

## CHAPTER II

# THEORETICAL BACKGROUND AND LITERATURE REVIEWS

### 2.1 Bioavailability

In soil, metals exist as a variety of chemical species in a dynamic equilibrium governed by soil physical, chemical and biological properties (Lasat, 2002). The mobility and bioavailability of individual trace metals are metal specific (Lee, 2006). Speciation is of the major importance to provide the qualitative information on their reactivity under changing conditions (Sparks, 1995) and crucial information about ecotoxicity characteristics (reactivity, bioavailability, toxicity) of contaminants (Herreweghe et al., 2003). According to several studies (Gobran, Lombi, and Wenzel, 2001; Nigam and Srivastava, 2003; Žemberyová et al., 2006), it has shown that the form the metal in soil is of much greater importance than the total concentration with regard to the bioavailability of metals. The behavior of the elements in the environment cannot predict on the basis of their total concentration because it overlooks the fact that not all of the metal may be labile or available for uptake (Gobran et al., 2001) due to the different and complex distribution patterns of metals among various chemical species of solid phases. The total metal content of a soil is distributed among all possible chemical forms (speciation) in the solid, liquid or the biotic phases (Vig et al., 2003). Use of total concentration as a criterion to assess the potential risks may be misleading as the risks may be over-estimated, implies that all forms of a given metal have an equal impact on the environment (Tessier et al., 1979).

Despite this, the availability of metals and the toxicity of metals are more closely related to available metal, rather than total metal concentrations in soil (Bettinelli et al., 2000). Bioavailability can be defined as the total metals that are available for incorporation into biota (bioaccumulation) (John and Leventhal, 1995) and/or the availability of chemicals to living receptors (plant roots) through direct absorption or uptake. For most metals, uptake into roots takes place from the aqueous phase. Strong binding to soil particles and or precipitation render significant soil

metal fraction insoluble, and largely unavailable for plant uptake (Lasat, 2002). This suggests that there is a continuum between 0% bioavailability and 100% bioavailability which vary depending on the nature of the environment. Commonly small percentage is present in the form in which can be taken up by the plant. The bulk is present in the soil solid phases, as unavailable to the plant (Sparks, 1995). Speciation of the metal ions in the soil solution may play a significant role in its bioavailability (Vig et al., 2003). Thus, it is therefore, more important to know the distribution of each trace metals in the forms than just the total contents to understand the processes whereby metals are held in soils and the conditions under which they can be released.

## 2.2 Sequential Extraction

A number of chemical extractions have been developed to determine element behavior including single and sequential extraction in order to fractionate the metals into specific phases. Single and sequential extraction procedures have been commonly applied to soils and sediment in the environmental studies, enable broader forms or phases to be measured.

Single extraction uses only one solvent aiming directly for the determination of the available form for the plant. The extractants commonly used include H<sub>2</sub>O, KNO<sub>3</sub>, EDTA, DTPA and HNO<sub>3</sub>. Each extractant is targeted on a single form of each metal, for example H<sub>2</sub>O for soluble, KNO<sub>3</sub> for exchangeable, EDTA for carbonate and sulfide precipitation and HNO<sub>3</sub> for residual form of each metal (Iwegbue et al., 2007). Alternatively, several schemes of sequential extraction procedures have been proposed frequently to characterize and predict the mobility and bioavailability of heavy metals in soils (Iwegbue, et al., 2007), for example the methods of Tessier et al. (1979), the Standard Measurement and Testing Programme (SM&T, formerly BCR), Förstner, and Meguellati, etc. The use of sequential extraction furnishes detailed information about origin, mode of occurrence, mobilization and transport of trace metals (Žemberyová et al., 2006). The principle of this procedure is based on the selective extraction of heavy metals in different physicochemical fractions using a series of extracting reagents of increasing strengths to fractionate elements in solid materials into several groups of varying mobility. At

first, the most mobile fractions will be extracted followed by increasingly absorbed fractions and finally those fractions that are strongly attached to the soil matrix (Manz et al., 1999). The resulting extract is operationally defined based on the proposed chemical association between the extracted species and solid phases in which it is associated. Procedures differ mainly in the nature of reagents used, volume and the time required for optimum extraction (Alomary and Belhadj, 2007). Sequential extractions are considered to be a great tool in the determination of metal distribution because the results obtained may give the answer or information about geochemical phases within an element exist. And such knowledge allows for a better insight into the mechanism of heavy metal retention and released thus providing the evaluation of availability, mobility or persistence.

In this study, the three-step BCR sequential extraction procedure according to the Standards, Measurements and Testing Programme of the European Union (SM&T) was more preferable here. This scheme consists of three successive extractions (Table 2.1). The first step (BCR1), acetic acid extraction, portions of metals found in this fraction are weakly absorbed and easily solubilized which become readily available to plant. Acetic acid extracts ion-exchangeable forms of elements, acid soluble fraction and forms bound to carbonates, which are held by electrostatic adsorption (Narwal and Singh, 1998). The second step (BCR2), with hydroxylamine hydrochloride as an extractant, will solubilize the remaining carbonates not previously leached out in the first step and metals associated with reducible species mainly Fe/Mn oxides (Pérez-de-Mora et al., 2007). The third step (BCR3) (hydrogen peroxide and ammonium acetate) extract the metals that bound to the various forms of organic matter and sulfides. Mobility and bioavailability of the metals decrease approximately in the order of the extraction sequence (Dudka et al., 1996). Each successive form represents less availability (Iwegbue et al., 2007). Exchangeable (BCR1) and reducible (BCR2) fractions indicate the fraction of metals that are most mobile and available for plant uptake, while those in the latter stages of the extraction schemes are less available to plants (Davidson et al., 1998; Ipolyi et al., 2002; Tokaliolu et al., 2003; Obrador, 2007). Therefore, only the first-two steps of the BCR sequential extraction were focused and carried out in this study with the intension for determination of the availability of metals, particularly Cd.

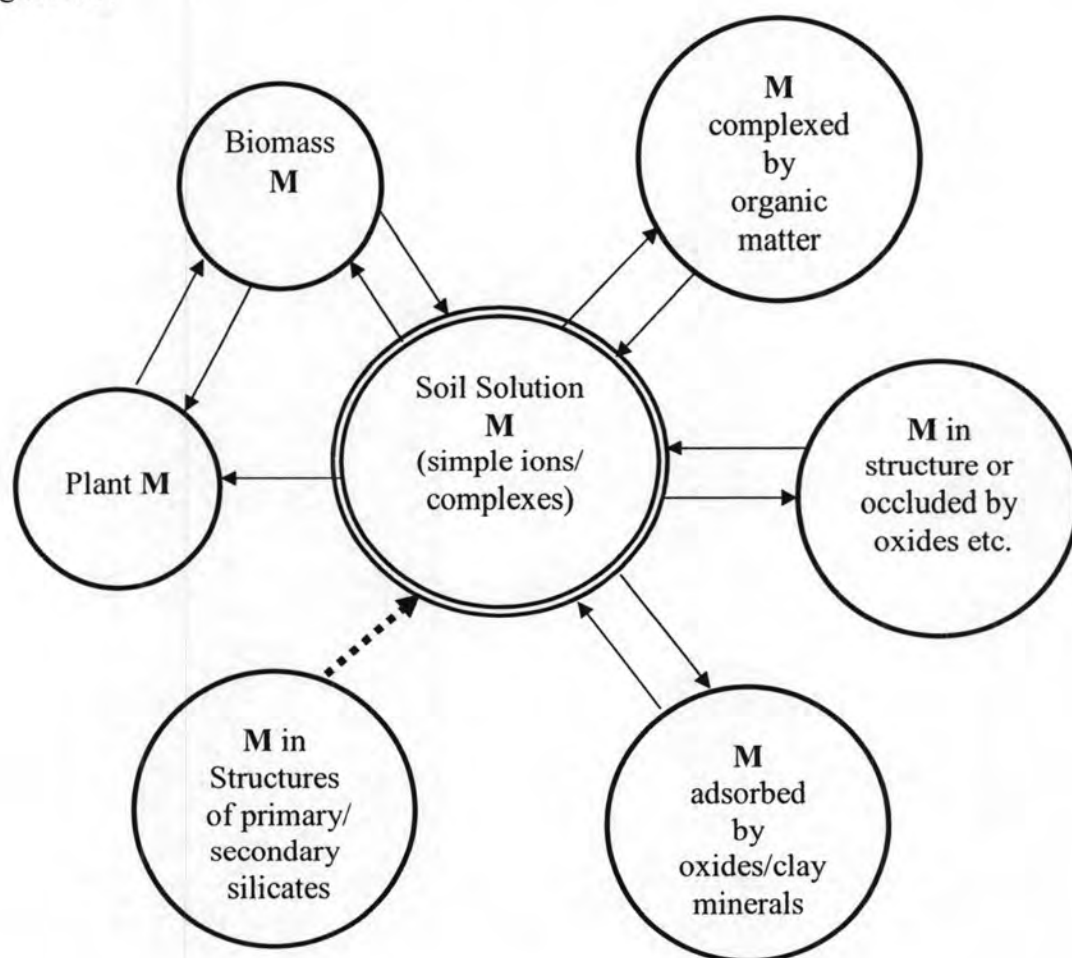
**Table 2.1** The three-step BCR sequential extraction (modified from Pérez and Valiente, 2005)

Fractions	Reagent	Metal species/association
1. Exchangeable (BCR1)	0.11 mol/L CH <sub>3</sub> COOH (acetic acid)	Bound to colloidal/particulate material or soil surface by relatively weak electrostatic interactions, susceptible to change of pH, released by changes in ionic composition and affected by production or consumption of protons
2. Reducible (BCR2)	0.5 mol/L HONH <sub>2</sub> -HCL hydroxylamine hydrochloride	Bound to iron and manganese oxides, instability under anoxic conditions and dissolution of metal-oxide phases under controlled ORP and pH conditions
3. Oxidizable (BCR3)	8.8 mol/L H <sub>2</sub> O <sub>2</sub> (hydrogen peroxide) and 1.0 mol/L NH <sub>4</sub> OAc (ammonium acetate)	Bound to the various forms of organic matter and sulfides. The degradation of organic matter under oxidizing conditions is responsible for releasing trace elements.

Based on results of numerous studies, investigations and observations (He and Singh, 1993; Manz et al., 1999; Kashem and Singh, 2002b; Liu et al., 2003; Wang et al., 2007), bioavailability of metals is a complex function of many factors such as the pH, oxidation-reduction potential (ORP), organic matter (OM), and influence of other soluble ionic species. Thus, identification of the major soil parameters affecting metal mobility in soils is requisite to prediction of metal behavior (Selim and Kingery, 2003).

## 2.3 Soil Parameters Affecting Metal Uptake

Knowledge of the factors affecting the concentrations of elements dissolved in the solution phase of soils is central to understanding the behavior of elements in the soil system (McLaughlin and Singh, 1999). Once metals enter the soil, they can be taken up by the standing crop, remain in soil in soluble and/or insoluble form or even leach to groundwater (Kashem and Singh, 2002a). A soil solution composition provides an important index of the availability of different ions to plants (Manz et al., 1999). It is a dynamic and mobile system acting as a chemically reacting system. The soil solution reflects the processes controlled by mineral equilibria, cation exchange, sorption/desorption reactions and complexation with organic matter (Lee, 2006). The association of trace metals in soil solution is given in Figure 2.1.



**Figure 2.1** The association of micronutrient (M) in soil solution (modified from McLaren and Cameron, 1997)

Different chemical, physical and biological processes acting on soil media control the fate of heavy metals in soil (Kashem and Singh, 2001a). The major factor governing metal behavior to plants in soils is the solubility of the metal associated with the solid phase (Cataldo and Wildung, 1978). The factor influencing solubility and the distribution of metals between the specific forms varies widely according to the individual metal and the characteristics of soils (Sparks, 1995). Overall, the most important factors influencing the form of available metal species in soil are: soil pH, oxidation-reduction potential (ORP) and soil organic matter content (OM) (He and Singh, 1993; Sparks, 1995; Manz, 1999; Kashem and Singh, 2001b). These factors vary seasonally and temporally. Most factors are interrelated as changing one factor may affect others (John and Leventhal, 1995), as this influence the kinetics of sorption reaction, metal concentration in solution and the form of soluble and insoluble chemical species (Cataldo and Wildung, 1978).

Thus, knowledge of the soil properties and interactions between other metals are important to assess the bioavailable Cd uptake by sugarcane. This in turn related to food chain contamination and the health risk. The following selected soil factors affecting metal bioavailability (particularly Cd) focused in this study were:

- Soil pH
- Organic Matter (OM)
- Oxidation-reduction potential (ORP)
- Presence of other metals (Cu, Fe, Mn, Pb and Zn)

### **2.3.1 Soil pH**

Soil pH is considered as the most important factor affecting metal speciation (Selim and Kingery, 2003) and behavior in soil systems both directly and indirectly. The influence of soil pH on metal availability is due mainly to its effect on the reactions controlling concentrations of metal in the soil solution (Sparks, 1995). Metal speciation changes with pH, affecting metal sorption/desorption on solid surfaces as well as the solubility of metal hydroxide minerals. Metals tend to be adsorbed at different pH values. This results in precipitation of different metals over a large range of pH units. For example, Cd and Zn tend to have adsorption edges at higher pH than Fe and Cu, and consequently they are likely to be more mobile and more widely dispersed (Kabata-Pendias and Pendias, 2001). The most mobile

fractions of ions occur at a lower range of pH and at a lower oxidation-reduction potential (Sparks, 1995).

### **2.3.2 Oxidation-reduction potential (ORP)**

Oxidation-reduction processes are expected to play a major role in the mobilization/immobilization processes of trace elements that can occur under various oxidation states in soil conditions (Selim and Kingery, 2003). Redox potential provides information on conditions that favorable for increased bioavailability and distribution of species. Metals with multiple oxidation states may present as any more species than simple cations. Typically the higher oxidation states predominate under oxidizing conditions, while the lower oxidation states predominate under reducing conditions. Iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) for example are complex cations occur in several oxidation states ( $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mn}^{3+}$ ,  $\text{Mn}^{4+}$ ). Changes in the oxidation state of the metals associated with the oxides can greatly affect their solubility and mobility in soil and aqueous environments (Lee, 2006). As soils become anaerobic, the redox potential decreases and transformation to the more soluble reduced forms of metals ( $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) take place. This effectively results in the dissolution of oxide minerals and the substantial increases in solution concentrations ( $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) and therefore plant availability of these nutrients is enhanced (Sparks, 1995).

### **2.3.3 Soil organic matter (OM)**

Soil organic matter influences many of the physical, chemical and biological properties of soils. Organic matter is of important in the transportation and accumulation of metallic ions (Kabata-Pendias and Pendias, 2001) which affects the availability of micronutrient (Quevauviller, 2002) due to it act as an important sorbent of plant macronutrients, micronutrients, heavy metal cations (particularly of copper and manganese) (Sparks, 1995) and organic material. Interaction between organic matter and metals has been described as ion exchange, surface sorption, chelation, coagulation and peptization. Organic matter may change the solid/liquid phase equilibrium through their effect on sorption, solubility or dissociation of metals (McLaughlin and Singh, 1999). All reactions lead to the formation of water-soluble and/or water-insoluble complexes (Kabata-Pendias and Pendias, 2001).

The uptake and availability of plant nutrients, particularly micronutrients such as Cu and Mn, are generally affected by organic matter (Sparks, 1995). Soil colloids responsible for the specific adsorption of micronutrients are principally the oxides and hydrous oxides of iron, aluminum and manganese and soil organic colloids. The presence in the soil of these colloids able to adsorb micronutrients will therefore have a major influence in controlling micronutrient availability to plants. The greater the number of potential micronutrient adsorption sites, the lower will be solution micronutrient concentrations and plant availability (Sparks, 1995).

## 2.4 Six interested metals

### 2.4.1 Cadmium (Cd)

Cadmium is a non-essential trace metal pollutant and considered as one of the most important elements for food-chain contamination that results from various agricultural, mining and industrially activities (Pinto et al., 2004). Cadmium is an element that occurs naturally in the earth's crust and not usually present in the environment as a pure metal, but as a mineral combined with other elements such as oxygen (cadmium oxide), chloride (cadmium chloride) or sulfur (cadmium sulfate, cadmium sulfide) (Christensen, 1987). It always occurs in combination with zinc and also similar in many respects but it forms more complex compounds. Cd is much more mobile than other heavy metals as a result of its weak affinity for soil colloids and might be easily transferred to crops (Renella et al., 2004). Cd in soil solution can be present in different physico-chemical forms, varying in size and charge (McLaughlin and Singh, 1999). Dissolved Cd can be present as free, hydrated cations or as several complex ions ( $\text{CdCl}^+$ ,  $\text{CdOH}^+$ ,  $\text{CdHCO}_3^+$ ,  $\text{CdCl}_3^-$ ,  $\text{CdCl}_4^{2-}$ ,  $\text{Cd(OH)}_3^-$  and  $\text{Cd(OH)}_4^{2-}$ ) and organic chelates, however, the most important valence state in the natural environment is  $\text{Cd}^{+2}$ . Cd in plants is relatively very mobile, 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 (Kabata-Pendias and Pendias, 2001).



### 2.4.2 Copper (Cu)

Copper is essential for plants and animals and known to be one of the least mobile heavy metal in soil. It occurs as free and complexed ions in soil solutions of all types of soil. Chelation and complexing are the key reactions governing Cu behavior in most soils (Kabata-Pendias and Pendias, 2001). The natural concentration of copper found in plants usually ranges from 5 to 25 mg/kg (dry weight). A concentration below this level will render the plant deficient in copper, although a concentration above 25 mg/kg might promote phytotoxicity (Haroun et al., 2006). The most common mobile Cu in the surface environment is believed to be the cation with the valence of +2 which held very tightly on both inorganic and organic exchange sites depending on the surface charge carried by the absorbents. Under neutral to weak alkaline conditions there exist higher fractions of organically complexed copper (soluble organometallic complexes) in the soil. Soil retains copper through exchange and specific adsorption. Copper retention and partitioning in soils is related to the presence of organic matter, Fe and Mn oxides, and clay minerals soils through exchange and specific adsorption mechanism (Balasoïu et al., 2001). The bioavailability of soluble forms of Cu depends most probably on both the molecular weight of Cu complexes and on the amount present. The concentrations of Cu in soil solutions are principally controlled by both the reaction of Cu with active groups at the surface of the solid phase and by reactions of Cu with specific substances (Kabata-Pendias and Pendias, 2001).

### 2.4.3 Iron (Fe)

Iron plays significant roles in the biochemistry of plants as the geochemistry is very complex by the easy change of its valences states in response to the physicochemical conditions. In soils, Fe is believed to occur mainly in the forms of oxides and hydroxides as small particles and/or associated with the surfaces of other minerals. Both mineral and organic compounds of Fe are easily transformed in soils. Organic matter appears to have a significant influence on the formation of Fe oxides. Any iron species may become attached to soil components to form more species. The content of soluble Fe in soils is extremely low in comparison with the total Fe content. Soluble inorganic forms include  $\text{Fe}^{3+}$ ,  $\text{Fe}(\text{OH})^+_{2}$ ,  $\text{FeOH}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}(\text{OH})^-_{3}$  and  $\text{Fe}(\text{OH})^{2-}_{4}$ , most commonly iron is the ferrous ( $\text{Fe}^{2+}$ ) or ferric ( $\text{Fe}^{3+}$ ). Changes between these two depend on the soil's pH and ORP. Fe plays a special role

in the behavior of several trace elements and of some macronutrients. The degree to which soil Fe is responsible for trace metal solubility and availability is strongly governed by several soil factors. Conversely, heavy metals are also known to influence the bioavailability of Fe (Kabata-Pendias and Pendias, 2001).

#### 2.4.4 Manganese (Mn)

Manganese is one of the most abundant trace elements in the lithosphere, and forms various oxide and hydroxide species and chelates with many components. Mn may form a number of simple and complex ions in solution and also several oxides of variable composition. It exists in multiple oxidation states:  $Mn^{2+}$ ,  $Mn^{3+}$ , and  $Mn^{4+}$  but its oxidation state +2 is the most frequent in the rock-forming silicate minerals. All Mn compounds are very important soil constituents because this element is essential in plant nutrition and controls the behavior of several other micronutrients. It was found that Mn uptake is metabolically controlled which was similar to that of divalent cation species such as  $Mg^{2+}$  and  $Ca^{2+}$  and known to replace the sites of some divalent cations ( $Fe^{2+}$ ,  $Mg^{2+}$ ) in silicates and oxides (Kabata-Pendias and Pendias, 2001).

The Mn compounds are known for their rapid oxidation and reduction under variable soil environment and thus oxidizing condition may reduce the availability of Mn and associated micronutrients, whereas reducing conditions may lead to the ready availability of these elements even up to toxic range. The complex mineralogical and chemical behavior of Mn results in the formation of the large number of oxides and hydroxides which give a continuous series of composition of stable and meta-stable arrangements of atoms. Colloidal Mn oxides reveal a great affinity for adsorption of cationic and anionic forms elements as well as inorganic and organic substances (Kabata-Pendias and Pendias, 2001).

#### 2.4.5 Lead (Pb)

Lead is not essential for plants and exists in three oxidation states: 0, +2(II), and +4(IV). Lead exists in all soils and all crops which tend to accumulate in the soil surface. The natural Pb content of soils is reported to be the least mobile among the heavy metals and occurs mainly as  $Pb^{2+}$  (Kabata-Pendias and Pendias, 2001). Only a small proportion of soil lead is in a form available to plant roots and only a small amount taken by plant roots is translocated to the shoots (Chlopecka and

Adriano, 1997). The major portion is usually solid or adsorbed onto soil particles as long as reducing conditions are maintained. Plants tolerance to soil lead is very high because it is easily adsorbed, once incorporated into the soil; lead tends to have a low mobility, resulting in a long residence time (Haroun et al., 2006).

The great variation of Pb contents of plants is influenced by environmental factors which are known to promote both Pb uptake by roots and Pb translocation into plant top (Kabata-Pendias and Pendias, 2001). The concentration of lead in plants depends on their total and available concentrations in soil, soil properties, and plant species, age, and cultivar, plant parts, plant species and the type of amendments (Chlopecka and Adriano, 1997). A high soil pH may precipitate Pb as hydroxide, phosphate, or carbonate, as well as promote the formation of Pb-organic complexes which are rather stable. Increasing acidity increases the Pb solubility (Kabata-Pendias and Pendias, 2001). Lead is tightly bound under strongly reducing conditions by sulfide mineral precipitation and complexation with insoluble organic matter, and is very effectively immobilized by precipitated iron oxide minerals under well-oxidized conditions.

#### 2.4.6 Zinc (Zn)

Zinc is essential for plants and animals and is considered to be readily soluble relative to the other heavy metals in soils (Kabata-Pendias and Pendias, 2001). Zinc is unique among the quartet of metals (Cu, Fe, Mn, and Zn) as zinc does not exhibit multiple valences and is not subject to oxidation-reduction in the soil plant system (Miller and Gardiner, 2001). Zn is relatively active in biochemical processes and is known to be involved in several biological and chemical interactions with several elements (Kabata-Pendias and Pendias, 2001).

A zinc tissue concentration of less than 15 mg/kg (dry weight) leaves a plant deficient, whereas a concentration over 400 mg/kg (dry weight) is potentially phytotoxic. Toxicities of zinc are seldom observed until plant tissue levels in excess of 1000 mg/kg are reached (Haroun et al., 2006). The most common and mobile Zn in soil is believed to be in forms of free and complexed ions in soil solutions (Kabata-Pendias and Pendias, 2001). Zinc may form complexes, for example  $Zn(OH)_2$ ,  $Zn(OH)_3^-$ , and  $Zn(OH)_4^{2-}$  with  $OH^-$  depending on the pH and metal concentration (Ören and Kaya, 2006). As a result of high pH value, zinc hydroxyl species may participate in the adsorption and precipitation. Zinc forms complexes with inorganic

and organic ligands, which will affect its adsorption reactions with the soil surface. The adsorption of  $Zn^{2+}$  can be reduced at lower pH by competing cations and these results in easy mobilization and leaching of Zn from light acid soils.

## 2.5 Interaction of Cadmium with Other Metals

Contamination of soil by a single metal is rare; where one metal is highly concentrated, there are usually others. Metal interaction can be difficult to predict and depend on the nutritional status of the plant (Shute and Macfie, 2006). It has been known that the availability of micronutrients to plants may be influenced strongly by interaction between nutrients and/or metals. The plant uptake of one can be affected by the levels of another in the soil. These interactions are extremely complex (John and Leventhal, 1995) and controlled by several factors (Kabata-Pendias and Pendias, 2001). In case of multi-element contamination, interaction between metals may occur both at root surface, affecting uptake, and within the plants, affecting translocation and toxicity (Luo and Rimmer, 1995).

The interaction of Cd with other elements (most commonly Zn), in the environment has been observed which can modulate the resultant metal toxicity. The uptake and distribution of mineral nutrients might be affected by the presence of Cd, and altered the nutrient balances of plant (Hernández et al., 1998). Vig et al. (2003) stated that the presence of different mineral nutrients, cations or anions in the soil alter the toxicity of cadmium by complexation, sorption or desorption processes. The soil micronutrient availability to plants is therefore clearly complicated by the existence of these interactions.

Copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) are of the selected metals in this study because they are similar multi-element contaminants found in large amounts in the sources (sewage sludges disposal, soil and mining waste) (Luo and Rimmer, 1995) and are potentially toxic to plants, animals and /or humans. The interaction can result both in synergism and antagonism effect between different micronutrients and/or macronutrients (Sparks, 1995). The description of multi-element interaction is given below:

Synergism (+,+): It occurs when uptake of one metal induces synthesis of binding sites that affect accumulation both metals (John and Leventhal, 1995) and

the combined effects of elements is greater (Kabata-Pendias and Pendias, 2001). In other word, the presence of one element can influence the uptake of another.

Antagonism (+,-): It occurs when the combined physiological effect of two or more element is less than sum of their independent effects. As the presence of one element inhibit the uptake of another. It is commonly observed in simultaneous exposure to several metals (John and Leventhal, 1995), most often in two ways: the macronutrient inhibits trace element absorption and, in turn, the trace element may inhibit absorption of a macromutrient (Kabata-Pendias and Pendias, 2001).

## **2.6 Principal Component Analysis (PCA)**

To determine the effect of other metals (Cu, Fe, Mn, Pb and Zn) and soil properties (pH, organic matter content, oxidation-reduction potential) influence on available Cd uptake to sugarcane, Principal Component Analysis (PCA) and correlation analysis were used in this study.

Among the multivariate statistical approaches, Principal Component Analysis (PCA), a particular type of factor analysis, was chosen in this study to reduce the number of variables to a smaller set of factors making it easier to interpret by displaying the correlations existing among the original variables (Morillo et al, 2004). Principal Component Analysis (PCA) is a variable reduction technique to determine the main components (PCs) accounting for the largest proportion of variability in the score of all of variables (Girden, 2001). Variables that are correlated with one another, which are largely independent of other subsets of variables, are combined into factors which represent the underlying process that create the correlations among these variables (Muller et al., 2008). It is often used in exploratory data analysis to study the correlations among a large number of interrelated quantitative variables by grouping the variables into a few factors; after grouping, the variables within each factor are more highly correlated with variables in that factor than variables in other factors (Kartal et al, 2006).

This statistical technique is widely applied to various environmental solid and/or liquid matrices such as street dust, soil, sediment and water (Kartal et al, 2006). A principal component analysis (PCA) is therefore more preferable here to determine the possible influence of soil properties (pH, organic matter content,

oxidation-reduction potential) and the presence other metals on available Cd uptake to sugarcane.

## 2.7 Literature Reviews

Several studies have been conducted to determine metal levels in soil, a number of the investigations described below have concentrated on assessing plant-available Cd in highly polluted soils where Cd has been added to soils either as Cd salts (to stimulate pollution) or in the form of sewage sludge, and crop uptake assessed by pot experiments. However, in this study the determination of Cd has been assessed under realistic field condition to be truly predictive for the contaminated area, Mae Sot district, Tak Province.

He and Singh (1993) studied the distribution and plant uptake of soil Cd as influenced by organic matter in a greenhouse experiment using ryegrass (*Lolium multorum* L.) as a test crop. Soil Cd was analyzed by a sequential extraction technique and the results showed that the concentrations in both cuts of ryegrass decreased with increasing amounts of organic matter added. The plant Cd was highly but negatively correlated to soil CEC. The decrease in Cd concentration of ryegrass was in the order: sand > sandy loam > clay loam.

Luo and Rimmer (1995) studied the effects of metal interactions on spring barley. A greenhouse experiment was conducted in which spring barley was grown in soil to which known amount of metal salts (Cd, Cu, Pb, and Zn) was added singly and in combination. The most consistent effect on plant growth was the interaction between Cu and Zn. Their studies led to the conclusion that the growth of barley was controlled principally by the amount of plant available zinc, which depended on the amounts of both added Zn and added Cu. The effect of added Cu was to increase the toxicity of the added Zn, such that overall the effect was rather small.

Qian et al. (1995) studied trace metals (Ni, Co, Cu and Pb) in soils collected from 10 different rural regions of China using a sequential extraction procedure. The metals were partitioned into Mg (NO<sub>3</sub>)<sub>2</sub> extractable (F1), CH<sub>3</sub>COONa extractable (F2), NH<sub>2</sub>OH·HCL extractable (F3), HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> extractable (F4) and residual (F5) fractions. The chemical fractionation showed that F1 fraction of the

metals was less than 1% and residue was the dominant form of Cu and Ni in all samples. Significant interrelationships of the fractions varied considerably with the different metals. The results of this study demonstrated that the sequential extraction procedure, in conjunction with multiple regression models using a combination of correlated fractions as an independent variable, may be useful for prediction plant absorption of trace metals in soils.

Dudka (1996) studied the forms of Cu, Ni, and Zn in the contaminated soils of the Sudbury mining/smelting district (central Ontario, Canada) to assess metal mobility and plant availability. Soil, tufted grass (*Deschampsia caespitosa* (L.) Beauv.), tickle grass (*Agrostis scabra* Willd.), dwarf birch (*Betula pumila* L. var. *glandulifera* Regel) and white birch (*Betula papyrifera* Marsh.) leaf and twig samples were taken from 20 locations around three Cu-Ni smelters. The water-soluble, exchangeable, sodium acetate-soluble, and total concentrations of the metals in the soils were analyzed. The mobility of Cu and Zn, expressed as the proportion of metals in BCR1 and BCR2 forms, increased with soil pH decrease. Concentrations of Cu and Ni in birch twigs showed a good linear relationship with exchangeable forms of the metals in soils. No significant correlation was found between Zn concentrations in the soils and plants. The authors concluded that strong variability of the soil metal mobility requires any reclamation effort be site-specific.

Kashem and Singh (2001b) also studied the solubility of Cd, Ni, Zn in soil solution in relation to changes in ORP and pH values caused by continuous flooding and organic matter application in three contaminated soils: tannery, city sewage and alum shale soils. In all soils, organic matter treated soil showed lower ORP and higher pH values compared to untreated soil. The results showed that the soluble metal concentration in all three soils was lower in organic matter treated soils.

Davidson et al. (1998) applied the BCR (the European community Bureau of Preference) three-step sequential extraction procedure to determine the metal contents (Cd, Cr, Cu, Pb, Mg, Ni, and Zn) in samples of made-up ground from layers of a trial pit excavated from industrially contaminated site. The study illustrated the importance of considering metal speciation when assessing the mobility of potentially toxic elements in industrially-contaminated land. The total metal content and the BCR three-step sequential extraction procedure were used and applied to the layer of trial excavated and "made-up ground" material, respectively to assess potential metal mobility. The results showed that the amount of metal extracted in the

sequential extraction (Step 1 + Step 2 + Step 3 + Residual) did not agree well with total digestion. Various layers of the trial pit contained significant levels of contaminant metals, but these were not always in easily mobilized forms. Cadmium was released at all steps of sequential extraction but the more easily mobilized form (acid exchangeable and reducible fractions) was predominant in most layers. Copper and zinc were present in all fractions, although the proportions varied down the pit profile. Near the surface, the metal was found mainly in association with residual fraction but, at depth, reducible species were dominant. Lead was present in various forms throughout the pit profile, but always with a significant proportion of the total in the residual phase.

Hernández et al. (1998) studied the effects of Cd on the pea plants (*Pisum sativum* L. cv. Argona), tissue accumulation and subcellular distribution of Mn and Fe in long term and short term experiment. Cd was supplied continuously with 0, 10, or 50  $\mu\text{M}$  Cd for 10 days and 50  $\mu\text{M}$  Cd was supplied for 72 hr in long term and short term treatment, respectively. In both experiments, they found that there was a reduction in the content and tissue distribution of manganese in the presence of cadmium, as well as a decline in the tissue accumulation of cadmium when the concentration of manganese was decreased. Cadmium inhibited the uptake of Mn. The relative distribution (%) of Mn between shoots and roots revealed that more Mn was found in shoot as cadmium concentration increased in the nutrient solution (from 20% to 50% in Cd-treated plants). The tissue content of manganese was the most affected among other cationic micronutrients studied (Fe, Cu, and Zn) by the exposure of several plant species to cadmium. Iron concentration and uptake showed less correlation with the cadmium treatments.

Moreover, several studies have confirmed that heavy metal bioavailability can be influenced by a variation in soil. For example, MaGowen and Basta, (2001a) and Wilkens and Loch (2003) studied the distribution of Cd and Zn from smelter emissions in acid soils. Their researches centered on soil chemical components potentially responsible for retention and/or movement of metals in soils. These included aluminum, iron, and manganese oxides, organic matter, clay, carbonates, and soil pH. They found that the organic fraction is the fraction best correlated with Cd and Zn distribution. The effect of low pH in the study soils was also noted to have decreased the retention of metals by the oxide fractions.



Metal partitioning of metal fractions to determine metal transport and release from mine and smelter waste has also been studied by MaGowen and Basta (2001b) and Li and Shuman (1996) by using a sequential extraction. The scheme divided into five phases: exchangeable, organic, Mn-oxide, amorphous Fe-oxide, crystalline Fe-oxide and residual. The results showed that the exchangeable Zn fraction became the dominant fraction. Compared among Zn, Cd and Pb movement, the authors concluded that Cd and Pb sorption to the organic fraction.

Liu et al. (2003) used different genotypes of 20 rice cultivars (*Oryza sativa* L.) to investigate the relationship of the adsorption and accumulation of Cd, Fe, Zn, Cu, Mn, and Mg under Cd stress under pot experiment. Complicated relations were observed between Cd, Fe, Zn, Cu, Mn, Mg in absorption and translocation in rice plants according to their concentrations in the roots and leaves. The statistic analysis showed that for their contents in roots, significant positive correlations between Cd and Fe, Cd and Zn, Cd and Mn, Cd and Cu were existed, but no significant correlation between Cd and Mg at both heading and ripening period. In the leaves, Cd also showed significant positive correlation with Fe, Zn and Cu but a significant negative correlation with Mn and no significant correlation with Mg. These results suggested that there were cooperative absorption between Cd and Fe, Mn, Cu in rice plants.

Pinto et al. (2004) also studied the effects of organic matter (OM) on Cd uptake and distribution by sorghum by applied with 0, 0.1, 1 and 10 Cd mg/dm<sup>3</sup> nutrient solution for 20 days. The results showed that sorghum biomass was decreased at 10 Cd mg/dm<sup>3</sup> due to Cd toxicity. The presence of OM further increased biomass production. The authors concluded that the presence of OM decreased the bioavailability of Cd that was partially retained in solution by OM ligands. OM promoted the translocation of Cd to shoots. However, the presence of OM decreased the uptake of Cu, Fe and Zn.

Zarcinas et al (2004) studied 318 soil (0–15 cm depth) and 122 plant samples for the assessment of heavy metal pollution of agricultural soils and crops of Thailand. Arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) were determined in soils using *aqua regia* digestion, and in plants using nitric acid digestion. Organic carbon (C), pH, electrical conductivity (EC) and available phosphorus (P) were determined on the soil samples. Regression analysis between the concentrations of metals in soil (*aqua*

*regia* extractable) and edible plant parts indicated a small but positive relationship for Cd in all the plants ( $R^2 = 0.081$ ,  $p < 0.001$ ). There was also a positive relationship between soil and plant Cd concentrations in rice ( $R^2 = 0.242$ ,  $p < 0.010$ ), and negative relationships for Zn in rice ( $R^2 = 0.385$ ,  $p < 0.001$ ), and Cu ( $R^2 = 0.355$ ,  $p < 0.001$ ) and Zn ( $R^2 = 0.122$ ,  $p < 0.026$ ) in glutinous rice. Principal component analysis of the soil data suggested that concentrations of As, Co, Cr, Cu, Hg, Ni and Pb were strongly correlated with concentrations of Al and Fe, which is suggestive of evidence of background variations due to changes in soil mineralogy. On the other hand, Cd and Zn were strongly correlated with organic matter and concentrations of available and *aqua regia* extractable P.

Parada (2005) studied the distribution of cadmium in stream sediment and suspended solids from Mae Tao and Mae Ku creeks in Mae Sot District, Tak Province. Twenty eight stream sediment and eleven suspended solids samples with surrounding water samples were collected and analyzed. Regarding sequential analyses, cadmium in stream sediments from Mae Tao contained mostly extractable forms (BCR1 and BCR2), while those from Mae Ku were significantly characterized by BCR2 and final residual forms.

Abollino et al. (2006) studied a contaminated soil from Piedmont, Italy in order to point out the availability and extractability of a series of metal ions and consequently their potential release into other environmental compartments. A sequential extraction procedure has been adopted in order to subdivide total concentrations into five operational fractions with different potential toxicity. High concentrations of the main pollutants, namely Cu, Pb and Zn, were extracted into the exchangeable, carbonate-bound/specifically adsorbed and Fe-Mn oxide bound fractions, showing the risk of release of these metals into the environment. The authors concluded that a remobilization of Cr, Mn and Ni could also take place, but to a lesser extent. Al, Fe, La, Sc, V and Ti were mainly bound to the residual and, in some samples, to the Fe-Mn oxide bound fractions. The high availability of several potentially toxic metals shows that the soil needs remediation.

Angelova and Ivanov (2006) used a sequential extraction procedure to investigate the chemical distribution of metals (Pb, Cd and Zn) in different fractions of the soils, industrially polluted by a metallurgical factory. The results showed that potential bioavailability of heavy metals are strongly controlled by their chemical forms related to solubility. Exchangeable and carbonate forms are the most important

form of heavy metal uptake by plants. They found that the highest amount of Pb was bound with hardly available forms for plants (Fe/Mn oxides and residual fraction) whereas, cadmium was found in available forms. The apparent mobility and potential metal bioavailability for this polluted soil were  $Cd > Pb > Zn$ . Regression analysis showed a significant correlation exists between Pb, Cd and Zn concentration in soil and that in peanuts. They concluded that exchangeable exhibited stronger influence on uptake than total concentration in soil.

Žemberyová et al. (2006) also used the BCR three-step sequential extraction for the fractionation of heavy metals to the Slovak reference materials of soils (soil orthic luvisols, soil rendzina and soil eutric cambisol) in Slovakia. The sum of the element contents in the three fractions plus aqua-regia extractable content of the residue was compared to the aqua-regia extractable content of the elements in the origin soils. The accuracy obtained by comparing the determined contents of the elements with certified values, using BCR CRM 701, certified for the extractable contents (mass fractions) of Cd, Cr, Cu, Ni, Pb and Zn in sediment following a modified BCR-three step sequential extraction procedure, was found to be satisfactory. The authors found that copper was mainly in association with aqua-regia extractable fraction (66-85%), lead presented as acid exchangeable species (55-77%), zinc was predominantly in aqua-regia extractable fraction (65-74%) and cadmium was 31-51% and 38-61% in the first and second fraction, respectively.

Wang et al. (2007) studied the effects of different concentrations of Cd on growth of maize (*Zea mays* L.) and metal uptake. The correlation among the distribution, accumulation of metals and the effect of Cd on Mn, Fe, Cu and Zn uptake in the cultivars of maize (*Zea mays* L.) were also investigated. The results showed that the Mn levels in the plantlets of the two cultivars (Nongda No. 108 and Linyu No.6) decreased with increasing cadmium concentration ( $10^{-4}$ - $10^{-6}$  M.) and duration of treatment. All Fe levels of treated groups were higher than the control. Zinc accumulation showed the same trend as decreased significantly ( $P < 0.05$ ) with different concentrations of cadmium in nutrient solution. Copper levels of Nongda No. 108 increased with an increase in Cd concentration, except for the group treated with  $10^{-4}$  M Cd. The Cu concentration in Linyu No.6 exposed to  $10^{-4}$  M Cd was lowest. However, all Fe and Cu levels of the treated groups were higher than the control, except for Nongda No. 108 treated with  $10^{-4}$  M cadmium.

Žemberyová et al. (2007) also applied the single extraction procedures validated by the BCR extraction procedure, extraction with 0.43 mol/L acetic acid and 0.05 mol/L EDTA to reference materials of soils to determine bioavailable contents of Cd, Cu, Ni, Pb and Zn. It was obvious that the sequence of EDTA-extractable elements is different from the sequence of acetic acid-extractable elements. The amounts decreased in the order Cd > Pb > Cu > Ni > Zn for EDTA extraction, while the availability for plant uptake in acetic acid extracts decreased in the order Cd > Ni > Zn > Cu > Pb.