Ecotoxicological Studies of Soil Using Analytical and Biological Methods: A Review

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Abstract—Soil is a complex physical and biological system that provides support, water, nutrients and oxygen to the plants. Apart from these, it acts as a connecting link between inorganic, organic and living components of the ecosystem. In recent years, presence of xenobiotics, alterations in the natural soil environment, application of pesticides/inorganic fertilizers, percolation of contaminated surface water as well as leachates from landfills to subsurface strata and direct discharge of industrial wastes to the land have resulted in soil pollution which in turn has posed severe threats to human health especially in terms of causing carcinogenicity by direct DNA damage. The present review is an attempt to summarize literature on sources of soil pollution, characterization of pollutants and their consequences in different living systems.

Keywords—Soil Pollution, Contaminants, Heavy metals, Pesticides, Bioassays.

I. INTRODUCTION

COIL is the outer, loose material of the earth's surface that Dis distinctly different from the underlying bedrock. In other words, it is naturally occurring unconsolidated or loose covering of broken rock particles and putrefying organic matter on surface of earth. Soil is also defined as the collection of natural bodies occupying portions of earth's surface that supports plants and other living organisms. Soil is healthy, if it consists of roughly 40 % minerals, 23 % air, 6 % organic and 8 % living organisms [1]. The physico-chemical properties of soil differ due to integrated effects of climate and living matter acting upon parent material, as conditioned by relief, over periods of time [1], [2]. It takes more than thousands of years for the formation of a thin layer of soil [3], [4]. The study of soil is of immense importance because it not only supports the plants but also acts as a major ecosystem supporting the survival of many other living beings.

The soil, in recent years, has been polluted through various sources like application of pesticides [5]-[16] industrial effluents [17]-[21] wastewater discharges [22]-[25] which further have threatened the life of various organisms including human beings. Furthermore, different heavy metals like arsenic, cadmium, copper, cobalt, lead, manganese, mercury, nickel and zinc are also reported to be equally responsible for soil pollution [26]-[34]. The literature survey revealed a large number of reports on different aspects of soil pollution;

therefore, the review given under has been categorized accordingly.

A. Physico-Chemical Analysis of Soil Samples

1. Pesticides

After The World War-II, the use of pesticides turned out to be an obligatory input to agricultural food industry in order to meet the demands of increasing population [35]. The versatile use of various kinds of pesticides has resulted in contamination of agricultural field soils [5]-[15], [36]-[41]. The summary of literature on estimation of pesticides in different soil samples is given in Table I.

Feng [42] examined organochlorine pesticides residues (DDT and HCH) in soils of China. OCP residues in the soil samples were found in the range of $0.1-4.5\mu g/kg$ for DDT and $0.1-4.5\mu g/kg$ for HCH, respectively. They revealed that the above concentration of OCPs was far below the maximum residue limits (<0.05mg/kg) of National Environmental Standards for agricultural soils for both DDT and HCH. Gong [7] studied the level and distribution of DDT in surface soils from Tianjin, China. They collected 188 samples and estimated content of DDT and its metabolites in those samples. All the samples were subjected for p,p'-DDE, p,p'-DDD, p,p'-DDT, o,p'-DDE, o,p'-DDD, and o,p'-DDT detection. The results revealed that p,p'-DDD, p,p'-DDE were predominant contaminants in all the surface soil samples with mean concentrations of 27.5ng/g and 18.8ng/g, respectively.

Fuentes [43] analyzed four different pesticides viz. trifuralin, metolachlor, chlorpyrifos and triadimefon in agricultural soils using gas chromatography-electron capture used microwave-assisted detection (GC-ECD). They extraction and partitioning method (MAEP) through an aqueous medium and simultaneously cleaned the samples by partitioning with hexane. Apart from this, they also applied MAEP method to determine an additional group of pesticides (triallate, acetochlor, ensosulfan I and II, endrin, methoxychlor, and tetradifon) where a good recovery range was observed. They recommended the MAEP method as a simple, convenient and sensitive method to determine hydrophobic pesticides even at very low levels. Li [8] studied the concentration, enantiomeric compositions and source of hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethanes (DDTs) and chlordane in 74 soil samples, consisting of 37 crop soils, 14 paddy soils and 23 natural soils, collected from Pearl River delta of South China. They estimated various pesticides using gas chromatographymass spectrometry (GC-MS) and observed that the mean concentrations of total HCHs and DDTs in various samples

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descended in order: crop soils> paddy soils> natural soils. The total HCH concentration in crop soils ranged from 0.05-24.1mg/g while DDT ranged from 0.52-414mg/g.

Hao [9] estimated the contents of pesticides viz. hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethanes (DDTs), dichloro-2,2-bis(pchlorophenyl)-ethane (p,p'-DDD), 1,1 dichloro-2,2-bis(pchlorophenyl) ethylene (p,p'-DDE), endosulfan, dieldrin, hexachlorobenzene endrin. (HCB), and pentachloronitrobenzene (PCNB) in soil samples collected from paddy and vegetable field in Yicheng town, Yixing city. All soil samples were collected from surface layer (0-15cm) and sublayer (15-30cm). Each sample was a composite of 8-10 sub samples that were mixed, sieved (2mm) and freeze dried. The pesticides were estimated using GC/µECD gas chromatography. They reported that the concentration of total OCPs in all soil samples ranged from 15.5-56.8µg/kg while that of DDTs (including α , β and γ) in all soil samples ranged from 6.2-36.9µg/kg. The value of total HCHs was in the range of 5.7-12.3µg/kg.

Wong [40] studied the contamination of soil with organochlorine pesticides of Southern Mexico. The dominant organochlorines (OCs) detected were dichlorodiphenyltrichloroethanes (DDTs) and toxaphene (TOX), which together accounted for 83-100% of the total OCs measured. DDTs in soil samples ranged from 0.057-360ng/g whereas TOX ranged from 0.06-69ng/g. Westbom [10] estimated the content of organochlorine pesticides in soil samples collected from 12 farms of Upper Awash Agro Industry Enterprises (UAAIE), Ethopian state. They performed both quantitative and qualitative analysis using a dual column gas chromatographyelectron gas capture detection system (GC-ECD) and a GC equipped with a mass spectrometer (MS) and found different persistent organic pollutants (POPs), currently used insecticides and low concentrations of OCPs (aldrin, dieldrin, endrin and hepatachlor). Endosulfan and DDTs were also detected with concentration of 56000ng/g and 230ng/g. Nishina [44] studied pesticide residues in agricultural soil samples near Red River delta of northern Vietnam. These soils were found to contain a great content of dicofol. The range of this pesticide was more prevalent in clayey soils as compared to sandy soils. They also studied their transference pattern in the crop samples. Although the level of pesticides in both soil and crop samples was below the permissible limit but the study provides an alarm evidence about the pesticide contamination of soils of Vietnam.

Fang [12] examined organochlorine pesticide (OCPs) residues in soil/sediment using isotope dilution gas chromatography-mass spectrometry (GC-MS). Soil samples were subjected to Soxhlet extraction, sulfur removal with copper powder and cleaned up with gel permeation chromatography (GPC) and a florisil column of solid phase extraction (SPE). The analytes were separated on an HP-MS capillary column, detected in selected ion monitoring (SIM) mode and quantified using internal standard calibration curves of isotope dilution technique. They reported that content of OCPs ranged from 0.20-10.3µg/kg. Gonzalez [13] observed

the contamination of ground water due to leaching of organochlorine pesticides from agricultural fields of Pampa and Patagonia of Argentina. They also estimated the contents of pesticides in soil samples and reported that the contents of OCP were very high ranging from $4.65-38.1\mu g/g$. The predominance of p, p'-DDE residues reflected an extensive use of DDT. Pampean soil showed lower OCP levels (0.039-0.07 $\mu g/g$) but was found to be polluted with endosulfan. Huang [14] also reported the occurrence of organochlorine pesticides in soil using isotope dilution-high resolution gas chromatography (HRGC).

Oyekunle [15] carried out the estimation of organochlorine pesticides in 40 agricultural soil samples of Oke-Osun farm settlement, Osogbo, Nigeria. Samples were sieved through a mesh of 2.0mm pore size after air drying. They used solidliquid extraction to extract OCP from the soil. Qualitative identification and quantitative evaluation of the OCPs were carried out with the aid of a Perkin Elmer gas chromatograph coupled with electron capture detector (GC-ECD). The study revealed that agricultural soil samples of Oke-Osum farm were contaminated with persistent organochlorine pesticides and the content was more in dry season samples as compared to rainy season samples. Zhang [41] studied OCPs in soil and sediment samples of southeast China. Different OCPs detected were: a-HCH, β-HCH, Y-HCH, o,p'-DDT, p,p'-DDE, p,p'-DDD, p,p'-DDT, α-endosulfan, β-endosulfan, cis-chlordane and trans-chlordane. All the pesticides were observed in a significant range. Among all the pesticides studied, DDT was found to be the most dominant source of contamination.

2. Heavy Metals

Apart from contamination of soil by continuous application of pesticides, the reports are also available on contamination of soil by heavy metals. The following part of review deals with the reports on contamination of soils due to direct/indirect application of heavy metals on to the land (Table II). Most of the soils all over the world have been found to be polluted due to heavy metals [45]. Heavy metals in soil are of special concern because they do not degrade naturally and can retain in soil even after thousands of years. The ultimate fate of these heavy metals is that either they leach into ground water or surface water thereby contaminating them or can enter the food crops. Many studies and surveys have been conducted to assess the heavy metal content in various types of soils [46]-[48] as well as to study the effects of various heavy metals like arsenic, cadmium, copper, cobalt, lead, manganese, mercury, nickel and zinc [49]-[54]. Some reports also convey that although traces of some of these metals are required for the plant growth, but prove fatal, if present more than their maximum permissible limits [55]-[58]. Many scientists have reported the occurrence of high contents of heavy metals in various soil samples [59]-[63].

Srivastava [17] estimated heavy metal content in industrial sludge amended soils. All samples were found to be showing high heavy metal contents which were in range of Cu (0.99- 1.19μ g/g), Zn (1.68- 2.97μ g/g), Cd (0.026- 0.045μ g/g) and Pb

(0.30-1.72µg/g). Abrol [20] studied edaphic impacts of sewage and industrial effluents on soil resources. Field survey was carried out to determine the nutritive status of different sewage and industrial effluents. Heavy metal contents in corresponding soil samples were found to be as Cd (14 mg/kg), Cu (2.35mg/kg), Fe (16.04mg/kg), Mn (5.83mg/kg), and Zn (1.42mg/kg). Gimeno-Garcia [26] documented the presence of heavy metals in rice farming soils. They reported that among various fertilizers used for rice farming soils, superphosphate contained maximum concentration of Cd, Co, Cu and Zn as impurities. However, they also reported that the most significant heavy metals that soil received from inorganic fertilizers were Mn, Zn, Co and Pb. Giller [59] showed the toxic behavior of heavy metals in microorganisms and various microbial processes in agricultural soils. Mudakavi [27] reported the heavy metal contamination of some of the soils of some regions of India and found a high range of toxic heavy metals in all the samples studied.

Tariq [21] evaluated the effects of chrome and vegetable tanning effluents of two tanning units of Kasur and Mian Channun of Pakistan. They collected effluent and soil samples from 16 tanneries from both regions and determined the levels of selected metals viz. Na, K, Ca, Mg, Fe, Cr, Mn, Co, Cd, Ni, and Zn by using flame atomic Ph absorption spectrophotometer under optimum analytical conditions. They found that the concentrations of various heavy metals were higher in soil of Kasur sample when compared to that of sample of Mian Channun. Burman [28] estimated high concentrations of heavy metals in wheat, mustard and weeds grown in the agricultural fields irrigated with industrial effluents. Srinivas [29] studied the heavy metal content in various agricultural soil samples collected from different regions of Vishakhapatnam. The content of Pb was found to be in the range of 0.8-45.0mg/g, Cd (0.16-5.4mg/g), Zn (3.8-60mg/g), Ni (30-70mg/g), Cu (2.6-72mg/g), Mn (482-535 mg/g), and Fe (0.39-0.48mg/g). Romic [60] reported heavy metal distribution in agricultural topsoils in an urban area. They estimated different heavy metals and found their mean concentrations in samples studied as Cd (0.66mg/g), Cu (20.8mg/g), Fe (27.041mg/g), Mn (613mg/g), Ni (49.5mg/g), Pb (25.9mg/g), and Zn (77.9mg/g). The data revealed that the agricultural soils were polluted by occurrence of above mentioned heavy metals.

Vidhya [18] estimated heavy metal content in eleven agricultural soil samples collected from different sites near industry. Concentrations of heavy metals in soils were found to be in range of Cd (0.51-2.01mg/kg), Co (3.9-17.7mg/kg), Cr (31.2-70.8mg/kg), Ni (11.5-41.7mg/kg), Pb (6.5-19.5 mg/kg), and Zn (123-356mg/kg). In most of the samples, heavy metal concentrations were found to be more than their permissible limits. Abollino [64] also studied heavy metal concentration in agricultural soils of Piedmont, Italy and found significant content of all the metals analyzed. Aleem [31] reported accumulation of toxic substances like heavy metals and polychlorinated substances in soils and crops that were irrigated with raw sewage. Singhal [65] studied absorption, mobility and distribution of three heavy metals (Pb, Cr and

Cd) in two soils, one from riverbed and other from agricultural land and reported that the agricultural soil exhibited higher metal sorption capacity as compared to the river sand and the order of mobility of different metals studied was observed as Cr > Cd > Pb. Yokel [32] estimated arsenic and lead concentrations in orchid soil of Hansford site in Washington State (USA) which was contaminated due to application of lead arsenate pesticides. They reported that As and Pb concentrations were found to be higher than their background levels.

Song [66] studied the heavy metal concentration in soils of China. All the collected soil samples were exposed for the estimation of Cd, Cu, Zn, Pb, Ni, and Cr metals. Concentration of total Cd was found to be in the range of 1.4-2mg/kg exceeding the maximum allowable limits (MAL) of 1mg/kg. Cu concentration varied from 45.9-116mg/kg below the MAL value of 400mg/kg. Other metals viz. Zn, Pb and Ni were studied in the range of 175-473mg/kg, 87.5-565mg/kg and 26.6-68.8mg/kg, respectively also showing lower concentration than MAL values.

Mico [67] assessed heavy metals and their sources in agricultural soils of Spain. 54 soil samples were collected from Alicante province (Spain), a representative area of European Mediterranean region to determine the content of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The mean values of all the metals studied were found in the order of Zn > Cr > Pb >Cu > Ni > Co > Cd. The content of Co, Cr, Fe, Mn, Ni and Zn was correlated with the parent rocks whereas the content of Cd, Cu and Pb were associated with anthopogenic activities and found to be more persistent in soil cores. Mico [68] also analyzed heavy metal content of 29 agricultural soil samples in a Mediterranean semiarid area of Spain and found mean values of all the metals (mg/kg) as Cd (0.38), Co (7.9), Cr (28.3), Cu (21.6), Fe (15274), Mn (320), Ni (23.7), Pb (19.6) and Zn (57.8), respectively. This report concluded the contamination of agricultural lands of Mediterranean semiarid area with different heavy metals.

Luo [69] examined the speciation of different heavy metals in agricultural soils of China. All the samples studied covered a wide range of metal concentration with Cu (280-1931 mg/kg), Cd (0.13-2.84mg/kg), and Zn (27.4-704mg/kg).Shi [34] detected 273 soil samples of Shangai, China with high contents of Cd, Cr, Cu, Ni, Pb and Zn. They reported that the main sources of Cu, Pb and Zn were traffic emissions. Cd was from industrial emissions, whereas Cr and Ni were related to atmospheric deposition. Luo [70] analyzed seven different heavy metals (Cu, Zn, Pb, Cd, As, Ni and Cr) and found all the metals to be in the order of Cd > Cr > Zn > As > Cu > Ni >Pb. They concluded that use of phosphate fertilizers, waste water discharge and sludge application were the primary sources of Cd and Zn in soils whereas high concentration of As, Cu, Cr and Ni was due to natural rock weathering. Pb concentration was associated with the vehicular emission as well as the excessive use of inorganic fertilizers. Lado [71] reported similar observations in top soils of Italy, Europe as they detected critical concentrations of eight different heavy metals studied (As, Cd, Cr, Cu, Hg, Pb and Zn).

Katnoria [33] estimated heavy metal content of agricultural soils in four soil samples of Amritsar, India. Two soil samples were collected from the agricultural field of Fatehgarh Churian, one from Chabba and one sample from the Botanical garden of Guru Nanak Dev University, Amritsar. Various heavy metals in all the samples were found to be in the range of Cu (0.123-5.312mg/g), Cr (0.161-3.117mg/g), Co (0.056-2.709mg/g), Mn (0.244-0.274mg/g), Hg (0.004-0.0150mg/g), Ni (0.619-5.017mg/g), and Zn (0.414-0.489mg/g). Bai [35] studied heavy metal content in Chinese plantation land soils from different zones. In this report, they found significant content of all the metals studied. In the eastern region, the metal concentration was studied in the order of Cd > As > Zn> Cu > Cr > Pb. Eastern region was found to contain maximum toxicity where the major contaminants were Cd, Hg and Zn. In the middle region, As and Cd were main pollutants and Hg, Zn and Cu to some extent. In the western region, contamination was due to Cd and As. Among all the regions studied, maximum contamination was seen in the waste water irrigated land whereas, Cd, Hg and Zn were present at very high concentration and level of Hg was almost 21 times higher than the recommended value.

Janos [72] examined heavy metal concentration in soils of Czeth Republic. They estimated Cd, Cu, Pb and Zn concentration in all the samples studied. Cadmium concentration was observed to be maximum for all the samples. Sollito [73] documented heavy metal contamination in soils of Zagreb region (Northwest Croatia). Concentration of different heavy metals analyzed (Zn, Pb, Cd, Cu and Ni) were correlated with the different sources of anthropogenic contamination whereas high content of Ca was related to the lithology and parent material components. Katnoria[45] estimated four heavy metals viz. Co, Cr, Ni and Zn in two soil samples contaminated by effluents from zinc coating industry (S-I) and copper sulphate coating industry (S-II)) of Amritsar (India) and reported that S-I sample contained Co (5.05mg/g), Cr (4.49mg/g), Ni (6.86mg/g), and Zn (6.53mg/g), while S-II sample contained Cu (32.86mg/g), Co (6.85mg/g), Ni (9.66mg/g) and Zn (5.41mg/g). Bai [74] analyzed heavy metal content in agricultural soils of China. They collected 148 soil samples from four land use patterns and exposed to the analysis of Cr, Ni, Cu, As, Cd, Pb and Zn using ICP and ICP-Mass. A wide variation was found in the accumulation pattern of all metals in soils under different land patterns except Pb. The study revealed that accumulation of heavy metals like Cr. Ni, Cu, As, Cd and Zn was significantly affected by the different land use patterns and concluded that it was probably due to the excessive use of chemical fertilizers, organic fertilizers and pesticides containing very high content of heavy metals.

Chanda [75] assessed Cr, Pb and Hg contamination in agricultural soils of Kolkata (India) which were irrigated using metropolitan sewage effluent. They found Cr and Pb concentration below the MPL value (40.2-105.2mg/kg and 18.90-79.7mg/kg, respectively) whereas Hg was found to be showing ten times higher concentration than the MPL value (0.19-9.65mg/kg). The study focused on the level of Hg

contamination of agricultural lands of Kolkata. Flores-Magdaleno [76] studied heavy metals in agricultural soils and irrigation wastewater of Mixquiahuala, Hidalgo, Mexico. The concentration of extracted metals in all the samples was found in the order of Pb > Ni > Cd > As > Cr > Hg. Kebir [61] estimated different heavy metal concentrations in agricultural soils. They also determined the concentration of different heavy metals in plants growing near dumping site of Ghazaouet, Algeria. All the samples showed Pb (7.95-56.02 mg/kg), Zn (119.17-539.01mg/kg), Ni (5.12-34.70mg/kg), Cu (5.57-29.65mg/kg), Cd (1.97-24.15mg/kg), Mn (74.77-677.25 mg/kg), Cr (6.12-65.05mg/kg), Fe (3915-29750mg/kg), Mo (5.42-5.61mg/kg), and As (3.8-50.60mg/kg).

Masona [62] assessed different heavy metals in agricultural soils of Zimbabwe. They studied the effect of long term irrigation with wastewater on to agricultural lands and found that all the metals detected (Zn, Cu, Mn, Cd, Pb, Ni, Fe and Cu) were present beyond their maximum permissible limits. Pb was found to contain maximum transfer factor (0.59) whereas Fe having the least one (0.025). Singh [77] studied accumulation of heavy metals in soil samples near Ramgarh Lake, Gorakhpur, UP, India. Different metals analyzed were Cd, Cr, Cu, Pb, Zn, As and Mn. Out of all the metals analyzed, highest concentrations of Zn, Cr, Mn, Cu and Pb were found in the study area. Metal concentration was found in the order of Zn > Cr > Mn > Cu > Pb > As > Cd > Hg within the MPLvalues. Liu [63] detected concentration of different heavy metals (Cd, Hg, As, Pb, Cr, Cu, Zn and Ni) in 149 vegetable soils of Shandong Province, China. The average concentration of all the metals studied was below the threshold values. But a great heterogeneity was observed for metals like Cd, Hg and Cu which showed coefficient of variation of 103.42, 69.59 and 68.94 %, respectively.

3. pH

pH is considered as one of the most important parameters of soil analysis. It is a measure of total hydrogen ion concentration. Measured on a logarithmic scale, a soil at 5 pH is 10 times more acidic than a soil at pH 6 and 100 times more acidic than a soil at pH 7 [78]. The soil at pH of 7 is considered as neutral soil. pH of the soil has direct influence on nutrient's availability to the plants [49]. If the pH is above the permissible limit for a plant, the nutrients may not be soluble i.e. absorbable to the plants or if the pH is below 7, nutrients may be so soluble that they start leaching and become phytotoxic. Such soils with abnormal range of pH cause severe toxicity in living beings through consumption of crops or vegetables which are sown in such soils [79]. It is documented that plants show symptoms of toxicity or nutrient deficiency even when accurate quantity of fertilizers are applied to them [49]. In nature, acidic soils (pH 5 to 6.5) are derived from graphite rocks while alkaline soils (pH 7.5 to 8) are derived from limestone. Various anthropogenic activities resulted in a change in pH of the soil which also enhanced the toxicity of soil. Sindhu [80] reported that soil acidity leads to reduced supply of nutrients like Ca²⁺ and other cations. It also increases bioavailability of metal ions like Al, Mn, Cr, Cu, Ni,

Zn etc., which are toxic to plants at higher concentrations. Some reports also convey that if pH decreases below 5.4, the soil solution contains increasing concentrations of aluminum ions which displace other ions that result in low contents of Ca and Mg and ultimately lead to reduction in supply of nutrients to the plants. Various studies have demonstrated the dependence of certain metals availability of plants on soil pH [81]-[83]. Table III summarizes the various reports on physicchemical characterization of different soil samples studied.

Various reports have reported the variation of pH of different soil samples [33], [84]-[88]. Selvaraj [84] reported pH range of 7.3-7.9 among polluted soils and 5.8-6.4 among non-polluted soils of TNPL-Pulgar area. Barano [85] analyzed three soil samples comprising of two rural samples and one garden sample of Spain and reported the pH in the range of 4.4-8.2. Srinivas [29] estimated the physicochemical parameters of agricultural soils of Vishakhapatnam and found pH of all soil samples in the range of 7.34-8.25. Kelly-Quinn [88] studied physicochemical characteristics of soil samples collected from sides of Caher River. All soil samples were found to be slightly alkaline in nature showing pH in the range of 8.29-8.34. Katnoria [33] documented agricultural soil pH varying from 6.11-8.07. Chanda [75] detected similar variation of pH (5.41-7.81) in agricultural soils of Kolkata (India). Many reports are also available showing wide variation in pH [89-92].

4. Alkalinity

Alkalinity and acid neutralizing capacity of a solution is equal to stochiometric sum of the bases in solution. In nature, the carbonate alkalinity makes most of the total alkalinity due to presence of carbon dioxide in the atmosphere and common occurrence and dissolution of carbonate rocks. Apart from this, some of the common natural components viz. borate, hydroxide, phosphate, silicate, nitrate and sulphide also make up alkalinity [78]. In natural water, the carbon dioxide, carbonate, and bicarbonate alkalinity equilibrium determine and control the pH of the water. In water chemistry, the alkalinity equilibrium is measured and reported as ppm P alkalinity and ppm T alkalinity using color indicators such as phenolphthalein and bromcresol green methyl red that show a distinct color change with changes in pH. P alkalinity exists at pH > 8.3 e.g. boiler water. T alkalinity exists when the pH is greater than 4.3. T alkalinity represents all of the hydroxide, all of the carbonate, and 2/3 of the phosphate and other alkali producing material present in the sample above a pH of 4.3. Although the P and T alkalinity do not bear any direct relationship to pH, the readings can be used to determine the carbonate and bicarbonate concentrations in sample [78]. The alkalinity determinations represent the following:

If P alkalinity = 0, all of the alkalinity is bicarbonate

T alkalinity -2P alkalinity = carbonate alkalinity

2P – T alkalinity = hydroxide alkalinity

As the pH of natural water/soil is normally < 8.3, there exists no P alkalinity in nature and similarly as they do not contain strong mineral acids, the pH is never below 4.3. However, various anthropogenic activities like use of

pesticides; inorganic fertilizers etc. can alter the alkalinity of soil/water.

A number of studies have reported the differences in alkalinity of soil samples [29], [33], [93], [94]. Pande [93] analyzed and reported alkalinity of different sampling sites of Ramganga river sediments at Moradabad and found it to be in the range of 340-460mg/100g. They correlated high alkalinity of their studied samples with presence of excess carbonates, bicarbonates, metal hydroxides and free hydroxyl ions. Tyagi [94] found alkalinity in recycled water irrigated soil in the range of 1.49-2.33mg/l and in tube well irrigated soil as 1.25-1.81mg/l. Srinivas [29] analyzed alkalinity of agricultural soils of four different areas of Vishakhapatnam and found it in the range of 0.103-0.14mg/g. Kelly-Quinn [88] found alkalinity of all the samples studied to be in the range of 7.4-8.75ml/l (CaCO)₃. Katnoria [33] analyzed the physicochemical characteristics of agricultural soils of Fatehgarh Churian (FC-I and FC-II), Chabba (CB) and Botanical garden (BG) of Guru Nanak Dev University of Amritsar and reported alkalinity of all the samples to be in the range of 0.13-0.96meq/100g.

5. Soil Texture

Soil texture is a soil property used to describe the relative proportion of different particle sizes present in a soil. These particles are grouped according to their size into soil separates such as clay, silt and sand. Coarse textured soils contain a large proportion of sand; medium textures are dominated by silt and fine textures by clay [95]. The variation in soil texture has been documented earlier by various reports [18], [96]-[97]. Mohapatra [96] estimated different physicochemical parameters of harbor sediments of Pardip port, East coast of Asia. The textural studies of all the samples conducted by them revealed two distinct size classes of soil i.e. clayey silt and silty sand. They found content of sand, silt and clay fractions in the range of 14.0-75.0%, 17.40-62.85% and 7.6-29.22%, respectively. Kumari [97] reported different types of soil textures viz. clayey loam, sandy loam and sandy in soil fertility survey of forest soil of Guntur district. Vidhya [18] found clay soil in the range of 9.0-310%, silt (4.5-26%) and sand (52-79%) in soils irrigated with effluents from a smallscale chemical industry.

The healthy or fertile soils provide human beings the bountiful crops, forests, product rangelands, diverse wild life as well as beautiful landscapes and all these are possible only if the soil possesses the nutrient in sufficient/required quantity. In nature, at least 16 elements are essential for normal growth of any plant which includes macronutrients as nitrates, phosphates and potassium [98].

6. Nitrogen

Nitrogen (NO₃⁻) is an essential nutrient for plant growth, but excess of it in soil can increase the risk of contamination of ground as well as surface water. Nitrates impart early vigor, vegetative growth and dark green color. However, their deficiency results in yellowing of plants, stunted growth and thinned stems. Srinivas [29] studied physicochemical characteristics of agricultural soils of Vishakhapatnam and found nitrate content in the range of 0.061mg/g (Sheela Nagar)-0.294mg/g (Agraharam). Katnoria [33] analyzed the physicochemical characteristics of agricultural soils of Fatehgarh Churian (FC-I and FC-II), Chabba (CB) and Botanical garden (BG) of Guru Nanak Dev University, Amritsar, Punjab (India) and found nitrate content in the range of 0.180-0.450mg/g. Udotong [79] studied total nitrogen content of wetland soils in Eket, Nigeria and found it in the range of 0.09-0.12%. Chanda [75] reported nitrogen content of agricultural soils of Kolkata and found it in the range of 29.3-383.1 mg/kg.

7. Phosphorous

Phosphorous (PO_4^{3-}) is another important macronutrient for plant growth required for development of strong roots and fruits as well as for disease resistance by plants. Pande [93] reported the content of total phosphates in the range of 0.5-0.85mg/100g in Ramganga river sediments of Moradabad. Srinivas [29] estimated phosphate content in the range of 0.555-3.340mg/g among different types of soils of Vishakhapatnam. Daly [99] detected the phosphorus content in soil sample collected from seven grassland sites on the Johnstown Castle Estate in south-east Ireland. They found total phosphate content to be ranging from 2.1-157mg/g in 2 cm depth soil, 1.0-140mg/g in 5cm and 0.8-135mg/g in 10cm depth soil. Katnoria [33] analyzed the physicochemical characteristics of agricultural soils of Fatehgarh Churian (FC-I and FC-II), Chabba (CB) and Botanical garden (BG) of Guru Nanak Dev University of Amritsar. They estimated the content of phosphates in the range of 0.750-6.900mg/g. Asadi [89] studied phosphorous content of studied samples in the range of 15-37%. Chanda [75] reported phosphorous content of agricultural soils of Kolkata and found it in the range of 8.7-40 mg/kg.

8. Potassium

Potassium is a necessary macronutrient for the plant growth and is necessary for the plant's ability to create sugars to resist diseases and survive in cold temperatures. Selvaraj [84] estimated content of potassium of polluted and non-polluted sites at TNPL-Pulgar area, Thriruchiaplli, Tamilnadu and reported increase in potassium in polluted sites, which they considered due to effluents from TN Newsprints and Papers Ltd. Potassium ranged from 142-219kg /acre in non-polluted areas. They specified that K<140.7kg/acre was considered as low, 140-281kg/acre as medium and >281.6kg/acre as high. Kelly-Quinn [88] studied 0.84 mg/l potassium content in Caher River. Katnoria [33] analyzed the physico-chemical characteristics of agricultural soils of Fatehgarh Churian (FC-I and FC-II), Chabba (CB) and Botanical garden (BG) of Guru Nanak Dev University of Amritsar, Punjab (India). They found potassium in the range of 0.160-0.253mg/g in all soil samples. Udotong [79] studied potassium content of wetland soils of Eket, Nigeria to be in the range of 0.04-0.12%. Asadi [89] reported content of potassium of soil samples in the range of 62-98%. A high range of potassium (1.47-5.44mg/g) was reported by Doi [90]. Joshi [91] also estimated high content of potassium (0.21-0.29meq/l) in farm site soils of Rajkot, Gujrat, India. Chanda [75] studied high potassium content of agricultural soils of Kolkata and found it in the range of 130-305mg/ kg.

9. Sodium

Sodium is an important cation widely present in all types of soils. However, the excess of sodium cause the saline conditions which are normally not suitable for plant growth. Udotong [79] studied sodium content in the wetland soils of Eket, Nigeria and found it in the range of 0.06-0.12%. Doi [90] reported content of calcium in the range of 0.38-1.65 mg/g in soil samples studied. Joshi [91] reported content of sodium in the range of 3.7-4.2meq/l of all the samples studied.

10. Calcium

Calcium is an essential element required for all living organisms. It is an essential part of plant cell wall structure supplied to regulate the transport and retention of other elements in the plant. It controls the effects of alkali salts and organic acids within the plant. In plants, calcium is essential for the growth of meristems and root tips and tends to accumulate in leaves as calcium pectate. But in excess, it causes various disturbances in the organic acid metabolism of calcium-sensitive plants [1]. Srinivas [29] studied analysis of agricultural physicochemical soils of Vishakhapatnam. They found calcium content in the range of 2.352-8.097mg/g in all the samples studied. Kelly-Quinn [88] reported calcium in the range of 38.95-53.75mg/l in water and sediment samples collected from different locations of Caher River. Udotong [79] analyzed calcium content in the wetland soils of Eket, Nigeria and found it in the range of 0.96-4.56%. Doi [90] reported content of calcium in the range of 1.47-5.44 mg/g in studied soil samples. Kebir [61] found 0.24-1.23g/kg of calcium in agricultural soils near dumpsite of Ghazaouel, Algeria.

11. Magnesium

Magnesium is an important part of the chlorophyll in all green plants and essential for photosynthesis. It also helps to activate many enzymes needed for the plant growth. Soil minerals, organic material, fertilizers and dolomite limestone are sources of magnesium for plants. Srinivas [29] estimated magnesium content in agricultural soils of Vishakhapatnam and found it in the range of 0.753-2.53mg/g. Kelly-Quinn [88] studied physicochemical characteristics of water and sediment samples collected from different locations of Caher River and found magnesium in the range of 1.92-2.15mg/l. Udotong [79] analyzed magnesiun content in the wetland soils of Eket, Nigeria and found it in the range of 0.48-2.10%. Doi [90] reported content of magnesium in the range of 6.68-34.1mg/g in studied soil samples. Kebir [61] analyzed physicochemical parameters in agricultural soils near dumpsite of Ghazaouel, Algeria and found magnesium in the range of 0.88-4.89g/kg in all the samples studied.

B. Ecotoxicological Effects of Contaminated Soils Using Different Bioassays

The pollutants present in the soil upon entering the living systems causes the deleterious effects to all the living organisms present in soil ecosystem. These pollutants not only cause the general health effects, but also can potentially damage the gene pool. Various scientists all over the world have reported the deleterious effects of different pollutants of soil samples [100]-[104]. Many other scientists have explored the mutagenic/genotoxic effects of polluted soil using various bioassays viz. bacterial bioassays [105]-[107], animal bioassays [108]-[113] and plant bioassays [114]-[116]. The following part of the review deals with the mutagenic/genotoxic effects of soil contaminants using different bioassays. Table IV summarizes the literature available estimation of mutagenic potential of different soil samples using different bioassays.

1. Bacterial Bioassays

Various scientists have recommended the bacterial bioassays for the evaluation of genotoxicity in different samples [105]-[107]. Among various bacterial assays, Ames assay has been most widely used and considered as one of the most reliable bioassay by various workers to study the toxic behavior of various environmental contaminants [105], [106]. Ames test is a short term bacterial reverse mutation assay especially designed to evaluate the mutagenic potential of wide range of chemical substances [107]. The test was developed by Dr. Bruce Ames in early 1970's and was found to be very sensitive to wide range of mutagenic and carcinogenic chemicals. The test employs several histidine dependent Salmonella strains like TA 97a, TA 98, TA 100, TA 102 and TA 104 each carrying different mutation in specific gene in the histidine operon. These mutations acted as hot spots for mutagens and caused DNA damage via different mechanisms [106]. When the Salmonella tester strains were grown on a minimal media agar plate containing a trace of histidine, only those bacteria that reverted to histidine independence were able to form colonies. The number of spontaneously induced revertant colonies per plate was relatively constant. However, when mutagenic sample was added to the plate, the number of revertant colonies per plate was increased [107]. Malachova [117] demonstrated that bacterial systems for mutagenicity detection could not only be applied for the immediate screening of the genotoxic risk of contaminated soils but also for assessment of the course and character of long term changes occurring in the environment.

A number of reports have traced the presence of different mutagenic substances in different soils using Ames test [118]-[120]. Goggleman [118] found mutagenic activity in extracts of soils with different cultures in Europe using Ames test. Matsushita [119] reported that soil near some cities in Asia like Tokyo, Bangkok, Chaing Mai and Manila induced mutagenic effects. Brown [120] evaluated the mutagenic potential of three agricultural soils of Bastrop, Norwood and Sassafrab employing *Salmonella typhimurium* and *Aspergillus nidulans*. The extracts of soils exhibited mutagenic response in

both bioassays. At a dose level of 1000 micrograms per plate, the organic extract of the Bastrop clay induced 434 net revertants; while at the same dose level, the Norwood sandy clay and the Sassafrass sandy loam induced 35 and 178 revertants, respectively, in the *Salmonella* assay with metabolic activation. In the *Aspergillus* assay, the extract of the Norwood and Bastrop soils induced a positive response without metabolic activation; this effect was reduced or eliminated in the presence of metabolic activation.

Jones [121] used Ames mutagenicity test to estimate the mutagenic potential of soils of Welsh region. They used Salmonella typhimurium strains TA 98 and TA 100 for the experiment and found that net revertants were in the range of 6-19g and 24-38g of dried soil, respectively. Edenharder [122] employed Ames test for estimation of mutagenic potential of soil samples collected from Mainz (urban area, exposed to anthropogenic pollution) and Corsica (rural area, unexposed to anthropogenic pollution), Germany and reported that soil sample collected from Mainz were highly mutagenic. Ehrlichman [123] evaluated genotoxicity of aqueous soil extracts using three bacterial bioassays: the umu test with Salmonella typhimurium TA1535/pSK1002, the NM2009 test with Salmonella typhimurium NM2009 and SOS Chromotest with Escherchia coli PQ37. The soil samples included sandy contaminated with samples explosives viz. 2,4,6trinitrotoulene and nitroaromatic compounds, sandy soil samples contaminated with heavy metals and soil samples from a coal mine. The aqueous extracts of soil contaminated with nitroaromatic compounds exhibited the maximum genotoxic potential in all the genotoxic tests.

Watanbe [124] examined the mutagenicity of surface soil samples from five geographically different regions viz. Hikkaido, Kanto, Chubu, Kinki and Kyusyu of Japan employing the Ames/Salmonella assay. Among 20 samples collected at Osaka city in the Kinki region, two soil samples showed strong mutagenic potential in TA 98 and TA 100 strains of Salmonella typhimurium. Watanbe [125] also estimated the mutagenic potential of 62 surface soil samples collected from Kinki region of Japan by the Ames/Salmonella assay. All the samples were mutagenic for TA 98 in the presence and absence of mammalian metabolic activation system (S9 mix). On the other hand, in the absence of S9 mix, all the samples were mutagenic for TA 98 strain while 73 % (45/62) of that were for TA 100 strain. The results revealed that the surface soils in the Kinki region were highly polluted with mutagens.

Katnoria [33] used Ames test to evaluate the mutagenic potential of agricultural soils of Fatehgarh Churian (FC-I and FC-II), Chabba (CB) and Botanical garden (BG) of Guru Nanak Dev University of Amritsar. They reported that all the samples were found to be non-mutagenic for TA 98 tester strain with and without S9. Mutagenic potential of soil in TA 100 strain was observed in the order of FC-I>FC-II>CB>BG without supplementation of S9 mix and FC-I>CB>FC-II>BG with S9 mix.

2. Animal Bioassays

Animal bioassays have been widely used to evaluate the mutagenic activity of different environmental contaminants using different animal cells/tissues by number of scientists [108]-[110]. Many animal bioassays viz. *Rattus* bone marrow micronucleus test, *Rattus* chromosomal aberration assay, Sister chromatid exchange assay and Comet assay have been employed by various scientists worldwide to assess the mutagenicity of different chemicals [111]-[113].

Akogbeto [126] studied the effects of pesticides residues from soil and water samples of agricultural settings which were exposed to high contents of pesticides. Two strains viz. the pyrethroid susceptible Kisumu strain and the resident Ladyi strain of Anopheles gambiae were used for the investigation. The mosquito larvae were grown on the bed of these samples. The authors reported that the growth rate was found to be decreased in the bed site prepared from pesticides containing sample as compared to control samples. Mouchet [111] monitored the genotoxic potential of aqueous extracts of soils using the comet and micronucleus tests in amphibian (Xenopus laevis) larvae. They reported the genotoxicity of soil extracts using both bioassays. Vernile [127] evaluated the bioavailability of pentachlorophenol of contaminated soil and estimated toxicity on coelomocytes of Eisenia andrei. E. andrei were exposed to contaminated soil for 7 and 14 days. They reported the toxicity of pesticides in terms of reduction of lysosomal membrane of E. andrei.

Leitgib [128] estimated the toxicity of contaminated soil and its extract using five bioassays viz. bioluminescence of *Vibrio fischeri*, the dehydrogenase activity of *Azomonas agelis*, the reproduction inhibition of *Tetrahymena pyriformis* and *Panagrellus redivivus*, the mortality of *Folsomia candida*, the root and shoot elongation of *Sinapis alba*. The measured endpoints were the bioluminescence inhibition of *Vibrio fischeri*, the dehydrogenase activity of *Azomonas agelis*, the reproduction inhibition of *Tetrahymena pyriformis* and *Panagrellus redivivus*, the mortality of *Folsomia candida*, the root and shoot elongation inhibition of *Sinapis alba* and the nitrification activity inhibition of an uncontaminated garden soil used as "test organisms". All the soil samples were found to be causing high genotoxicity in all the bioassays used.

Antunes [11] studied the structural effects of bioavailable fraction of pesticides from soil. They recovered four different pesticides viz. chloropyrifos, glyphosate, vinclozin and endosulfan. The ecotoxicological evaluation of all the four different pesticides was carried out using a standard battery of aquatic bioassays. The bioassays used to test the soil elutriates were: Daphnia magna acute and chronic toxicity; Vibrio fescheri- Microtox(®) and Pseudokirchneriella subcapita growth inhibition assay. Among all pesticides, endosulfan caused the maximum toxicity in Daphnia magna where the EC50 concentration was observed to be 36.8 % lethal. In third bioassay i.e. Pseudokirchneriella subcapita growth inhibition assay, all the pesticides resulted in total inhibition of growth. Bartha [5] monitored the stability and effects of 29 pesticides in Nixon sandy loam soil (a soil typical of those areas that are agriculturally productive in New Jersey). They determined the influence of pesticides on CO_2 production and nitrification by soil microorganisms and reported that pesticides had negative effects on production of CO_2 and nitrification.

Lors [129] used different bioassays viz. inhibition of lettuce germination and growth, earthworm mortality, inhibition of springtail population growth, avoidance by springtails to study the effects of polyaromatic hydrocarbons (PAHs) and heavy metal contaminated soils. Several end points were combined for each bioassay in an 'ecoscore' which is a measure of test sensitivity. The results depicted that the most of the organisms were highly sensitive to the concentration of 3-ring PAHs in soil. Springtail *Folosomia candida* showed highest ecoscores indicating most sensitivity of this organism towards soil contamination.

3. Plant Bioassays

Plant bioassays are considered to be the utmost important parameters to assess the toxicity of various complex mixtures like surface/ground waters, landfill leachates, waste water/sludge and industrial wastes in different types of soils including agricultural soils. These are the most efficient and less expensive when compared to bacterial and animal bioassays. Apart from this, different gene mutation assays and animal cytogenetic assays are thought to be inadequate for the detection of genotoxic effects of various pollutants in complex environmental mixtures [130]-[134]. As complex environmental samples contain both hydrophilic and lipophilic chemicals inside them, the bioassays used for the evaluation of their genotoxicity must be sensitive enough to detect the effects of both types of chemicals i.e. hydrophilic as well as lipophilic. Since, 1970s, higher plant bioassays have been recommended for the test the genotoxic potential of different environmental contaminants by various organizations [135]-[143].

Three well known bioassays viz. Allium cepa root chromosomal aberration assay (A/RCAA), Tradescantia micronucleus (Trad-MCN) assay and Tradescantia stamen hair mutation (Trad-SHM) assay have been validated by United Nations Environment Programme (UNEP) and International Programme on Chemical Safety (IPCS) for the chemical screening and in situ monitoring for genotoxicity evaluation of environmental pollutants [114]-[116]. While some other bioassays viz. Arabidopsis assay, Vicia faba root chromosomal aberration assay, Vicia faba root tip micronucleus assays and Allium sativum root chromosomal aberration assay have also been recommended by various scientists [144]-[146]. All these bioassays have their own importance and are widely used for the testing of genotoxicity of different environmental samples viz. waste water, wastewater sludge and agricultural soil samples [33], [147], [148].

Gichner [149] employed *Arabidopsis* assay for assessing the mutagenic potential of five compounds viz. ethyl methanesulfonate (EMS), *N*-methyl-*N*-nitrosourea (NS), azidoglycerol (AG), sodium azide (SA) and maleic hydrazide (MH) on *Arabidopsis thaliana*. Out of the five chemicals studied, EMS, NS and AG were reported to be mutagenic

while other two compounds viz. SA and MH were reported to be weakly mutagenic. Grant [150] suggested higher plant bioassays to be the most efficient bioassays to detect the environmental mutagens. He recommended two bioassays viz. Tradescantia stamen hair assay for mutations and Tradescantia micronucleus assay for chromosomal aberrations to be ideal for in situ monitoring and testing of airborne and aqueous mutagenic agents. He also recommended other higher plant genotoxicity bioassays like Arabidopsis thaliana, Allium cepa, Hordeum vulgare, Vicia faba and Zea mayz to test the genotoxic agents which have a large number of genetic markers. He further supported that higher plant systems were recognized as the excellent indicators of the cytotoxic, cytogenetic and mutagenic effects of environmental chemicals and had unique advantages for in situ monitoring and screening.

Kanaya [115] studied Vicia faba chromosomal aberration assay to determine the sensitivity, efficiency and reliability of this bioassay. Six laboratories participated in the study using a standard protocol. Mutagenic effects of four chemicals viz. azidoglycerol (AG, 3-azido-1,2-propanediol), N-methyl-Nnitrosourea (MNU), sodium azide (SA) and maleic hydrazide (MH) were studied using Vicia faba root tip meristem chromosomal aberration assay. Of the four chemicals, MH, MNU and AG were found to be showing clastogenic effects and gave a concentration related response whereas SA was found to be weakly mutagenic. The study depicted the sensitivity of Vicia faba root tip meristem chromosomal aberration assay to study the mutagenic potential of various contaminants present in different ecosystems viz. air, water or soil. Ma [151] employed Tradescantia stamen hair mutation assay for its efficiency and reliability and studied mutagenic effects of four chemicals viz. azidoglycerol (AG, 3-azido-1,2propanediol), N-methyl-N-nitrosourea (MNU), sodium azide (SA) and maleic hydrazide (MH). Different mutagenicity levels of these chemicals were evaluated by Tradescantia stamen hair mutation assay. The effective doses of chemicals studied were: AG (50-100ug/ml), MH (1-45ug ml), MNU (10-80µg/ml), and SA (3-80µg/ml). The study revealed the reliability of Tradescantia stamen hair mutation assay for chemicals from different components screening of environment including soil for their potential mutagenic effects.

Kovalchuk [152] performed *Allium cepa* root chromosomal assay to evaluate the genotoxicity of soils of inhabited areas in Ukraine contaminated by the Chernobyl accident. High toxicity and genotoxicity of radioactivity polluted sites in the used bioassays confirmed the fidelity of *Allium cepa* test as a quick and inexpensive biological test for ecological and genetic risk assessment. Gichner [153] used *Tradescantia* stamen hair and micronucleus assays to monitor the genotoxicity of soil extracts from two heavily polluted sites in Prague, the capital of Czech Republic. The results showed that the *Tradescantia* micronucleus assay was more sensitive to detect the genotoxic agents than the stamen hair assay.

Kong [154] evaluated the genotoxicity of contaminated soils and shallow well water samples employing three bioassays viz. Allium root anaphase aberration assay (Al-Tradescantia micronucleus (Trad-MCN) and RAA), Tradescantia stamen hair mutation (Trad-SHM) tests. The results of Al-RAA test showed a similar pattern of positive response as the Trad-MCN test. Cabrera [116] assessed genotoxicity of soil from farmland irrigated with wastewater using three plant bioassays viz. Allium root chromosome aberration (Al-RAA) assay, the Tradescantia micronucleus (Trad-MCN) assay and the Tradescantia stamen hair (Trad-SHM) mutation assay. These plant bioassays have proven to be efficient tests for chemical screening and especially for in situ monitoring for genotoxicity of environmental pollutants. Soil irrigated with wastewater was sampled and monitored for the presence of genotoxic agents using the above three bioassays viz. Allium root chromosome aberration (Al-RAA) assay, the Tradescantia micronucleus (Trad-MCN) assay and the Tradescantia stamen hair (Trad-SHM) mutation assay. Extracts from soil samples were made using distilled water and organic solvents by shaking the sample for about 12 h under a relatively low temperature (15-20°C). Plant cuttings of Tradescantia and 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 and were found to be equally genotoxic.

Monarca [155] studied the effects of aqueous extracts of soil samples collected from the different depths of a site near an industrial plant using chromium compounds. They analyzed the samples for micronuclei in roots of Vicia faba and showed a correlation between genotoxicity and concentration of chromium in the samples. Majer [156] evaluated the effects of heavy metal contaminated soils on micronuclei induction in Tradescantia. They also investigated correlation between genotoxic effects and changes of microbial parameters caused by metal contamination in soils. They examined 20 soils from nine locations for genotoxicity, metal content and physico-chemical parameters. Α pronounced induction of micronuclei was observed in Tradescantia micronucleus (Trad-MCN) assay with increased metal concentration of soils. However, no correlation was found between metal content and genotoxicity of soils from different locations. The microbial parameters (dehydrogenase, arylsulfatase activity, biomass C and biomass N) showed inconsistent results for different soils studied indicating that it was not possible to define a specific marker enzyme for metal contamination.

Song [66] reported the genotoxicity of soil samples of Northern China that are irrigated for a long-term with wastewater including industrial effluent mixed with the municipal waste water. They collected soil samples from six different sites and prepared soil extracts by dissolving 50g of soil in 250ml of double distilled water at room temperature and keeping it on mechanical shaker at 125rpm for 24h. Ma [151] prescribed *Vicia faba* root tip micronucleus test for evaluation of genotoxicity of soil systems. They observed that micronuclei frequency had elevated in all soils when compared to the control. The frequency of micronuclei ranged from 3.5-8% while the mean value of control was 1.58%. Katnoria [33] estimated the genotoxic potential of agricultural soils of Fatehgarh Churian (FC-I and FC-II), Chabba (CB) and Botanical garden of Guru Nanak Dev University (BG) of Amritsar employing *Allium cepa* root anaphase aberration asaay (*Al*RAAA). They followed two modes of treatments viz. *In situ* and root dip treatment for the evaluation of genotoxicity of soil samples. All soil samples showed percent aberrant cells as FC-I (6.90%), FC-II (5.21%), CB (6.06%) and BG (4.57%) in *in situ* treatment while FC-I (7.50%), FC-II (5.34%), CB (5.92%) and BG (3.20%) at maximum concentration (100%) in root dip treatment.

Jan-hui [157] evaluated the genotoxic potential of soil samples taken from an electronic-waste recycling area by employing *Vicia faba* root cells micronucleus assay in China. The soil extracts were prepared by dissolving the soil in liquid in 1:10 w/v. The solution was kept on a shaker with a speed of 120rpm for 8h. The soil solution was then centrifuged at 3500rpm for 10min and filtered through a 0.45μ m membrane filter. They observed that the induction of micronuclei in root tip cells of *Vicia faba* treated with the soil samples were higher than those which were treated with negative control.

Marcato-Romain [158] examined genotoxicity of soil using Vicia faba micronucleus test. They collected two contaminated soil samples from an industrial site and from near a coke works in France. Soil extracts were prepared by 24h extraction procedure. Heavy metals were estimated by digesting the soil samples in aqua regia. Soil genotoxicity was estimated by (i) testing of aqueous extracts of the different soils (ii) different contact methods between soils and roots. Seedlings were grown by soaking the seeds for 24h in deionized water for the germination of primary roots. Primary roots were suspended in Hogland's solution for the germination of secondary roots. Secondary roots were exposed to aqueous soil extracts for 30h. In direct contact method, effect of duration of seedling exposure to soil was studied for 2. 5 or 7 days. For the second treatment, Vicia faba roots were directly exposed to different concentrations (10%, 40%, 70%, and 100 %) of the aqueous extract. Roots were collected for the further investigation. In both modes of treatments, both soil samples induced significant toxicity. Testing of aqueous extracts showed 18.5 and 9.0 micronucleus per 1000 cells while direct contact after 2, 5 and 7 days treatment showed 0.42-0.84 micronucleus per 1000 cells.

Feretti [159] evaluated the genotoxic effects of gaseous emissions and percolates from three landfills using two plant bioassays viz. *Tradescantia* micronucleus assay and *Allium cepa* root chromosomal aberration assay. They suggested the usefulness of plant bioassays for the *in situ* monitoring of environmental genotoxins and their importance for the prevention of environmental pollutants. Cerniene [160] estimated the soil surface genotoxicity of military and urban territories, Lithuania by using *Tradescantia* bioassays. The specific character of the soil- surface concentration was shown in a series of *Tradescantia micronucleus* (*Trad*-Mn) and Stamen hair mutation (*Trad*-SHM) bioassays. *Trad*-Mn test has shown maximum effectiveness towards soil genotoxicity. Foltete [161] suggested *Vicia faba* micronucleus test as the most reliable bioassay to assess the genotoxic potential of pure substances, effluents or water extracts from soil. They also recommended it as a relevant and easiest biological tool to detect mutagens in the soils. Apart from the agricultural soils, plant bioassays are also being widely used to evaluate the genotoxicity of other soils like roadside soils, industrial soils. Various studies have demonstrated the genotoxicity of roadside and industrial soils [152], [153], [162] using plant bioassays.

S.No.	Description of site	Type of pesticide studied	Technique used	Results obtained	Reference
1.	Surface soils of Tianjin, China.	p,p`-DDE, p,p`-DDD, p,p`-DDT, o, p`-DDE, o,p`-DDD and o, p`- DDT	GC-MS	p,p'- DDE, p,p'- DDD were the predominant contaminants with mean concentrations of 27.5 and 18.8ng /g, respectively.	Gong [7]
2.	74 Soil samples of delta of Pearl River, South China.	HCHs, DDTs and chlordane	GC-MS	Mean concentrations of total HCHs and DDTs were found to be present in the order: crop soils> paddy soils> natural soils	Li [8]
3.	Soil samples of paddy and vegetable field of Yicheng town, Yixing city.	HCHs, DDTs, p,p'-DDE, p,p'- DDD, endosulphan, dieldrin, endrin, HCB and PCNB	GC/µECD gas chromatography	Total OCPs (15.5-56.8µg/kg), DDTs (6.2-36.9µg/kg) and HCHs (d 5.7-12.3µg/kg)	Hao [9]
4.	Soil samples of farms of Upper Awash Agro Industry Enterprises, Ethopian State	OCPs (aldrin, dieldrin, endrin and hepatochlor), POPs, endosulfans and DDTs.	GC-ECD and GC-MS	Significant concentrations of OCPS, Endusulfans and DDTs (56000ng/g and 230ng/g, respectively)	Westbom [10]
5.	Soil samples of China	OCPs	GC-MS	HCHs> DDTs > HCB > α -Endosulfan	Fang [12]
6.	Agricultural soils of Argentina.	OCPs, OCs, p,p'DDE, p,p'DDT, endosulfan	GC-MS	OCP levels (0.039-0.07µg/g OC)	Gonzalez [13]
7.	Soil sample of Beijing, China	OCPs	Isotope dilution HRGC	High range of all the pesticides studied	Huang [14]
8.	Agricultural soil of Oke- Osun farm settlement, Osogbo, Nigeria	OCPs	GC-MS	OCPs (13.09 \pm 21.68 beta -BHC,) p (') - DDT- (42.01 \pm 17.50 p µg/kg) in soil and 30.74 \pm 17.38 alpha - BHC, -82.88 \pm 32.24 p, p (') -DDT in rainy season samples, respectively.	Oyekunle [15]
9.	Soil samples of Southern Mexico	DDTs, TOX	GC-MS	DDTs (0.0567 - 360ng g) and TOX (0.06-69ng/g)	Wong [40]
10.	Soil samples of China	OCPs	GC-MS	Significant range of all the pesticides studied	Zhang [41]
11.	Soil samples of China	DDT andHCH	GC-MS	DDT (0.1-4.5µg/g) and HCH (0.1-4.5µg/g)	Feng [42]
12.	Agricultural soils of Chile	Trifuralin, metolachlor, chloropyrifos and triadimefon	GC-ECD and MAEP	Very high concentrations of all the pesticides studied.	Fuentes [43]
13.	Agricultural soil samples near Red River delta, Northern Vietnam	OCPs	GC-MS	All the pesticides studied were below the MRL value	Nishina [44]

TABLE I SUMMARY OF LITERATURE ON CONTAMINATION OF SOILS WITH PESTICIDES

TABLE II

			TABLE II	
S. No.		SUMMARY OF LITERATURE ON CO Heavy metal studied	NTAMINATION OF SOILS WITH HEAVY METALS Results obtained	Reference
1.	Industrial sludge amended soils of India	Heavy metal content, organic matter (OM) and chemical oxygen demand (COD)	Cu (0.99-1.19µg/g), Zn (1.68-2.97µg/g), Cd (0.026-0.045µg/g) and Pb (0.30-1.72µg/g); OM (58000-328100µg/g); COD (47.10- 269.20µg/g).	Srivastava [17]
2.	Agricultural soils near industry of Vishakhapatnam, India	Cd, Cu, Cr, Ni, Pb and Zn	Cd (0.51-2.01 mg/kg), Co (3.9-17.7 mg/kg), Cr (31.2-70.8 mg/kg), Ni (11.5-41.7 mg/kg), Pb (6.5-19.5 mg/kg) and Zn (123-356 mg/kg)	Vidhya (18)
3.	Soil samples of Italy	Cd, Cu, Fe, Mn and Zn	Cd (14 mg/kg), Cu (2.35 mg/kg), Fe (16.04 mg/kg) and Zn (1.42 mg/kg)	Abrol [20]
4.	Soil samples of Korea	Heavy metal content	Presence of high concentrations of different heavy metals	Tariq [21]
5.	Rice farming soils of Albufera Natural Park (Valencia, Spain)	Cd, Co, Cu, Mn and Zn	Mn,>Zn> Co>Pb	Gimeno-Garcia [26]
6.	Indusrial effluent irrigated soils of Lucknow, India	Pb, Cd, Zn, Cu, Mn and Fe	Pb (0.8-45.0 mg/g), Cd (0.16-5.4 mg/g), Zn (3.8-60 mg/g), Cu (2.6-72 mg/g), Mn (482- 35 mg/g) and Fe (0.39-0.48 mg/g).	Burman [28]
7.	Agricultural soils of Vishakhapatnam (India)	Pb, Cd, Zn, Ni, Cu, Mn and Fe	Pb (0.8-45 mg/g), Cd (0.16-5.4 mg/g), Zn (3.8-60 mg/g), Ni (30- 70 mg/g), Cu (2.6-7.2 mg/g), Mn (482-535 mg/g) and Fe (0.39- 0.48 mg/g)	Srinivas [29]
8.	Orchid soil of Hansford site in Washington state	As and Pb	High contents of As and Pb	Yokel [32]
9.	Agricultural soils of Amritsar	Cu, Cr, Co, Mn, Hg, Ni and Zn	Cu (0.123 - 5.3118 mg/g), Cr (0.1607 - 3.1170 mg/g), Co (0.056 - 2.7090 mg/g), Mn (0.244 - 0.2742 mg/g), Hg (0.0037 - 0.0150 mg/g), Ni (0.6193 - 5.0175 mg/g) and Zn (0.414 - 0.4897 mg/g).	Katnoria [33]
10.	273 soil samples of Shangai, China	Pb, Zn, Cu, Cr, Cd and Ni	Highconcentrations of all heavy metals studied	Shi [34]
11.	Soils of China	As, Cd, Cr, Cu, Pb, Zn	Cd > As > Zn > Cu > Cr > Pb	Bai [35]
12.	Agricultural soils of United Kingdom	Cd, Co, Cu and Mn	Occurrence of high contents of all heavy metals studied	Giller [59]

S. No.	Type of soil	Heavy metal studied	Results obtained	Reference
13.	Agricultural top soil in urban area Philippines	, Cd, Cu, Fe, Mn, Ni, Pb and Zn	Cd (0.66 mg/g), Cu (20.8 mg/g), Fe (27.041 mg/g), Mn (613 mg g/g), Ni (49.5 mg g/g), Pb (25.9 mg/g) and Zn (77.9 mg/g).	Romic [60]
14.	Agricultural soils near dumpsite of Ghazaouet, Algeria	Pb, Zn, Ni, Cu, cd, Mn, Cr, Fe, Mo and As	Pb (7.95-56.02 mg/kg), Zn (110.17-539.01 mg/kg), Ni (5.12-34.70 mg/kg), Cu (5.57-29.65 mg/kg), Cd (1.97-24.15 mg/kg), Mn (74.77-677.25 mg/kg), Cr (6.12-65.05 mg/kg), Fe (3915-29750 mg/kg), Mo (5.42-5.61 mg/kg) and As (3.8-50.60 mg/kg).	Kebir [61]
15.	Agricultural soils of Zimbabwe	Zn, Cu, Mn, Cd, Pb, Ni, Fe and Cu	All metals were present beyond permissible limits	Masona [62]
16.	Soil samples of Shandong Province, China	Cd, Hg, As, Pb, Cr, Cu, Zn and Ni	Great heterogeneity was studied for Cd, Hg and Cu having coefficient of variation of 103.42, 69.59 and 68.9%, respectively	tLiu [63]
17.	Soil samples of China	Cd, Cu, Zn, Pb, Ni and Cr	Cu (45.9-116mg/kg), Zn (175-473mg/kg), Pb (87.5-565mg/kg) and Ni (26.6-68.8mg/kg)	Song [66]
18.	Agricultural soil samples of Spain	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn	All metals studied were found in the order: Zn>Cr>Pb>Cu>Ni>Co>Cd.	Mico [67]
19.	Agricultural soil samples of Mediterranean semiarid area,	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn	All the metals were having mean values of Cd (0.38), Co (7.9), Cr (28.3), Cu (21.6), Fe (15274), Mn (320), Ni (23.7), Pb (19.6) and Zn	Mico [68]
20	Spain Soils of China	Cu Zn Dh Cd An Ni and Cn	$\begin{array}{l} (57.8) \\ Cd > Cr > Zn > As > Cu > Ni > Pb \end{array}$	Contd
20.		Cu, Zn, Pb, Cd, As, Ni and Cr		Luo [70]
21.	Soils of Italy	As, Cd, Cr, Cu, Hg, Ni, Pb and Zn	Critical concentration of all the metals studied	Lado [71]
22.	Soils of Zagreb region (Northwest Croatia)	t Zn, Pb, Cd, Cu and Ni	All metals were found to be present al high concentration	Sollito [73]
23.	Agricultural soils of China	Cr, Ni, Cu, As, Cd, Pb and Zn	All metals were present in significant concentration.	Bai [74]
24.	Agricultural soils of Kolkata (India)	Cr, Pb and Hg	Cr (40.2-105.2 mg/kg), Pb (18.9-79.7 mg/kg) and Hg (0.19-9.65 mg/kg)	Chanda [75]
25.	Agricultural soils of Mexico	As, Cd, Cr, Hg, Ni and Pb	Pb > Ni > Cd > As > Cr > Hg	Flores- Magdaleno [76]
26.	Soil samples near Rangarh Lake, Gorakhpur (India)	Cd, Cr, Cu, Pb, Zn, As and Mn	Zn, Cr, Mn, Cu and Pb were found to be present in the study area.	Singh [77]

	SUMMARY OF LITERATURE ON OTHER PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL SAMPLES					
S.No.	Description of site	Parameters studied	Results obtained	Reference		
1.	Soils ofAl-Khums city, Libya	Soil moisture content (SMC), water holding capacity (WHC), Soil texture, pH, Soil electrical conductivity (SEC) and total dissolved solids (TDS) and chloride content	SMC (0.02-1.04%) in air dried base soil and 2.61-3.64% in saturated wet soil base. pH (8.12-8.60). Moisture content (3.6-3.3 in/ft).SEC (410-766 mS/cm), TDS (246-455 ppm) and chloride (0.43-1.42 mg/g)	Zaiad [4]		
2.	Eleven soil samples from villages surrounding the factory, Vishakhapatnam, India	pH, electrical conductivity, calcium, magnesium, sodium and potassium	pH (6.3-7.9), electrical conductivity (0.104-1.273 mS), calcium (0.07-0.92 meq/100 g), magnesium (0.07-0.97 meq/100 g), sodium (0.08-2.24 meq/100 g) and potassium (0.01-0.10 meq/100 g)	Vidhya [18]		
3.	Soil sample of Jammu, India	Heavy metal content, pH, electrical conductivity, organic carbon and available nitrogen content	Cd (1.14 mg/g), Cu (2.35 mg/kg), Fe (16.04 mg/kg), Mn (5.83 mg/kg) and Zn (1.42 mg/kg)	Abrol [20]		
4.	Agricultural soils of Vishakhapatnam, India	pH, conductivity, organic carbon, alkalinity, chlorides, nitrates, phosphates, calcium and magnesium	pH (7.34-8.25), conductivity (0.2 -0.44 mMhos/cm), organic carbon (0.07 -0.132), chlorides (0.034-0.037 mg/g), nitrates (0.06 -0.294 mg/g), phosphates (0.513-3.340 mg/g), calcium (2.352- 8.097 mg/g) and magnesium (0.753-2.53 mg/g)	Srinivas [29]		
5.	Agricultural soils of Fatehgarh Churian, Chabba and Botanical garden, Amritsar	pH, alkalinity, water holding capacity, moisture content, bulk density, nitrates, phosphatesand potassium	pH (6.11-8.07), alkalinity (0.13-0.96 meq/100 g), water holding capacity (4.55-18.8%), moisture content (2.66- 6.10%), bulk density (1.05-1.38 g/cc), nitrates (0.180-0.450 mg/g), phosphates (0.750 - 6.900 mg/g) and potassium (0.160-0.253 mg/g)	Katnoria [33]		
6.	Agricultural soils near dumpsite of Ghazaouel, Algeria	pH, total organic carbon, nitrogen content, C/N ratio, Na, K, Ca, Mg and CEC	pH (5.05-7.29), total organic carbon, nitrogen content (1.25- 3.48 g/kg), C/N ratio (0.24-1.23 g/kg), Na (1.86-2.82, 0.89- 5.04 g/kg), K (1.35-12.05 g/kg), Ca (10-238.28 g/kg), Mg (0.88- 4.89 g/kg) and CEC (21.87- 32.01 c mol ⁺ /kg)	Kebir [61]		
7.	Wetland soils of Nigeria	pH, electrical conductivity, organic matter, nitrogen, calcium, magnesium, sodium and potassium	Soils were deficient in elements like nitrogen, phosphorous, potassium	Udotong [79]		
8.	Grassland sites on the Johnstown Castle Estate of south-east Ireland	Total phosphate content	2.1 - 157 mg/g in 2 cm depth soil, 1.0 - 140.0 mg/g in 5 cm and 0.8-135.0 mg/g in 10 cm depth soil	Daly [99]		

 TABLE IV

 SUMMARY OF LITERATURE ON ECOTOXICOLOGICAL EFFECTS OF SOILS USING DIFFERENT BIOASSAYS

 (i) BACTERIAL BIOASSAYS

S. No.	Description of sample	Test used	Bacteria used	Mutagenic effects	Reference
1.	Agricultural soils of Fatehgarh Churian,	Ames mutagenicity test	Salmonella typhimurium		Katnoria [33]
	Chabba and Botanical Garden, Amritsar		TA 98	Negative	
			TA 100	Positive	
2.	Soils samples of Europe	Ames mutagenicity test	Salmonella typhimurium	Positive	Goggleman [118]
3.	Soil of Tokyo, Bangkok, Chaing Mai and Manila	Ames mutagenicity tes	Salmonella typhimurium	Positive	Matsushita [119]
4.	Agricultural soils of Bastrop, Norwood and Sassafrab	Ames mutagenicity test	Salmonella typhimurium,Aspergillus nidulans	Positive Positive	Brown [120]
5.	Soils samples of Welsh region	Ames mutagenicity test	Salmonella typhimurium	Positive	Jones [121]
6.	Soils of Mainz and Corsica, Germany	Ames mutagenicity test	Salmonella typhimurium	Positive	Edenharder [122]
7.	Soil samples of Germany	Umu test, NM2009 and	Salmonella	Positive	Ehrlichman
	· ·	SOS Chromotest	typhimurium,Escherchia coli	Positive	[123]
8.	Surface soil samples of Hikkaido, Kanto, Chubu, Kinki and Kyusu, Japan	Ames mutagenicity test	Salmonella typhimurium	Positive	Watanbe [124]
9.	Surface soils of Kinki region, Japan	Ames mutagenicity test	Salmonella typhimurium	Positive	Watanbe [125]

(ii) ANIMAL BIOASSAYS

S. No.	Description of sample	Test used	Animal used	Results obtained	Reference
1.	Soils of France	Comet and micronucleus test	Xenopus laevis	Positive	Mouchet [111]
2.	Soil of Bénin, Africa	Bioassay using Anopheles gambiae	Anopheles gambiae	Positive	Akogbeto [126]
3.	Soils of Italy	Bioassay using Eisenia Andrei	Eisenia andrei	Positive	Vernile [127]
4.	Soil samples of Hungary	Bioluminescence of Vibrio fischeri, dehydrogenase activity of Azomonas agelis and reproduction inhibition of Tetrahymena pyriformis and Panagrellus redivivus, mortality of Folsomia candida.	Vibrio fischeri, Azomonas agelis, Tetrahymena pyriformis, Panagrellus redivivus and Folsomia candida.	Positive in all animals tests	Leitgib [128]
5.	Soils contaminated with polyaromatic hydrocarbons and heavy metals of France	Earthworm mortality, inhibition of springtail population growth	Pheretima posthuma	Positive	Lors [129]

s.	Description of sample	Bioassay used	Plant used	Results obtained	Reference
No.				~	
1.	Agricultural soils of Fatehgarh Churian, Chabba and Botanical Garden, Amritsar	Allium cepa root anaphase aberration assay	Allium cepa	Positive	Katnoria [33]
2.	Soil samples of Northern China irrigated with long-term wastewater	Vicia faba root micronucleus test	Vicia faba	Positive	Song [66]
3.	Soil of farmland irrigated with wastewater, Queretaro, Mexico	Allum cepa root chromosomal aberration assay, Tradescantia stamen hair mutation assay and Tradescantia micronucleus assay	Allium cepa,Tradescantia	Positive	Cabrera [116]
4.	Soils of Ukraine contaminated by Chernobyl accident	Allium cepa root chromosomal aberration assay	Allium cepa	Positive	Kovalchuk [152]
5.	Soils from heavily polluted sites of Prague, Czech Republic	<i>Tradescantia</i> stamen hair mutation assay and <i>Tradescantia</i> micronucleus assay	Tradescantia	Positive	Gichner [153]
6.	Contaminated soils and shallow well water samples, USA	Allum cepa anaphase aberration assay, Tradescantia stamen hair mutation assay and Tradescantia micronucleus assay	Allium cepa, Tradescantia	Positive	Kong [154]
7.	Soil samples contaminated by chromium, Brescia, Italy	Vicia faba root choromosomal aberration assay	Vicia faba	Positive	Monarca [155]
8.	Heavy metal contaminated soils of Vienna, Austria	Tradescantia micronucleus assay	Tradescantia	Positive	Majer [156]
9.	Soil samples of electronic-waste recycling area, Hangzhou, China	Vicia faba root micronucleus test	Vicia faba	Positive	Jan-hui [157]
10.	Soil samples from industrial site, France	Vicia faba root micronucleus test	Vicia faba	Positive	Marcato- Romain [158]
11.	Military and urban territories, Lithuania	<i>Tradescantia</i> stamen hair mutation assay and <i>Tradescantia</i> micronucleus assay	Tradescantia	More genotoxicity in <i>Tradescantia</i> micronucleus test	Cerniene [160]
12.	Soil samples of France	Vicia faba micronucleus test	Vicia faba	Positive	Foltete [161]

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II. SUMMARY

In recent years, soil has been contaminated via various sources viz. (i) release of industrial discharges (ii) dumping of solid wastes (iii) mining activities (iv) vehicular emissions and modern agricultural practices such as indiscriminate use of inorganic fertilizers and pesticides. All these have contributed to the degradation of soil ecosystem. It was observed during the study that anthropogenic activities all over the world have altered the physico-chemical as well as biological characteristics of soil ecosystems that further had resulted in the severe toxicities to various living organisms by ultimately targeting the gene pool. Hence, it is mandatory to analyze the soil quality in comprehensive manner by evaluating its physico-chemical and biological characteristics along with estimation of their probable toxic effects employing various bioassays.

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