Chapter 2

REVIEW OF LITERATURE

Soil is naturally occurring loose covering of broken rock particles that exists on earth’s surface. It is defined as collection of natural bodies occupying earth’s surface that supports plants and other living beings by providing primary requirements of life (Hesse, 1994). Soil consists of around 40% minerals, 23% air, 6% organic matter and 8% living organisms (Zaid, 2010). Soil is formed by a process called weathering where mineral rocks are broken down by various physical, chemical and biological processes. Physical processes involve freezing and thawing, heating and cooling, wetting and drying, erosion, chemical decomposition as oxidation, reduction, solution, hydrolysis and carbonation whereas biological actions include action of microorganisms, plants, animals and man (Donahue et al., 1971). The microorganisms also help in formation of soil grains from parent materials by direct or indirect actions (Alexander, 1961; Treshew, 1970).

It takes more than thousand years to from a thin layer of soil. Soil formation is largely affected by the type of parent material from which soil has been derived (Tan, 2014). Donahue et al. (1971) have classified the development of soils from parent materials as residual (that remain into place long enough for soil development), transported (mineral and rock fragments that move via different agents like water, wind, ice and gravity to the site of soil development) and cumulose (peats and mucks that develop in a place from plant residues).

The study of soil is of immense importance as it acts as a major ecosystem supporting the survival of all living beings. However, in recent years, soil has been exposed to various contaminants like application of pesticides (Maliszewaska-Kordybach and Smreczak et al., 2003; Gong et al., 2004; Li et al., 2006; Hao et al., 2008; Westbom et al., 2008; Antunes et al., 2010; Fang et al., 2010; Gonzalez et al., 2010; Hernandez-Soriano et al., 2000; Huang et al., 2010; Oyekunle et al., 2010), industrial effluents (Srivastava, 2001; Vidhya et al., 2001; Sharma et al., 2002; Abrol et al., 2003; Tariq et al., 2009), wastewater discharges (Aleem and Malik, 2003, 2005; Alam et al., 2009; Bacaloni et al., 2005), which 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 reported to be accumulated in the soil through these sources (Gimeno-Garcia et al., 1996; Mudakavi and Narayana, 1998; Burman et al., 2000; Srinavas and Kumar, 2001; Vidhya et al., 2001; Watanabe and Hirayama, 2001; Aleem and Malik, 2003; Aleem et al., 2003; Yokel and Delistraty, 2003; Katnoria and Nagpal, 2004; Shi et al., 2007; Katnoria et al., 2008; Alam et al., 2009; Chahal et al., 2014).

Further, heavy metals leach either into ground water or enter surface water thereby contaminating them or directly enter into the growing food crops (Janos et al., 2010). Crops and vegetables grown in soils contaminated with heavy metals are also reported to contain significant amount of heavy metals than those grown in uncontaminated soil (Marshall et al., 2007; Singh et al., 2011). It has also been revealed that plants have inherent tendency to uptake toxic substances including heavy metals from the soil that are subsequently transferred along the normal food chain. From here, heavy metals find their way into the human body by direct or indirect usage of respective crops.

Contaminants upon entering the human beings pose potential health hazards in terms of hypertension, fever, kidney disorders, DNA damage, cramps and respiratory disorders (Chandra et al., 2005; Bertin and Averbeck, 2006; Suciu et al., 2008). In addition to genetic damage, soil contaminants are reported to cause significant changes in DNA methylation, histone modifications and epigenetic silencing of gene expression (Salnikov and Zhitkovich, 2008). The literature survey revealed a large number of reports on different aspects of soil pollution, therefore, the review given under has been categorized accordingly.

2.1. Physico-chemical Analysis of Soil

The physico-chemical properties of soil vary due to integrated effects of climate and living beings acting upon parent material over periods of time (Grossman and Reinsch, 2002). It is well established that the study of physico-chemical characteristics of soil system is important to explore the composition of soil (Kelly-Quinn et al., 2003; Katnoria and Nagpal, 2004; Daly and Casey, 2005; Katnoria et al., 2008; Zaiad, 2010; Kebir and Bouhadjera, 2011; Pujar et al., 2012). The summary of literature on
estimation of physico-chemical parameters in different soil samples is described in Table 1.

The physico-chemical parameters have also been reported to largely influence the enzymatic behavior of soils. Aon and Colaneri (2000) also supported the fact that physico-chemical parameters viz., organic carbon, total nitrogen and water-filled pore spaces exhibited strong relationships with the enzymatic activities (acid and alkaline phosphatases, β-glucosidase, urease, FDA hydrolytic activity, dehydrogenase). Vekemans et al. (1989) suggested that physico-chemical characteristics such as organic carbon, total nitrogen, clay content and cation exchange property (CEC) were closely related to the biological measurements.

Bentham et al. (1992) estimated physico-chemical parameters of 12 soil samples of London. Different physico-chemical parameters of soil samples were found to be in the range as total carbon content (14 - 60 µg/ml), total nitrogen content (0.1 - 2.9 µg/ml), moisture content (0.12 - 0.72 g/ml), bulk density (0.54 - 0.98 g/ml), pH (4.0 - 7.2) and soil texture (4 - 40 w/w sand; 6 - 66 w/w silt; 2 - 40 w/w clay). McKee (1993) studied pH of soil samples collected from mangrove forest in south-west Florida at Rookery Bay near Naples and observed it to be in the range of 6.7 - 7.0. Cambardella et al. (1994) analyzed various physico-chemical characteristics of field soils of Boone Country, Iowa. Mean contents of all parameters were found to be as organic carbon (5377 g/m²), total nitrogen (454 g/m²), K (37 g/m²), Mg (94 g/m²), Ca (666 g/m²), bulk density (1.32 g/m³), pH (6.25) and soil texture (sand - 33 %, silt - 34 % and clay - 33 %).

Srinavas and Kumar (2001) carried physico-chemical analysis of agricultural soils of Vishakhapatnam. Different parameters were found in the ranges as pH (7.34 to 8.25), conductivity (0.23 - 0.44 mMhos/cm), organic carbon (0.077 - 0.132 mg/g), chlorides (0.034 - 0.037 mg/g), nitrates (0.061 - 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). Kelly-Quinn et al. (2003) studied physico-chemical characteristics of soils of Iran and documented the results as temperature (11.2 - 11.4 ºC), pH (8.29 - 8.34), conductivity (424 - 442 µS/cm), alkalinity (7.4 - 8.75 mg/l CaCO₃), total hardness (105.18 - 142.38 mg/l.
CaCO$_3$), dissolved oxygen (10.4 - 11.1 mg/l), oxygen saturation (96 - 105 %), 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).

Katnoria and Nagpal (2004) analyzed the physico-chemical parameters of 2 agricultural soils of Amritsar (India). They reported the results of their study as pH (7.32 and 7.58), alkalinity (0.80 and 0.72 meq/100 g), bulk density (1.25 and 0.66 g/cc), moisture content (5.59 and 18.9 %), water holding capacity (6.79 and 49.88 %), nitrates (0.39 and 0.12 mg/g), phosphates (1.86 and 1.58 mg/g) and potassium (0.07 and 0.14 mg/g). Daly and Casey (2005) estimated the phosphate content in soil samples collected from 7 grassland sites on the Johnstown Castle Estate in south-east Ireland. Soil samples were taken from different depths viz., 2, 5 and 10 cm depth. Total phosphate content was found to be in the range of 2.1 - 157 mg/g in 2 cm, 1.0 - 140.0 mg/g in 5 cm and 0.8 - 135.0 mg/g in 10 cm of soil depth.

Iqbal et al. (2005) studied physico-chemical characteristics of soil samples of Perthshire. They found all the parameters as available water content (9.27 – 11.56 %), sand (24.4 – 28.28 %), silt (58.54 – 60.99 %), clay (11.32 – 14.88 %) and organic matter (0.93 – 28.35 %). Katnoria et al. (2008) analyzed the physico-chemical characteristics of 5 agricultural soils of Amritsar (India). Different physico-chemical parameters were found in the range of 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).

Different physico-chemical parameters viz., pH, electrical conductivity, organic matter, nitrogen, phosphorous, calcium, magnesium, sodium and potassium of wetland soils of Nigeria were estimated by Udotong et al. (2008). They reported that soils were deficient in elements like nitrogen, phosphorous and potassium. Physico-chemical properties of 4 farm site soils in surrounding areas of Gujrat (India) were estimated by Joshi et al. (2009). Correlation between physico-chemical and microbial data for soil under rice cultivation was conducted by Doi and Ranamukhaarachchi (2009). They analyzed physico-chemical parameters like moisture, bulk density, pH, P, Ca, Mg, Na,
Al, H, organic matter and reported direct correlation of physico-chemical parameters with soil fertility and soil microbes.

Rabah et al. (2010) studied different physico-chemical parameters of soil samples of Sokoto, Nigeria. They reported the contents of different parameters as pH (7.36), temperature (33 °C), nitrogen (0.23 mg/g), magnesium (2.67 mg/g), phosphorous (5.60 mg/g), potassium (1960 ppm), calcium (76 ppm), organic matter (12 %) and cation exchange capacity (18.53 %). Zaiad (2010) carried physico-chemical analysis of soils from Al-Khums city, Libya. The range of different parameters was observed as soil moisture content (0.02 – 3.64 %), pH (8.12 - 8.60), soil electrical conductance (410 - 766 mS/cm), total dissolved solids (246 - 455 ppm) and chloride content (0.43 - 1.42 mg/g). All the samples were found to be of loamy sandy nature.

Kebir and Bouhadjera (2011) analyzed physico-chemical parameters of agricultural soils of Ghazaouel, Algeria. pH was found to be in the range of 5.05 - 7.29. Other physico-chemical parameters like total organic carbon, nitrogen content, C/N ratio, Na, K, Ca, Mg and CEC were observed in the range of 1.25 - 3.48 g/kg, 0.24 - 1.23 g/kg, 1.86 - 2.82, 0.89 - 5.04 g/kg, 1.35 - 12.05 g/kg, 10 - 238.28 g/kg, 0.88 - 4.89 g/kg, and 21.87 - 32.01 mol/kg, respectively. Pujar et al. (2012) carried out physico-chemical analysis of soil samples of Karnataka (India). They found different parameters to be in the range as pH (7.9 – 8.4), electrical conductivity (0.22 – 0.30 ds/m), nitrogen (135 – 160 Kg/ha), phosphorous (8.0 – 10.1 Kg/ha) and potassium (295 – 355 Kg/ha).

2.2. Analysis of Pesticides in Soil

Use of various pesticides has vigorously increased in agricultural crops after world war-II in order to enhance the world food production. Since then, there has been marked development of enormous pesticides belonging to different groups (Zhang et al., 2011). 98% of the sprayed insecticides and 95% of the herbicides were reported to reach destination other than their target species and thereby contaminate land, air and water (Vryzas et al., 2009). It is well documented that pesticides hinder the nitrogen fixation in soil which is the primary requirement for plant growth. Insecticides like DDT and its metabolites, methyl parathion and pentachlorophenol interfere with legume-rhizobium chemical signaling which decrease rate of nitrogen fixation and reduced crop yields (Lv
et al., 2010; Yang et al., 2012; Akan et al., 2013a). Most of the pesticides have been banned due to their carcinogenicity and endocrine disrupting properties (Westbom et al., 2008; Yang et al., 2012)

Different pesticides like HCHs (hexachlorohexanes), DDTs (dichlorodiphenyltrichloroethanes), aldrin, dieldrin, endrin, chlordane, heptachlor have been reported to cause cancer, nervous system damage and reproductive disorders including damage of immune system in human beings (Westbom et al., 2008; Rissato et al., 2006; Yang et al., 2012). Table 2 summarizes the reports available on contamination of soils by pesticides.

2.2.1. Analytical techniques for Detection of Pesticides in Soil

The literature survey has revealed many reports on estimation of pesticides in soil samples using various analytical methods from different regions of world. Among different analytical techniques used, gas chromatography and liquid chromatography were explored as most convenient for detection of pesticides.

2.2.1.1. Gas Chromatography (GC)

It is the most extensively used technique worldwide for estimation of different groups of pesticides in soil (Lesueur et al., 2008). There are number of reports conducted worldwide which reflect the fidelity of gas chromatography technique for estimation of a large group of pesticides (Gonzalez et al., 2010; Lv et al., 2010; Yang et al., 2012). In a comparative study, Lesuer et al. (2008) analyzed 24 pesticides in soil samples of Austria using gas chromatography as well as liquid chromatography and stated that limits of detection (LODs) achieved with gas chromatography were much lower than those achieved with liquid chromatography.

Feng et al. (2003) examined organochlorine pesticides residues (DDT and HCH) in soils of China using GC-MS. OCP residues in the soil samples were found to be in the range of 0.1 – 5.3 µg/kg for DDT and 0.4 - 4.5 µg/kg for HCH. The study revealed that the concentrations of OCPs were far below the maximum residue limits (< 0.05 mg/kg) of National Environmental Standards for agricultural soils for both DDT and HCH. Gong et al. (2004) studied the level and distribution of DDT in surface soils from
Tianjin, China using gas chromatography. They collected 188 samples and estimated content of DDT and its metabolites. 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 27.5 ng/g and 18.8 ng/g, respectively.

Zhu et al. (2005) estimated organochlorine pesticides (DDTs and HCHs) in soils of China. They collected soil samples from shallow as well as deeper layers of soil and found that the residue levels of DDTs and HCHs in the deep layer soils were lower than those in the shallow subsurface soils. Li et al. (2006) detected the concentration, enantiomeric compositions and source of HCHs, 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 chromatography-mass spectrometry (GC-MS) and observed that the mean concentrations of total HCHs and DDTs in various samples descended in order of crop soils > paddy soils > natural soils. The total HCH concentration in crop soils ranged from 0.11 – 1.08 ng/g while DDT ranged from 0.46 – 20.9 ng/g.

Hao et al. (2008) estimated the contents of pesticides viz., hexachlorocyclohexanes (HCHs), dichlorodiphenyltrichloroethanes (DDTs), dichloro-2,2-bis (p-chlorophenyl)-ethane (p,p'-DDD), 1,1 dichloro-2,2-bis (p-chlorophenyl) ethylene (p,p'-DDE), endosulfan, dieldrin, endrin, hexachlorobenzene (HCB) and pentachloronitrobenzene (PCNB) in soil samples collected from paddy and vegetable field of Yicheng town, Yixing city in 2003. All soil samples were collected from surface layer (0 - 15 cm) and sub layer (15 - 30 cm). Each sample was a composite of 8 - 10 sub samples that were mixed, sieved (2 mm) and freeze dried. The pesticides were estimated using GC/µECD. The concentration of total OCPs in all soil samples ranged from 15.5 - 56.8 µg/kg while that of DDTs (including \( \alpha \), \( \beta \) and \( \gamma \)) in all soil samples ranged from 6.2 - 36.9 µg/kg. The value of total HCHs was in the range of 5.7 - 12.3 µg/kg.

The contamination of soil with organochlorine pesticides of Southern Mexico was estimated by Wong et al. (2008). 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 - 360 ng/g whereas TOX ranged from 0.06 – 69.0 ng/g. Westbom et al. (2008) estimated the content of organochlorine pesticides in soil samples collected from 12 farms of Upper Awash Agro Industry Enterprises (UAAIE), Ethiopia. They performed both quantitative and qualitative analysis using a dual column gas chromatography-electron 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 hepatachlord). The mean concentrations of Endosulfan and DDTs were reported as 56000 ng/g and 230 ng/g, respectively.

Nishina et al. (2010) analyzed pesticide residues in agricultural soil samples near Red River delta of northern Vietnam and found high range of DDT (0.03 – 0.30 ng/g). The contents of pesticide were found to be high in clayey soils as compared to sandy soils. They further studied the uptake of DDT in the crop samples. Although, the level of DDT in crop samples was low, the study created an alarm evidence of pesticide contaminated soils of Vietnam. Gonzalez et al. (2010) observed the contamination of ground water due to leaching of organochlorine pesticides from agricultural fields of Argentina. They also estimated contents of pesticides in soil samples and reported that levels of DDT were very high ranging from (46.5 - 38.1 µg/g). The predominance of p,p’-DDE residues reflected an extensive use of DDT. Pampean soil showed lower OCP levels as 0.039 - 0.07 µg/g. Huang et al. (2010) also reported the occurrence of organochlorine pesticides in soil using isotope dilution-high resolution gas chromatography (HRGC).

Lv et al. (2010) assessed 20 organochlorine pesticides (OCPs) in 80 suburban soil samples of Tianjan, China using GC-ECD. They revealed that seven pesticides viz., p,p’-DDE, p,p’-DDD, o,p’-DDT, o,p’-DDD, hexachlorobenzene, dicofol and β-HCH were frequently detected in all soil samples while frequencies of other pesticides were observed in the range of below detection limit (aldrin, dieldrin and endrin) - 34.5 % (p,p’-DDT). Estimation of organochlorine pesticides (OPPs) in 40 agricultural soil samples of Oke-Osun farm settlement, Osogbo, Nigeria was done by Oyekunle et al.
Solid-liquid extraction was used to extract OCPs 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 *et al.* (2011) studied OCPs in soil and sediment samples of southeast China. Different OCPs detected were $\alpha$-HCH, $\beta$-HCH, $\gamma$-HCH, $o,p'$-DDT, $p,p'$-DDE, $p,p'$-DDD, $p,p'$-DDT, $\alpha$-endosulfan, $\beta$-endosulfan, cis-chlordane and trans-chlordane. Among all the pesticides studied, DDT was found to be the most dominant source of contamination. Kumar *et al.* (2011) analyzed persistent organochlorine pesticides (OCPs) and polychlorinated biphenyls in intensive agricultural soils of north India. The average concentration of OCPs was detected as 37.67 ng/g while 0.95 ng/g, 0.16 ng/g and 13.44 ng/g for endosulfan, dieldrin and PCBs, respectively.

Yang *et al.* (2012) estimated residues of OCPs in 93 agricultural soils of Zhangzhou city, China. DDT was found to be in the range of 0.64 - 78.07 ng/g and in the order of paddy fields > vegetative lands > tea plantations > orchards while HCHs were found to be in the range of 0.72 - 30.16 ng/g and in the order of and tea plantations > orchards > paddy fields > vegetative lands. Akan *et al.* (2013) analyzed organochlorine pesticides (dichlorvos, dianzinon, chlorpyrifos and fenitrothion) in agricultural soil samples of Alau dam and Gongulong areas of Borno state, Nigeria using GC-ECD. Concentrations of all pesticides were observed to be maximum at a depth of 21 - 30 cm while minimum at a depth of 0 - 10 cm. Contents of pesticides in all soil samples were found to be present at alarming rates i.e. 0 - 10 cm (57.98 - 232.98 µg/g), 11 - 20 cm (78.92 - 264.98 µg/g) and at 21 - 30 cm (88.98 - 287.89 µg/g).

Although the techniques play a major role for estimation of pesticides in different environmental mixtures but their proper extraction is very important. For analysis of pesticide residues, the proper extraction method can help to extract the pesticides residues even if they are present in traces. In context to this, different extraction techniques have been optimized for the extraction of sample. The most
extensively used extraction methods for detection of pesticides are categorized as follows:

2.2.1.1. Sample Preparation Methods

Different sample preparation methods were used by authors for extraction of pesticide residues are as follows:

2.2.1.1.1. Soxhlet Extraction (SOE)

Soxhlet extraction method was invented by Frenz von Soxhlet in 1879. This method is generally required where the desired compound has a limited solubility in a solvent due to impurities present in the analyte. This method is an exhaustive and one of the traditional techniques used for the extraction of various pesticides in soil and has been applied for many years to analyze a large group of analytes (Rissato et al., 2005). Due to high recovery rates, it has been widely adopted as a reference method for soil extraction (van der Hoff and van Zoonen, 1999). Fang et al. (2010) examined organochlorine pesticide (OCP) 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) along with 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. The content of OCP was reported to be in the range of 0.20 - 10.3 µg/kg.

However, this technique was found to have certain limitations as it was more laborious, time and solvent consuming (Rissato et al., 2005). Later on, various conventional methods came into existence such as sonication, ultrasonic solvent extraction (USE), superficial fluid extraction (SFE), pressurized liquid extraction (PLE), microwave assisted extraction (MAE), single drop microextraction (SDME) etc. which were found to have more precision, low solvent consuming, less laborious with good recovery rates and were economic (Goncalves et al., 2006; Mohammed et al., 2011; Salemi et al., 2013).
2.2.1.1.1.2. Superficial Fluid Extraction (SFE)

Superficial Fluid Extraction (SFE) involves the separation of extractant from the matrix using superficial fluids as extracting solvent. This method has emerged as a conventional technique for estimation of organic compounds in solid samples (Goncalves et al., 2006; Sunarso and Ismadjik, 2009). This method exhibits several unique properties viz., high selectivity, less solvent consumption. Rissato et al. (2005) studied the fidelity of SFE method in extraction of multiresidues (including organohalogen, organonitrogen, organophosphorous and pyrethroids) in soil samples of California using gas chromatography-mass spectrometry (GC-MS) coupled with electron-capture detector (ECD) by adopting three extraction methods viz., solid liquid extraction (SLE), SFE and solid pressurized extraction (SPE). The authors considered SFE as the most reliable technique for estimation of a wide range of pesticide residues in soil and ECD as the best detector in conjugation with proposed method to develop fast, efficient and consistent results as it reflected good precision (RSD < 7 %) and with average recoveries of > 80 %.

Goncalves et al. (2006) also optimized SFE technique for the extraction of pesticide residues (both OPPs and OCPs) in soils of Portugal using gas chromatography. The method resulted in high precision (4.2 - 15.7 %) and extraction efficiency rates (80.4 - 106.5 %) of selected pesticides. LODs were reflected with excellent detection capacity as 0.1 - 3.7 µg/kg. The study suggested SFE as an attractive technique for the estimation of mutiresidues in soil samples when coupled with GC-MS-MS. However, this method has been left behind by many authors due to evaporation of collected solvent at the end of extraction to get high preconcentration factor (Rezaee et al., 2006; Berijani et al., 2006; Mohammed et al., 2011). Also, this procedure has been recognized to be more time-consuming and degradation of collected analytes (Mohammed et al., 2011).

Later on, some studies were conducted with certain modifications of SFE technique especially SFE in conjunction dispersive liquid-liquid microextraction (DLLME) which provided more constructive results such as consuming less organic solvent and resulting in very high enrichment factors (Rezaee et al., 2006; Berijani et al., 2006). DLLME
involves the rapid injection of suitable dispersive solvent containing extractant solvent into aqueous phase. Combination of SFE and DLLME was primarily used by Rezaee et al. (2006) for the extraction of poly aromatic hydrocarbons in marine samples. Since then, the combination was adopted for extraction of pesticides from solid samples.

Mohammed et al. (2011) combined SFE and DLLME techniques for the first time to extract OPPs (o,o,o-triethyl phosphorothioate, thionazin, sulfotep, disulfoton, methyl parathion, parathion and famphur) from soil samples of Iran. Coupling of both the methods resulted in very high extraction recovery and pre-concentration factor. The extraction recovery rate for all the target analytes were found in the range of 44.4 - 95.4 % with relative standard deviation (RSD) below 7.5 %. The method was validated as the most efficient to study selected pesticides as it provided LODs in the range of 0.001 - 0.009 mg/kg.

2.2.1.1.1.3. Single Drop Microextraction (SDME)

SDME has been proved to be a very proficient liquid phase microextraction technique as it combines extraction and concentration in a minimum amount. It is quick, easy to handle, affordable technique that consumes very little solvent (Salemi et al., 2013). This method involves the suspension of a micro-drop of the solvent at the tip of needle of a GC micro-syringe. Salemi et al. (2013) determined five organophosphorous pesticides (thionazin, sulfotep, dimethoate, disulfoton and parthion) in soil samples of Iran. They developed SDME extraction method for detection of organophosphorous pesticides by using gas chromatograph with nitrogen-phosphorous detector (NPD). They reported SDME as a versatile procedure for analysis of OPPs in soil media when coupled with GC-NPD which provided LODs and LOQs in the range of 0.1 - 2.0 ng/g and 0.3 - 6.6 ng/g, respectively. Enrichment factors were obtained as maximum for sulfotep (12.7) and minimum for parathion (1.4). SDME was developed as the most promising, sufficiently sensitive and highly reproducible technique for estimation of OPPs.

2.2.1.1.1.4. Head Space-Solid Phase Micro extraction (HS-SPME)

Solid Phase Microextraction (SPME) emerged as a solvent-free analytical technique which was widely accepted as a reliable method for quantitative detection of volatile organic compounds as well as polycyclic aromatic hydrocarbons (PAHs) (Potter and
Pawliszyn, 1992; Arthur et al., 1992; Langenfeld et al., 1996). The fidelity of this method persisted in its simplicity to extract compounds, low detectable limits (LODs) and good reproducibility (Doong and Liao, 2001).

SPME is of increasing interest for the detection of a large group of pesticides in aqueous solutions (Beltran et al., 1998) but the literature with reference to soil is very scarce. Only few reports are available for determination of OPPs and herbicides in soil samples (Ng et al., 1999; Hernandez et al., 2000). The probable reason for the same was coded as fiber instability and less sensitivity of the proposed method in soil systems (Guan et al., 1998). Therefore, to overcome all these defects, Head Space-Solid Phase Micro extraction (HS-SPME) technique has been developed for analysis of pesticides in soil. Doong and Liao (2001) determined 18 OCPs and their metabolites in soil samples of Taiwan and compared the results obtained with Soxhlet extraction method. The proposed method provided a great linearity of OCPs between 0.2 and 4 ng/g and relative standard deviation was also found to be lower than 25 %. The mean concentration of all studied analytes was found in the range of 16.5 - 1459.6 mg/kg with recovery rates of 68 - 127 %.

2.2.1.1.5. Microwave-assisted Extraction (MAE)

MAE method was first introduced by Ganzler et al. (1986) and has been successfully implemented to extract organic compounds from various environmental mixtures. The proposed extraction procedure is simple, time and cost effective (Perez et al., 2012; Merdassa et al., 2013). Fuentes et al. (2006) analyzed four different pesticides viz., trifuralin, metolachlor, chlorpyrifos and triadimefon in agricultural soils using gas chromatography electron capture detection (GC-ECD). They used microwave-assisted 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 tetradiodon) and observed high recovery range. They recommended the MAEP method as a simple, convenient and sensitive method to determine hydrophobic pesticides even at very low levels.
Al-Wabel et al. (2011) monitored pesticide residues in agricultural soils of Saudi Arabia. They collected 15 soil samples from analyzed various groups of pesticides viz., organochlorines, organophosphorous, carbamates, pyrethroids and herbicides. 12 pesticides were detected in soil samples of selected regions which were found in the range as dimethoate (0.007 - 0.700 mg/kg), chloroneb (0.011 - 0.400 mg/kg), methomyl (0.018 - 0.575 mg/kg), oxamyl (0.014 - 0.150 mg/kg), toxaphen (0.006 - 0.162 mg/kg), p,p'-DDT (0.080 - 0.575 mg/kg), p,p'-DDE (0.1 - 0.525 mg/kg), monocrotophos (0.021 - 0.047 mg/kg), chlorpyrifos (0.12 - 0.24 mg/kg), diazinon (0.024 mg/kg), cypermethrin (0.012 - 0.205 mg/kg) and lindane (0.290 - 394.0 mg/kg). LODs of all pesticides were observed to be from 0.0001 - 0.004 mg/l while RSD was below 2.48 %. MAE was concluded as a versatile method for the extraction of 13 selected pesticides of various groups in soil.

Merdassa et al. (2013) also determined OPPs and fungicides in soils of Ethiopia using MAE method by gas chromatography-mass spectrometry (GC-MS). The precision of this technique was evaluated in terms of repeatability and reproducibility. The extraction method provided recovery ranges of 70 - 120 % for all selected analytes. The method was found to be linear over 1 - 250 ng/g with correlation coefficient (r²) of 0.991 and 0.996 and LODs between 0.10 - 0.12 ng/g. They suggested MAE as an ideal technique for the extraction of OPPs and fungicides from different soil samples.

2.2.1.1.6. Sonication Extraction (SE)

Sonication offered a more efficient contact between the solid and solvent over other methods of extraction (Babic et al., 1998). Sanchez-Brunete et al. (2004) examined 52 mutiresidues in soils of Spain by sonication method using gas chromatography-mass spectrometry (GC-MS). They found that the proposed method was capable to detect all the pesticides at good recovery rates (87.0 - 106.2 %) as well as LODs (0.02 - 1.6 µg/g). Also, the developed method was linear over the range of analytes, 25 - 1000 µg/L with determination coefficients greater than 0.999. Further, no subsequent cleanup was required as samples were extracted with very low volume of solvent with negligible interferences. The proposed method was recommended to be fit for analysis of various groups of pesticides in agricultural soils.
2.2.1.1.7. Ultrasonic-solvent Extraction (USE)

Although sonication provided good detection results but the same technique had certain limitations as it was time consuming (Babic et al., 1998). Therefore, the modern techniques have been implicated to solve the time and solvent consuming problems as an alternative to traditional methods. USE is an extraction technique which is proficient to extract organic analytes from the soil system but very rare reports are available focused on this method (Banjoo and Nelson, 2005). Tor et al. (2006) successfully applied the proposed method for detection of organochlorine pesticides (OCPs) in soil. They studied 13 different OCPs namely α-, β-, γ- and Δ-HCH, heptachlor, aldrin, o,p’-DDE, dieldrin, p,p’-DDE, p,p’-DDT, methoxychlor and mirex in soils of Turkey by using gas chromatography (GC) equipped with electron capture detector (ECD) and found recoveries above 88 % with relative standard deviation (RSD) below 6 %. They also compared the results obtained with shake-flask to those from soxhlet extraction methods and concluded that the proposed technique was more rapid and conventional than the previous ones. The authors suggested that the time and solvent consumption was reduced up to 82 % and 67 %, respectively.

2.2.1.1.8. Pressurized Liquid Extraction (PLE)

PLE technique was developed by Richter (1995) and has been emerged as a convenient method for the extraction of organochlorine pesticides from different matrices including soil system over other methods due to its fidelity to extract residues with very low LODs (Gan et al., 1999; Hubert et al., 2000). Concha-Grana et al. (2004) estimated the contents of 21 organochlorine pesticides in soils of Spain using three different methods of extraction viz., PLE, MAE and Soxhlet method using gas chromatography coupled with ECD. PLE was observed as most efficient method as it provided recoveries in the range of 0.1 - 1.0 µg/g for all the pesticides analyzed than those obtained in rest two methods. PLE provided most satisfactory results for the extraction of OCPs in soil samples.

Hussen et al. (2006) analyzed three OCPs viz., α-endosulfan, β-endosulfan and endosulfan sulfate in contaminated soils of Ethiopia using PLE and Soxhlet extraction methods. They found PLE as more exhaustive technique than Soxhlet extraction as it
provided highly reproducible results for the extraction of endosulfans. Apart from OCPs, the PLE technique has also been applied to extract some OPPs. Diaz-Cruz and Barcelo (2006) studied two OPPs (chlorpyrifos and diazinon) in the sludge and sludge-fertilized agricultural soils of Spain. The authors optimized PLE as a less time consuming and cost-effective extraction approach and found that the proposed method showed good recovery yields for target pesticides (>92%). LODs were also detected in the range of 4 - 62 ng/g and 3 - 31 ng/g for sludge and sludge-fertilized soils, respectively. Schreck et al. (2008) also developed PLE as a rapid multiresidue method for determination of 7 pesticides in agricultural soils of France. The target compounds were 2 insecticides (chlorpyrifos-ethyl and λ-cyhalothrin), 2 herbicides (flazasulfuron and flumioxazin) and 3 fungicides (metalaxyl, folpet and myclobutanil) from different groups of chemical compounds. The proposed method was quite efficient to extract selected analytes and gave recovery of 53.8 - 99.9%. LODs were observed in the range of 1.4 - 4.6 µg/kg. The authors validated PLE as a sensitive, easy to perform and cost-effective.

Although PLE has been established as a successful technique for the estimation of OCPs in soil but the interferences in sample cleanup was emphasized as the major drawback of this technique (Hussen et al., 2007) which required further steps for sample purification (Tekel and Hatrik, 1996; Mukherjee and Gopal, 1996). Many scientists have recommended Selective Pressurized Liquid Extraction (SPLE) for the extraction of samples over offline cleaning (Gomez-Ariza et al., 2002; de la Cal et al., 2003; Sporring and Bjorklund, 2004). Hussen et al. (2006) determined organochlorine pesticides (β-endosulfan, endosulfan sulfate, p,p’- DDT and p,p’- DDE) in Ethiopian soils using SPLE extraction method. They examined that recovery rates were over 80% in selected pesticides with PLE method as compared to simple offline cleanup. They obtained similar results in reference to recovery rates (80 - 90%) by applying SPLE method for other 13 OCPs. They suggested SPLE as a suitable method for multiresidue analysis of OCPs that increased the automation with no further requirement of sample clean up. Westbom et al. (2008) assessed 13 OCPs in Ethiopian soils using SPLE technique. SPLE was confirmed as an affordable technique due to its precision to detect endosulfans, cyclodienes, HCHs and chlorodiphenyl aliphats with good recovery rates.
2.2.1.1.9. Liquid Solid Extraction (LSE)

Liquid Solid Extraction (LSE) method has been adopted for the extraction of various classes of pesticides in soil. Durovic et al. (2012) carried out multiresidue analysis in soil samples of Serbia using LSE. They validated LSE method for detection of four pesticides (diazon, acetochlor, aldrine and carbofuran) and found it quite efficient to study the selected analytes. They reported recovery values and LODs of pesticides to be in the range of 89 - 95 % and 0.73 - 3.95, respectively.

2.2.1.1.10. QuEChERS Method

QuEChERS (quick, easy, cheap, efficient, rugged and safe) method was first introduced by Anastassiades et al. (2003). Anastassiades et al. (2003) analyzed the extracted samples using gas chromatograph with NPD detector. They concluded that recovery values for all the pesticides were quite high (45 - 96 %) than those obtained in basic method. QuEChERS method is based on acetonitrile extraction of samples and one of the most efficient methods used for the extraction of pesticides in soil (Rashid et al., 2010). It successfully met the alternative technique for extraction of pesticide residues in soil using gas chromatography as well as liquid chromatography (Lesueur et al., 2008). This method was validated to detect a large group of pesticides when used in conjugation with mass spectrometer (Shi et al., 2009).

Lesueur et al. (2008) estimated 24 pesticides in soil samples of Austria using four different extraction methods viz., ultrasonic solvent extraction, European norm DIN 12393, QuEChERS and pressurized liquid extraction. They recommended QuEChERS method as the most adapted method to study different types of pesticides as it presented the highest recoveries in recommended range of 70 - 110 %. Asensio-Ramos et al. (2010) evaluated a modified QuEChERS method for the extraction of 11 organophosphorous pesticides from soils of Spain. They extracted all the samples by slightly modifying the recommended doses of respective chemicals.

Rashid et al. (2010) studied 19 organochlorine pesticides in soils of United Kingdom by adopting QuEChERS method and Soxtec extraction. QuEChERS method provided mean recoveries of 70 - 100 % with RSD values at or below 20 % of all pesticides studied. The authors concluded that the QuEChERS method was more
sensitive as it produced cleaner extracts as compared to the Soxtec method. Further, it was also found to be less time and solvent consuming as it produced extracts in less than 3 h while those produced in Soxtec method was in 40 h. Therefore, the authors validated the QuEChERS method as an efficient extraction technique to analyze highly lipophilic compounds (organochlorine pesticides) at very low LODs. The cited reports prove the versatility of QuEChERS method to analyze the polar (organophosphorous) as well as non-polar (organochlorine pesticides) compounds (Lesueur et al., 2008; Asensio-Ramos et al., 2010; Rashid et al., 2010).

2.2.1.1.11. Matrix Solid Phase Dispersion (MSPD)

MSPD technique of sample extraction was introduced by Barker et al. (1989). This technique integrates the extraction and clean up procedures thereby reducing time and labor via omitting preliminary steps like filtration, centrifugation and moisture removal etc. The proficiency of MSPD in the sample extraction for estimation of different analytes has been supported by various reports (Kristenon et al., 2006; Pena et al., 2007). Literature survey has also revealed MSPD as a superior method to MAE for sample extraction as it was less affected by soil type and remained unchanged at optimum efficiency level (Pena et al., 2007; Salemi et al., 2012). Salemi et al. (2012) optimized MSPD coupled with gas chromatography-electron capture detector for determination of chlorinated pesticides in soil. They analyzed 16 different PAHs and OCPs following same extraction method and found that MSPD was capable to extract all the selected compounds. Recoveries for proposed analytes were calculated as > 75 % except for endrin aldehyde (59 %) and endrin sulfate (62 %). Further, the proposed method provided satisfactory results by consuming very less amount of sample (0.5 g) as well as solvent (< 10 ml).

2.2.1.1.12. Dispersive Solid Phase Extraction (DSPE)

Various drawbacks of the traditional method of extraction led to the standardization of modern techniques to analyze pesticides of various groups with more precision, limited time and solvent consumption. DSPE is one of the contemporary procedures for valid extraction of pesticides from various environmental mixtures (Anastassiades et al., 2003; Lehotay et al., 2005). However, the application of DSPE technique in the analysis
of agricultural soils was found to have certain limitations. Lesueur et al. (2008) used DSPE method for the estimation of 24 pesticides in soils using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-ion trap-mass spectrometry. Rashid et al. (2010) validated DSPE as a proficient alternative technique for the extraction of 19 OCPs from soil samples.

Pham et al. (2014) determined 103 trace pesticides including organophosphates, organochlorines, carbamates and pyrethroids in soil using DSPE extraction coupled to GC-MS. The study revealed that DSPE method was quite efficient to resolve the selected group of pesticides and produced recoveries of 65 - 117 % with RSDs below 19 %. LODs and LOQs were detected in the range of 1 - 13 µg/kg and 3 - 38 µg/kg, respectively. Further, the proposed method required less time and solvent, safer and easier for routine analysis of pesticides in soil systems as compared to other methods like Soxhlet extraction (SOE), Superficial fluid extraction (SFE), Microwave assisted extraction (MAE), Ultra sonication extraction (USE), Liquid solid extraction (LSE) and Pressurized liquid extraction (PLE).

2.2.1.2. Liquid Chromatography (LC)

Although majority of reports has revealed gas chromatography as an ideal technique for the estimation of pesticides in various environmental mixtures including soil (Paleologus et al., 2003; Partrolecco et al., 2004). Yet, the literature survey has reflected some studies where a large group of pesticides were detected using liquid chromatography (Herrera-Herrera et al., 2010; Rezae et al., 2010). Further, several groups of pesticides are more accurately detected using liquid chromatography in conjugation with different detectors (Sanchez-Brunete et al., 2003). This particular segment of review deals with the reports concerning exploration of pesticides using liquid chromatography (LC). LC also involves various extraction procedures before injecting to analytical instruments. The following part of review summarizes the reports on estimation of pesticides from contaminated soils using different extraction methods with liquid chromatography technique.

2.2.1.2.1. Sample Preparation Methods

The details of some of the sample preparation methods are as here under.
2.2.1.2.1.1. Sonicated Assisted Extraction (SAE)

Sonication is one of the most traditional extraction procedures used since ages for the determination of a large group of pesticides. Sanchez-Brunete et al. (2003) studied six carbamate pesticides (oxamyl, methomyl, propoxur, carbofuran, carbaryl and methiocarb) in agricultural soils of Spain. They preferred High-performance liquid chromatography (HPLC) over other techniques like GC as carbamate compounds have a great tendency to break down under column GC conditions due to their highly instable nature. Further, they opted fluorescence detector (FD) coupled with HPLC after post column derivatisation. The selected technique provided good response linearity ($r^2 > 0.98$), high precision recovery (82 - 99 %) and very low detection limits (1.6 - 3.7 µg/kg). The proposed method was remarked as a rapid and sensitive method for the extraction and analysis of carbamate insecticides in agricultural soils.

2.2.1.2.1.2. Microwave-assisted Solvent Extraction (MASE)

Elbert et al. (2001) followed MASE method for extraction of ten acidic pesticides of different chemical classes (bentazone, bromoxynil, metsulfuron, metsulfuron-methyl, 2,4-D, MCPA, MCPP, 2,4-DP, 2,4,5-T, a,4-DB and MCPB) in soils of Netherland by reverse-phase liquid chromatography (RPLC) coupling with UV detector. They revealed that the combination of MASE and couples-column RPLC-UV by using an analytical restricted access medium (RAM) was a realistic approach for the quantitative detection of acidic group of pesticides. MASE was also found to offer an improved selectivity and hindrance during extraction as it provided recoveries between 60 - 90 % with relative standard deviation (RSDs) of 5 - 25 %.

2.2.1.2.1.3. Microwave-assisted Micellar Extraction (MAME)

Microwave-assisted (MA) methodologies have been adopted for various scientific applications for past three decades since Ganzler et al. (1986) used microwave ovens for extraction of anti-nutritive compounds from plant materials. Later on, microwave assisted extraction (MAE) was developed as a conventional method for detection of a large group of chemical compounds from several mediums due to less time consumption (Garmouma and Poissant, 2004).
However, later on MAME technique was found to be inappropriate because it required more organic solvents. In order to avoid the more consumption of these extractants, biodegradable micellar media was used as an extractant with MAE (Paleologus et al., 2003; Partrolecco et al., 2004). This combination with MAE with micellar media (MAME) was proved to be a simple, fast, low cost and affordable alternative for the extraction of different contaminants from solid matrices including soil as well (Pino et al., 2000). Padron-Sanz et al. (2005) pursued MAME for the determination of 8 organophosphorous pesticides (OPPs) in soil samples of Spain using liquid chromatography with UV detector. All the studied samples were observed to show > 70 % recovery for selected analytes with RSDs below 2.6 %.

Moreno et al. (2006a) extracted six organochlorine pesticides (OCPs) viz., 4,4’-DDD, dieldrin, 4,4’-DDT, 2,4’-DDT, 4,4’-DDE and aldrin in agricultural soils of Spain using MAME method by HPLC-UV. The method was quite successful to extract all the selected OCPs inspite of their lipophilicity and hydrophobic nature. The recovery rates of selected analytes with LODs varied between 80 - 800 ng/g. In another report, Moreno et al. (2006b) determined 5 organochlorine pesticides in soil samples of Spain by combining MAME with solid phase microextraction (SPME) using HPLC-UV. The coupling technique was proved to be more efficient as it increased the proficiency of recovery (70 - 110 %), reproducibility and low LODs. The combination of MAME using surfactant as extractant with SPME proved to be a viable approach over other techniques as the developed methodology was less time and solvent consuming and was cost effective.

### 2.2.1.2.1.4. Ionic-liquid Dispersive liquid-liquid Microextraction (IL-DLLME)

A suitable derivatization is required to detect fluorescent compounds. IL-DLLME technique has been developed considering these circumstances and is relatively young LPME technique (Herrera-Herrera et al., 2010; Rezae et al., 2010). In liquid chromatography, most commonly used detectors are ultraviolet detector (UV) and diode array detection (DAD). While in HPLC, Fluorescent detector (FD) is used to detect the selective group of pesticides which are fluorescent in nature (Asensio-Ramos et al., 2011). However, this method has been rarely applied for extraction of organic
analytes from soils (Wu et al., 2009; Hu et al., 2009). Asserino-Ramos et al. (2011) first applied IL-DLLME method for the extraction of 6 native fluorescent pesticides (carbendazim/benomyl, thiabendazole, fuberidazole, carbaryl and triazophos) and their key metabolites in soils of Spain using LCMS coupled with fluorescent detector (FD). The authors observed that proposed method resulted in recoveries of pesticides in the range of 90 - 119 %. LODs and LOQ values were also examined to be very low 0.02 - 27.07 ng/g and 0.06 - 90.25 ng/g, respectively. They validated the proposed method to be a very simple, quick, effective, highly repeatable and reproducible with proficient precision.

2.3. Heavy Metal Analysis of Soil

Contamination of soils by accumulation of heavy metals from different sources has engrossed a great attention worldwide in recent years (Katnoria et al., 2011). Heavy metals in soil are of special concern because they do not degrade naturally and retain in soil even after thousands of years (Vidhya et al., 2001; Abrol et al., 2003). Apart from this, some proportions of heavy metals leach into ground water or enter surface water bodies and agricultural lands through surface run offs, thereby contaminating the food crops (Srivastava, 2001; Alam et al., 2009). Many studies and surveys have been conducted to assess the heavy metal content in soil ecosystem (Ho and Tai, 1988; Fatoki, 1996; Sanchez-martin et al., 2007; Kebir and Bouhadreja, 2011; Masona et al., 2011; Liu et al., 2011a; Chanda et al., 2011). The emphasis has been given on the hazardous effects of various heavy metals like arsenic, cadmium, copper, cobalt, lead, manganese, mercury, nickel and zinc on living beings by some authors (Alam et al., 2009; Tariq et al., 2009).

Human activities have been documented to be the major cause for heavy metal contamination of the soil ecosystem (Al-Khashman, 2006; Banat et al., 2005; Chen et al., 2009; Kasassi et al., 2008). Mining processes, iron and steel industries, transportation, open disposal of waste and evitable use of inorganic fertilizers, pesticides on to the agricultural lands are some of the anthropogenic activities that directly influence the content of heavy metals in soil system (Hutton and de Meeûs, 2001; Hansen et al., 2002, Lado et al., 2008). In addition to this, emissions from
volcanoes, degassing processes in the earth's crust, forest fires or chemical composition of the parent material are other factors responsible for contamination of soil with heavy metals (Lokke *et al.*, 1996; Palumbo *et al.*, 2000; Luo *et al.*, 2007).

Heavy metal pollution is more prevalent in agricultural soils as compared to other soil types due to continuous application of agrochemicals viz., fertilizers and pesticides that are used in order to obtain high yield of the food crops. The problem of heavy metal contamination is further severe in agricultural fields near industries that release industrial effluents rich in acidifying compounds and metal ions (Salemaa *et al.*, 2001; Lin *et al.*, 2002; Clemente *et al.*, 2003). Heavy metals present in industrial waste migrate *via* different routes viz., water, soil sediments and air to nearby agricultural lands and thus become a source of heavy metal pollution in agricultural soils (Cappuyns *et al.*, 2004; de Vries *et al.*, 2005). The metals like Cd, Cu, and Zn have been reported to be transported into the soil through industrial emissions by Schindler *et al.* (1980) and Steinner (1990). Vehicular pollution also participates in enhancing heavy metal pollution of soil ecosystem (Nadal *et al.*, 2004). Although traces of some metals are required for the plant growth, but prove fatal, if present beyond their maximum permissible limits (Freitas *et al.*, 2010).

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 in different parts of the world.

The presence of heavy metals in rice farming soils was documented by Gimeno-Garcia *et al.* (1996). The authors reported that among various fertilizers used for rice farming soils, superphosphates contained maximum contents of Cd, Co, Cu and Zn as impurities. They also documented that the applied inorganic fertilizers to be rich in Mn, Zn, Co and Pb. Romic and Romic (2003) studied heavy metal distribution in agricultural top soils in an urban area. They estimated 8 heavy metals and found mean contents of metals as Cd (0.66 mg/g), Cu (20.8 mg/g), Fe (27.041 mg/g), Mn (613 mg/g), Ni (49.5 mg/g), Pb (25.9 mg/g) and Zn (77.9 mg/g).

Srivastava (2001) estimated heavy metal content in industrial sludge amended soils of India. All samples were found to contain high heavy metal contents which were in
range of Cd (0.026 - 0.045 µg/g), Cu (0.99 - 1.19 µg/g), Pb (0.30 - 1.72 µg/g) and Zn (1.68 - 2.97 µg/g). Abrol et al. (2003) estimated heavy metal contents in soils of Jammu (India) and observed average concentration as Cd (14 mg/kg), Cu (2.35 mg/kg), Fe (16.04 mg/kg), Mn (5.83 mg/kg) and Zn (1.42 mg/kg). Srinivas and Kumar (2001) studied the heavy metal content in various agricultural soils of Vishakhapatnam (India). The content of Pb was found to be in the range of 0.8 - 45.0 mg/g, Cd (0.16 - 5.4 mg/g), Zn (3.8 - 60 mg/g), Ni (30 - 70 mg/g), Cu (2.6 - 72 mg/g), Mn (482 - 535 mg/g) and Fe (0.39 - 0.48 mg/g).

Vidhya et al. (2001) estimated heavy metal content in 11 agricultural soil samples of Vishakhapatnam (India). Concentrations of different heavy metals in soils were found to be in range of Cd (0.51 - 2.01 mg/kg), Co (3.9 - 17.7 mg/kg), Cr (31.2 - 0.8 mg/kg), Ni (11.5 - 41.7 mg/kg), Pb (6.5 - 9.5 mg/kg) and Zn (123 - 356 mg/kg). Singhal et al. (2003) studied absorption, mobility and distribution of three heavy metals (Pb, Cr and Cd) in two soils, one from riverbed and other from agricultural land of India. They reported that the agricultural soil exhibited higher metal sorption capacity as compared to the river sand. The order of mobility of different metals studied was observed as Cr > Cd > Pb.

Abollino et al. (2002) analyzed heavy metal contents in 5 agricultural soils of Piedmont, Italy. They analyzed 10 heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti and Zn) and found significant content of Pb and Cr in all the soil samples analyzed. Aydinalp and Marinova (2003) studied distribution of different heavy metals viz., Cd, Cu, Pb, Mn, Ni and Zn in agricultural soils of Bursa, Turkey. They detected various heavy metals following a fractional scheme based on sequential extraction developed by Emmerich et al. (1977). They observed that total heavy metal content of soil was higher than the threshold levels reported in literature from similar soils. Summary of literature on estimation of heavy metals in different soil samples is described in Table 3.

Vidal et al. (2004) studied the origin and behavior of heavy metals in agricultural soils of Spain for 6 subsequent years. During their study, Pb concentration was found to be lower in samples collected during initial samplings i.e. 19.4 ppm in 1993 and 16.4 ppm in 1996 but the same samples contained very high content of Pb.
(685 ppm) during last sampling in 1999. The authors stated that soil samples were previously unaffected by any anthropogenic inputs of Pb which were later on enhanced by substantial increase in application of fertilizers and traffic emissions. Cicek and Koparal (2004) estimated the heavy metal contents in soils of Turkey. They observed the contents of heavy metals to be in the range of Cd (1.4 - 12.7 mg/kg), Cr (32 - 277 mg/kg), Cu (12.6 – 261.3 mg/kg), Fe (564- 1650 mg/kg), Pb (15 – 401 mg/kg), Ni (20.1 - 372 mg/kg) and Zn (18 - 784 mg/kg).

Sichorova et al. (2004) estimated the contents of heavy metals in soils of Czech Republic. They observed that the contents of Cd and Pb were as much as 13 mg/kg and 2500 mg/kg, respectively in top soil whereas 6 mg/kg and 400 mg/kg in deep layer of soil, respectively. Cui et al. (2004) studied the levels of heavy metal contamination in soils of Nanjing, China. They observed the contents of heavy metals to be in the range of Cd (0.87 - 22.06 mg/kg), Cu (4.01 - 38.97 mg/kg), Fe (9669 – 14612 mg/kg), Pb (16.1 – 991.59 mg/kg) and Zn (29.7 - 596.7 mg/kg). They further reported that the concentration of Pb was found to be more than the threshold limits decided by Unites States Environmental Protection Agency (USEPA) in 2007.

Lokeshwari and Chandrappa (2006) analyzed seven heavy metals (Cd, Cr, Cu, Fe, Pb, Ni and Zn) in soils of Bangalore (India). They reported that the average concentrations of Cd and Cu were 6 and 2.5 fold higher than the natural concentration. The authors stated that inputs of sewage-contaminated water from nearby water body were primary sources of contamination of soil samples with Cd and Cu. Song et al. (2006) analyzed the heavy metal concentration in soils of China. All the collected soil samples were analyzed for estimation of Cd, Cr, Cu, Ni, Pb and Zn metals. Concentration of Cd was found to be in the range of 1.4 - 2.0 mg/kg which was beyond the maximum allowable limits (1 mg/kg) according to Chinese Environmental Quality Standard (Standard Beaureau, 1995). Other metals viz., Cu, Ni, Pb and Zn were studied in the range of 45.9 - 116 mg/kg, 26.6 - 68.8 mg/kg, 87.5 - 565 mg/kg and 175 - 473 mg/kg, respectively.

Mico et al. (2006a) assessed heavy metals and their sources in agricultural soils of Spain. They collected 54 soil samples from Alicante province (Spain) to determine
the contents of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The mean values of all metals were found in the order of Zn > Cr > Pb > Cu > Ni > Co > Cd. In another report, Mico et al. (2006b) analyzed heavy metal content of 29 agricultural soil samples of Mediterranean semiarid area of Spain. The mean values of all the metals studied were observed as Cd (0.38 mg/kg), Co (7.9 mg/kg), Cr (28.3 mg/kg), Cu (21.6 mg/kg), Fe (1527 mg/kg), Mn (320 mg/kg), Ni (23.7 mg/kg), Pb (19.6 mg/kg) and Zn (57.8 mg/kg).

Speciation of different heavy metals in agricultural soils of China was examined by Luo et al. (2006). All the samples covered a wide range of metal concentration with Cd (0.13 - 2.84 mg/kg), Cu (280 - 1931 mg/kg) and Zn (27.4 - 704 mg/kg). Shi et al. (2007) analyzed 273 soil samples of Shangai, China and found to contain high contents of Cd, Cr, Cu, Ni, Pb and Zn. The main sources of metal pollution were observed as Cu, Pb and Zn (traffic emissions), Cd (industrial emissions) whereas Cr and Ni (atmospheric deposition). Qishlaqi and Moore (2007) reported the accumulation pattern and sources of heavy metals in agricultural soils of Iran. They collected 40 topsoils for the detection of Ca, Cd, Cr, Cu, K, Mg, Ni, Pb and Zn and found high contents of Cr, Ni and Pb in all the samples studied. They correlated high levels of above metals with the extensive use of organic/inorganic fertilizers, soil manures and various anthropogenic activities.

Zhao et al. (2007) studied seven heavy metals viz., As, Cd, Cr, Cu, Hg, Pb and Zn in 102 agricultural soils of China. They found mean content of metals as As (14.3 mg/kg), Cd (0.14 mg/kg), Cr (58.6 mg/kg), Cu (40.4 mg/kg), Hg (0.16 mg/kg), Pb (46.7 mg/kg) and Zn (112.9 mg/kg). Luo et al. (2007) analyzed 7 different heavy metals (Cu, Zn, Pb, Cd, As, Ni and Cr) in agricultural soils of Nanjing, China. All metals were observed to be in the order of Cd > Cr > Zn > As > Cu > Ni > Pb. The authors 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 correlated to natural rock weathering. They also correlated concentration of Pb with the vehicular emission as well as the excessive use of inorganic fertilizers. Similar observations were documented by Lado et al. (2008) in top soils of Italy, Europe as they observed critical concentrations of eight different heavy metals (As, Cd, Cr, Cu, Hg, Pb and Zn) studied.
Qislaqui and Moore (2007) estimated heavy metals in agricultural soils of Iran. They observed the mean concentrations of all detected metals as Cd (5.2 mg/kg), Cr (124.5 mg/kg), Cu (96.9 mg/kg), Ni (171.4 mg/kg), Pb (254.6 mg/kg) and Zn (117 mg/kg). Khan et al. (2008) analyzed heavy metals in wastewater irrigated soils of Beijing, China. The concentration of metals was found to be in the range of Cd (0.41 - 0.71 mg/kg), Cr (58.3 - 62.5 mg/kg), Cu (21.5 – 48.7 mg/kg), Ni (24.2 – 25.4 mg/kg), Pb (47.7 – 52.6 mg/kg) and Zn (136 – 176 mg/kg). They further concluded that Cd concentration was higher than the permissible limits given by State Environmental Protection Agency (2005). Covelo et al. (2008) studied the absorption pattern of 6 heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) in soils of Spain.

Zeng et al. (2008) studied different heavy metals in soil samples collected from plantation land of China. Average concentrations of all the metals were observed as As (8.03 mg/kg), Cd (0.28 mg/kg), Cr (49.79 mg/kg), Cu (29.97 mg/kg), Hg (0.12 mg/kg), Pb (29.52 mg/kg) and Zn (99.86 mg/kg). Heavy metal contamination in soils of Zagreb region (Northwest Croatia) was documented by Sollito et al. (2010). Different heavy metals analyzed (Cd, Cu, Ni, Pb and Zn) were found to have average concentrations of Cu (32.86 mg/g), Co (6.85 mg/g), Ni (9.66 mg/g) and Zn (5.41 mg/g). Bai et al. (2010) analyzed heavy metal content in agricultural soils of China. They collected 148 soil samples from four land use patterns and detected seven metalsviz., As, Cd, Cr, Cu, Ni, Pb and Zn using ICP (Inductively Coupled Plasma) and ICP-Mass (Inductively Coupled Plasma-Mass Spectrometry).

A wide variation was found in the accumulation pattern of Cr, Ni, Cu, As, Cd and Zn which was further reported to be significantly affected by the different land use patterns. Katnoria et al. (2008) estimated heavy metal content of agricultural soils in four soil samples of Amritsar, India. Various heavy metals in all the samples were found to be in the range of Cr (0.161 - 3.117 mg/g), Co (0.056 - 2.709 mg/g), Cu (0.123 - 5.312 mg/g), Hg (0.004 - 0.015 mg/g), Mn (0.244 - 0.274 mg/g), Ni (0.619 mg/g - 5.017 mg/g) and Zn (0.414 mg/g - 0.489 mg/g). Heavy metal contamination in agricultural soils of Korea was studied by Ko et al. (2010). They collected 600 soils samples from 58 paddy fields near closed metal mines during the years 2000, 2004 and 2008, respectively. The average metal concentrations were studied as As (3.7 mg/kg),
Cd (0.6 mg/kg), Cu (17.9 mg/kg) and Pb (22.6 mg/kg) during year 2000; As (1.9 mg/kg), Cd (0.46 mg/kg), Cu (13.9 mg/kg) and Pb (13.9 mg/kg) in 2004 and As (1.9 mg/kg), Cd (0.4 mg/kg), Cu (13.3 mg/kg Pb (14.0 mg/kg), Ni (22.0 mg/mg) and Zn (132.4 mg/kg) during last sampling i.e. 2008.

Tang et al. (2010) studied sources of heavy metals and their associated risks in response to agricultural intensification in soils of East China and observed high contents of Cd, Pb and Zn. They further concluded that the intensive use of phosphate fertilizers was responsible for increase in concentrations of above metals. Bhuyian et al. (2010) estimated the heavy metal pollution of agricultural soils affected with coal-mine in Bangladesh. Concentrations of different heavy metals were found to be in the range as As (8.25-24.4 mg/kg), Fe (26000 – 78000 mg/kg), Mn (530 – 3255 mg/kg), Nb (70 – 120 mg/kg), Pb (155 – 521 mg/kg), Rb (98 – 308 mg/kg), Sr (70 – 555 mg/kg), Ti (2900 – 19700 mg/kg), Zn (60 – 470 mg/kg) and Zr (19.5 – 1240 mg/kg).

Flores-Magdaleno et al. (2011) studied heavy metals in agricultural soils of Mixquiahuala, Mexico. The concentration of extracted metals in all the samples was found to be in the order of Pb > Ni > Cd > As > Cr > Hg. Kebir and Bouhadjera (2011) estimated different heavy metal contents in agricultural soils of Ghazaouet, Algeria. All the samples contained As (3.8 mg/kg - 50.60 mg/kg), Cd (1.97 mg/kg - 24.15 mg/kg), Cr (6.12 mg/kg - 65.05 mg/kg), Cu (5.57 mg/kg - 29.65 mg/kg), Fe (3915 mg/kg - 29750 mg/kg), Mn (74.77 mg/kg - 677.25 mg/kg), Mo (5.42 mg/kg - 5.61 mg/kg), Ni (5.12 mg/kg - 34.70 mg/kg), Pb (7.95 mg/kg - 56.02 mg/kg) and Zn (119.17 mg/kg - 539.01 mg/kg). Masona et al. (2011) assessed different heavy metals in agricultural soils of Zimbabwe. They studied the effects of long term irrigation with wastewater on to agricultural lands and found that contents of all metals as Cd (1.5 – 4.8 ppm), Cr (55.2 – 936.5 ppm), Cu (8.5 – 94.4 ppm), Fe (21247 – 31808 ppm), Mn (61.5 – 77.5 ppm), Ni (6.6 – 55.6 ppm), Pb (16.7 – 94 ppm) and Zn (32.5 – 285.4 ppm).

Estimation of different heavy metal contents (Cd, Hg, As, Pb, Cr, Cu, Zn and Ni) in 149 vegetable soils of Shandong Province, China was carried out by Liu et al. (2011b) like Luo et al. (2007). 22 soil samples were found to be severely contaminated with Cd, Cu, Ni and Hg. The statistical analysis of this study also confirmed that parent
rocks influenced the contents of As, Cr and Ni whereas anthropogenic activities like vehicular/industrial emissions and wastewater irrigation contributed in high contents of Hg and Pb in soil. Further, contents of Cd, Cu and Zn were found to be affected by use of agrochemicals. Wei et al. (2011) assessed the heavy metal contents in soils of China. Different heavy metals estimated were observed to be in the range of Cd as 0.5 – 3.2 mg/kg, Cr as 6.8 - 101.8 mg/kg, Cu as 47.6 – 805.7 mg/kg, Ni as 12.6 – 43.9 mg/kg, Pb as 37.7 – 43.8 mg/kg and Zn as 42.3 -148.3 mg/kg.

Katnoria et al. (2011) analyzed 4 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 (5.05 mg/g), Cr (4.49 mg/g), Ni (6.86 mg/g) and Zn (6.53 mg/g), while S-II sample contained Cu (32.86 mg/g), Co (6.85 mg/g), Ni (9.66 mg/g) and Zn (5.41 mg/g). Chanda et al. (2011) 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 maximum permissible limit (MPL) value (40.2 - 105.2 mg/kg and 18.90 - 79.7 mg/kg, respectively) whereas Hg was found to be showing ten times higher concentration than the MPL value (0.19 - 9.65 mg/kg). Singh et al. (2011) studied accumulation of heavy metals in soil samples of Gorakhpur, India. Different metals analyzed were As, Cd, Cr, Cu, Mn, Pb and Zn and all the values were found in the order of Zn > Cr > Mn > Cu > Pb > As > Cd > Hg within the MPL values. Pujar et al. (2012) analyzed heavy metal contents of soil samples of Karnataka (India). Different metals analyzed were found to be in the range of Cd (0.02 – 0.054 ppm), Cu (3.1 – 4.0 ppm), Fe (1.07 – 1.27 ppm), Mn (2.85 – 3.9 ppm), Ni (0.18 – 0.22 ppm) and Zn (1.15 – 1.45 ppm).

Bahrampour et al. (2013) studied contents of Cd, Ni and Pb in 131 farming and non farming soils of Moghan. They found that the accumulation pattern of Cd, Ni and Pb were 3, 20 and 5 times higher in farming soils as compared to non farming soils which the authors correlated to the excessive use of fertilizers, pesticides and anthropogenic activities onto the agricultural lands. Khan et al. (2013a) detected heavy metal content viz., Cd (0.09 - 0.11 mg/kg), Cr (0.29 - 0.64 mg/kg), Mn (1.41 - 10.05 mg/kg), Ni (0.28 - 0.72 mg/kg) and Zn (0.20 - 0.52 mg/kg) in agricultural soils of
Pakistan and quoted various agricultural practices such as addition of manures, fertilizers and pesticides as a major cause of contamination.

Shan *et al.* (2013) studied the sources of heavy metals in agricultural soils of China and reported that different farming practices were the major sources of soil contamination. They found that Cd content (0.241 mg/kg) was almost three times higher than its background value since 20 years in Chinese soils. Except for Cd, the mean values of all other metals were found to be less than the maximum permissible limits. The obtained values reflected 40.1% of total variance using Principal component analysis (PCA).

Khan *et al.* (2013b) analyzed different heavy metals (Cd, Cu, Fe, Mn, Ni, Pb and Zn) in soils of Lahore (Pakistan). Metal concentrations were found to be as Cd (0.05 - 0.52 mg/kg), Cu (1.06 - 5.42 mg/kg), Fe (8.6 - 35.03 mg/kg), Mn (8.7 - 37.07 mg/kg), Ni (0.28 - 1.76 mg/kg), Pb (2.11 - 30.86 mg/kg) and Zn (1.3 - 8.02 mg/kg).

Wang *et al.* (2014) estimated heavy metal concentrations in agricultural soils of China using visible and near-infrared reflectance spectroscopy. Among 30 soil samples studied, mean content of heavy metals was observed to be as As (21.90 mg/kg), Cu (26.38 mg/kg), Pb (37.60 mg/kg) and Zn (117.94 mg/kg). They also compared the Partial least squares regression with genetic logarithm (GA-PLSR) with PLSR method and concluded that GA-PLSR was more promising method to predict the low concentrations from visible and near-infrared (VNIR) reflectance.

Kelepertzis (2014) studied the accumulation of heavy metals in 132 agricultural soils of Peloponese, Greece. Median concentration of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were found as 7.1, 0.45, 65.23, 72.3, 20.6, 27.10, 956.5, 120.3, 20.10 and 72.75 mg/kg, respectively. The report concluded that the prolonged use of fertilizers, pesticides and fungicides led to the accumulation of Cd, Cu, Pb and Zn whereas parent material influenced the accumulation of Co, Cr, Ni and Fe in agricultural fields.

Lucchini *et al.* (2014) analyzed heavy metals in agricultural soils of Abergwyngregyn, Wales for two subsequent years (2011 and 2012). The average contents of metals were As (11.6 mg/kg), Cd (0.7 mg/kg), Cu (15.1 mg/kg), Ni (12.1 mg/kg), Pb (24.9 mg/kg) and Zn (88.1 mg/kg) in 2011 while As (9.5 mg/kg), Cd (0.43
mg/kg), Cu (11.2 mg/kg), Ni (8.8 mg/kg), Pb (19.5 mg/kg) and Zn (65.1 mg/kg) in year 2012. Santiago-Martin et al. (2014) studied heavy metal concentration in agricultural soils of Spain. Total heavy metal content was found to be in the range of Cu (7 - 13.2 mg/kg), Pb (14 - 55.8 mg/kg) and Zn (45.2 – 74.9 mg/kg).

2.4. Heavy Metal Analysis in Plants

Plants have inherent tendency to uptake toxic substances including heavy metals from the soil that are subsequently transferred along the normal food chain. Increasing heavy metal accumulation in soil ecosystem not only damages the food crops and vegetables but also pose the adverse effects on human beings (Singh et al., 2011). Karatas et al. (2006) have directly correlated the uptake of heavy metals with mortality rates of living beings. Therefore, it becomes necessary to evaluate their transference pattern into the various food crops. Kaasalainen and Yli-Halla (2003) have also stressed on estimation of heavy metals in food crops as well as soils to check rate of metals uptaken by the growing plants. The significant uptake of heavy metals has been observed in plants growing in contaminated soils than those grown in uncontaminated soils (Sharma et al., 2007; Marshall et al., 2007). Various studies have been conducted across the world to demonstrate the transfer of different heavy metals into growing vegetables and crops from contaminated soils (Angelova et al., 2004; Rattan et al., 2005; El-Arby et al., 2006; Lokeshwari and Chandrappa, 2006). Table 4 summarizes the reports conducted on estimation of heavy metals in plants.

Burman et al. (2000) estimated high concentrations of heavy metals in wheat, mustard and weeds grown in the agricultural fields irrigated with industrial effluents. They observed high levels of heavy metals in different plants uptaken through contaminated soils. Angelova et al. (2004) also supported the fact of transference of heavy metals from soils using fibre crops (flax, cotton and hemp) of Bulgaria. The authors observed distribution of metals along the plant axis which was found to be in the order as roots > stems > leaves > seeds in flax and hemp while leaves > seeds > roots > stems in cotton plant. Cui et al. (2004) studied the transfer of metals from soils to vegetables in an area near a smelter in China. They reported that plant samples collected from villages near by the smelter were highly contaminated as compared to
those which were collected away from the smelter. Mean concentrations of metals studied in vegetables were observed to be as Cd (0.24 mg/kg) and Pb (0.45 mg/kg).

Rattan et al. (2005) estimated the metal transfer factor in sewage effluents irrigated soils and crops of New Delhi, India. They observed the range of metals as Cu (5.42 – 57.2 mg/kg), Fe (122 – 711 mg/kg), Mn (15.3 – 208 mg/kg), Ni (8.78 – 20.6 mg/kg) and Zn (46.7 – 91.9 mg/kg). El-Arby et al. (2006) accessed the availability of heavy metals in wastewater irrigated soils of Egypt and their uptake by plants. The authors studied the accumulation pattern of heavy metals in different plants as Jojoba > Khaya > Axodium > Italian cypress. They further observed the uptake levels of different heavy metals in Jojoba trees as Co > Pb > Ni > Cu > Fe = Zn > Mn. Impact of heavy metal contaminated soils of Bangalore on cultivated crops was studied by Lokeshwari and Chandrappa (2006). Khan et al. (2008) evaluated the health risks in food crops growing under heavy metal contaminated soils of China. They observed that plants grown on irrigated soils contained significantly higher metal concentrations than those grown on reference soils. They further concluded that all the metals exceeded permissible limits given by SEPA (State Environmental Protection Agency) in China and the World Health Organization (WHO).

Lacatusu and Lacatusu (2008) studied heavy metal contents in vegetables of Romania. They observed that in edible parts of fresh root vegetables, contents of metals exceeded up to 2.5 times (Cd) and 11.5 times (Pb) higher than the maximum allowable limits. The contents of Cd and Pb in leafy vegetables were found to be 7 times and 17 times, respectively higher than the normal concentration. Zeng et al. (2008) estimated the heavy metal contents in vegetables grown in sewage irrigated soils of China. The authors observed all vegetable samples to be highly contaminated with As, Cd, Hg, Pb and Zn. Singh et al. (2010) assessed total heavy metal contents of food crops contaminated with wastewater irrigated soil. They reported that the levels of metals (Cd, Cr, Cu, Ni and Pb) analyzed in studied food crops were several folds higher than those grown in uncontaminated soils. They further demonstrated that the content of metals like Cd, Ni and Pb crossed the safe limits of Indian and World health organization/Food agricultural organization (WHO/FAO) standards.
Surdyk *et al.* (2010) studied the uptake of heavy metals of plants through wastewater irrigated soils of Belgrade (Serbia). They observed that Cu, Mn and Zn were the predominant metals and were significantly taken up by the growing crop. Similar study was conducted by Akbar *et al.* (2010) on wastewater irrigated (Peshawar) and relatively clean water irrigated (lower Dir) soils of Pakistan. The authors demonstrated that transference of heavy metals in food crops grown on wastewater irrigated soils were higher than those grown on reference soils. The concentrations of different metals analyzed (Cu, Mn, Ni and Zn) was detected within the WHO/FAO permissible limits except for Zn. Felix-Henningsen *et al.* (2010) observed the uptake of heavy metals by food crops from highly contaminated soils of Georgia. The studied crops indicated a high uptake of Cd, Cu and Zn, where Cd and Cu exceeded the tolerance thresholds for plants. Guala *et al.* (2010) studied the dynamics of heavy metal uptake in food crops of Spain grown under contaminated soils. They reported that among the different metals studied (Cd, Cu and Zn), the plants showed maximum absorbing capacity for Zn metal.

Auda *et al.* (2011) reported the accumulation of heavy metals in crops grown under contaminated soils of Palestine. They observed that the contents of all metals (Cd, Fe, Pb and Zn) were found to be within the normal ranges except Pb. They further demonstrated that different growth characters like root length, shoot length, fresh and dry weights of shoots were observed to decrease in case of high Cd concentration. Kalaskar (2012) analyzed the levels of Cd, Cr, Cu and Pb in vegetables collected from Amravati, India. Studied metals were found to be in the range as Cd (0.28 – 1.923 mg/kg), Cr (0.358 – 3.765 mg/kg), Cu (10.78 – 29.27 mg/kg) and Pb (7.022- 11.70 mg/kg). Ali and Al-Qahtani (2012) assessed the contents of heavy metals in vegetables, cereals and fruits of Saudi Arabia and found that contents of metals (Cd, Cu, Fe, Hg, Mn, Pb and Zn) studied exceeded the recommended maximum acceptable limits proposed by Joint WHO/FAO.

Mohammed and Khamis (2012) estimated the heavy metal contents in vegetables of Tanzania. The authors observed that all 13 elements (Al, Ag, Br, Cd, Cl, Cr, Cu, Fe, I, Mn, Ni, Pb and Zn) analyzed were present above their maximum allowable limits as per Energy Dispersive X-ray Fluorescence (EDXRF) system. They further reported that the contents of As and Co were very high as compared to the
previous studies conducted in that area. Elbagermi et al. (2012) analyzed the heavy metal concentrations in fruits and vegetables of Libya. They observed the concentrations of metals in the range of Cd (0.01 – 0.362 mg/kg), Co (0.141 – 1.168 mg/kg), Cu (0.75 – 6.21 mg/kg), Ni (0.19 – 5.143 mg/kg), Pb (0.02 – 1.824 mg/kg) and Zn (0.042 – 11.4 mg/kg).

Singh et al. (2012) studied accumulation pattern of heavy metals in 16 vegetable crops grown in contaminated soils of New Delhi (India). The order of accumulation of different heavy metals was recorded in the order of Zn > Cu > Ni > Cd. All metals were found to be in the range of Cd (1 – 36 µg/g), Cu (11 – 96 µg/g), Ni (1 – 49 µg/g) and Zn (22 – 96 µg/g). Among all the vegetables studied, leafy vegetables (spinach, amaranthus, mustard and fenugreek) were recorded to contain higher accumulation of heavy metals. Kumar et al. (2013) estimated the accumulation of different heavy metals in weed species grown in industrial effluent contaminated soils of Lucknow (India). They reported the concentration of Cr, Cu, Ni, Pb and Cd (µg/g) in root tissues as 3.97 – 67.72, 3.44 – 45.47, 2.52 – 14.19, 6.13 – 58.28 and 0.32 – 3.96 whereas 5.45 – 125.67, 2.87 – 19.60, 1.81 – 15.39, 5.18 – 44.28 and 0.23 – 3.17 in shoot tissues, respectively.

Khan et al. (2013b) reported the effects of heavy metal contaminated soils on growing vegetables in Lahore (Pakistan). They observed that the contents of all metals (Cd, Cu, Fe, Ni, Pb and Zn) analyzed were found to be higher than the WHO/FAO recommended permissible limits in different edible parts (leaves and fruits) of growing crops. Akan et al. (2013a) determined the contents of heavy metals in vegetables of Nigeria. They observed the studied metals to be in the range of Cd (0.34 – 5.44 mg/kg), Cr (0.23 – 3.22 mg/kg), Cu (0.21 – 3.22 mg/kg), Mn (0.23 – 3.45 mg/kg), Ni (0.21 – 3.54 mg/kg), Pb (0.25 – 4.56 mg/kg) and Zn (0.87 – 8.34 mg/kg).

Mahmood et al. (2014) also studied the accumulation pattern of heavy metals in vegetables collected from contaminated soils of Lahore (Pakistan). Among the different metals (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) analyzed, the accumulation pattern of Co was found to be higher than the permissible limits by European Union, 2002. Huang et al. (2014) analyzed the heavy metal contents in 343 vegetables grown under contaminated soils of Zhejiang, China. They studied mean levels of all metals as As
(0.009 mg/kg), Cd (0.015 - 0.009 mg/kg), Hg (0.003 - 0.009 mg/kg) and Pb (0.022 - 0.009 mg/kg). They further stated that the samples with Cd (4.37 %), Hg (2.62 %) and Pb (3.79 %) exceeded the maximum allowable limits by Chinese legislation.

### 2.4.1. Heavy Metal Contamination in Rice Crops

Zhuang et al. (2009) evaluated different heavy metals in food crops and vegetables in soils near Dabaoshan mine, South China. They observed that rice crop accumulated high concentrations of Cu and Pb in grains. Accumulation of heavy metals in different vegetables was observed to be in the order of Cd > Zn > Cu > Pb. The Target Health Quotient (THQ) for different metals was observed to be as Cu (0.66 – 8.89), Zn (0.48 – 0.60), Pb (1.43 – 1.99) and Cd (2.61 – 6.25). They further concluded that concentrations of Cd and Pb in rice and vegetables exceeded the maximum permissible limits proposed by WHO/FAO in China.

Zhao et al. (2010) estimated the heavy metal accumulation in rice crop of Wenling, China. They also observed the spatial distribution of heavy metals in relation to different soil properties of paddy field. During their study, the authors observed high content of Cd as compared to other metals (Cd, Cu, Pb, Ni and Zn) in rice crops. They also correlated the soil properties like soil types (acrisols, lixisols and regosols), low pH and high sand content of contaminated area with the high Cd metal concentration. Chung et al. (2011) estimated heavy metal contents in brown rice (*Oryza sativa*) grown in fields that were irrigated with wastewater in Korea. They observed the mean concentrations of all metals as Cd (0.011 mg/kg), Cu (0.340 mg/kg), Pb (0.370 mg/kg) and Zn (2.05 mg/kg). Fang et al. (2014) evaluated the contents of heavy metals 92 rice samples of China. They reported that except Hg, all other metals As (2.2 %), Cd (3.3 %) and Pb (4.3%) were higher than the maximum allowable limits.

### 2.4.2. Heavy Metal Contamination in Wheat Crops

Lavado et al. (2001) analyzed heavy metal contents in wheat crops of Argentina. They observed that the accumulation of heavy metals (Cd, Cu, Pb, Mo, Ni and Zn) were higher in wheat grains than roots except for Cr which was 1.77 mg/kg in grains and 19.70 mg/kg in roots. Jamali et al. (2009) analyzed the heavy metal contents in wheat grown in sludge amended soils. They observed that the edible parts of wheat crop
contained significant amount of all heavy metals studied. The uptake pattern of different heavy metals was found in the order of Zn > Pb > Cu > Ni > Cd > Cr. The authors also reported a significant correlation between heavy metal contents of soil samples as well as the corresponding wheat grains.

Castaldi et al. (2009) studied the accumulation of three heavy metals (Cd, Pb and Zn) in wheat crops from polluted soils of Italy. They observed that high contents of Cd, Pb and Zn were accumulated in roots of wheat crop than the shoots. The translocation factor of three metals in wheat crops was found to be in the range of Cd (0.025 - 0.085 %), Pb (0.060 – 0.068 %) and Zn (0.100 – 0.324 %). Accumulation and bioavailability of Cu and Ni in wheat crops from contaminated soils of China were estimated by Wang et al. (2009). The accumulation of both metals in wheat plant parts was observed to be in the order of roots > leaves > shells > stocks > grains. Authors reported that the bioaccumulation factor (BCF) and translocation factor (TF) of Cu was higher than those of Ni. The mean concentrations of both metals were found to be higher than the permissible limits suggested by soil Environmental Quality Standards in China (USEPA, 2007).

Bermudez et al. (2011) estimated the heavy metal contents in wheat grains collected from Argentina. They observed that metal concentrations in wheat grains were dependent on the metals in source soils. They demonstrated that mean concentrations of As, Cd, Ni and Pb were found to be within the maximum tolerance limits as per international legislations whereas metals like Cr, Cu, Fe, Mn and Zn crossed these limits. Al-Othman et al. (2013) analyzed metal contents in wheat crops grown under wastewater irrigated soils of Saudi Arabia. They reported that the accumulation pattern of heavy metals in different plant parts was observed to be as roots > vegetative parts > grains. Further, the general order of metal deposition in wheat was found to be as Mn > Zn > Cu > Ni > Cr > As > Pb > Cd.

2.5. Biochemical Analysis of *Allium cepa*

Although the literature survey has revealed very few reports on studies related to the biochemical aspects of *Allium cepa* grown in heavy metal contaminated soils (Fatima et al., 2005; Csiszar et al., 2007), yet, no report was seen that would indicate the
correlation of pesticides with that of biochemical activities. Different pollutants present in soils can cause the degraded effects like direct damage of proteins, lipids and enzymes thereby causing the biochemical changes in living systems (Mannervik and Axelsson, 1975, 1980; Stajner et al., 1998; Plummer, 2004; Fatima and Ahmad, 2005; Gao et al., 2010). This section of review focuses the various reports available on antioxidant defence system of *Allium cepa* under the stress of different contaminant.

Stajner et al. (1998) studied the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione-peroxidase (GPX) in leaves, stalks and roots of *Allium cepa* L. and *Allium fistulosum* L. Among different plants studied, *Allium fistulosum* L. was found to show abrupt changes in SOD, CAT and GPX activities in all plant parts. Fatima and Ahmad (2005) studied some antioxidant enzymes viz., glutathione- S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) of *Allium cepa* and considered these antioxidant enzymes as biomarkers for the detection of heavy metals in wastewater. The enzymes were estimated in onion bulbs exposed to heavy metals such as Cd, Cr, Cu, Hg, Pb and Zn. GST, GR, SOD and CAT did not show the increased pattern. APX, GPX and MDHAR had shown a sharp decline after exposure to higher concentrations of heavy metals while GST, APX, GPX and MDHAR exhibited abrupt changes with increase in the concentration of individual heavy metals.

Csiszar et al. (2007) studied antioxidant defense mechanism of *Allium cepa* root and shoot cells under heavy metal stress. They estimated variability in activities of antioxidant enzymes like SOD, CAT, POD, GR and GST and further observed a strong correlation of change in enzymatic activities with contents of heavy metals like. Achary et al. (2008) reported aluminium induced oxidative stress in roots cells of *Allium cepa* L. They studied activities of different antioxidant enzymes viz., catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX), glutathione peroxidase (GPX) and found that aluminium inhibited CAT activity whereas significantly enhanced activities of SOD, GPX and APX was observed with respect to the control.

Gao et al. (2010) studied the response of soil enzyme activities (dehydrogenase, phosphatase and urease) at different levels of Cd and Pb in present soil samples. They
reported that Cd and Pb had negative effect on the soil enzyme activity. Olorunfemi and Lolodi (2011) studied the effects of effluents on antioxidant enzyme activities of *Allium cepa* L. They measured the activities of SOD and CAT in root cells of *Allium cepa*. SOD activity was found to increase at lower concentration but decreased with increase in concentration of effluent. Catalase activity was observed to be inconsistent initially but exhibited drastic increase at high concentration of the effluent.

2.6. Genotoxicity/mutagenicity of Contaminated Soils

Various contaminants alter the natural composition of soil systems and pose deleterious health effects upon entering the living systems. Therefore, the evaluation of genotoxic/mutagenic potential of contaminated soils using different living systems is equally mandatory. Various studies have been conducted worldwide to explore the genotoxic/mutagenic potential of polluted soils using battery of different bioassays viz., bacterial bioassays (White and Claxon, 2004; McCann and Ames, 1976; Mortelmans and Zeiger, 2000; Singh *et al.*, 2007; Aleem and Malik, 2005; Vargas *et al.*, 2001), animal bioassays (Augustyniak *et al.*, 2006; Rank *et al.*, 2007; Hseu *et al.*, 2008; Mouchet *et al.*, 2006; Frenzilli *et al.*, 2009; Azqueta *et al.*, 2009) and plant bioassays (Katnoria *et al.*, 2008; Tkalec *et al.*, 2009; Turkoglu, 2007; Akinboro and Bakare, 2007; Pereira *et al.*, 2009; Carita and Marin Morales, 2008; Vujosevic *et al.*, 2008; Srivastava and Mishra, 2009; Yildiz *et al.*, 2009).

Among different bioassays adapted for estimation of genotoxicity/mutagenicity of soils samples, plant bioassays are much preferred being more 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 (Rossman, 1995; IARC, 1976; 1980; 1987; 1990). The following part of the review deals with the mutagenic/ genotoxic effects of soil contaminants using plant bioassays.

2.6.1. 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. 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. Since, 1970s, higher plant bioassays have been recommended for testing the genotoxic potential of different environmental contaminants by various organizations viz., Royal Swedish Academy of Sciences (1973); Committee 17 (1975) of Environment Mutagen Society; World Health Organization (1985) and National Swedish Environmental Protection Board (1989) (Fiskesjo, 1993a,b; 1994; 1995). The summary of literature on estimation of genotoxic potential of different soils samples using plant bioassays is described in Table 5.

Three well known bioassays viz., *Allium cepa* root chromosomal aberration assay (AlRCAA), *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 (Cabrera and Rodrigues, 1999) 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 (Gichner et al., 1994; Cottele et al., 1999; Saxena et al., 2009; Srivastava and Mishra, 2009). All these bioassays have their own importance and are widely used for the testing of genotoxicity of different environmental samples viz., waste water (Grover and Kaur, 1999), wastewater sludge (Rank and Nielson, 1998) and soil samples (Katnoria nad Nagpal, 2004).

Gichner et al. (1994) employed *Arabidopsis* assay for assessing the mutagenic potential of 5 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 5 chemicals studied, EMS, NS and AG were reported to be mutagenic while other 2 compounds viz. SA and MH were reported to be weakly mutagenic. Grant (1994) 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 mays to test the genotoxic agents. He further supported that higher plant systems were the excellent indicators of the cytotoxic, cytogenetic and mutagenic effects of environmental chemicals and had unique advantages for in situ monitoring and screening.

Kanaya et al. (1994) 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., 3-azido-1, 2-propanediol or azidoglycerol (AG), N-methyl-N-nitrosourea (MNU), sodium azide (SA) and maleic hydrazide (MH) were studied using Vicia faba root tip meristem chromosomal aberration assay. Of the 4 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 environmental contaminants.

Ma et al. (1994) employed Tradescantia stamen hair mutation assay for its efficiency and reliability and studied four coded chemicals viz., azidoglycerol (AG, 3-azido-1,2-propanediol), 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 - 100µg/ml), MH (1 - 45 µg/ml), MNU (10 - 80 µg/ml) and SA (3 - 80 µg/ml). The study revealed the reliability of Tradescantia stamen hair mutation assay for screening chemicals for their potential mutagenic effects.

Knasmuller et al. (1998) assessed genotoxic potential of 4 heavy metal salts viz., Cr(VI)O₃, Cr(III)Cl₃, Ni(II)Cl₂ and Sb(III)Cl₃ using micronucleus tests with pollen tetrad cells of Tradescantia clone #4430 (Trad-MN assays) and in meristematic root tip cells of Vicia faba. The authors concluded that Trad-MN assay was found to be more simple and fast biomonitoring assay as a valuable supplement to analytical analysis of
contaminated soils. Gichner and Veleminsky (1999) used *Tradescantia* stamen hair and micronucleus assays to monitor the genotoxicity of soil extracts from two heavily polluted sites in Prague, Czech Republic. *Tradescantia* micronucleus assay was found to be more sensitive to detect the genotoxic agents than the stamen hair assay. Monarca *et al.* (2002) 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 *et al.* (2002) studied the effects of heavy metal contaminated soils on micronuclei induction in *Tradescantia*. They examined 20 soils from nine locations for genotoxicity. A pronounced induction of micronuclei was observed in *Tradescantia* micronucleus (*Trad*-MCN) assay with increased metal concentration of soils. They also investigated correlation between genotoxic effects and changes of microbial parameters caused by metal contamination in soils. However, no correlation was found between metal content and genotoxicity of soils from different locations. The microbial parameters (dehydrogenase, arylsulfatase acticity, biomass C and biomass N) had shown inconsistent results for different soils studied indicating that it was not possible to define a specific marker enzyme for metal contamination.

Song *et al.* (2006) reported the genotoxicity of soil samples of Northern China using *Vicia faba* micronucleus test. They observed that the frequency of micronuclei was in the range of 1.6 – 76.4 % in all soil samples studied. *Vicia faba* root tip micronucleus test was performed using the protocol prescribed by Ma *et al.* (1994). It was 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 %. Jan-hui and Hang (2009) evaluated the genotoxic potential of soil samples taken from an electronic-waste recycling area by employing *Vicia faba* root cells micronucleus assay in China. 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 *et al.* (2009) evaluated 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.
They observed that both soil samples induced significant toxicity in both modes of treatment.

Cerniene et al. (2010) 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 et al. (2011) 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 (Kovalchuk, 1998; Gichner and Veleminsky, 1999; Abdel Migmid et al., 2007) using plant bioassays.

2.6.1.1. Genotoxic Potential of Soil Sample using Allium cepa Root Chromosomal Aberration Assay

Among different plant bioassays, Allium cepa root chromosomal aberration assay (Al-RCA) has been developed as a most reliable protocol for evaluation of genotoxic/mutagenic potential of soil systems. Al-RCAA bioassay is highly preferred over other bioassays because of low cost and easy availability of the plant in all seasons, low chromosome number (2n=16) and less time consuming. The fact has been supported by various scientists across the world (Kong and Ma, 1994; Cabrera and Rodriquez, 1999).

Kong and Ma (1999) evaluated the genotoxicity of contaminated soils and shallow well water samples employing three bioassays viz., Allium root anaphase aberration assay (Al-RAA), Tradescantia micronucleus (Trad-MCN) and Tradescantia stamen hair mutation (Trad-SHM) tests. The authors reported Al-RCAA to be more reliable and less time consuming over other bioassays. Cabrera and Rodriguez (1999) assessed genotoxicity of soil from farmland irrigated with wastewater using three plant bioassays viz., Allium root chromosome aberration (Al-RAA) assay, Tradescantia
micronucleus (*Trad*-MCN) assay and *Tradescantia* stamen hair (*Trad*-SHM) mutation assay. *Al*-RAA was proved to be efficient test for chemical screening and especially for *in situ* monitoring for genotoxicity of environmental pollutants. Kovalchuk *et al.* (1998) performed *Allium cepa* root chromosomal assay to evaluate the genotoxicity of soils of Ukraine. 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.

Katnoria and Nagpal (2004) estimated the genotoxic potential of agricultural soils of Malah and Rajasansi of Amritsar employing *Allium cepa* root chromosomal aberration assay (*AlRCAA*). They treated onions in different concentrations (10, 25, 50, 75 and 100 %) of soil extracts. Both the samples induced significant number of aberrations (6.5 % in Malah and 8.1 % in Rajasansi soil sample) at maximum concentration (100 %) of the soil extract. In another report, Katnoria *et al.* (2008) 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 assay (*AlRAAA*). They followed two modes of treatments *viz. in situ* and root dip treatment for the evaluation of genotoxicity of soil samples. All soil samples have shown percent aberrant cells in the range of 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. Feretti *et al.* (2009) evaluated the genotoxic effects of gaseous emissions and percolates from 3 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.

Chahal *et al.* (2012) evaluated the genotoxic potential of a soil sample form agricultural field of Amritsar (India) employing *Allium cepa* root anaphase aberration assay. The soil sample was found to be significantly genotoxic showing 9.2 % and 12.81 % total aberrant cells following *in situ* and root dip treatment, respectively. In another report, Chahal *et al.* (2013) accessed the genotoxic potential of an agricultural
soil sample of Nangli village of Amritsar (India) under rice cultivation. The authors observed that the sample induced significant chromosomal aberrations (both physiological and clastogenic) in root tip cells of *Allium cepa*. In situ treatment was found to induce 12.03 % total aberrant cells whereas root dip treatment resulted in 12.63 % total chromosomal aberrations.

2.6.1.2. Genotoxic Potential of Contaminated Soils using *Allium cepa* Chromosomal Aberration Assay

Although evaluation of genotoxic potential of various environmental mixtures by using pollen mother cells of *Allium cepa* is an old technique but the literature available on soil systems is very limited. Levan (1939) studied the effects of colchicine on meiotic division in *Allium cepa* L. The colchicine affected both first and second divisions of meiosis and induced various chromosomal abnormalities. They further observed that colchicine restricted the cell division and converted first pollen division to a typical c-mitosis. Fisun and Rasgele (2009) studied genotoxic effects of a fungicide (raxil) on pollen mother cells of *Allium cepa* L. using *Allium cepa* chromosomal aberration assay. The meiotic studies showed maximum abnormality of 3.05 % which included different chromosomal abnormalities like chromatin bridge/s, stickiness, laggard/s, micronuclei, fragment/s, synchronization disorder and formation of univalent/s and quadrivalent/s in pollen mother cells of *Allium cepa*.

2.7. Tumor Inducing Potential of Contaminated Soils

Along with assessment of genotoxic/mutagenic potential of contaminated soils, evaluation of tumor inducing potential of various soil contaminants is equally important. Potato disc tumor assay has been developed as a very proficient method for evaluation of antitumor induction potential of different plant extracts and drugs in past few decades. Since then, this bioassay has been used for antitumor induction of various drugs and extracts (Anand and Heberlin, 1977; Ferrigni et al., 1982). Coker et al. (2003) used potato disc tumor assay to verify the detection of antineoplastic activity of various drugs (camptothecin, paclitaxel, vinblastine, podophyllin and vincristine). They evaluated potato disc tumor induction assay as an effective indicator of antitumor activity of drug action. As no study has been conducted to evaluate the tumor inducing
potential of complex environmental mixtures, the objective of tumor induction by soil samples was initiated by Kaur et al. (2013). The authors evaluated tumor inducing potential of roadside soils samples of Golden Temple and Putlighar of Amritsar (India) and observed that both samples induced mean number of tumors as 13 (Golden Temple) and 14.4 % (Putlighar) at maximum concentration of soil extract (100 % of 1 : 2 :: w/v; soil : water).