollution of the aquatic environment has many sources such as sewage disposal, land run off, atmospheric fallout and industrial wastes. This review concentrates on the impact of surface water toxicity with special reference to oil refinery wastes. Contamination of water resources from diverse origins with oil refineries is one of the major global problems (Gupta and Ahmad 2011). The total quantity of aqueous effluent that is being discharged by oil refineries has decreased over the years, for example European refineries discharged 3119×10^6 t year^{-1} from 80 refineries in 1969 reducing to 2543×106 t year^{-1} from 84 refineries in 2000. The decrease between 1974 and 1978 is thought to be due to more refineries using air cooling and recirculation cooling water systems. Refineries can be categorized into four different types depending on their complexity (Concawe 2004). Over the years the complexity of refineries has increased and since 1969 there has been the introduction of more effective treatment systems. The three main treatment processes for effluent before its discharge are gravity separation (API separators, tank separation), advanced treatment (flocculation, sedimentation, filtration) and biological treatment (biofilters, activated sludge, aerated ponds) (Concawe 2004). The percentage of refineries that have all three treatment processes has increased over the years from only 23% (of 82 refineries) in 1969 to 91% (of 84 refineries) in 2000 (Wake 2005).

As not all refineries have the same processes, the effluents that are produced will have different chemical compositions depending on the type of treatment they receive (Lehtinen 1986).

Petroleum refinery wastewaters are made up of many different chemicals which include oil and greases, phenols (creosols and xylenols), sulphides, ammonia, suspended solids, cyanides, nitrogen compounds and heavy metals like chromium, iron, nickel, copper, molybdenum, selenium, vanadium and zinc (Cote 1976; Gupta and Ahmad 2011). Oil consists of five types of components, saturated non-cyclic hydrocarbons (paraffins), cyclic hydrocarbons (cycloalkanes), olefinic hydrocarbons (alkenes), aromatics and non-hydrocarbons (sulphur compounds, nitrogen-oxygen compounds and heavy metals) (Cote 1976). 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 (Tatem et al. 1978; Gupta and Ahmad 2011).

Mutagenic/genotoxic compounds, including carcinogens, whether known or unknown, become the components of complex environmental mixtures that can have adverse health effects on humans and indigenous biota (Fatima and Ahmad 2006; Tabrez and Ahmad 2010). We know quite a lot about identified contaminants, and it is relatively easy to study the sources and fate of those contaminants that have been identified as priorities for concern and control. Post-emission fate and behavior of polycyclic aromatic hydrocarbons (PAHs) in complex mixtures including surface waters have widely investigated throughout the world, because PAHs are identified contaminants and are relatively easy to study the sources and fate (White 2002). However, few studies have investigated the identification of novel putative mutagens and the quantification of their response concentrations. On the other hand, the use of short-term bioassays, which can detect a wide range of chemical substances that may produce genetic damage, has permitted the quantification of mutagenic hazard without a prior information about identity or physical–chemical property. Various studies on surface waters toxicity from various sources like pulp industries, lock industries, pesticides manufacturing plant and refinery waste effluent have been done by Malik and Ahmad 1995; Fatima and Ahmad 2005; Tabrez and Ahmad 2010; Gupta and Ahmad 2012a, by using different animal and plant systems. Plants and animals, as living bioindicator species, play an increasingly important role in the monitoring of water pollution because they respond with great sensitivity to changes in the aquatic environment (Gupta and Ahmad 2016). Studies conducted on the mutagenicity/genotoxicity of surface water and aquatic biota, Parry et al. (1976) reported on mutagenicity studies on the tissue of the mussel *Mytilus edulis* in the marine environment, and Pelon et al. (1977) reported on the mutagenicity/genotoxicity of Mississippi River water samples by the Salmonella assay developed by Ames et al. (1975). Cytogenic damage in fish exposed to the industrially contaminated Rhine River was also observed (Alink et al. 1980). Since 1980, many researchers have assessed mutagenicity/genotoxicity of surface waters using a variety of bioassays and analytical methods from the standpoint of determining the potential contribution to the mutagenic hazards of treated drinking water and potential ecological hazard. The purpose of this review is to summarize the state of the current literature on mutagenicity/genotoxicity data for surface waters with special reference to refinery waste effluent and to lead the most profitable directions for future research in order to control and manage effectively our water environment. In this review, we will focus on a synopsis of the mutagenicity/genotoxicity assay data in surface waters in the scientific literature published. Subheading of this review will be focus on the refinery as a major source of water pollution, various mutagenic/genotoxic bioassays and suspected or identified mutagens in waste water.

Consequences of the Refinery Effluent

The effect of oil refinery effluent on environment is based on the conditions and hydrodynamics of the receiving water which released into the environment. 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 that around a petroleum refinery in the Gulf of Fos (South France), there were three zones of contamination of the sediment.

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).

Oil Refinery as a major source of water bodies contamination

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, phenol, 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 quantity 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 wastewaters are accounted in the wastewater system. The quantity of wastewater generated and their characteristics depend on the process configuration, as a general rule, approximately 3.5–5m^3 of waste water are generated per ton of crude oil processed when cooling water is recycled (Dold 1989; Al Zarooni and Elshorbagy 2006; Ponce-Ortega et al. 2011).

Petroleum refineries as a source of water pollution in India

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; Gupta and Ahmad 2011).

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, metallic (Zn, Cr, Va, Ni, Pb,Cu) and non-metallic constituents. High levels of phenol, nitrogen, total grease and oil, total hydrocarbons, floride, chloride, calcium have also been reported in this refinery waste effluent (Solanki et al. 2011).

Chaudhary et al. (2011) analyzed and compared different soil 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 activity) were estimated in the soil samples. Among all the samples, sewage sludge soil showed maximum activity of enzymes,

microbial biomass carbon and most probable number of polycyclic aromatic hydrocarbon (PAH) degraders in soils spiked with three- to four-ring PAHs at 50 ppm.

Behavioral dynamics of pollutants

Organisms living in chronically polluted sites supposed to have exposure with low concentrations of xenobiotics for long periods of time in the natural environment. On other hand, organisms may be abruptly exposed to higher level of toxicants upon the outfall of a pollutant in coastal waters. Xenobiotics in the aquatic ecosystem can partition between land, sediment, sediment–water interface, interstitial waters, biota and the air–water interface. Thus, the dynamic behavior of pollutants in the environment is hypothetically under the influence of water and atmospheric conditions and, biotic and abiotic (sediments) materials. Although the physical–chemical sorption of xenobiotics onto solid phases is subject to a vast range of factors, sediments.

Correlations and functional relationships must be established between abiotic and biotic levels of pollution exposure in order to make early and realistic environmental risk assessments (ERA). Persistent hydrophobic chemicals and heavy metals may also be the most substantive source of environmental pollutants (Pe´ rez-Ruzafa et al. 2000). Persistent hydrophobic chemicals and heavy metals may accumulate in aquatic organisms through different mechanisms: directly from water, via uptake of suspended particles, or by the consumption of lower trophic level organisms (Binelli and Provini 2003; Van der Oost et al. 2003). An essential point to consider during the application of an ERA program is the food chain structure, since bioaccumulation and biomagnification of xenobiotics in the organisms are critical factors in evaluating adverse effects on ecosystems. The study of physiological and biochemical alterations, as well as the identification and quantification of pollutants in basal-level trophic organisms are an essential diagnostic tool (Van Gestel and Van Brummelen 1996; Handy and Depledge, 1999; Handy et al., 2003). The presence of chemical compounds in isolated sediments does not, by itself, indicate injurious effects to organisms (Wang et al. 1998), as bioavailability of these materials should also be taken into account. On the other hand, the detection of pollutants (quantitative analysis) (Baum et al. 2004) or their effects (biochemical biomarkers) (Aksmann and Tukaj 2004; Geoffroy et al. 2004) in plants like onion and other photosynthetic organisms such as micro and macroalgae are early and timely indicators of potential hazard in aquatic systems.

Major pollutants of refinery waste effluent

According to Marcia et al. (2009), petroleum refinery wastewaters may contain a wide range of organic and metallic pollutants such as oil and greases, phenols, sulfides, ammonia, suspended solids, 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 wastewater (Avci et al. 2005).

Polycyclic aromatic hydrocarbons

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 (PAH) 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) currently identifies 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).

Phenols

Besides the presence of PAH, phenols are also a major component of refinery wastewaters. The phenols are produced in the cracking process and special gasoline washeries (Al Zarooni and Elshorbagy 2006). Phenol and its vapors are corrosive to the eyes, the skin, and the respiratory tract. Repeated or prolonged skin contact with phenol may cause dermatitis, or even second and third-degree burns due to phenol's caustic and defatting properties (Lin et al. 2006). Inhalation of phenol 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

phenol may be the formation of phenoxyl radicals (Corwin 2006).

Polychlorinated biphenyls

Another class of persistent environmental pollutants is the polychlorinated biphenyls (PCBs) marketed worldwide under trade names such as Aroclor, Askarel, Clophen, Therminol, etc. PCBs comprise mixtures of 209 possible synthetic organic chemicals (congeners), ranging from oily liquids to waxy solids (Borja et al. 2005). Because of their non-inflammable nature, chemical stability, and insulating properties, commercial PCB mixtures have been used in many industrial applications, especially in capacitors, transformers, and other electrical equipment (USEPA 1996). Biodegradation processes, including dechlorination, can transform PCBs, effectively altering their potential toxicity. On the other hand, dechlorination reactions are usually slow, while altered PCB mixtures can persist in the environment for many years (Borja et al. 2005). Based on long-term persistence in the environment and their toxicity, the commercial production and use of PCBs is believed to have finally ceased in the mid-1980s in the USA and in Northern Europe (USEPA 1996; HELCOM 2001).

Heavy metals

A number of trace metals are used by living organisms to stabilize protein structures, facilitate electron transfer reactions and catalyze enzymatic reactions (Ash and Stone 2003). For example, copper (Cu), zinc (Zn), and iron (Fe) are essential as constituents of the catalytic sites of several enzymes (Allan 1997). Heavy metals are known to cause irreversible damage to a number of vital metabolic constituents and important biomolecules (Gupta and Ahmad 2012 b). Other metals, however, such as lead (Pb), mercury (Hg), andcadmium (Cd) may displace or substitute for essential trace metals and interfere with proper functioning of enzymes and associated cofactors. Metals are usually present at low or very low concentrations in the oceans (Ash and Stone 2003). In coastal waters, metals can occur at much higher concentrations, probably due to inputs from river systems (Morillo et al. 2004). Close to urban centers, metal pollution has been associated with sewage outlets (Chen et al. 2005). Although, there have been several successful programmes of phasing out lead in the developing world modeled on the programs of industrialized countries (Singh and Singh 2006), with important emission reducing by improved control to replace leaded petrol by unleaded petrol (AMAP 1997, 2002). A major source of air contamination is the non-ferrous metals industry, which emits Cd, Pb, Ni, As, Cu, Se, and Zn (Blake et al. 2007). Coal burning is the major source of Hg, As, chromium (Cr), and Se (Guijian et al. 2007), while combustion of oil is the most important source of Ni and vanadium (V) (USEPA 2002).

Toxicity with reference to genotoxicity and its assessment

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 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 short and long term genotoxicity.

Salmonella mutagenicity test

Among the microbial bioassays, the 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 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 may be attributable to the pesticides used in the upstream region. Fatima and Ahmad (2006)

compared genotoxic potential of wastewater 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).

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 genotoxins in surface waters (Langevin et al. 1992; White et al. 1979). 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).

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 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).

Single cell gel electrophoresis/ comet assay

Comet assay is used as an important tool for monitoring genotoxicity in aquatic environments (Gupta and Ahmad 2014) and 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 possibility 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 performing the test (Angerer et al. 2007).

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).

Chromosome aberration assay

Chromosome aberrations include structural aberrations such as fragments or intercalations and numerical aberrations. Cytogenetic effects 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). Gupta and Ahmad (2012a) have reported the induction of chromosomal aberration in *Allium cepa* cells exposed to the river water being contaminated by petroleum waste.

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 micronuleus 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) but the costs of equipment are high. An induction in chromosomal aberration and micronuclei formation in *Allium cepa* cells, exposed to Atibaia River water, being contaminated by petroleum refinery waste was noticed by Hoshina et al. (2009). The genotoxicity of refinery waste was also found to be positive by

the formation of micronuclei and binucleated cells in *Allium cepa* cells by Leme and Marin-Morales (2008), Gupta and Ahmad (2012a).

Other genotoxicity assessment methods

Sister chromatid exchange (SCE) assay, UDS assay, DNA adduct formation and the Tradescantia stamen hair muatation 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. Among the tests that can be conducted routinely for the genotoxic evaluation of water samples plasmids nicking assay has gained a reasonable level of popularity (Gupta and Ahmad 2014).

The efforts on the identification of putative mutagens in surface waters by bioassay-directed chemical analysis should be further extended for better understanding of risk of adverse effect for humans and indigenous biota.

Conclusion

Mutagenicity/genotoxicity test of complex mixtures such as surface waters using variety of bioassays demonstrates that these environmental mixtures contain many unidentified and unregulated toxicants which may have carcinogenicity and a risk of unknown magnitude. It can be concluded that the analysis of surface waters proved to be an essential stage of the study to identify areas potentially contaminated by genotoxic compounds from the different sources. The main implication from the studies on the effects of refinery effluents is that generalisations cannot be made. Each refinery is made up of different plants, which produce different effluents that can vary from day to day. The fate of the effluent is dependent on environmental conditions, i.e. weather and the recipient. Volatile compounds are lost from the effluent into the atmosphere where as the majority of the non-volatile compounds, like the hydrocarbons, end up in the sediment. Sediment analysis for hydrocarbon concentrations can be useful in determining the history of the input into that area. Toxicity tests are very useful indicators of the possible impacts that refinery effluents may have on aquatic organisms. Lethal tests are good for indicating the relative toxicities of different chemicals and the differences between different species. Whereas sublethal tests are more realistic in that they consider the impacts on communities, not just to the individual, but to the population through effects to the reproductive success and growth.

References

[1] Al Zarooni M, Elshorbagy W (2006) Characterization and assessment of Al Ruwais refinery waste water. Journal of Hazardous Materials, 136: 398-405.

[2] Allan R (1997) Mining and metals in the environment. journal of geochemical exploration, 58:95-100.

[3] Alink GM, Frederix-Wolters EMH, Van der Gaag MA, Van der Kerkhoff JFF, Poels CLM (1980) Induction of sister chromatid exchanges in fish exposed to Rhine water. Mutation Research, 78: 369-374.

[4] Angerer J, Ewers U, Wilhelm M (2007) Human biomonitoring: state of the art. International Journal of Hygiene and Environmental Health, 210:201-228

[5] AMAP-Arctic Monitoring and Assessment Programme (1997) A state of the Arctic environment report, Oslo, Norway, 188pp.

[6] AMAP-Monitoring and Assessment Programme (2002) Persistent organic pollutant, heavy metals, radioactivity, human health and changing pathways, Oslo, Norway, 122pp.

[7] Armannsson H, Burton JD, Jones GB, Knap AH (1985) Trace metals and hydrocarbon in sediments from the Southampton Water region, with particular reference to the influence of oil refinery effluent. Marine Environmental Research, 15:31-44.

[8] Ash C and Stone R (2003) Metals: impacts on health and the environment. A question of dose. Science 300, 925.

[9] Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalianmicrosome mutagenicity test. Mutatation Research, 31:347-364.

[10] Aksmann A and Tukaj Z (2004) The effect of anthracene and phenanthrene on growth, photosynthesis, and SOD activity of the green algae (Scenedesmus armatus) depends on the PAR irradiance and CO_2 level. Archives of Environmental Contamination and Toxicology, 47:177–184.

[11] Avci A, Kac–maz M, Durak I (2005) Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicology and Environmental Safety, 60: 101-105.

[12] Blake WH, Walsh RP, Reed JM, Barnsley MJ, Smith J. (2007). Impacts of landscape remediation on the heavy metal pollution dynamics of a lake surrounded by non-ferrous smelter waste. Environmental Pollution, 148:268-280.

[13] Bakare AA, Lateef A, Amirda OS, Afolabi RO (2003) The aquatic toxicity and characterization of chemical and microbiological constituents of water samples from Oba River, Odo-oba, Nigeria. Asian Journal of Microbiology, Biotechnology & Environmental Sciences, 5: 11–17.

[14] Baum A, Ledin A, Reitzel LA, Bjerg PL, Christensen TH (2004) Xenobioticorganic compounds in leachates from ten Danish MSW landfills-chemical analysis and toxicity tests. Water Research 38: 3845–3848.

[15] Bhattacharryya JK and Shekdar AV (2003) Treatment and disposal of refinery sludges: Indian scenario. Waste Management and Research, 21: 249–61.

[16] Binelli A and Provini A. (2003). The PCB pollution of Lake Iseo (N. Italy) and the role of biomagnification in the pelagic food web. Chemosphere, 53: 143–151.

[17] Borja J, Taleon DM, Auresenia J, Gallardo S. (2005). Polychlorinated biphenyls and their biodegradation. Process Biochemistry 40, 1999–2013..

[18] Campana MA, Panzeri AM, Escalants AH, Moreno VJ, Dulout FN (2001) Micronucleus test in fish from a pampasic pond (Argentina): an estimation of the presence of genotoxic compoundsournal of Environmental Pathology, Toxicology and Oncology . 20: 325-331.

[19] Chen Z and Gallie DR (2006) Dehydroascorbate reductase affects leaf growth, development, and function. Plant Physiology, 142: 775-787.

[20] Chung KT, Chen SC, Wong TY, Li YS, Wei CI, et al. (2000) Mutagenicity Studies of Benzidine and Its Analogs: Structure-Activity Relationships Toxicological Science, 56: 351-356.

[21] Chung KT, Chen SC, Wong TY, Wei CI (1998) Effects of benzidine and benzidine analogues on growth of bacteria including *Azotobacter vinelandii.* Environmental Toxicology and Chemistry, 17: 271-275.

[22] Conney AH (1982) Induction of Microsomal Enzymes by Foreign Chemicals and Carcinogenesis by Polycyclic Aromatic Hydrocarbons: G. H. A. Clowes Memorial LectureCancer Research, 42: 4875–4917.

[23] Concawe (2004) Trends in oil discharged with aqueous effluents from oil refineries in Europe: 2000 survey. Concawe (address as above) Report no. 4/04, 9 pp.

[24] Corwin H, McKarnsb SC, Smith C J, Doolittle DJ (2000) Comparative QSAR evidence for a free–radical mechanism of phenol–induced toxicity". Chemico-Biological Interactions, 127: 61-72.

[25] Cote, R.P. 1976. The effects of petroleum refinery liquid wastes on aquatic life, with special emphasis on the Canadian environment. National Research Council of Canada. NRC Associate Committee on Scientific Criteria for Environmental Quality, Ottawa, Ontario, Canada K1A 0R6, publication number 15021, 77 pp.

[26] Cranthorne B, Roddie B, Bolton C, Jonston D, Munro D. (1989).Collaborative research programme: the effects of oily discharges upon the Forth estuary. Water Research Centre, Marlow, Bucks,Report no. PRS 2001-M/3, 71 pp.

[27] Cupples CG, Miller JH. (1988) Effects of Amino Acid Substitutions at the Active Site in Escherichia Coli β-Galactosidase. Genetics, 120: 637-644.

[28] Dixon DR, Pruski AM, Dixon LRJ, Jha AN (2002) Marine invertebrate eco-genotoxicology: a methodological overview. Mutagenesis, 17: 495-507.

[29] Dold PL (1989) Current practice for treatment of petroleum refinery waste water and toxics removal. Water Pollution Research Journal of Canada, 24: 363–390.

[30] Duan C, Hu Z, Wang Z, Wen C, Yan S, et al. (1999) *Tradescantia* bioassays for the determination of genotoxicity of water in the Panlong River, Kunming, People's Republic of ChinaMutation Research, 426: 127–131.

[31] Fatima RA and Ahmad M (2005) Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater. Scienc of the total environnent, 346: 256–273.

[32] Fatima RA and Ahmad M (2006) Genotoxicity of industrial wastewaters obtained from two different pollution sources in northern India: A comparison of three bioassays. Mutation Research, 609: 81-91.

[33] Fucic A, Zeljezic D, Kasuba V, Kopjar N, Rozgaj R, et al. (2007) Stable and Unstable Chromosome Aberrations Measured after Occupational Exposure to

Ionizing Radiation and Ultrasound Croatian Medical Journal, 48: 371–377.

[34] Gana JM, Ordóñez R, Zampini C, Hidalgo M, Meoni S, et al. (2008) Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentina. Journal of Hazardous Materials, 155: 403-406.

[35] Geoffroy L, Teisseire H, Couderchet M, Vernet G (2002 Effect of oxyfluorfen and diuron alone and in mixture on antioxidative enzymes of (Scenedesmus obliquus). Pesticide Biochemistry and Physiology, 72: 178-185.

[36] Grahl-Nielsen, O (1987). Hydrocarbons and phenols in discharge water from offshore operations. Fate of the hydrocarbons in the recipient. Sarsia, 72:375-382.

[37] Griffiths HR, Møller L, Bartosz G, Bast A, Bertoni–Freddari C, et al. (2002) Biomarkers. Molecular Aspects of Medicine, 23: 101-208.

[38] Grummt T (2000) In: Grummt T (Eds.) Erprobung, Vergleich, Weiterentwicklung und Beurteilung von Gentoxizitätstests für Oberflächenwasser. Forschungszentrum Karlsruhe GmbH, Projektträger Wassertechnologie und Entsorgung, Außenstelle Dresden, Dresden. p. 217.

[39] Guijian L, Liugen Z, Duzgoren-Aydin NS, Lianfen G, Junhua L, Zicheng (2007). Health effects of arsenic, fluorine, and selenium from indoor burning of Chinese coal. Reviews of Environmental Contamination and Toxicology, 189: 89-106.

[40] Gupta AK and Ahmad M (2011) Study of the effect of refinery waste water exposure on *Allium cepa's* enzymatic antioxidant defense: probing potential biomarkers. Toxicological and Environmental Chemistry, 93:1166–1179.

[41] Gupta AK and Ahmad M (2012a) Assessment of cytotoxic and genotoxic potential of refinery waste effluent using plant, animal and bacterial systems. Journal of Hazardous Materials 201-202: 92- 99.

[42] Gupta AK and Ahmad M (2012b) Effect of refinery waste effluent on tocopherol, carotenoid, phenolics and other antioxidants content in *Allium cepa.* Toxicology and industrial health, 29:652-661.

[43] Gupta AK and Ahmad M (2014) Genotoxicity of refinery waste assessed by some DNA damage tests. Ecotoxicology and environmental safety, 114:250-6.

[44] Gupta AK and Ahmad M (2016) Cytochrome P450 System as a Toxicity Biomarker. Imperial Journal of Interdisciplinary Research, 9:1151-1159.

[45] HELCOM–HELSINKI Commission–Baltic Marine Environment Protection Commission (2001). Polychlorinated biphenyls, Helsinki, Finland, 25pp.

[46] Hoshina MM, Marin–Morales MA (2009) Micronucleus and chromosome aberrations induced in onion (Allium cepa) by a petroleum refinery effluent and by river water that receives this effluent. Ecotoxicology and Environmental Safety,72 :2090-2095.

[47] Klobucar GIVM, Pavlica M, Erben R, Papes D (2003) Application of the micronucleus and comet assays to mussel Dreissena polymorpha haemocytes for genotoxicity monitoring of freshwater environments. Aquatic Toxicology. 64:15–23.

[48] Knap AH, Williams PJL. (1982). Experimental studies to determinethe fate of petroleum hydrocarbons from refinery effluent on an estuarine system. Environmental Science and Technology, 16:1-4.

[49] Knap AH, Williams PJLeB, Lysiak E (1982) Petroleum hydrocarbons in sediments of Southampton Water estuary. Marine Environmental Research, 7:235249.

[50] Kohlpoth M, Rusche B, Nusse M. (1999) Flow cytometric measurement of micronuclei induced in a permanent fish cell line as a possible screening test for the genotoxicity of industrial waste waters. Mutagenesis, 14: 397-402.

[51] Kummarow F, Rech CM, Coimbras CA, Roubicek DA, Umbuzeiro G, et al. (2003) Comparison of the mutagenic activity of XAD4 and blue rayon extracts of surface water and related drinking water samples. Mutation Research, 541: 103–113.

[52] Kutlu M, Aydoğan G, Mumcu E. (2007) Mutagenicity analysis of water samples from Seydisuyu (Kirka, Turkey) stream under the influence of boron production complex. Food and Chemical Toxicology, 45: 2064-2068.

[53] Le Dreau Y, Jacquot F, Doumenq P, Guiliano M, Bertrand JC, Mille G. (1997). Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum wastes of a refinery (from 1956 to 1992). Marine Pollution Bulletin 34, 456-468.

[54] Langevin R, Rasmussen JB, Sloterdyk H, Blaise C (1992) Genotoxicity in water and sediment extracts from the st-lawrence-river system, using the sos chromotest. Water Research, 26: 419-429.

[55] Lehtinen CM (1986) Environmental impact studies of refinery effluents – a challenge to the analytical chemist. In: Giam, C.S., Dou, H.J.M. (Eds.), Strategies and Advanced Techniques for Marine Pollution Studies: Mediterranean Sea. Springer-Verlag, Berlin, pp. 315-326.

[56] Leme DM and Marin–Morales MA (2009) *Allium cepa* test in environmental monitoring: a review on its application. Mutation Research, 682: 71–81.

[57] Lin TM, Lee SS, Lai CS, Lin SD (2006) Phenol burn. Journal of the International Society for Burn Injuries 32: 517–21.

[58] Ponce-Ortega JM, Napoles-Rivera F, El-Halwagi MM, Jimenez-Gutierrez A (2011) An optimization approach for the synthesis of recycle and reuse water integration networks. Clean Technologies and Environmental Policy, 14:133-151.

[59] Malik A, Ahmad M (1995) Genotoxicity of some wastewaters in India. Environmental Toxicology, 10: 287-293.

[60] Morillo J and Usero J Gracia, I (2004) Heavy metal distribution in marine sediments from the Southwest Coast of Spain. Chemosphere, 55: 431-442.

[61] Moore CG, Murison DJ, Mohd-Long S, Mills DJL (1987). The impact of oily discharges on the meiobenthos of the North Sea. Philosophical Transactions of the Royal Society of London B 316, 525–544.

[62] Oda Y, Nakamura S, Oki J, Kato T, Shingawa H (1985) Evaluation of the new system (*umu*-test) for the detection of environmental mutagens and carcinogens. Mutation Research 147: 219-229.

[63] Parry JM, Tweats DJ, Al-Mossawi MAJ (1976) Monitoring the marine environment for mutagens, Nature, 264 :538-540.

[64] Pelon W, Whitman BF, Beasley TW (1977) Reversion of histidine- dependent mutant strains of Salmonella typhimurium by Mississippi River water samples. Environmental Science & Technology, 11:619-623.

[65] Pe´ rez-Ruzafa , Navarro S, Barba A, Marcos C, Ca´mara MA, Salas F, Gutie´ rrez JM (2000) Presence of pesticides throughout trophic compartments of the food web in the Mar Menor Lagoon (SE Spain). Marine Pollution Bulletin, 40:140-151.

[66] Pratt IS, Barron T (2003) Regulatory recognition of indirect genotoxicity mechanisms in the European Union. Toxicology Letters, 140: 53-62.

[67] Quillardet P, Huisman O, d'ari R, Hofnung M (1982) SOS chromotest, a direct assay of induction of an SOS function in *Escherichia coli* K-12 to measure genotoxicity. Proceedings of the National Academy of Sciences, 79: 5971-5975.

[68] Rehana Z, Malik A, Ahmad M (1996) Genotoxicity of the Ganges water at Narora (U.P.), India. Mutation Research, 367: 187-193.

[69] Reifferscheid G, Ziperle J. (2000) In: Grummt T. (Eds.) Erprobung, Vergleich, Weiterentwicklung und Beurteilung von Gentoxizitätstests für Oberflächenwasser. Forschungszentrum Karlsruhe GmbH, Projektträger Wassertechnologie und Entsorgung, Außenstelle Dresden, p. 217.

[70] Russo C, Rocco L, Morescalchi MA, Stingo V (2004) Assessment of environmental stress by the micronucleus test and comet assay on the genome of teleost populations from two natural environments. Ecotoxicology and Environmental Safety, 57: 168-174.

[71] Singh AK and Singh M (2006) Lead decline in the Indian environment resulting from the petrol-lead phase-out programme. Science of the total Environment, 368:686-694.

[72] Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175:184-191.

[73] Solanki K, Singh H B, Bhadauria S (2011) A study for Assessment of Physico-chemical Analysis of Oil Refinery Effluent. Research Journal of Agricultural Sciences, 2: 125-129.

[74] Spies RB, Stegeman JJ, Rice DW, Woodin B, Thomas P, et al. (1990) In: McCarthy JF, Lee LR. (Eds.) Lewis Publishers, Boca Raton, FLA. p. 87–127.

[75] Stamato TD, Denko N (1990) Asymmetric field inversion gel electrophoresis: a new method for detecting DNA double-strand breaks in mammalian cells. Radiat Research, 121: 196-205.

[76] Tabrez S, Ahmad M (2010) Components of antioxidative system in *Allium cepa* as the toxicity monitor of trichloroethylene

(TCE) Toxicological and Environmental Chemistry, 93: 73-84.

[77] Talsi T (1987) Effects of oil refinery effluents on the receiving waters and sediments: a case study of Neste Oy's Oil refinery in Porvoo, Finland. Baltic Sea Environmental Proceedings 22, 81-91.

[78] Tatem HE, Cox BA, Anderson, JW (1978) The toxicity of oils and petroleum hydrocarbons to estuarine crustaceans. Estuarine, Coastal and Shelf Science, 6: 365-373.

[79] Umbuzeiro GA, Roubicek DA, Rech CM, Sato MIZ, Claxton LD (2004) Investigating the sources of the mutagenic activity found in a river using the Salmonella assay and different water extraction procedures. Chemosphere, 54: 1589-1597.

[80] United States Environmental Protection Agency (USEPA, 1989). Risk Assessment Guidance for Superfund Volume 1 Human Health Evaluation Manual (Part A) EPA/540/l –89/002.

[81] United States Environmental Protection Agency (USEPA, 1993). Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. EPA/600/R–93/089.

[82] United States Environmental Protection Agency (USEPA, 1996). PCBs: cancer dose–response assessment and application to environmental mixtures. Report No. EPA 600/P-96/001F, Washington, DC.

[83] United States Environmental Protection Agency, (USEPA 2002). Health assessment document for diesel engine exhaust. Report No. EPA/600/8-90/057F, Washington, DC.

[84] United States Environmental Protection Agency (USEPA, 2007) "The National Water Quality Inventory: Report to Congress for the 2002 Reporting Cycle – A Profile." EPA 841–F–07–003.

[85] US Environment Protection Agency (USEPA, 1989) Short term methods for estimating the chronic toxicity of effluents and receiving waters to fresh water organisms, Cincinnati, OH. EPA/600/4–89/001.

[86] Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: A review Environmental Toxicology and Pharmacology, 13: 57-59.

[87] Van Gestel CA and Van Brummelen TC (1996) Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicology, 5:217-225.

[88] Varanasi U, Stein JE, Nishimoto M, Reichert WL, Collier TK (1987) Chemical carcinogenesis in feral fish: uptake, activation, and detoxication of organic xenobiotics. Environmental Health Perspective, 71: 155-170.

[89] Vargas VMF, Motta VEP, Henriques JAP (1993) Mutagenic activity detected by the Ames test in river water under the influence of petrochemical industries. Mutation Research, 319: 31-45.

[90] Wake H (2005) Oil refineries: a review of their ecological impacts on the aquatic environment. Estuarine, Coastal and Shelf Science, 62:131-140.

[91] Wang Z and Fingas MF (2003) Development of oil hydrocarbon fingerprint and identification techniques. Marine Pollution Bulletin, 47:423-452.

[92] Warner MA, Harper JV (1985) Cardiac dysrhythmias associated with chemical peeling with phenol. Anesthesiology, 62: 366-367.

[93] White AE, Eger EI, Wolff S, Steven WC (1979) Perfusion Index: An Indicator of Success During Endoscopic Thoracic Sympathectomy for Hyperhidrosis. Anesthesiology, 50: 426-430.

[94] White PA (2002) The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. Mutation Research, 515:85-98.

[95] Woo S, Kim S, Yum S, Yim UH, Lee TK (2006) Comet assay for the detection of genotoxicity in blood cells of flounder (Paralichthys olivaceus) exposed to sediments and polycyclic aromatic hydrocarbons. Marine Pollution Bulletin, 52: 1768-1775.

[96] World Health Organization/International Labour Organization (2006) International Chemical Safety Cards.

[97] Zeiger E (2001) Mutagens that are not carcinogens: faulty theory or faulty tests?. Mutation Research, 492: 29-38.

[98] Zimmermann FK. (1984) In: Kilbey BJ, Legator M, Nichols W, Rmel C. (Eds.) Elsevier Biomedical Press, Amsterdam, p. 215–238