CHAPTER II
THEORETICAL BACKGROUNDS AND
LITERATURE REVIEW

2.1 Zinc

2.1.1 Zinc in Soil

The solubility of Zn minerals during weathering produces mobile Zn^{2+}, especially in acid and oxidizing environments. Zn is, however, also easily adsorbed by mineral and organic components and thus, in most soil types, its accumulation in the surface horizons.

Reactions with soil components

The most common and mobile Zn in soil is believed to be in forms of free and complex ions in soil and solutions, but several other ionic species that occur as nonspecifically and specifically adsorbed cations can also be easily mobilized in soils (Figure 2.1). The important factors controlling the mobility of Zn in soils are very similar to Cu, but Zn appears to occur in more readily soluble forms. Many studied of Zn adsorption and relation in soils were reviewed by Lindsey (1972), and it has been shown that clays and soil organic matter are capable of holding Zn quite strongly; thus the solubility of Zn in soils is less than that of $Zn(OH)_2$, $ZnCO_3$, and $Zn(PO_4)$ in pure experimental systems. Processes involved in Zn adsorption are not yet completely understood; there are two different mechanisms of Zn adsorption: one in acid media related to cation exchange sites, and the other in alkaline media that is considered to be chemisorption and is highly influenced by organic ligands. The adsorption of Zn^{2+} can be reduced at lower pH (<7) by competing cations and this results in easy mobilization and leaching of Zn from light acid soils. At the higher pH values, while an increase of organic compounds in soil solution becomes more evident, Zn-organic complexes may also account the solubility of this metal (Figure 2.2). In sandy, acid soils, on the other hand, organic matter seems to be the most important soil component for binding Zn, while oxides (hydro) of Al, Fe, and Mn appear to be of minor importance.
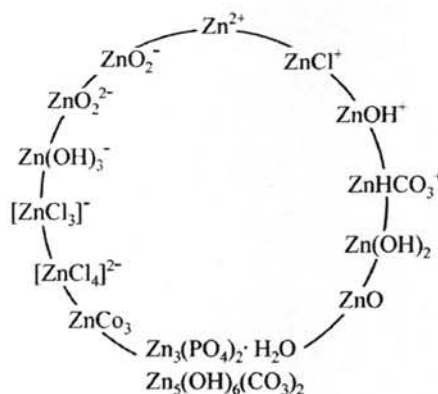
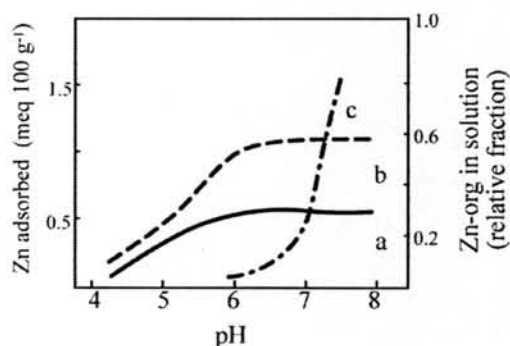**Figure 2.1** Ionic species and compounds of Zn occurring in soils (Kabata Pendias and Pendias, 2001)

Figure 2.2 Influence of soil pH on adsorption of Zn at (a) 20 ppm (b) 40 ppm Zn levels in initial solutions and on formation of soluble Zn-organic complexes in the solution of loamy silt soil (c) (McBride and Blasiak, 1979)

Abd-Elfattah and Wada (1981) found the highest selective adsorption of Zn by Fe oxides, hallaysite, allophone, and imogolite and the lowest by montmorillonite. Thus, clay minerals, hydrous oxides, and pH are likely to be the most important factors controlling Zn solubility in soils, while organic complexing and precipitation of Zn as hydroxide, carbonate, and sulfide compounds appear to be of much lesser importance. Zn can also enter some layer lattice silicate structure and become very immobile. Soil organic matter is known to be capable of bonding Zn in stable forms; the Zn accumulation in organic soil horizons and in some peat.

However, stability constants of Zn¯organic matter in soils are relatively low, but a high proportion of Zn is bound to organic matter in mineral soils. The addition of sewage sludge to these soils modifies the distribution pattern of Zn, increasing significantly two Zn species-easily soluble and exchangeable (Figure 2.3).
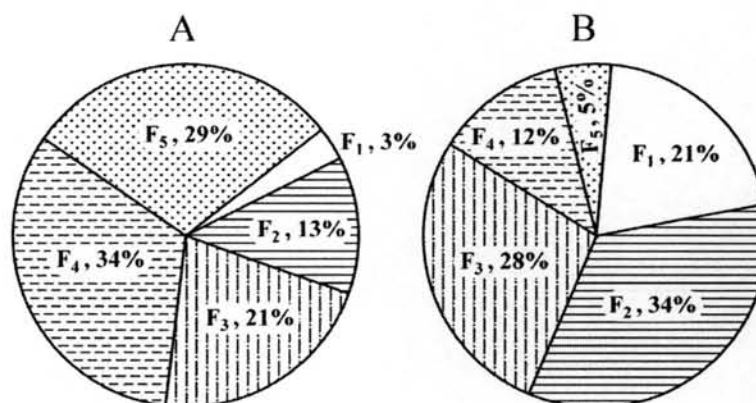

Figure 2.3 Variation in Zn species in soils under sludge application: (A) unsludged soil, (B) sludged soil. Zn species: (F_1) easily soluble ;(F_2)exchangeable; (F_3)associated with hydrous oxides; (F_4) bound to organic matter; and (F_5) residual (Dudka and Chlopecka, 1990)

Zn is considered to be readily soluble relative to the other heavy metals in soils. The Zn concentrations in soil solution are ranging from 4 to 270 ug/l, depending on the soil and the techniques used for obtaining the solution. However, in natural but very acid soils (pH<4), Zn concentration in solution was reported to average 7,137 ug/l. Although Zn is highly soluble in solution of peaty soils, it occurs mainly in colloidal forms (up to 60% of total content). Zn anionic organic and inorganic complexes account for up to 40%, whereas cationic forms are present only below 10%. Zn is most readily mobile and available in acid light mineral soils. Solubility and availability of Zn is negatively correlated with Ca-saturation and P compounds present in soils. This relationship may reflect both adsorption and precipitation processes, as well as interactions between these elements. However, soluble Zn-organic complexes and complex anionic forms of Zn (Figures 2.1 and 2.2) may account for the relative solubility and availability of Zn in soils with a high pH range.

The immobilization of Zn in soils rich in Ca and P, in well-aerated soils with S compounds, and in soils containing enhanced amounts of certain Ca-saturated minerals, as well as hydrous oxides, has an important practical impact on the Zn deficiency of plants. Zn deficiencies most frequently result from management practices used during crop production (e.g., over-liming, P fertilization, organic matter amendment).

Contamination of soils

The anthropogenic sources of Zn are related, first of all, to the non-ferric metal industry and then to agricultural practice. Contemporarily observed soil contamination with Zn has already brought Zn to an extremely high accumulation in top soils in certain areas. Additional problems related to Zn pollution are changes in the metal speciation. Amelioration Zn-contaminated soils are commonly based on controlling its availability by the addition of lime or organic matter or both. Soluble Zn-organic complexes that occur particularly in municipal sewage sludge are very mobile in soils and therefore are easily available to plants. Zn contamination of soils may create an important environmental problem.

2.1.2 Zinc in plants

Absorption and Transport

Soluble forms of Zn are readily available to plants and the uptake of Zn has been reported to be linear with concentration in the nutrient solution and in soils. The rate of Zn absorption differs greatly among both plant species and growth media. The composition of the nutrient solution, particularly the presence of Ca, is of great importance. The form in which Zn is absorbed by roots has not been precisely defined. There is, however, general agreement on the predominating uptake of both hydrated Zn and Zn^{2+}. Several other complex ions and Zn-organic chelates may also be absorbed. Fractions of Zn bound to light organic compounds in xylem fluids and in other plant tissue extracts may suggest its high mobility in the plant. Some authors regard Zn as highly mobile, while others consider Zn to have intermediate mobility. Indeed, when given luxury supplies of Zn, several plant species have mobilized appreciable quantities of this metal from old leaves to generative organs; but when under Zn-deficiency conditions, the same species have mobilized little, if any, Zn from old leaves. Hence, in summary of several findings it may be stated that Zn is

likely to be concentrated in mature leaves. Roots often contain much more Zn than tops, particularly if the plants are grown in Zn-rich soils. With luxury levels of soil Zn, this element may be translocated from the roots and accumulated in the tops of the plant.

Plant species and varieties differ widely in their susceptibility to Zn deficiencies. Although these deficiencies are relatively common, their diagnosis is rather complex, and the best diagnose are obtained when based on visual symptom, plant analyses, and soil testing together. It must be emphasized, however, that for certain crops and soil, two chelating extractants, DTPA and EDTA, give linear relationships between Zn in the plant and the soluble Zn pool in the soil.

The most important factors contributing to Zn deficiency as follows (Lindsay, 1972):

1. Low soil Zn content
2. Calcareous soils and pH above 7
3. Soils low in organic matter
4. Microbial in activation of Zn in soil
5. Limited Zn uptake by roots due to restricted root zone and too cool spring seasons
6. Differential responses of plant species and genotypes
7. Antagonistic effects

Zn toxicity and Zn tolerance in plants have been of special concern because the prolonged use of Zn fertilizers, as well as its input from industrial pollution, is reflected in enhanced Zn content of surface soils.

Zn-Cd interactions appear to be somewhat controversial, since there are reports of both antagonism and synergism between the two elements in the uptake-transport processes. Kitagishi and Yamane (1981) explained the observed synergism in rice plants in terms of Zn competition for the Cd sites, resulting in an increase in Cd solubility, and in Cd translocation from the roots to tops. Wallace et al (1980) reported a high Cd accumulation in roots of plants at a high Zn level and at a low pH of solution. Earlier findings, however, antagonism between these cations in the uptake-transport process have been observed. It may be stated that the ratio of Cd to Zn in plant media controls the occurrence of synergism and antagonism between these cations.

Environmental Zn pollution greatly influences the concentrations of this metal in plants. In ecosystems where Zn is an airborne pollutant, the tops of plants are likely to concentrate the most Zn. On the other hand, plants grown in Zn-contaminated soils accumulate a great proportion of the metal in the roots.

2.1.3 Factors Affecting Mobility and Bioavailability of Zn

Since Zn^{2+} ions are intercepted by plant roots primarily through diffusion, any factor inducing changes in the solubility or affinity of Zn for soil constituents is important relative to its fate in the soil-plant system.

pH

Among factors affecting the solubility of metals in soils, pH is likely to be the most easily managed. In general, the solubility and phytoavailability of metals are inversely related to soil pH. At soil pH above 7.0 the bioavailability of Zn is substantially reduced. Severe Zn deficiency is often associated with alkaline soil pH. In calcareous soils, Zn deficiency can also be prevalent. It is difficult to correct Zn deficiency in calcareous soils because the added Zn precipitates in soil rapidly under this condition. There are numerous reports elucidating the depression of Zn uptake by crops in alkaline or calcareous soils (Adriano, 1986).

Zinc deficiency of rice is widespread throughout Asia on neutral to alkaline, calcareous soils especially those containing 1% or more OM. The incidence of Zn deficiency has been correlated positively with high pH (Forno et al.,1975); Yoshida et al.,1973). When soil pH is low, a low content of extractable Zn is not necessarily associated with Zn deficiency. In controlled experiments, Zn uptake by rice plants under either aerobic or anaerobic conditions progressively decreased as soil pH was raised from 5.0 to 8.0 (Jungsujinda and Patrick, 1977). There was a sharp decrease in uptake under aerobic conditions at pH 7.0 to 8.0. Lowland rice growing in calcareous or limed soils often exhibits Zn deficiency (Ponnamperuma, 1972).

While elevated soil pH is expressed as decreased metal content in plant tissue, its effect is directly expressed in the soil capacity to adsorb metals. In general, there is direct correlation between adsorption and soil pH (Harter, 1991). In soils, adsorption of Zn dramatically increased above pH 7.0 to 7.5 (Harter, 1983). At this pH range, Zn^{2+} and $ZnOH^+$ are expected to be the predominant species adsorbed.

The importance of pH on the chemical speciation of Zn and its adsorption by soil constituents has been discussed by Harter, (1991). It is well known that raising the pH by limiting apparently is the most efficient method for reducing plant uptake of potentially toxic metals (Albasel and Cottenie, 1985). In calcareous soils where Zn deficiency of crops and fruit trees is common, acidification of the root zone may prove an efficient method to increase the bioavailability of Zn to plants (Fenn et al., 1990).

Organic Matter

Zinc bioavailability to plants is generally low in organic soils. This can be attributed to the formation of insoluble Zn-organic complexes or adsorption by insoluble soil OM that renders Zn unavailable to plants. In comparing two Coastal Plain soils, Zn uptake by soybeans was lower from higher OM soil than the lower OM soil (White and Chaney, 1980). Other evidence linking the importance of OM to Zn retention by soils is the decreased adsorption capacity for soils where OM was removed (Shuman, 1991).

The role of OM on the mobility of Zn is influenced by pH, the solubility of soil OM increasing pH, which in turn affects the stability of metal-humic complexes.

Redox Potential

Redox condition has also a pronounced effect on the solubility of Zn and hence its bioavailability to rice plants. The effect is not directly related with Zn since it cannot be reduced under low redox conditions. In constantly flooded soils, insoluble zinc sulfide may be formed under strongly reducing conditions. This occurs at about -150 mV redox potential (Connell and Patrick, 1968), which may render Zn nonavailable to rice plants. However, the activity of rice plant roots may be involved in metal absorption and translocation from flooded soils averting potential nutritional problems (Chino, 1981a).

2.2 Cadmium

2.2.1 Cadmium in soils

There is growing environmental concern about Cd as being one of the most ecotoxic metals that exhibit highly adverse effects on soil biological activity, plant metabolism, and the health of humans and the animal kingdom. The behavior of Cd in the environment and related health aspects has recently been reviewed by (Kabata-Pendias and Pendias, 1999 and Stoeppler, 1991). The abundance of Cd in magmatic and sedimentary rocks does not exceed around 0.3 ppm, and this metal is likely to be concentrated in argillaceous and shale deposits. Cd is strongly associated with Zn in its geochemistry, but seems to be a stronger affinity for S than Zn, and also exhibit a higher mobility than Zn in acid environments. Cd compounds are known to be isotopic with corresponding compounds of such cations as Zn^{2+}, CO^{2+}, Ni^{2+}, Fe^{2+}, Mg^{2+}, and in some cases, of Ca^{2+}. During weathering, Cd goes readily into solution and, although known to occur as Cd^{2+}, it may also form several complex ions ($CdCl^+$, $CdOH^+$, $CdHCO_3^+$, $CdCl_3^-$, $Cd\,Cl_4^{2-}$, $Cd(OH)_3^-$, and $Cd(OH)_4^{2-}$ and organic chelates. However, the most important valence state of Cd in the natural environmental is +2, and the most important factors which control Cd ion mobility are pH and oxidation potential. Under conditions of strong oxidation, Cd is likely to form minerals (CdO, $CdCO_3$) and is also likely to be accumulated in phosphate and in biolith deposits.

The main factor determining the Cd content of soil is the chemical composition of the parent rock. The average contents of Cd in soils lie between 0.06 and 1.1 ppm. The calculated worldwide mean is 0.53 ppm Cd in surface soils, and apparently all higher values reflect the anthropogenic impact on Cd status in top soils (Kabata-Pendias and Pendias, 1999).

Reaction with soil Components

The sorption of Cd species by soil components has recently been widely studied. Farrah and Pickering (1977) stated that competitive adsorption by clays is the predominant process in Cd bonding. Also, findings of Tiller et al. (1979) and Soon (1981) support the opinion that adsorption, rather than precipitation, controls Cd concentrations in soil solutions until a threshold pH value is exceeded. The pH solubility diagram (Figure 2.4) indicates that above pH 7.5, Cd sorbed in soils is not easily mobile; therefore, the solubility of $CdCO_3$, and possibly of $Cd(PO_4)_2$,

would control the Cd mobility in soils. The solubility of Cd appears to be highly dependent on the pH; however, the nature of sorbent surfaces and of organic ligands is also of importance.
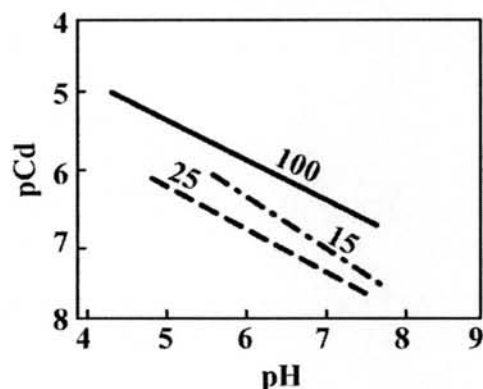

Figure 2.4 Solubility of Cd^{2+} in soils as a function of soil pH, equilibration with 15, 25, and 100 µg Cd per 2 g of soil (Soon, 1981).

As John (1972) reported the coefficient of bonding energy of Cd adsorption was higher for organic matter than for soil clays. Abd-Elfattah and Wada (1981), on the other hand, stated that Fe oxides, allophone, and imogolite reveal the highest affinity for the selective adsorption of Cd. Hydroxides appear to be minor importance in soil at low pH (<5.5). Cd sorption in acid soil is highly influenced by composition of the liquid phase. All the finding lead to some generalizations: in all soil, Cd activity is strongly affected by pH; in acid soils, the organic matter and sesquioxides may largely control Cd solubility, and that in alkaline soil, precipitation of Cd compounds is likely to account for Cd equilibria. The amount of Cd bound to organic matter and in residual fraction seems to be relatively stable in the soil, while its exchangeable forms increase significantly under sludge application (Figure 2.5).

Cd is most mobile in acidic soils within the range of pH 4.5 to 5.5, whereas in alkaline soil Cd is rather immobile. However, as the pH is increased in the alkaline range, monovalent hydroxy ion species are likely to occur (e.g., $CdOH^+$), which could not easily occupy the site on cationic exchange complexes. The sorption of Cd is known to be the fast process. Cd concentration in soil solution is relatively low and is reported to range from 0.2 to 6 µg/l.
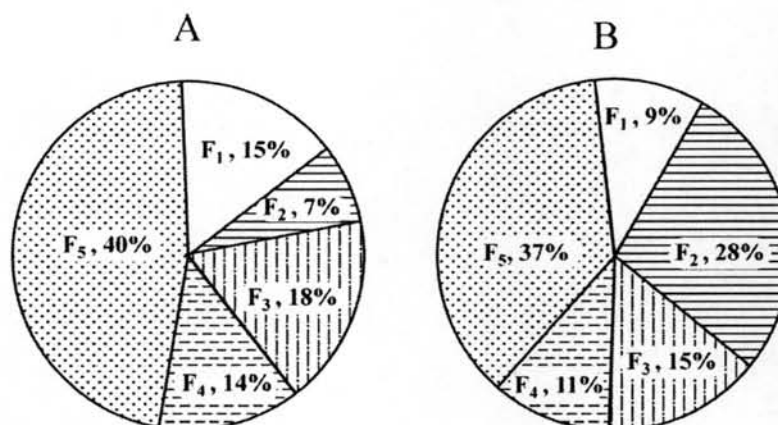
A   B

Figure 2.5 Variation in Cd species in soils under sludge application: (A) unsludged soil, (B) sludged soil. Cd species: (F_1) easily soluble; (F_2) exchangeable; (F_3) associated with hydrous oxides; (F_4) bound to organic matter; and (F_5) residual (Dudka and Chlopecka, 1990)

The solubility of Cd is closely related to the acidity of the soil solution. The critical acidity in mineral soils is within the pH range of 4.0 to 4.5, at which a drop in pH of merely 0.2 units results in a 3 to 5 times increase in Cd concentration. Asami (1984) found that a half or more of the total Cd in paddy soils is readily extractable by 0.05 M $CaCl_2$. Among the other trace metals, Cd is known to be most mobile under conditions of different soils. The strongest leaching was achieved by neutral solution containing Ca^{2+}, K^+, NO_3^- and SO_4^{2-} ions due to the alkalinity increase, Cd adsorption decreases, probably due to the competition from Ca^{2+} and Mg^{2+} ions. These have important implications for the development of realistic reclamation techniques for the management of Cd-enriched soils. In soil developed under the influence of humid climate, migration of Cd down the profile is more likely to occur than its accumulation in the surface horizon; thus, the enrichment in Cd content observed so commonly in top soils should be related to contamination effects.

Contamination of soil

Soil contamination with Cd is believed to be a most serious health risk. Under man-induced conditions, Cd is likely to build up in surface soils. However, elevated levels of Cd in soil can also be of lithogenic (geogenic) origin. The present concentration of Cd in top soils is reported to be very high in the vicinities of Pb and Zn mines and, in particular, smelting operations. Sewage sludges and phosphate fertilizers are also known as important sources of Cd. A significant source of Cd in soils is phosphate fertilizers. However, phosphate added to soils immobilizes the Cd. The exchangeable fraction of Cd decreases with the presence of phosphate in soils, while carbonate and oxide fractions increase. Phosphate fertilizers are a continuous source of Cd in soils, consequently this contributes the continuous increase of Cd in soils with the decrease of soil pH, and therefore the transfer of Cd to the food chain will grow significantly with time. Because of the environmental significance of the Cd accumulation in soil, several techniques for the management of Cd-enriched cropland have been investigated. Similarly, as in the case of Zn-contaminated soils, these techniques are based on increasing the soil pH, it is not effective for all soils and plants.

2.2.2 Cadmium in Plants

Absorption and Transport

Although Cd is considered to be a nonessential element for metabolic processes, it is effectively absorbed by both the root and leaf systems, and also highly accumulated in soil organisms. In nearly all the publications on the subject, soil pH is listed as the major soil factor controlling both total and relative uptake of Cd. However, there are contradictory results which show that when Cd becomes more mobile in alkaline soil due to the formation of complexes or metal chelates, the plant uptake of Cd may be independent of the pH. Kitagishi and Yamane (1981) described that when the redox potential of soils decrease to about - 0.14 V, the proportion of soluble Cd decrease, corresponding to the reduction of sulfate to sulfides. Although soil characteristics other than the pH can also cause differences in the Cd absorption by roots, it may be stated that soluble species of Cd in soil are always easily available to plants. The content of clay fraction proved to be the most important factor, among

other soil parameters, controlling the Cd uptake by plants. In some cases, however, soil pH and carbonates also influenced the Cd phytoavailability.

Chloride forms strong complexes with Cd (e.g., $CdCl^+$, $CdCl_2^0$, $CdCl_3^-$, and $CdCl_4^{2-}$) and therefore mobilizes and increases its phytoavailability. Data from several experiments indicate that soil salinity effects may dominate soil pH effects on the Cd uptake by plants.

A great proportion of Cd is known to be accumulated in root tissues, even when Cd enters the plant via foliar systems. When the amount of Cd is increased in the growth medium, the concentration of this metal in roots exceeds its content in the tops by 100 times. It may be concluded that the Cd in plants is relatively very mobile, although the translocation of Cd through the plant tissues may be restricted because Cd is easily held mainly in exchange sites of active compounds located in the cell walls. The Cd content of plants is, however, of great concern as a Cd reservoir and as the pathway of Cd to man and animals. Thus, tolerance and adaptation of some plant species to higher Cd levels, although important from an environmental point of view, create a health risk. Cd-Zn interactions are commonly observed, in most cases, Zn reduces the uptake of Cd by both root and foliar systems (Kabata and Pendias, 2001).

2.2.3 Factors Affecting Mobility and Bioavailability of Cd

The primary factors that affect mobility and bioavailability of Cd in soil are pH, texture, OM, Cd concentration, Cd species, Zn status, and salinity.

pH

Soil pH is the most important single soil property that determines Cd bioavailability to plants. Consequently, it is recommended that soil pH be maintained at pH 6.5 or greater in land receiving biosolids containing Cd. In general, Cd uptake by plants almost always increases with decreasing pH. Cadmium uptake by plants is usually higher in acidic than in alkaline or calcareous soils. Higher Cd concentrations were obtained for lettuce and Swiss chard grown on acid soils (pH 4.8 to 5.7) than on calcareous soils (pH 7.4 to 7.8) (Mahler et al., 1978). This pH effect on Cd uptake was attributed, in part, to competition between Ca^{2+} and Cd^{2+} ions at the root surface. Similarly, increasing the pH decreased the Cd uptake of radish plants grown on Cd-contaminated soils due to a decrease in mobility of Cd in the soil (Lagerwerff, 1971)

Soil pH was also negatively correlated with Cd content of rice (Bingham et al., 1980; Chino, 1981b; Takijima et al., 1973a). Total Cd uptake and shoot uptake increased with an increased in redox potential and a decrease in pH. They suggested that increased uptake by the rice plant under low soil pH conditions may also be due to the increased solubility of solid phases of Cd such as the carbonates, hydroxides, and phosphates, and increased concentrations of Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and H^+ which may then compete for exchange sites.

In addition to decreased solubility of Cd in soils associated with the formation of carbonates and phosphates under increasing pH, raising the pH of soil solution can lead to the formation of hydrolysis products that have a different affinity for sorption sites.

In the case of soils containing greater amounts of Ca^{2+}, the difference is considered to be due to ion competition between Ca^{2+} and Cd^{2+} ions at the root surface or interaction within the plant.

In general, metals are more soluble in soils at low pH due to the dissolution of the carbonates, phosphates, and other solid phases. Low pH also weakens the sorption of metals to specific adsorption sites on mineral surfaces and lowers the CEC of OM. There are exceptions, however. At pH above 7, solubility or uptake of Cd can be enhanced due to facilitated complexation of Cd with humic or organic acids (Naidu and Harter, 1998).

Organic Matter

The presence of OM in soils affects the biogeochemical processes in these systems. OM adsorbs metal ions by the ion exchange mechanism rendering them less mobile, therefore less bioavailable. Organic matter has both the cation exchange property and the chelating ability. It has been established that trace metals in sludge are taken up less readily by plants than are trace metals added to sludge as inorganic salts or trace metals added directly to soil in the same concentrations as present in sludge (Kirkham, 1977). The sorption ability of OM for Cd is predominantly through its CEC rather than chelating ability.

The amount of clay in relation to the amounts of silt and sand determines the soil texture, which in turn influences the CEC of soils. In general, the higher the clay content, the higher the CEC. In addition to CEC, soil humus has chelating ability, and certain metals have a tendency to combine with certain chelating

groups. In general, when Cd is added with sewage sludge, it is taken up less by plants than when added as inorganic Cd alone.

Because of the importance of organic acids from OM decomposition and root exudates on the solubility of micronutrients and metals in the rhizosphere (Mench and Martin, 1991), it was recently demonstrated that low-molecular-weight organic acids (e.g., oxalic, citric, etc.) have some ability to desorb Cd from soils, with malate, fumarate, and succinate being more effective than the others (Naidu and Harter, 1998).

Redox Potential

The moisture content of soils influences their retention for trace metals through oxidation-reduction reactions. In oxidized soils, redox potential may range from about +400 to +700 mV. In sediments and flood soils, redox potential may range from around -400 (strongly reduced) to +700 mV (well oxidated) (Gambrell and Patrick, 1978). Under reducing conditions, sulfides of metals can form. The metal-bearing sulfides are quite insoluble, so that metal mobility and bioavailability are considerably less than would be expected under oxidized soils. Elemental concentrations in solution extracted from sludge-treated soil indicate the decreased solubility of Cd, Cu, and Zn and increased solubility of Mn and Fe under reducing conditions (Bingham et al., 1976). Chino (1981b) indicated that activity of the rice roots may be involved in Cd absorption and translocation from flooded soil. Cadmium placed in the subsurface layer was absorbed by the roots in that layer but was not transported to the shoots, whereas Cd placed in the surface layer was transported efficiently. This implies that roots in reductive subsurface layer may be too inactive to transport Cd.

2.3 Extraction Procedure for Bioavailability Metals

A number of extraction methods have been suggested in recent years for the evaluation of trace element concentrations in soils. In this study, they can be classified into separate groups as follows: weak acid (acetic acid in the first step of BCR), chelating agents (EDTA and DTPA) and neutral salt solutions ($CaCl_2$).

2.3.1 Single Extraction Procedures

The extraction procedures for extractable heavy metals in soils have been developed and modified. In general, two groups of tests must be considered: the single reagent extraction test- one extraction solution and one soil sample- and the sequential extraction procedures – several extraction solutions are used sequentially on the sample. However, in this study used the only first step of the European Commission's Bureau Communautaire de Reference (BCR) which can be considered as a single reagent extraction. Both types of extractions are applied, using not only different extraction schemes but also different laboratory conditions. This leads to the use of reagent deal of extraction procedures. Extraction procedures using a single extractant are widely used in soil science. These procedures are designed to dissolve element contents correlated with the availability of the element to the plants. The approach is also applied to predict the plant uptake of essential elements, to determine element deficiency or excess in soil, to study the physical-chemical behavior of elements in soil or in survey purposes. Quevauviller et al (2002) reported that the method is also applied, to a lesser extent, to elements considered as pollutants, such as heavy metals. The application of extraction procedures to polluted or naturally contaminated is mainly focused to ascertain the potential availability and mobility of the pollutants and their migration in a soil profile, which is usually connected with groundwater problem. As far as soil is concerned, single extraction procedures are always restricted to a reduced group of elements and they are applied to a particular type of soil.

Chelating agents offer great promise for assessing readily available micronutrient cations in soils. These agents combine with free metal ions in solution forming soluble complexes and thereby reduce the activities of the free metal ions in solution. In response, metal ions desorb from soil surfaces or dissolve from labile solid phases to replenish the free metal ions in solution. The amount of chelated metals that accumulates in solution during the extraction is a function of the activity of metal ions in the soil (intensity factor) and the ability of the soil to replenish those ions (capacity factor). Both factors are important in determining the availability of elements to plants (Lindsay and Norvell, 1978).

Neutral salt solutions are often called 'soft' or 'mild' extractants and are based on un-buffered salt solutions, which mainly dissolve the cation

exchangeable fraction, although in some cases the complexing ability of the anion can play a certain role. Additionally, several studies have demonstrated a good correlation between metal concentration in these extraction media and metal concentration in plants. All these studies justify the use of un-buffered salt solutions to estimate the soil trace metal availability and define guide values for risk assessment (Pueyo, et al 2004).

EDTA (Ethylene diamine tetra acetic acid)

EDTA is a synthetic chelator that has been shown to substantially lower soil CEC. EDTA is a non-specific extractant, and it can extract labile and non-labile fractions. It has been used since the 1950s to alleviate iron deficiency, and has been used frequently to improve phytoextraction of metal contaminants, such as lead, from soil (Jorgenson, 1993). EDTA is not known to be particularly toxic per se in the environment. Its capacity to mobilize toxic metals in combination with its persistence is of environmental concern (Kikham, 2006). Oftentimes, a sudden increase in bioavailable metals resulting in EDTA application will be fatal to plants. This is overcome by growing plants up to a large biomass, then adding EDTA. The metal will become highly bioavailable, and will be taken up in large quantities by the plant for a short time before the plant dies. In this way, large amounts of metal can be extracted from soil. This strategy is referred to as chelator-assisted phytoextraction. EDTA has been reported to remove both the organically bound metals and metals occluded in oxides and secondary clay minerals in part (Perez et al., 1993). EDTA belongs to the best chelating agents, because it offers the most favorable combination of stability constants for the complexing of the studied elements. EDTA extracts represents the content of the elements sorbed and organically bound to the soils (Zemberyov´a et al., 2006).

DTPA (Diethylenetriamine-pentaacetic acid)

DTPA is one of the most commonly used methods to determine plant availability of heavy metals which was originally developed to extract Mn, Fe, Cu, and Zn from slightly acidic to alkaline soils (Lindsey and Norvell, 1978), but in some cases it can be used for other metals such as Ni, Cd, and Pb and for acid soils provided soil pH and perhaps other soils properties are used in interpreting the results. The DTPA is to extract soil metals by forming chelates with free metals in solution,

which lowers their ionic activities so that additional metal quantities are released from the soil until equilibrium is attained. DTPA was originally developed to identify near-neutral and calcareous soils with insufficient transition metals. Iron and manganese oxides are potentially extracted from acidic soils by DTPA (O'Connor, 1988). In this study, extractions are done for a fixed time interval because equilibrium may not be attainable within a reasonably short time period. The degree of chelation of various metals is a function of magnitudes of the metal-DTPA stability constants and also varies with pH. For DTPA extraction procedure, triethanolamine (TEA) is used to buffer the extracting solution to pH 7.3 in an attempt to optimize the extraction of various metals and calcium chloride is included in extracting solution to minimize dissolution of calcium carbonate minerals.

CaCl$_2$ (Calcium chloride)

CaCl$_2$ is recommended as an extractant for extraction of cations or anions. The CaCl$_2$ is proposed as a simulated solution phase of soil for the estimation of bioavailable trace element contents because of the labile Cd is solubilized through complexation by Cl$^-$ and competition for surface sites by Ca^{2+} and the formation of Cd-Cl complexes and the formation is attributed to increased solubility of Cd in soil solution and Cd uptake by plants (Kuo, Lai, and Lin., 2006). This final conclusion is that CaCl$_2$ extractant can be considered universal because it has more or less the same ionic strength (0.03 M) as the average salt concentration in many soil solutions. It cannot however, serve as a soil test to estimate both deficient and phytotoxic levels of trace metals. To do this, calibration to biological indices is necessary. Possible formation of complex ions with Cl$^-$ in certain soil conditions should be considered. Such complex ions (e.g., CdCl$^+$, CdCl$^-_3$) can increase the solubility of some trace metals, as observed in the case of Cd (Kabata Pendias and Pendias, 2001). Therefore, CaCl$_2$ is a more reasonable choice of a neutral salt extractant.

2.3.2 Sequential extraction (BCR)

The European Commission's Bureau Communitaire de Reference, BCR, three steps sequential extraction method is the procedures to harmonize the extraction schemes for the determination of extractable metals (Ure et al., 1993). The BCR protocol is a defined operational procedure using different reagents in order to

separate the metal fractions associated with different phases of the soil. The four fractions divided as the follows: (1) exchangeable fractions, water and acid soluble, (2) reducing conditions, (3) oxidizing conditions, and (4) residue fraction. In the first step, using acetic acid as the solvent, the mobile fraction of the metal, soluble in water or in weak acids, adsorbed or co-precipitated with carbonates, is obtained. In the second step, the metals bound to iron and manganese oxides are extracted with a solution of hydroxylamine ammonium chloride because of their thermodynamic instability under anoxic conditions and dissolution of metal-oxide phases under control pH and Eh conditions. While in the third step, the metal fraction bound to the organic matter or to sulfides is extracted with a solution of ammonium acetate after treatment with hydrogen peroxide. Lastly, the forth step, digestion with aqua regia (ISO 11466 protocol) is commonly used for determination the metal associated in the lattice of primary and secondary minerals. In this case possible changes in environment condition the release of metals from this fraction on a time-scale of several years (Pe'rez and Valiente, 2004).

2.4 Literature reviews

Jahiruddin et al. (1992) studied effects of soil properties on the extraction of zinc. The results showed that the extractants' ability to remove Zn followed the order: 0.1 M HCl > EDTA-$(NH_4)_2CO_3$, pH 8.6 > DTPA, pH 7.3~ 1 M NH_4OAc, set at soil pH > NH_4OAc, pH 4.8 > water. The influence of soil pH was much more evident in low organic matter soils ($\sim< 6.5\%$) than in soils with high organic matter contents ($> 6.5\%$). For soils with high organic matter contents, the amounts of Zn extracted by HCl, EDTA or DTPA were positively correlated with soil CEC, whereas the correlations were negative or insignificant for soils with low organic matter contents. DTPA and NH_4OAc both appear to extract labile Zn from the soil solid phase with comparable efficiency when soil pH is < 5.5. For these extractants and soils, soil pH was of no consequence. For low organic matter soils, soil pH was a key factor, and high clay content and/or CEC appeared, if anything, to decrease Zn extractability.

Pandeya et al. (1998) studied the labile pool of cadmium in sludge-treated soils by application of isotopic dilution principle under laboratory and green

house conditions using moong (Phaseolus aureus L.) as a test crop. The isotopic distribution coefficient (Kd) of ^{115}Cd in 0.1 M CaCl$_2$, labile pool (LP) of Cd in DTPA–CaCl$_2$–Na acetate and supply parameter (SP) using Kd as intensity and LP as capacity factor of Cd in soils, were computed to compare with actual uptake of Cd by the crop to test them as indices of Cd availability. The water soluble + exchangeable Cd and the 0.05 M EDTA extractable Cd were observed to be the two major chemical pools of Cd in soils responsible for supply of this element to plants. The amounts of soil Cd extracted by DTPA–CaCl$_2$–TEA (pH 7.3), EDTA–NH$_4$OAC (pH 7.3) and Mg(NO$_3$)$_2$ (pH 6.0) were significantly correlated with concentration of Cd in plants and with uptake of Cd by moong crop.

Maiz et al. (2000) studied evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. For the more labile metal fractions, the mobile (extracted by 0.01 mol/l CaCl$_2$) and the mobilisable (extracted by 0.005 mol/l DTPA), were evaluated using a short sequential procedure with two steps. The labile levels were compared with the results obtained using the Tessier sequential procedure. Factor analysis was used to check the associations between the total metal contents in soil and grass, as well as between the levels of the different sequential fractions and the total content in grass. The results showed that the metal lability in polluted soils and its availability to the plants was highly dependent of the association forms that the metal presented in soil and the type of plant. They found Cd, Cu and Zn labile levels were correlated with the total content of these metals in grass, indicating the suitability of the short procedure for availability studies of those elements. The labile levels obtained with the first four steps of the Tessier procedure showed a strong association with total contents of grass for Cd, Cu, Fe and Ni.

Kashem and Singh (2001) studied the effects of flooding and organic matter application on changes in Eh, pH and solubility of Cd, Ni and Zn in contaminated soils. The results showed that the effect of Eh and pH on metal solubility depends on metal type and soils. The behavior of Cd and Zn was similar in its solubility with the former metals. Adsorption of Cd and Zn on Fe-Mn hydroxide fractions was the major mechanism of their solubility reduction in submerged conditions. The soluble metal concentration in all three soils was related to larger changes of Eh and pH values in these soils. Correlation coefficient calculations also

show that metal solubility decreased with decreased Eh and increased pH in the soil solution.

Pérez et al. (2002) applied theconventional four-step sequential extraction method (The Tessier) and the EDTA and acetic acid single extraction procedures to sewage sludge and sediment samples. The Tessier method compared with those supplied by the two single extractions to determine Cu, Cr, Ni, Pb, and Zn. As a result, Good agreement was found between the metal contents released in the first three fractions of the Tessier method and those leached by single extraction procedures. The extraction efficiency of the first three fractions of Tessier method in sewage sludge and CRM 483 samples provided satisfactory results for all the elements studied, except Cr and Pb. They concluded that single extraction procedures optimized in their work by the easiest mobilizable heavy metal in these samples with provided satisfactory results for all the elements studied, except for Cr in both methods and for Pb in acetic acid extracts.

Ayoub et al. (2003) investigated the distribution of labile Cd and Zn in two contrasting soils using isotopic exchange techniques and chemical extraction procedures from Great Billings, Northampton and an unamended soil, Aberdeen, UK were used. The extractable metal content of the soils was also quantified by extraction using 0.1 M $NaNO_3$, 0.01 M $CaCl_2$, 0.5 M NaOH, 0.43 M CH_3COOH and 0.05 M EDTA at pH 7.0. Between 1.3 and 68% of the total Cd and between 1 and 50% of the total Zn in the Great Billings soil was extracted by these chemicals. For the Countesswells soil, between 6 and 83% of the total Cd and between 0.1 and 7% of the total Zn was extracted. 0.05 M EDTA and 0.43 M CH_3COOH yielded the greatest concentrations for both soils but these were less than the isotopic estimates.

Fernández et al. (2004) applied the procedure of BCR sequential extraction to five samples from two unpolluted soils in southern Spain for determination of As, Cu, Cd, Co, Cr, and Pb. The results showed that for Cu, Co, Cr and Zn, the comparison of the results did not provide definitive conclusions concerning the capability of BCR in measuring total concentrations. On the other hand, a certain correlation was found between the concentrations measured and some soil characteristics, especially the clay, organic-matter.

Pueyo et al. (2004) studied three different extraction procedures including $CaCl_2$, $NaNO_3$ and NH_4NO_3 to estimate soil contamination and trace metal availability to plants. The soil-reference material BCR CRM 483, with indicative

values for $CaCl_2$, $NaNO_3$, NH_4NO_3 extractable metals, was analyzed for quality control purposes. The three methods were also applied to 10 contaminated soils and the extracted metals (Cd, Cu, Pb, and Zn).. The results showed that the metal extraction efficiency obtained with each procedure was slightly different, and the three methods provided equivalent information while predicting the relative trace-metal mobility (Cd > Zn > Cu > Pb) in the soils studied. They concluded that the 0.01 mol/l $CaCl_2$ extraction procedure seems to be the most suitable method for performing a harmonization process, since this procedure combines an appropriate extraction capacity for this type of studies with the lowest salt concentration in the extracts and, consequently, with a more simple matrix for metal determination.

Feng et al. (2005) compared a rhizosphere-based method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ extraction methods for the evaluation of bioavailability of heavy metals in soil to barley. The results showed that the proposed rhizosphere-based method was suitable for acidic, neutral and near alkaline soils. In contrast, the other extraction methods were restricted to soil types. The DTPA and EDTA extraction methods were suitable only for calcareous or for acidic soils, respectively. The $CaCl_2$ and $NaNO_3$ extraction methods were only suitable for exchangeable metals.

Ping et al. (2005) studied relationships between fractions of Pb, Cd, Cu, Zn and Ni and soil properties urban soils. The results showed that Pb, Ni and Cu were mainly associated with the residual and organic forms; most of Cd was concentrated in the residual and exchangeable fractions. Zn in residual and carbonate fraction was the highest. The activities of the heavy metals probably declined in the following order: Cd, Zn, Pb, Cu and Ni. Soil properties had different influences on the chemical fractions of heavy metals to some extent and the main factors influencing Cd, Zn, Pb, Cu and Ni fractionation and transformation were apparently different.

Ettler et al. (2007) studied four operationally defined single extractions tests: deionised water, 0.01 mol/l $CaCl_2$, 1 mol/l NH_4NO_3 and 0.005 mol/l DTPA in highly polluted forest/tilled soils and stream sediments from mining and smelting area. The DTPA procedure extracted the highest amounts of metals, but found to be unsuitable for highly organic acidic forest soils, where anionic metal–DTPA complexes are assumed to be re-adsorbed on the positively charged surfaces of soil organic matter and oxides. The NH_4NO_3 extraction solution yielded similar values for the extracted concentrations for all the studied elements as the 0.01 mol/l $CaCl_2$

extraction The 0.01 mol/l CaCl$_2$ solution seems to be the most suitable extraction agent for highly contaminated soils and sediments. The authors concluded that both water and CaCl$_2$ extracts with low ionic strength are suitable for subsequent determinations by analytical techniques with relatively low matrix effects.

Moreno et al. (2007) applied the BCR (Community Bureau of Reference) sequential extraction scheme (exchangeable + weak acid soluble, reducible, oxidizable, and non-extractable metal fractions) to volcanic soils to determine partitioning of zinc and copper between various solid-phase constituents, along with the major elements Al, Fe and Mn. Zn and Cu contents of the soils were generally large, total amounts extracted (the potentially mobilizable fraction) were small, especially for Zn and for soils with crystalline secondary minerals. The fraction of the total Cu which was potentially mobilizable generally exceeded that of Zn. In the potentially mobilizable Cu, the oxidizable fraction was generally dominant. The BCR sequential extraction appears to be a valuable tool for studying metal dynamics in soils with acidic properties.

Madrid et al. (2007) studied metal accumulation and DTPA-extractability in a sandy soil after three successive applications of municipal solid waste compost (MSWC) under intensive farming conditions. The results showed that increases in DTPA-extractable (available content) concentrations of metal contents were observed at higher rates than in aqua-regia extractable contents. They suggested that metals added with compost were more available than native metal in soils.

Cappuyns and Swennen (2008) compare and evaluate different methods to determine the influence of acid conditions on heavy metal release from soils, sediments and waste materials. The extractions with NaOAc (1 M, pH 5), HOAc (0.11 M), HOAc (0.43 M) and pH$_{stat}$ titrations (at pH 2, 4 and 6) were carried out on soils, sediments and waste materials with different physico-chemical properties and a different degree of contamination. The results showed pH played an important role in explaining the release of metals from the contaminated soils, sediments and waste materials. The pH-shift after extraction with the different acetic acid solutions (0.11 M and 0.43 M) was both explained by the initial pH of the sample and its acid neutralizing capacity. The pH of the NaOAc extract was well buffered and the release of elements from solid matrices by NaOAc both the result of the complexation with

acetate and pH (pH 5). Generally, a linear correlation is found between the amount of Zn and Cd extracted by 0.11 M HOAc, 0.43 M HOAc and 1 M NaOAc. As can be expected, a 0.43 M HOAc solution extracts more metals than a 0.11 M HOAc solution. The amounts of Zn and Cd extracted with HOAc (0.11 M and 0.43 M) were comparable with amounts of Zn and Cd released during pH_{stat} leaching at pH 4.