

Monitoring of Water Pollution and Its Consequences: An Overview

N. Singh, N. Sharma, J. K. Katnoria

Abstract—Water a vital component for all living forms is derived from variety of sources, including surface water (rivers, lakes, reservoirs and ponds) and ground water (aquifers). Over the years of time, water bodies are subjected to human interference regularly resulting in deterioration of water quality. Therefore, pollution of water bodies has become matter of global concern. As the water quality closely relate to human health, water analysis before usage is of immense importance. Improper management of water bodies can cause serious problems in availability and quality of water. The quality of water may be described according to their physico-chemical and microbiological characteristics. For effective maintenance of water quality through appropriate control measures, continuous monitoring of metals, physico-chemical and biological parameter is essential for the establishment of baseline data for the water quality in any study area. The present study has focused on to explore the status of water pollution in various areas and to estimate the magnitude of its toxicity using different bioassay.

Keywords—Genotoxicity, Heavy metals, Mutagenicity, Physico-chemical analysis.

I. INTRODUCTION

WATER is considered as a precious gift from God that provides the earth with the capacity of supporting life. It covers almost 70.9% of earth surface, out of which, about 97% marine water is present in oceans while only 3% fresh water is present in rivers, lakes, streams etc [1]. It is a universal solvent and has been a commodity required for day to day activities since time immortal. However, it is observed that in past few decades due to increase in urbanization and industrialization, water has been polluted continuously [2]-[5]. In fact the fresh/drinking water has become a limited reserve due to alteration of its physical, chemical and biological properties [6], [7]. Increasing population and its necessities have lead to the deterioration of not only the surface water or sub surface water systems but also the ground water. The continuous extraction of water from underground sources has led to another problem of water depletion table in most of developing countries including India [8], [9].

In short, it can be summarized that the over utilization of water resources has not only lead to its scarcity but also resulted in the indiscriminant pollution of available resources. Considering the various aspects related to the water resources, this review accordingly have been categorized as (i) uses of water (ii) sources of pollution (iii) physico-chemical analysis

Navdeep Singh, Naveen Sharma, Dr. Jatinder Kaur Katnoria, are with the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar-143005, India (e-mail: navdeep183@gmail.com jkat08@yahoo.com).

of water samples (iv) effects of water pollution in different test systems.

Uses of Water

Water is an important component of life. Besides drinking, it is used in various day to day activities. A cheap, plentiful water supply is especially important where people are engaged in small skill productive uses of water such as garden irrigation and keeping live stocks [10]. There are many reports concerning the use of water for irrigation purposes [11], [12], industrial purposes [4], [13], domestic [14], [15] and other purposes [16]-[20].

A. Sources of Water Pollution and Their Consequences

Alteration in physical, chemical and biological conditions of water which makes it unfit for human consumption and various other life forms is known as water pollution. Water pollution has been a major issue these days due to the fact that it is one of the essential components for survival of any living beings [21], [22]. The accumulation of recalcitrant elements in natural ecosystem including water resources have been documented by Gaur [14]. The addition of heavy metals to water bodies as well as their sediments through drains carrying domestic and industrial effluents was also emphasized [14].

Along with water, sediments are also ecologically important components of aquatic habitat and are a reservoir of contaminants that play a very important role maintaining the trophic status of any water body [14]. Gaur [14] stated that depending upon the limnological condition; the sediments can act both as source as well as sink for nutrients and other elements. Therefore, some scientists have directly correlated the pollution of sediments with that of pollution of water. Increased use of surface water as a source of drinking water is producing growing concern about the presence of genotoxic/carcinogenic substances in rivers and lakes [15], [16]. Kolawole [23] reported that effluents entering the water bodies carried pathogenic organisms that transmitted diseases to human beings and other animals; contained organic matter that caused odor and nuisance problem; held nutrients that resulted in eutrophication of receiving bodies.

There are numerous consequences of genotoxins present in water which include possibility of cancer induction, acceleration of ageing processes (e.g. arteriosclerosis) and the appearance of heritable diseases in offspring or reduction in fertility [24]. Among all sources, industrial effluents are the main sources of direct and often continuous input of pollutants or toxicants into aquatic ecosystems with long term implications on ecosystem functioning [5]. It was reported that some industries e.g. pulp and paper mills, steel foundries and

organic chemical manufacturing factories discharge waste of remarkable genotoxic potential [25]. Textile wastewater includes a large variety of dyes and chemicals additions that make the environmental challenge for textile industry not only as liquid waste but also in its chemical composition [26].

During the past several years, wastewater from industrial effluents, domestic wastewater, sewage and agricultural soils of developing countries have shown ascending trend of water pollution as they contain pesticides residue and heavy metals [4]. Urban wastewater contains numerous pathogenic microorganisms and high content of organic matter; thereby posing potential risks for public health and the environment [27]. It is reported that the use of wastewater for irrigation of the agricultural fields harms the mitotic division of plants and in turn wiping out the plant [28]. The pollution of water has been also documented by Akintonwa [29].

Wastewater irrigation has been commonly utilized for agricultural lands in many developing Asian countries like India, China and Pakistan. The levels of persistent organic pollutants (POPs) in water and soils increased by sewage sludge have been reported [3]. Use of wastewater for irrigation has led to an increase in heavy metals like chromium, copper, manganese, nickel, zinc etc. of agriculture soils in many areas and in plants grown over polluted soil thus paving way for its entry into the food chain [30]. When improperly handled and disposed of, these industrial wastes and effluents also contaminate the soil with their genotoxic compounds. Many scientists all over the world have analyzed the physico-chemical properties of water and its sediments to explore the magnitude of pollution of various water reservoirs. The following part of the review is summarization of reports on physico-chemical analysis of water and its sediments samples.

B. Physico-Chemical Analysis of Water Samples

Kelly-Quinn [31]; analyzed different physico-chemical parameters *viz.*, temperature, pH, conductivity, alkalinity, total hardness, dissolved oxygen, oxygen saturation, chloride, sulphate, potassium, sodium, calcium, magnesium, phosphate, nitrite, nitrate and ammonia of water and sediment samples collected from different locations of Caher River. They reported a wide range of parameter including temperature (11.2-11.4°C), pH (8.29-8.34), conductivity (424-442 $\mu\text{S}/\text{cm}$), alkalinity (7.4-8.75 mg/l CaCO_3), total hardness (105.18-142.38 mg/l CaCO_3), dissolved oxygen (10.4-11.1 mg/l O_2), oxygen saturation (96-105.5 %), chloride (12.16-17.83 mg/l), sulphate (2.29-2.67 mg/l), sodium (11.54-13.18 mg/l), calcium (38.95-53.75 mg/l) and magnesium (1.92-2.15 mg/l).

Akan [32] determined physico-chemical properties in wastewater Channelin Kano Metropolis, Kano State, Nigeria. They collected wastewater from the Jakara wastewater channel near the Airport Road Bridge, Kano metropolis between the periods of November, 2007 to May, 2008 to determine the various parameters like pH, temperature, turbidity, chemical oxygen demand (COD), Biological oxygen demand (BOD), dissolved oxygen (DO), conductivity, total dissolved solid (TDS), total suspended solid (TSS), sulphate, nitrate, nitrite and phosphate sodium, potassium and calcium.

They also determined metals such as copper, cobalt, chromium, iron, manganese, magnesium, nickel cadmium and lead. They observed that pH, conductivity, temperature, nitrate, nitrite, sulphate, phosphate, TSS, TDS, DO, BOD and COD were higher than the maximum permissible limits set by Federal Environmental Protection Agencies (FEPA) Nigeria. The concentrations of the metals in the wastewater were also higher than limits set by WHO and the maximum contaminant levels (MCL).

Joshi [33] collected the soil samples from four farm sites of different area surrounding Rajkot India. Their results revealed different values of the physical and chemical properties like pH, specific gravity, bulk density, particle size, moisture content, organic matter, Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- and HCO_3^- . Ekundayo and Obuekwe [69] reported the effects of an oil spill on soil physico-chemical properties of a spill site of the Niger delta basin of Nigeria. They have shown that the total hydrocarbon content of top soil layers ranged from 18.6 to 23.6ppm in the heavy impact zone and the oil had penetrated to a depth of 8.4m. The concentration of hydrocarbons in the medium impact zone ranged from 10.04 to 10.38ppm while hydrocarbons were not detected in 85% of samples from the unimpacted reference zone. Heavy metal concentration measurements in the soil revealed a significant build-up ($P < 0.05$) of lead, copper and zinc in the heavy impact zone.

Chen [3] assessed contamination and genotoxicity of soil irrigated with wastewater. He carried out a field survey in farmlands that have been irrigated with effluents from a sewage treatment plant and untreated wastewater. The soil quality was evaluated using a battery of chemical and biological parameters to describe the integrated situations of the polluted sites. Organochlorine pesticides (OCPs) were quantified by GC-ECD and polycyclic aromatic hydrocarbons (PAHs) were analyzed by GC-MS with internal standard. Polyphenol oxidase and catalase activities were examined to investigate the soil functions. They concluded that both PAHs and OCPs have accumulated in the soils irrigated with wastewater. The main source of PAHs was from wastewater discharged from coal plant.

Haq [34] estimated that N, P, and K status in effluent irrigated soils of some selected sites of North-West Frontier Province (NWFP). 40 samples from each depth of 0-20 and 20-40cm of soil were collected which were contaminated by irrigation with industrial effluent while 8 samples (4 samples from each depth) were taken from soil irrigated with tube well water. The average values of N, P and K at 0-20cm soil depth were 26.22, 16.92, and 94.16 mg/kg while at 20-40cm soil depth the values were 20.38, 10.6, and 76.15 mg/kg, respectively.

Saif [35] analyzed different heavy metals in industrial effluents from an industry in Korangi Area of Karachi (Pakistan). They found that various heavy metals were in range of Zn as (0.005-5.5), Cu as (0.005-1.19), Fe as (0.04-5.58), Mn as (0.01-1.79), Cd as (0.004-2.4), Cr as (0.004-5.62), Ni as (0.02-5.35) and Pb as (0.05 to 2.25) mg L^{-1} in various waste water samples. Nagajyoti et al. [70] analyzed various heavy metals like Cr, Cu, Mn, Fe, Co, Ni, Cd and Pb

in soil samples irrigated by industrial effluent. The samples were analyzed for 10, 15, 20 and 25th day of irrigation. In another report, Adebisi et al. [71] reported Ibadan soils were polluted by industrial effluents.

Mary [36] analyzed different physico-chemical parameter of water samples from coconut husk retting area in Parakkani river near Thengapattanam. The data showed variation of investigated parameters in samples as follows: pH (6.72–7.71), conductivity (852–5787 $\mu\text{S}/\text{cm}$), turbidity (16–36 NT units), total dissolved solids (579–3935), alkalinity (32–184 mg/l CaCO_3), total hardness (140–1040 mg/l CaCO_3), dissolved oxygen (3.20–5.10 mg/l O_2), BOD (15–36 mg/l), COD (45–108 mg/l), calcium (35–168 mg/l), magnesium (12–187 mg/l), nitrate (3–12 mg/l), nitrite (0.05–1.51), sulphate (21–120 mg/l), phosphate (1.77–2.8 mg/l), fluoride (0.2–2.4), chloride (232–1720mg/l).

Baig [37] evaluated the geological and anthropogenic aspects of As pollution in surface and ground water resources of Jamshoro Sindh, Pakistan. They collected 309 water samples from 48 sampling points of Jamashoro, Pakistan in 2007 to determine various physico-chemical parameters. The concentration of various parameters were as follow pH (7.4–8.0), electrical conductivity (7.4–8.0), TDS (204–896), calcium (39.9–111mg/l), magnesium (16–41.1mg/l), sodium (304–520 mg/l), potassium (6.39–17.4 mg/l), iron (0.12–0.96 mg/l), carbonate (198–426 mg/l), chloride (173–330 mg/l), nitrite (0.65–2.03 mg/l), nitrate (6.64–17.3 mg/l), sulphate (334–740 mg/l), arsenic (10.0–49.0 mg/l) and were under recommended values set by WHO (2004) [72] in most surface and ground water samples.

Raji [38] investigated the physico-chemical characteristics of drinking water collected from tap, well and sachet in Sokoto metropolis in north western Nigeria. Conductivity and pH values were determined by standard methods while composition was analyzed using X-ray fluorescence spectroscopy. Majority of water samples had neutral pH (6.80–7.20), few were slightly alkaline and one was acidic. Heavy metals such as nickel (0.260–0.470 ppm), lead (0.300–0.540 ppm), chromium (0.67–1.500 ppm), arsenic (0.210–0.80 ppm), manganese (0.510–1.200 ppm), selenium (0.130–0.250 ppm), and bromine (0.100–0.180 ppm) were found above WHO recommended permissible limits in most of water samples with their attendant health implications.

Mohiuddin [39] investigated the trace metal pollution of water and sediment of downstream of Tsusmi River, Yokohama, Japan. Twenty samples of water and sediments were collected and analyzed for trace metal pollutants. Results show that mean concentration of chromium, copper and nickel in water greatly exceeded (>100 times) the surface water standard. The concentration of molybdenum and lead was also higher than standard values while iron and manganese was lower than of surface water standards. The mean concentration of zinc, copper, cadmium, lead, chromium, vanadium, bromide and iodide was 381.1, 133.0, 1.0, 40.8, 102.9, 162.0, 71.5, and 10.6 $\mu\text{g}/\text{g}$ sediments, respectively. Pollution load index values of the site of the studied area ranged from 1.24 to 7.65 that testified that the river sediments were polluted.

Ogoyi [40] evaluated heavy metal content in water, sediment and microalgae from Lake Victoria, East Africa. They collected samples of water, soil, sediment, during dry long and short period of 2008. The highest concentration of trace metals found was Zn (1.589ppm), Pb (0.823ppm), and Hg (0.00148ppm) in sediment, water and microalgae, respectively.

Yahaya [41] studied heavy metal content in surface water of Oke-Afa Canal Isolo Lagos, Nigeria. Nine surface water samples were collected from different points and analyzed for heavy metals. Heavy metals such as Cd (0.06mg/l), Ni (0.05mg/l), Pb (0.14mg/l), Zn (0.62mg/l) were found to be more than WHO recommended limits except Zn. Mahananda [42] investigated ground and surface water quality of Bargarh district of Orissa (India). After studying the various parameters like temperature, pH, total suspended solids, and total dissolved solids, alkalinity, dissolved oxygen, chemical Oxygen demand, nitrate, chloride, sodium, potassium, phosphate and fluoride, all the parameters were found to be below the pollution level.

Rai [43] studied different physico-chemical parameters *viz.*, pH, total dissolved solids, total hardness, calcium hardness, magnesium hardness, alkalinity, chloride and dissolve oxygen to evaluate the drinking water quality in Patna district. The results were compared with standards as prescribed by WHO and it was found that the values of pH, total dissolved solids, total hardness, calcium, hardness, magnesium hardness, alkalinity, chloride and dissolve oxygen are within the permissible standard limits.

Although physico-chemical characteristics are the direct index of pollution load, yet bioassays have their own importance in order to assess the toxicity. Some authors have reported the adverse effects of polluted water in various test systems. The following part of the review deals with the mutagenic/genotoxic effects of contaminants from various ecosystems including water, sediments and soil samples using different bioassays.

C. Effects of Polluted Water

1. Bacterial Bioassay

Various short-term screening methods have been developed to detect mutagenic/carcinogenic substances in environment complex mixtures such as air, soil or water ecosystem. Microorganisms have demonstrated several attributes that make them attractive for use in quick screening of various chemicals for toxicity [7]. The *Salmonella*/microsome assay developed by Ames and his co-workers is a widely used *in vitro* bacterial assay for screening of mutagenic properties of chemicals [44]-[46]. However, the use of Ames test for estimation of mutagenic potential of water samples has also been documented [29], [47].

Massod [48] evaluated the cytotoxic and genotoxic potential of tannery waste contaminated soils near the jajmau Kanpur India using Ames *Salmonella*/mammalian microsome test, DNA repair defective *Escherichia coli* K-12 mutants and *Allium cepa* chromosomal aberration assay. They found TA 98 was to be most sensitive strain to all the soil extracts tested

The highest mutagenic potential was observed using DCM extracts of soil as compared with hexane extracts for each strain of *Salmonella typhimurium*. DCM extract of the soil exhibited maximum damage to the cells at a dose of 40µl of soil extract/ml of culture after 6 hours of treatment. The survival was 23% in *polA*, 40% in *polA* and 53% in *recA* mutants when treated with DCM extract of site I. In *Allium cepa* assay all the test concentration of soil extract (5-100%) affected mitotic index in a dose dependent manner and several types of abnormalities were observed at different mitotic stages with the treatment: C mitosis, anaphase bridges, laggards, binucleated cells, stickiness, broken and unequal distribution of chromosome at anaphase stages of cell division. The soil is accumulating a large number of pollutants as result of wastewater irrigation and this practice of accumulation has adverse impacts on soil health.

Singh [49] demonstrated mutagenicity of leachates from industrial solid wastes using *Salmonella* reverse mutation assay. They analyzed leachates derived from dry wastes of the metal, tannery and dye industries of the state of Uttar Pradesh (India) for their mutagenic potential using reverse mutation assay. Both the spot and plate incorporation assays were conducted with four tester strains of *Salmonella typhimurium* (TA97a, TA98, TA100, and TA102). They also determined the metal concentrations in the samples and stated that the leachates derived from metal and tannery wastes possessed mutagenic properties.

Vargas [44] performed analysis of mutagenicity of waters under the influence of petrochemical industrial complexes by the Ames test (*Salmonella*/microsome). Water sample within the area of the three petrochemical industrial complexes at different points along the Cai River were tested for the presence of mutagens or carcinogens using the Ames test. Positive results were obtained for the TA 100 and TA 98 strains with and without microsomal activation in samples within the area of petrochemical industrial complex and at the Cai river sampling site close to the industrial complex. The authors observed the presence of mutagens causing frame shift and base-pair substitution mutations and suggested that there was need for continuous monitoring of the area.

Bekaert [50] studied the use of *in vitro* (Ames and Mutatox tests) and *in vivo* (Amphibian Micronucleus test) assays to assess the genotoxicity of leachates from a contaminated soil. Positive results were obtained with three tests which suggested that this contaminated soil might be a threat to the aquatic environment. The bacterial test sensitivity was shown to be influenced by the experimental conditions. The response was higher with the non filtered leachate than with 0.45µm filtered one. Incubation of bacteria in a liquid medium enhanced the sensitivity of the Ames test by increasing the bioavailability of pollutants in comparison with the agar plate method. The authors suggested that biological tests were useful complement to physicochemical analysis since it accounted for bioavailability and bioaccumulation of chemicals and interactions between pollutants.

Mathur [7] assessed mutagenicity of effluents from textile dye industry, Sanganer. A total samples were tested for their

mutagenicity by Ames assay using strain TA 100 of *Salmonella typhimurium*. Only 1 dye effluent i.e. Red 12 B showed absence of mutagenic activity while the remaining 11 dyes effluents were strongly mutagenic. The authors stated that this bioassay could be used as an initial screening test to analyze various dyes and dye containing effluents which were causing major damage to the aquatic environment.

Akintonwa [29] assessed mutagenicity of some pharmaceutical effluents. They evaluated the mutagenic potentials of effluents from six pharmaceutical companies in the Lagos province of Nigeria using modified Ames test and *Allium cepa* (Linn) assay. The modified Ames test which was with modification of the standard Ames test was done using *E. coli* (0157:H7) that has the phenotypic characteristics of glucose and lactose fermentation, motile, urease negative, indole positive and citrate negative. The authors observed that two effluents showed mutagenicity with modified Ames test. They also mentioned about the problems in the use of this bioassay. First, survival of *S. typhimurium* tester strains under some growth conditions was dramatically reduced relative to that in standard media used in laboratory. For e.g. the strains were particularly sensitive to water samples containing high salt concentrations. Secondly, sensitivity of the test was absolutely acceptable for assessing mutagenicity of known chemicals under laboratory conditions; however, it might be too low for the detection of low amounts of mutagens in environmental sample.

2. Animal Bioassay

Animal models have been used for a number of purposes in metal carcinogenesis studies (a) to detect carcinogenic activity (b) to estimate carcinogenic risk and (c) to investigate mechanisms of metal carcinogenesis [51]. Because of ease of handling and accumulated long-term experience, the most widely used animal models include rodents *viz.*, mice, rats and hamsters. Oyeniy [52] studied *in vivo* genotoxic effects of wastewaters from Agbara industrial estate, Osun State, Nigeria using the mouse sperm morphology assay. Two wastewater samples; before treatment and after treatment were collected and characterized for some physic-chemical properties in accordance with standard methods. Sperms of mice were examined for morphological abnormalities after 35 days from the exposure to test samples. Genotoxicity in mouse was investigated at 5 different concentrations of 1%, 5%, 10%, 20%, and 50% of the effluent sample. The results suggest that the tested industrial waste water contained chemicals that are potential germ cell mutagens.

Giri [53] studied genotoxic effects of Malathion (organophosphorus insecticide), using three mammalian bioassays *in vivo viz.*, chromosome aberration, sister chromatid exchange (SCE) and sperm abnormality assays in mice. Grisolia [54] estimated the genotoxicity of domestic sewage in fish species *Oreochromis niloticus* and *Tilapia rendalli* through the index of micronuclei in peripheral erythrocytes. *O. niloticus* and *T. rendalli* specimens were maintained in an aquarium continuously receiving the final effluent, in an open system, before being discharged into Lake

Paranoa. At all stages, the most concentrated sample was more toxic than the respective diluted samples. Different sampling of the same group of fish revealed no increase in the micronuclei frequency, regardless of the period of exposure.

Hrenovic [55] suggested the importance of prokaryotic and eukaryotic biotests to assess toxicity of wastewater from pharmaceutical sources. They used yeast toxicity test (YTT), based on the inhibition of saccharose fermentation by the yeast *Saccharomyces cerevisiae* using standard toxicants (copper sulphate, formaldehyde, sodium nitrite, sodium sulphite, phenol, and zinc sulphate) and found that repeated measurements of YTT accuracy with the standard toxicants showed EC₅₀ values characterized by low standard deviation and coefficient of variation (7.62%). They also stated that the toxicity of wastewaters measured by YTT agrees sufficiently with those measured by standard methods of the determination of toxicity such as inhibition of bioluminescence, TTC-dehydrogenase activity, aerobic bacterial growth and anaerobic sludge biogas production.

Bakare [56] studied genotoxicity of a pharmaceutical effluent using four bioassays. They investigated the potential genotoxicity of a pharmaceutical effluent using the *Allium cepa*, mouse-sperm morphology, bone marrow chromosome aberration (CA) and micronucleus (MN) assays. *A. cepa* and the animal assays were carried out at concentrations of 0.5, 1, 2.5, 5, and 10% and 1, 5, 10, 25, and 50% of the effluent, respectively. They have shown the genotoxic potential of the samples in terms of induction chromosomal aberration assay in *Allium cepa* while induction of micronucleus (MN) aberration in rats.

3. Plant Bioassay

Allium cepa is one of the plant species commonly used for evaluating the potential genotoxicity of environmental chemicals [57]. *Allium* test is a simple, sensitive and rapid bioassay that has been widely used as a standard for biomonitoring of environmental contaminants using various genotoxicity parameters. *Allium* test generally provides useful estimate of the total toxic effects resulting from the treatment of root tip cells by mixture of wastes [4]. Three well known plant bioassays *Allium* root chromosome aberration (AI-RCAA) assay, *Tradescantia* micronucleus (*Trad*-MCN) assay, and the *Tradescantia* stamen hair (*Trad*-SHM) mutation assay were validated in 1991 by the International Programme on Chemical Safety (IPCS) under the auspices of the World Health Organization and the United Nations Environment Programme (UNEP). These plant bioassays are proven to be efficient tests for chemical screening and especially for *in situ* monitoring for genotoxicity of environmental pollutants. As a result of this validation study, standard protocols of these three plant bioassays were used by some of the 11 participating countries in the IPCS to carry on genotoxicity tests on air, water and soil as a follow up activity [58].

Kataeva [59]; studied the cytotoxicity of 5 soil samples collected near of copper-smelters and Ni-enriched soil from an area of natural geochemical anomaly (the polar urals) using *Allium* root micronucleus test. They exposed *Allium* roots in

aqueous soil extract for 30h with recovery period of 20-44h and found an increase of frequencies of total nuclear anomalies in meristematic cells as compared to control samples, most frequent types of anomalies in all studied samples were extrusions. The frequency of micronuclei in cells of root tips increased up to 6.5 fold, total number of anomalies and contribution of extrusions among them were higher for contaminated soils with copper whereas high concentration of nickel leads to decrease in root growth and increasing of numbers of micronuclei. Samuel [5] stated that some of the plant bioassays; *Allium cepa*, *Vicia faba*, and *Tradescantia paludosa* have been in use for over 60 years, initially for studying the mutagenic effects of ionizing radiation and chemical mutagens but recently to evaluate the mutagenicity/clastogenicity of environmental pollutants. Owing to the simplicity of such assays and relatively low cost, their versatility and the minimum facility requirements make them practically desirable assays for environmental monitoring. The plant root is extremely useful in biological testing. Observation of the root tip system therefore constitutes a rapid and sensitive method for environmental monitoring.

Genotoxicity testing of wastewater sludge using the *Allium cepa* anaphase-telophase chromosome aberration assay was carried out by Rank [60]. Wastewater sludge was sampled during three winter periods from three Danish municipal wastewater treatment plants differing in size and industrial load. The toxicity of the sludge wastes using *Allium* root inhibition assay. It was found that the sludge samples from the smallest plant with the lowest industrial load induced significant chromosome aberrations [60]. Cabrera [58]; observed genotoxicity of soil from farmland irrigated with waste water using three plant bioassays. Extracts from soil samples were made using distilled water and organic solvents by shaking the sample for about 12h under a relatively low temperature (15–20°C). Plant cuttings of *Tradescantia* or the roots of *Allium* were treated by submerging them in the extracts. Three replicates of each sample were analyzed in each of the three bioassays. It was observed that extracts using DMSO, ethanol and distilled water showed a positive result in the three bioassays and there was no difference for the genotoxicity of the extracts with the different solvents.

Grover [2] detected genotoxicity of wastewater samples from sewage and industrial effluent using *Allium* root anaphase aberration and micronucleus assays. Industrial effluents were collected and stored in the form of sludge (semi-dried matter). The acetone extracts of the sludge samples were also used for treatment of the *Allium* roots. *Allium* root micronuclei tests on the sewage extracts showed no significant increase in the number of micronuclei in comparison with negative control while the extracts from industrial effluent showed positive responses both in the micronucleus and anaphase aberration assays. Amin [61] evaluated cytotoxic testing of sewage water treatment using *Allium cepa* chromosome aberrations assay. Sewage influent (untreated) and effluent (primary treated) were used to treat *Allium cepa* bulbs. They found that the treatment with both sewage waters and their dilutions decreased the mitotic index

and increased rate of dividing and non dividing cells significantly than the control sample. Sewage water from the west plant caused the maximum chromosomal abnormalities due to addition of effluents from industrial activities.

Rank [62] evaluated *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater contain five mutagenic/carcinogenic chemicals viz; sodium dichromate (25 μ M), benzene (100 μ M), dichloromethane (175 μ M) and 1, 1, 1-trichloromethane (175 μ M) and formaldehyde (1mM). Among all the chemicals formaldehyde (1 mM) was found to be non-mutagenic. The sensitivity of the *Allium* test was calculated to be 82% when compared to other bioassays used. They concluded that the *Allium* test was recommended for the screening of wastewater because it had a high sensitivity was cheap, rapid, easy to handle.

Radetski [63] evaluated of the genotoxic, mutagenic and oxidant stress potentials of leachates from municipal solid waste incinerator bottom ash. Aqueous leachates of a municipal solid waste incineration bottom ash (MSWIBA) were produced according to a European standardized method. During the analysis, they found relatively low concentrations (less than 1mg⁻¹) for four metals (iron, cadmium, lead and copper). No mutagenic activity was revealed after performing the *Salmonella*/microsome assay with and without microsomal activation. They observed a significant increase in micro nucleated cells in *Vicia* root tip micronucleus assay with increase in concentration of leachate. Significant and elevated antioxidant stress enzyme activities e.g. superoxide dismutase (SOD), catalase (CAT), peroxidase (PER) and glutathione reductase (GR) were detected in *Vicia* root tissues even at the lowest tested leachate concentration (i.e., 0.3%). Their results suggested that MSWIBA aqueous leachates need to be formally tested with genotoxic sensitive tests before recycling and support the hypothesis that plant genotoxicity is related to the cellular production of reactive oxygen species (ROS).

Grisolia [54] evaluated genotoxicity of domestic sewage in a municipal wastewater treatment plant. They carried out the study using *Allium cepa* root tip cells through cytological parameters such as aberrant cells in anaphase-telophase and the mitotic index. In the *Allium* test, each of the four stages of the wastewater treatment viz., crude sewage, primary effluent, secondary effluent, and tertiary effluent was analyzed, The results showed that the numbers of aberrant cells found in the *Allium* test did not differ among the four stages tested nor when compared with the control.

Lah [64] evaluated genotoxicity of water soil leachates by Ames test, comet assay and *Tradescantia* micronucleus assay. Soil samples were collected at six sampling points from industrial and agricultural region of Slovenian where contamination by heavy metals and sulphur dioxide (SO₂) were primarily caused by a nearby power plant. In their study Lah and his co-workers reported that all samples were genotoxic in all three bioassays used.

Krishnamurthi [65] studied genotoxicity of sludge, wastewater and effluents from three different industries. They used four genotoxicity assays namely chromosomal aberration, DNA strand break, DNA laddering and P53

accumulation tests in mononuclear blood cells. They observed an increase in chromosomal aberrations from 2.6 \pm 1.1 (aberrant cells in %; control) to 33.6 \pm 6.6 in a petrochemical plant, 29.4 \pm 3.3 in a petroleum refinery and 14.4 \pm 1.8 in a coke plant of steel industry. They stated that the first and second treatment plants in petrochemical industry and coke plant of steel industry eliminated genotoxicity of the wastewater. They concluded that genotoxicity tests allowed the identification of critical sources contributing to contamination of surface waters.

Vujosevic [9] evaluated cytotoxicity and genotoxicity of the river Rasina in Serbia using the *Allium* anaphase-telophase test. Inhibition of root growth relative to the negative control (synthetic water) was observed in all samples. Analysis of the genotoxic potential through scoring anaphase and telophase aberrations showed that in 7 of the 9 samples, the level of aberrations was significantly increased relative to the negative control but was lower than that of the positive control (methyl methane sulfonate). Changes in the relation between physiological and clastogenic types of aberrations were found in some samples indicating differences in the potential genotoxic substances present at the analyzed sites. They stated that *Allium* anaphase-telophase test was a monitoring system that could serve as the first alert for the presence of genotoxic environmental pollutants.

Gupta [66] evaluated genotoxicity of hospital wastewaters before and after treatment. They stated that some of the substances found in hospital wastewaters were genotoxic and were suspected to be a possible cause of the cancers. Genotoxicity tests are an excellent means to study the toxicity and the risk associated waste water from hospitals. However, they observed that the genotoxicity of hospital wastewaters was highly reduced after the treatment process and concluded that there was a need for establishment of advanced and effective effluent treatment plants in the hospitals which are merely dumping the wastewaters in the municipal sewage system.

Radic [67] evaluated surface and wastewater genotoxicity using the *Allium cepa* test. The simplified *Allium cepa* root assay was utilized to evaluate the possible cytotoxic and genotoxic effects of surface and wastewaters collected near the Sava River (Croatia) over a three-month monitoring period. Physicochemical characterization of the water samples included measurements of conductivity, chemical and biological oxygen demand, levels of suspended matter and salts, nitrate, nitrite, ammonium, total nitrogen and total phosphorus. Morphological modifications of the *A. cepa* roots, inhibition of root growth, cell division and induction of mitotic and chromosomal aberrations were observed. The most highly polluted water samples (industrial effluents) caused an inhibition of root growth of over 50%, a decrease in the mitotic index of over 40% and a considerable increase in chromosomal aberrations compared to the control. The measured biological effects of some water samples appeared related to the physicochemical characteristics. Therefore, mutagenicity/genotoxicity assays should be included along with conventional chemical analysis in water quality

monitoring programs. Their use would allow the quantification of mutagenic hazards in surface and wastewaters.

Rodrigues [68] evaluated genotoxicity of an industrial effluent from an oil refinery using plant and animal bioassays. Polycyclic aromatic hydrocarbons (PAHs) are genotoxic chemicals commonly found in effluents from oil refineries. The authors studied the genotoxic potential of an oil refinery effluent by means of micronucleus (MN) testing of *Allium cepa* which revealed no effect after 24h of treatment. They suggested strong associations of the two bioassays with these cell types; plant (*Allium cepa*) and mammal (HTC) cells for more accurately assessing genotoxicity of environmental samples. Sik [28] demonstrated genotoxic effects of industrial wastewater on *Allium cepa* L. They studied the effects of different concentrations of water on both incoming and outgoing in central biological and chemical wastewater treatment plant in Manisa (Turkey) organized industrial zone (MOIZ) on the *Allium cepa* L. root meristems which were rooted in distilled water for 48 h. The onion bulbs were kept in the 100% concentrations of the refined water (RW) and of 10, 25, 50 and 100% concentrations of unrefined water (UW). Distilled water was used for the control samples. It was determined that wastewater reduced the rate of the mitotic division of different concentrations and increased the mitotic anomalies. Mitotic index was found to be 33.8, 23.6 and 16.7% in the control group (RW) 10% and 25% concentration of the UW, respectively. On the other hand, it was found that the rates of Mitosis (Anaphase + Telophase) were 0.23, 0.42, 0.71 in the control group, 10% and 25% concentration of the UW, respectively. They observed that plant growth was interrupted in the 50 and 100% concentrations of the UW and the mitotic division was inhibited. No anomalies were encountered in the control group. In the RW, a low rate of abnormality was observed while in the different concentrations of the UW; chromosomal aberrations such as high frequency of lagging chromosome, irregular distribution, polar slips, horizontal division and sticky chromosome were observed.

Samuel [5] evaluated cyto/genotoxicity of two industrial effluents viz. that of textile and paint industries using *Allium cepa* assay. The cyto/genotoxic effects of the industrial effluents from paint (0, 7.2, 18, 36, and 72%) and textile (0, 1.6, 4, 8, and 16%) manufacturing were evaluated using root tip cells of *Allium cepa*. The root length and chromosomal aberration assays were used to determine the 96 h effective concentration, root growth inhibition, mitotic index and chromosome aberration rate. They found that textile effluent was 4.5 times more toxic than the paint effluent. They also found that the root growth inhibition was concentration dependent. The mitotic index (MI) decreased with increasing concentrations of paint and textile industrial effluents. Two industrial effluents induced chromosomal aberrations in root tip cells of *A. cepa* with vagrant chromosome, bridges and fragments and sticky chromosomes being most frequently observed. At lower concentrations, bridges and fragments were the most common aberrations. They stated the suitability

of *A. cepa* chromosomal assay as a tool for monitoring the genotoxic effects of industrial effluents and wastewater.

Similarly, majority of reports showing the mutagenic/genotoxic effects lacked the assessment of physico-chemical characteristics. Although all the studies have their own importance, yet, the studies with all biological, biochemical and physical parameters can be the best indicator of water pollution.

II. CONCLUSION

Increase in population and industrialization, has resulted in production of huge quantities of liquid waste that ultimately finds its way to water sources viz, rivers, lakes, streams and oceans. The contaminated water bodies further results in spreading of various diseases. As some pollutants are potent mutagens/carcinogen, the danger occurs in their consumption along with drinking water. Hence, it is mandatory to analyze different characteristics including physical, chemical and biological.

The present study thus focused on to explore the importance of different parameters of water pollution. It was seen during the study that many reports have shown physico-chemical characteristics but very few reports indicated the biological parameters along with physico-chemical parameters.

REFERENCES

- [1] A.N. Strahler, Modern Physical Geography. John Wiley & Sons, Inc., New York (1992).
- [2] I. S. Grover, and S Kaur, "Genotoxicity of Waste Water Samples from Sewage and Industrial Effluent Detected by the *Allium* Root Anaphase Aberration and Micronucleus Assays," *Mutation Research*. vol. 426, pp. 183-188, 1999.
- [3] Y. Chen, Z. Wang, and S. Huang, Assessment of the Contamination and Genotoxicity of Soil Irrigated with Wastewater. *Plant Soil*. vol 56, pp. 189-196, 2004.
- [4] S. Chandra, L. K. S. Chauhan, R. C. Murthy, P. N. Saxena, P. N Pande, and S. K. Gupta, "Comparative Biomonitoring of Leachates from Hazardous Solid Waste of Two Industries Using *Allium* Test", *Science of the Total Environment*., vol 347, pp. 46- 52, 2005.
- [5] O. B. Samuel, F. I. Osuala, and G. C. Odeigah, "Cytogenotoxicity Evaluation of Two Industrial Effluents Using *Allium cepa* Assay", *African Journal of Environmental Science and Technology*. vol. 4, pp. 21-27, 2010.
- [6] O. A. Shahaby, H. M. Abdel Migid, M. I Soliman, and I. A. Mashaly, "Genotoxicity Screening of Industrial Wastewater Using the *Allium cepa* Chromosome Aberration Assay", *Pakistan Journal of Biological Sciences*., vol. 6, pp. 23-28, 2003.
- [7] N. Mathur, and P. Bhatnagar, "Mutagenicity Assessment of Textile Dyes from Sangner (Rajasthan)", *Journal of Environmental Biology*. vol. 28, pp. 123-126, 2005.
- [8] R. Srivastava, D. Kumar, and S. K. Gupta, "Bioremediation of Municipal Sludge by Vermi Technology and Toxicity Assessment by *Allium cepa*", *Bioresources Technology*., vol. 96, pp. 1867- 1871, 2005.
- [9] M. Vujosevic, S. Anelkovic, G. Savic, and J. Blagojevic, "Genotoxicity Screening of the River Rasina in Serbia Using the *Allium* Anaphase-Telophase Test", *Environmental Monitoring and Assessment*., vol. 147, pp.75-81, 2008.
- [10] A. Duran, D. Herbas, M. Reynaga, and J. Butterworth, "Planning for Multiple Uses of Water: Livelihood Activities and Household Water Consumption in Peri-Urban Cochabamba, Bolivia", 2004.
- [11] S. C. Barman, G. C. Kisku, P. R. Salve, D. Misra, R. K. Sahu, P. W. Ramteke, and S. K. Bhargava, "Assessment of Industrial Effluent and Its Impact on Soil and Plants", *Journal of Environmental Biology*., vol. 22, pp. 251-256, 2001.

- [12] L. Leitgib, J. Kalman, and K. Gruize, "Comparison of Bioassays by Testing Whole Soil and Their Water Extract from Contaminated Sites", *Chemosphere*. vol. 66, pp. 428-434, 2007.
- [13] A. Prasad, and K.V.B. Rao, "Physico Chemical Analysis of Textile Effluent and Decolorization of Textile Azo Dye by *Bacillus endophyticus* Strain VITABR 13", *The IIOAB Journal*., vol. 2, pp. 55-62, 2010.
- [14] V. K. Gaur, S. K. Gupta, S. D. Pandey, K. Gopal, and V. Misra, "Distribution of Heavy Metals in Sediment and Water of River Gomti", *Environmental Monitoring and Assessment*., vol. 102, pp. 419-433, 2005.
- [15] M. M. Hoshina, and M. A. Marin Morales, "Micronucleus and Chromosome Aberrations Induced in Onion (*Allium cepa*) by a Petroleum Refinery Effluent and by River Water that Receives Its Effluent", *Ecotoxicology and Environmental Safety*., vol. 72, 2090-2095, 2009.
- [16] I. E. Akubugwo, C. J. Ofoegbu, and C. U. Ukwuoma, "Physico-Chemical Studies on Uburu Salt Lake Ebonyi State- Nigeria", *Pakistan Journal of Biological Sciences*., vol 10, pp. 3170-3174, 2007.
- [17] M. D. Kumar, A. Patel, R. Ravindranath, and O. P. Singh, "Water Harvesting and Artificial Recharge in Naturally Water-Scarce Regions", *Economic and Political Weekly*. vol. 43, pp. 61-70, 2008.
- [18] H. Roselene and Paneerselvam. "Physico-Chemical Analysis and Role of Phytoplanktons in Bellandur Lake", in: proceedings of the 12th World Lake Conference. (Eds. Sengupta, M. and Dalwani, R.). pp. 1729-1726, 2008.
- [19] R. Shyamala, M. Shanthy, and P. Lalitha, "Physicochemical Analysis of Borewell Water Samples of Telungupalayam Area in Coimbatore district, Tamilnadu, India", *E- Journal of Chemistry*., vol. 4, pp. 924-929, 2008.
- [20] G. M. Zaiad, "Physico-Chemical Analysis of Soils in Al-Khums city, Libya", *Journal of Applied Sciences Research*. vol 6, pp. 1040-1044, 2010.
- [21] V. S. Srivastava, "Correlation of Metal-Organic Fractions in Industrial Sludge Amended Soils", *Indian Journal of Environmental Protection*. vol. 21, pp. 428-430, 2001.
- [22] V. Vidhya, R. Babu, C. K. Kale, V. M. Kothandaraman, U. Manivel, R. Jayabalou, and Y. V. Subramaniam, "Irrigation Waters and Soils Quality with Reference to Operation of a Small Scale Chemical Industry", *Indian Journal of Environmental Protection*., vol. 21, pp. 409-415, 2001.
- [23] O.M. Kolawole, and T.O. Summonu, "Effect of Wastewater Treated with Metanolic Bark Extract of *Bridelia Ferruginea* Benth on Rat Kidney and Liver", *Journal of Applied Sciences in Environmental Sciences*., vol. 5, pp. 55-64, 2010.
- [24] B.J. Majer, T. Grummt, M. Uhl, and S. Knasmüller, "Use of Plant Bioassays for the Detection of Genotoxins in the Aquatic Environment", *Acta hydrochimica Hydrobiologica*., vol. 33, pp. 45-55, 2005.
- [25] T. Watanabe, and T. Hirayama, "Genotoxicity of Soil", *Journal of Health Science*. vol. 47, pp. 433-438, 2001.
- [26] A. Kdasi, A. Idris, K. Saed, and C.T. Guan, "Treatment of Textile Wastewater by Advanced Oxidation Processes- A Review", *Global Nest: The International Journal*., vol. 6, pp. 222-230, 2004.
- [27] I. Howard, E. Espigares, P. Lardelli, J. L. Martin and M. Espigares, "Evaluation of Microbiological and Physicochemical Indicators for Wastewater Treatment", *Environmental Toxicology*. vol. 19, pp. 241-249, 2004.
- [28] L. Sik, O. Acar, and C. Aki, "Genotoxic Effects of Industrial Wastewater on *Allium cepa* L.", *African Journal of Biotechnology*., vol. 8, pp. 1919-1923, 2009.
- [29] A. Akintonwa, O. Awodele, A.T. Olofinnade, C. Anyakora, G.O. Afolayan, H.A.B. Coker, "Assessment of the Mutagenicity of Some Pharmaceutical Effluents", *American Journal of Pharmacology and Toxicology*., vol. 4, pp. 144-150, 2009.
- [30] A. Gill, and M.I.S. Saggoo, "Mutagenic Potential and Nutritive Quality of Turnip Plants Raised over Chromium Amended Soils", *International Journal of Botany*., vol. 6, pp. 127-131, 2010.
- [31] M. Kelly-Quinn, C. Bradley, D. Murray, D. Tierney, P. Ashe, J. Bracken, and M. McGarrigle, "Physico-Chemical Characteristics and Macroinvertebrate Communities of the Caher River", *Royal Irish Academy*., vol. 103, pp. 187-196, 2003.
- [32] J.C. Akan, F.I. Abdulrahman, G.A. Dimari, V.O. Ogugbuaja, "Physico-Chemical Determination of Pollutants in Wastewater and Vegetable Samples along the Jakara Wastewater Channel in Kano Metropolis, Kano State, Nigeria", *European Journal of Scientific Research*., vol. 23, pp. 122-133, 2008.
- [33] V. D. Joshi, N. N. Palei, and P. R. Rachh, "Physicochemical Properties of Four Farm Site Soils in Area Surrounding Rajkot, Gujrat, India", *International Journal of Chem Tech Research*., vol. 1, pp. 709-713, 2009.
- [34] M. Haq, R. A. Khattak, H. K. Puno, and M. S Saif, "NPK Status in Effluent Irrigated Soils of Some Selected Sites of NWFP", *International Journal of Agriculture and Biology*., vol.2, pp. 264-267, 2004
- [35] M. S. Saif, M. Haq, and K.S. Memon, "Heavy Metals Contamination through Industrial Effluent to Irrigation Water and Soil in Korangi Area of Karachi (Pakistan)", *International Journal of Agriculture and Biology*., vol. 4, pp. 646-648, 2005.
- [36] P. A. Mary Helan, S. Jayasree, J. Antony Johnson, I. H. Belsha Jaya Edith, Chittrrasu, "Seasonal Variations in Physico-Chemical Parameters of Water in Coconut Husk Retting Area, Parakkani, Tamil Nadu", *International Journal of Environmental Sciences*., vol. 1, pp. 1056-1061, 2011.
- [37] J. A. Baig, T. G. Kazi, M. B. Arain, H. I. Afridi, G. A. Kandhro, R. A. Sarfraz, M. K. Jamal, A.Q. Shah, "Evaluation of Arsenic and other Physico-Chemical Parameters of Surface and Ground Water of Jamshoro, Pakistan", *Journal of Hazardous Materials*., vol. 166, pp. 662-669, 2009.
- [38] M. I. O. Raji, Y. K. E. Ibrahim, J. O. Ehinmidu, "Physico-Chemical Characteristics and Heavy Metal Levels in Drinking Water Sources in Sokoto Metropolis in North-Western Nigeria", *Journal of Applied Science and Environment Management*., vol. 14, pp. 81-85, 2010.
- [39] K. M. Mohiuddin, H. M. Zakir, K. Otomo, S. Sharmin, N. Shikazono, "Geochemical Distribution of Trace Metal Pollution in Water and Sediments of Downstream of an Urban River", *International Journal of Environmental Science and Technology*., vol.7, pp. 17-28 2010.
- [40] D. O. Ogoyi, C. J. Mwita, E. K. Naguu, P. M Shiunndu, "Determination of Heavy Metal Content In Water, Sediment and Microalgae from Lake Victoria, East Africa", *The Open Environmental Engineering Journal*., vol. 4, pp. 156-161, 2011.
- [41] A. Yahaya, A.A. Adegbe, and J.E. Emurotn, "Assessment of Heavy Metal Content in Surface Water of Oke-Afa Canal Isolo Lagos, Nigeria", *Archives of Applied Science Research*. vol. 4, pp. 2322-2326, 2012.
- [42] M. R. Mahananda, B. P. Mohanty, and N. R. Behera, "Physico-Chemical Analysis of Surface and Ground Water of Bargarh District, Orissa, India", *International Journal of Research and Reviews in Applied Sciences*., vol. 2, pp. 284-295. (2010).
- [43] A. K. Rai, B. Paul, and G. Singh, "A study on the Physico-Chemical Analysis of Water Quality Parameters of Patna District, Bihar, India", *Plant Archives*., vol. 11, pp. 389-392, 2011.
- [44] V. M. F. Vargas, V. E. P. Motta, and J. A. P. Henriques, "Analysis of Mutagenicity of Waters under the Influence Petrochemical Industrial Complexes by the Ames Test (*Salmonella* microsome)", *Brazil Journal of Genetics*., vol. 11, pp. 505- 518, 1988.
- [45] H. Schweikl, and G. Schmalz, "Evaluation of Mutagenic Potential of Root Canal Sealers Using the *Salmonella* Microsome Assay", *Journal of Material science*., vol. 2, pp. 181-185, 1991.
- [46] C. Novotny, N. Dias, A. Kapanen, K. Malachova, M. Vandrovцова, M. Itavaara, and N. Lima, "Comparative use of Bacterial, Algal and Protozoan Tests to Study Toxicity of Azo- and Anthraquinone dyes", *Chemosphere*., vol. 63, pp. 1436-1442, 2006.
- [47] Z. A. Alam, S. Ahmad, and A. Malik, "Genotoxic and Mutagenic Potential of Agricultural Soil Irrigated with Tannery Effluents at Jajmau (Kanpur), India", *Archives of Environmental Contamination and Toxicology*. vol. 57, pp. 463-476, 2009.
- [48] Massod and Malik, "Cytotoxic and Genotoxic Potential of Tannery Waste Contaminated Soils", *Science of the Total Environment*. vol. 444, pp. 153-160, 2013.
- [49] A, Singh, S. Chandra, S. K. Gupta, L. K. S. Chauhan, and S. K. Rath, "Mutagenicity of leachates from Industrial Solid Wastes Using *Salmonella* Reverse Mutation Assay", *Ecotoxicology and Environmental Safety*., vol. 6, pp. 210- 216, 2007.
- [50] C. Bekaert, C., Rast, V. Ferrier, A. Bispo, M.J. Jourdain, and P. Vasseur, "Use of *in vitro* (Ames and Mutatox Tests) and *in vivo* (Amphibian Micronucleus Test) Assays to Assess the Genotoxicity of Leachates from a Contaminated Soil", *Organic Geochemistry*., vol. 30, pp. 953-962, 1999.
- [51] T. Clarkson, R A. Goyer, G. L. Fisher, A. Furst, and G. Kazantzis, "Experimental Studies in Whole Animal Bioassay", *Environmental Health Perspectives*. vol. 40, pp. 21-26, 1981.

- [52] T. Oyeniyi, C. Latunji, "Industrial Effluents Induced Abnormal Sperm Cells In mice (*Mus musculus*)", *New York Science Journal*. vol, 5, pp. 60-64, 2012.
- [53] S. Giri, S. B. Prasad, A. Giri, and G.D. Sharma, Genotoxic Effects of Malathion: An Organophosphorus Insecticide, Using Three Mammalian Bioassays *in vivo*", *Mutation Research.*, vol. 514, pp. 223- 231, 2002.
- [54] C. K. Grisolia, A.B. Oliveira, H. Bonfim, and M.N. Klautau-Guimaraes, "Genotoxicity Evaluation of Domestic Sewage in a Municipal Wastewater Treatment Plant", *Genetics and Molecular Biology*. vol. 28, pp. 334-338, 2005.
- [55] J. Hrenovic, B. Stilinovic, and L. Dvoracek, "Use of Prokaryotic and Eukaryotic Biotests to Assess Toxicity of Wastewater from Pharmaceutical Sources", *Acta Chimica Slovenica.*, vol. 52, pp. 119-125, 2005.
- [56] A. A. Bakare, A. A. Okunola, O. A. Adetunji, and H. B. J Enmi, "Genotoxicity Assessment of a Pharmaceutical Effluent Using Four Bioassays", *Genetics and Molecular Biology*. vol. 32, pp. 373- 381, 2009.
- [57] H. Yi, L. Wu, and L. Jiang, "Genotoxicity of Arsenic Evaluated by *Allium*-Root Micronucleus Assay", *Science of the Total Environment*. vol. 383, pp. 232-236, 2007.
- [58] G.L. Cabrera, and D.M.G. Rodriguez, "Genotoxicity of Soil from Farmland Irrigated with Wastewater Using Three Plant Bioassays", *Mutation Research*. vol. 426, 211-214, 1999.
- [59] M. Kataeva, V. Kotseruba, N. Terekhina, N. Kutlunina, A. Beljaeva, "Allium Root-Micronucleus (*Allium*-MCN) Test on the Genotoxicity of Soil Samples Contaminated with Heavy Metals", *World Applied Sciences Journal.*, vol. 17, pp. 992-1000, 2012.
- [60] J. Rank, and Nielson, "Allium cepa Anaphase-Telophase Root Tip Chromosome Aberration Assay on N-Methyl-N-Nitrosourea, Maleic Hydrazide, Sodium Azide and Ethyl Methane Sulfonate", *Mutation Research.*, vol. 390, pp. 121-127, 1998.
- [61] A.W. Amin, "Cytotoxicity Testing of Sewage Water Treatment Using *Allium cepa* Chromosome Aberrations Assay". *Pakistan Journal of Biological Sciences*. vol. 5, pp. 184- 188, 2002.
- [62] J. Rank, and M. H. Nielsen, "Evaluation of the *Allium* anaphase-Telophase Test in Relation to Genotoxicity Screening of Industrial Wastewater", *Mutation Research/ Environmental Mutagenesis.*, vol. 312, 17-24, 1994.
- [63] C. M. Radetski, B. Ferrari, S. Cotelle, J. F. Masfaraud, J. F. Ferard, "Evaluation of the Genotoxic, Mutagenic and Oxidant Stress Potentials of Municipal Solid Waste Incinerator Bottom Ash Leachates", *Science of Total Environment.*, vol. 333, pp. 209-216, 2004.
- [64] B. Lah, T. Vidic, E. Glasencnik, T. Cepeljnik, G. Gorjanc, and R.M. Logar, "Genotoxicity Evaluation of Water Soil Leachates by Ames Test, Comet Assay, and Preliminary *Tradescantia* Micronucleus Assay", *Environment Monitoring Assessment*, vol. 139, pp. 107-118, 2007.
- [65] K. Krishnamurthi, S. S. Devi, J. G. Hengstler, M. Hermes, K. Kumar, D. Dutta, S. M. Vannan, T. S. Subin, R. R. Yadav, and T. Chakrabarti, *Archives of Toxicology.*, vol. 82, pp. 965-971, 2008.
- [66] P. Gupta, N. Mathur, P. Bhatnagar, P. Nagar, and S. Srivastava, "Genotoxicity Evaluation of Hospital Wastewaters", *Ecotoxicology and Environmental Safety.*, vol. 72, pp. 1925-1932, 2009.
- [67] S. Radic, D. Stipanicev, V. Vujcic, M. M. Rajcic, S. Sirac, and B. P. Kozlina, "The Evaluation of Surface and Wastewater Genotoxicity Using the *Allium cepa* Test", *Science of Total Environment*. vol. 408, pp. 1228-1233, 2009.
- [68] F.P. Rodrigues, J.P.F. Angeli, M.S. Mantovani, C.L.B. Guedes, and B.Q. Jordao, "Genotoxic Evaluation of an Industrial Effluent from an Oil Refinery Using Plant and Animal Bioassays", *Genetics and Molecular Biology.*, vol. 33 , pp. 169-175 2009.
- [69] E.O. Ekundayo and O. Obuekwe, "Effects of an oil spill on soil Physico-chemical properties of a Spill Site in Typic Udipsamment of Niger Delta Basin of Nigeria", *Environmental Monitoring and Assessment.*, vol.60, pp.235-249, 1999
- [70] P.C. nagajyoti, N. Dinakar, T.N.V.K.V. Prasad, C. Suresh and T. Damodharam, "Heavy metal toxicity: Industrial Effluent effect on Groundnut (*Arachis hypogaea L.*)Seedlings", *Journal of Applied Sciences Research.*, vol. 4, pp.110-121, 2008.
- [71] S.A. Adebisi, Fayemivo and K. Adnike, "Pollution of Ibadan soil by industrial effluents", *New York Science Journal.*, vol. 3, pp. 37-41, 2010.
- [72] WHO, Guideline for drinking water quality: *Recommendation World Health Organization, third ed.*, Geneva, 2004.