Evaluation of Physicochemical Characteristics and Bioavailability of Selected metals in Paddy soil by Sequential Extraction method

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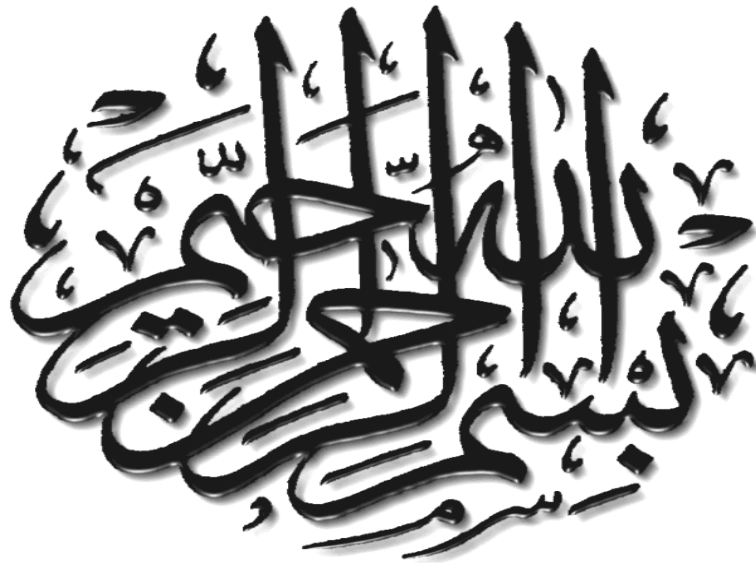
In

Analytical/Inorganic Chemistry

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IN THE NAME OF ALLAH,
THE MOST MERCIFUL,
THE MOST KIND

Dedicated to
My
Mother

Her Love is the Fuel for my life.

DECLARATION

This is to certify that this dissertation submitted by **Ms. Maria Hasan** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University Islamabad, Pakistan as satisfying the dissertation requirements for the degree of **Master of Philosophy** in **Chemistry**.

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ABSTRACT

The modified Community Bureau of Reference (mBCR) sequential extraction procedure was used in order to evaluate the mobility, bioavailability and persistence of selected metals in the paddy soil samples collected from district Sargodha, Pakistan. The operationally defined fractions isolated using BCR procedures were; exchangeable, reducible, oxidiseable and residual. Water soluble fraction of the soil was separated using single extraction method. The concentrations of selected metals in the soil samples were determined by flame atomic absorption spectrophotometer (FAAS), and the metal data were subjected to descriptive statistical and correlation study in order to evaluate the interrelationships among the metals in each fraction of soil. A number of significant correlations among selected metals in the soil samples were found. Multivariate cluster analysis was used to identify the plausible pollution sources and to find out the multiple relationships among the selected metals in all fractions of the soil. Significant anthropogenic intrusions of the metals were noted in the soil. Individual metal contents in different fractions of the soils were also compared. Of the selected metals Ca, Sr and Mn were more bioavailable. Enrichment of the selected metals by anthropogenic activities in the collected soil samples was also calculated, which revealed extremely severe enrichment of Cd whereas Ni, Zn, Ca, Co and Pb exhibited moderate to significant enrichment while Cr, Sr, Cu, Mg and Mn revealed minimal to moderate enrichment in the soil. Study regarding soil texture revealed predominantly silty loam texture of the area. Most of the soil samples showed slightly alkaline nature while organic matter was 8.37%, on the average. Piper diagram showed major contribution of Na and K with chloride and sulphate ions and minor contribution of Ca and Mg with carbonate and bicarbonate ions in the soil.

Chapter 1

INTRODUCTION

1.1   Nature and Composition of Soil

Soil is the most important part of the geosphere and it consists of finely divided layer of organic matter and weathered minerals upon which plants grow. It is formed by disintegrative weathering action of geochemical, biological and physical processes on rock (Manahan, 1999). Soil is the interface between the atmosphere and the lithosphere and strongly interacts with hydrosphere and the biosphere (Lal, 2005). It determines the behaviour of ecological land systems and affects water quality. It also plays an important role in the hydrologic cycle (Macutkiewicz *et al*., 2003). It is a mixture of organic matter, water and minerals. It has air spaces and generally has a loose texture. Typical productive soil contains 95% inorganic matter and 5% organic matter; however, peat soil may contain 95% organic materials. Soils have distinctive layers with increasing depth called horizons that results from complex interactions among processes during weathering which include bacterial decay of residual plant biomass, production of organic acids, complexing compounds and slightly acidic CO_2 that are carried by rainwater to lower horizons where they interact with minerals and alter their properties (Manahan, 1999).

As soil is formed by the weathering of parent rocks and minerals it eventually results in the formation of inorganic colloids. These colloids serve as repositories of plant nutrients and water, which are available to plants as needed. Inorganic colloids also serve in the way that these absorb toxic substances in the soil, thus playing a critical role in detoxification of substances. The quality and quantity of inorganic colloidal material in soil are important factors in determining the productivity of soil. Most common elements in earth's crust are magnesium, potassium, calcium, iron, sodium, silicon, aluminium and oxygen. Therefore, minerals that are composed of these elements in particular oxygen and silicon comprise major fraction of the soil. Common soil mineral constituent are finely divided orthoclase ($KAlSi_3O_8$), quartz (SiO_2), epidote (($4CaO•3(AlFe)_2O_3•6SiO_2•H_2O$), albite ($NaAlSi_3O_8$), magnetite ($Fe_3O_4$), geothite ($FeO(OH)$), oxides of manganese and titanium and magnesium carbonates ($CaCO_3$, $CaCO_3•MgCO_3$) (Manahan, 1999).

Soil organic matter constitutes less than 5% of total composition yet it largely determines the productivity of soil. It undergoes chemical reactions like ion exchange and

act as the food for micro-organisms. Organic matter also influences the physical properties of soil. A few organic compounds play role in weathering of mineral matter. For instance, oxalate ion, produced during fungi metabolism, occurs in soil as the calcium salts. Oxalates present in soil water dissolves minerals thus enhances nutrient ion availability and speeds up the weathering phenomenon. The weathering process involves oxalate complexation of either aluminium or iron. Temperature and oxygen availability also strongly influences the accumulation of organic matter in soil. As biodegradation is directly related to the temperature, in colder climates organic matter does not degrade rapidly and tends to accumulate in soil. In waterlogged soils and water, where anaerobic conditions prevails organic matter accumulates as decaying vegetation does not receive oxygen. In areas where plants grow and decay in soil saturated with water the organic content may reach 90% (Manahan, 1999).

1.2   Soil Pollution and Agriculture

Agriculture has pronounced effect on the overall quality of environment. The destruction of wildlife habitat, displacement of native plants, pesticide pollution, erosion and other agricultural aspects have tremendous potential for environmental destruction. For the survival of mankind agricultural practice should be environment friendly (Manahan, 1999). Among agricultural practices land disposal of waste water is the most common one that has been practiced for several years. In the long run it results in the excessive accumulation of toxic chemicals in the soils, which result in potential risk to human health due to accumulation of pollutants through food chain, poising health problem to humans (Cid *et al*., 2001, Ratuzny *et al*., 2009). Soil receives tremendous amount of fungicides and pesticides due to their application to increase the yield of agricultural products. Their ultimate environmental effects are determined by eventual fate and degradation of these agricultural supplements. Pesticides when sorbs into soil can leach into water, causing water pollution. These can affect the microbial and animal population in soil or these can degrade into more toxic products (Manahan, 1999).

There are many sources of pollutants in the environment such as, accidents, spills, and leaks from storage sites or industrial facilities (Marques *et al*., 2011). The soil is a long-term sink for heavy metals. Plant uptake and leaching losses are usually small compared to the total quantities entering the soil from agricultural and different diffuse sources. As a result, these potentially toxic elements gradually accumulate in the soil

profile over long periods of time. This could have long-term implications for the quality of agricultural soils, the maintenance of soil microbial processes, including phytotoxicity at higher concentrations and the transfer of zootoxic elements to the human diet from increased soil ingestion by grazing livestock or crop uptake (Nicholson *et al*., 2003).

Agricultural burning is another channel of soil contamination. Fuels that contain sulphur when burnt emit sulphur dioxide that ultimately ends up as soil sulphate. In the atmosphere oxides of nitrogen are converted to nitrates, eventually these are deposited on soil. Gases like NO and NO_2 are oxidized to nitrate in the soil. Soil residing bacteria and fungi convert carbon monoxide to biomass and CO_2. Elevated levels of particulate Pb are found in soil along heavily travelled roads and highways. Smelters and lead mines are among other sources of Pb in soil. Soil receives many harmful substances from lagoons, landfill leach ate and many other sources. Sometimes hazardous organic wastes are degraded and disposed of by land farming. The biodegradable material is worked into the soil and soil microbes bring about its degradation (Manahan, 1999).

Similarly, crude oil is another source of environmental pollution. It has been estimated that 2-9 million metric tons of hydrocarbon and crude oil enter into various segments of the environment annually. Detergents, monomers and polymers are derived from petroleum. Metal, oxygen, nitrogen and sulphur derivatives are also present in petroleum. As a result of petroleum combustion, compounds containing these elements enter into environment (Krause, 2002). Toxic compounds of mercury, cadmium and arsenic are released during coal combustion. A major source of sulphur in coal is pyrite that is not bound to it but occur within it. Anaerobic bacteria reduce sulphate in coal to sulphide. Combinations of these products produce pyrite (Krause, 2002). Oxides of nitrogen and sulphur entering the atmosphere from smelting, automobiles and other combustion sources enter the raindrop and reduce the pH of the rainwater that further affects the solubility, weathering reactions, biological processes in rivers, lakes and soils (Krause, 2002).

1.3 Metals in Soil

Soil is the reservoir for many harmful constituents, biological and elemental, including metals (Zimmerman and Weindorf, 2010). Among the most common environmental pollutants are trace elements especially the so called 'heavy metals'. Their occurrence in waters and biota indicate the presence of natural or anthropogenic

contamination (Bartoli *et al*., 2012). Commonly found heavy metals in the earth crust include Pb, Cd, Zn, Ni, Cr and Fe (Marques *et al*., 2011). Since long, heavy metals have been released from geogenic, anthropogenic sources and natural phenomenon into water reservoirs and soils (Namaghi *et al*., 2010). Anthropogenic sources include waste streams, urban habitation, mining excavations, atmospheric emissions, industrial process, tailings dams and fossil fuels combustion (Matthew *et al*., 2004). Naturally relatively low concentrations of heavy metals occur in soils. The metal concentrations inherited from the soil parent material are altered by biogeochemical processes, dust particles derived from soil, rocks and volcanic ash and most importantly, by anthropogenic sources (Marques *et al*., 2011). Natural weathering processes in soil like the organic degradation, mineralization or formation of secondary minerals have a profound effect on the bioavailability and mobility of the metals (Namaghi *et al*., 2010).

Some metals are essential for life providing essential cofactors for metallo-proteins and enzymes but at higher concentration these are toxic as these can block essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules. Some of the metals directly affect various physiological and biochemical processes, resulting in growth reduction, respiration and photosynthesis inhibition or degeneration of main cell organelles. These are similarly toxic to both micro-organisms as well as to multi-cellular organisms (Marques *et al*., 2011). Human exposure to the trace metals derives from a wide variety of sources, such as air, food and drinking water. The role of trace elements in the development of cancer has been established from literature. Arsenic exposure has been observed in relation to cancer risk generally focusing on exposure through drinking water, whereas Cd and Ni have been studied with a primary focus on work-place exposure (Stephanie *et al*., 2007). Cadmium once absorbed is retained in the body where it accumulates throughout life (Bernard, 2008). Studies regarding Zn show that it appears to be associated with cancer risk. Zinc deficient individuals appear to be protective compared to those who are Zn sufficient. Some of the metals (Cd, As and Ni) are generally associated with increased risk of many cancers. These have been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC) (Stephanie *et al*., 2007).

Trace elements can enter any agro-ecosystem through both anthropogenic and natural activities. Anthropogenic activities include input through use of compost, fertilizers, municipal or industrial wastes, wet/dry deposits and irrigation. Use of waste water, bio-solids, metal enriched materials like fertilizers, sewage sledges, farm wastes,

automotive emissions, industrial activities or mining, can cause toxicity to plants, contamination of the food chain and soil. In agriculture, fungicides and pesticides containing Zn, As, P and Cu are used to protect and increase production rate of strawberries, apples, citrus, peaches and many other fruit crops that result in an increase in these metal concentrations. Due to industrial activities and the use of fossil fuels, metal contamination may be widespread in urban areas (Mwegoha and Kihampa, 2010).

For many geochemical applications, total metal content of soils is useful but often the bioavailability of these metals is agriculturally more of an interest in terms of what is biologically extractable (Zimmerman and Weindorf, 2010). Bioavailability and mobility of elements in soils is controlled by many biochemical and chemical processes such as complexation-dissociation, precipitation-dissolution, adsorption-desorption and reduction-oxidation. These processes are affected by environmental factors, metal properties and soil properties e.g., soil pH, silt, clay, organic matter content, cation exchange capacity (CEC) and redox potential (Bakiricioglu *et al*., 2011). In the adsorption phenomenon, charged solute ions are attracted to the charged soil surface by electrostatic forces of attraction or through the formation of particular bonds. The complexation mechanism involves the formation of both organic and inorganic complexes between metals and a broad range of solutes in soils. In alkaline soils, precipitation appears to be the predominant process of metal immobilization, in anions presence such as carbonate, phosphate, hydroxide, and sulphate, especially when the concentration of the metal ion is high (Marques *et al*., 2011). In neutral and slightly alkaline conditions carbonate complexes dominate where as in acidic soils, usually simple cations and complexes of sulphates and chlorides exit (GLU *et al*., 2003).

In soil elements accumulate in various forms; carbonate bound, oxide-bound, organic matter bound, exchangeable, water soluble and residual (silicate bound). Bioavailability and mobility of metals varies in these forms. Under natural conditions residual fractions are immobile and mainly not available to plants and microorganisms; organic-matter-, carbonate- and oxide-bound fractions may be potentially bio-available while exchangeable and water soluble is readily released to the environment. In soil, metals can be transformed from one oxidation state or organic complex to another, as a result of which the metal may become either more water soluble, thus can be easily removed by leaching, inherently less toxic, less water soluble, therefore it can precipitates and then becomes less bio-available or volatilized and in way removed from the polluted area (Marques *et al*., 2011).

The distribution of chemical fractions and species according to bonding with specific soil subtracts of metals are important parameters for determining metal removal efficiency (Bakiricioglu *et al*., 2011). The accurate estimation of metal bio-availability in solid wastes and in soil is becoming more important in risk assessments. In this respect sequential and single extraction procedures are widely used (Olayinka *et al*., 2011). It is very difficult to separate various chemical fractions of elements. Thus for understanding association of trace elements with soil components selective chemical extraction methods are used. Such extractions aim to determine operationally defined metal fractions, toxic, potentially mobile, bio-available phases of a contaminant (Bakiricioglu *et al*., 2011). Sequential extraction methods provide more detailed information about the different availabilities of heavy metals among different geochemical phases. Partial extraction methods are comparatively cost-effective and simple way to investigate the labile metals in sediment and soils etc (Rao *et al*., 2010).

Living organism are affected by metals only when they biologically uptake fraction of the total metal present in the test media that can be soil, water, food and sediment. Although in many studies "total" metal concentrations are significantly correlated with biological effects, this might be a function of the study design, such as to compare lightly contaminated sites with grossly contaminated sites. It has been established that in a few environmental samples, none of the total metal may be bio-available. This is because as metals complex to varying degrees with so many different entities and their toxicity rapidly changes with complexation and speciation (Burton, 2010).

1.4   Single Extraction Procedure

Many extraction methods have been developed and modified for soil metals that differ in extracting schemes and laboratory conditions. In this respect, both sequential extraction and partial/single extraction procedures are widely used. In the partial extraction an extraction solution and a soil sample is used but several procedures and extraction solutions are used sequentially to the same sample in the sequential extraction procedures (Bakiricioglu *et al*., 2011). Among the approaches widely used to study metal mobility in the soils is the use of single extraction methods that make use of un-buffered salt solution that can be considered as good models for simulating flooding and raining events. The suitability of these extraction procedures for the prediction of plant uptake in soils have been demonstrated through several studies (Rao *et al*., 2008). When compared

to sequential extraction schemes, single extraction procedures are cost-effective as well as simple way to investigate labile metals in sediment, soils and sludges etc (Rao *et al*., 2010). Single extractants can be broadly classified into three main groups:

i. Strong acid extractable fraction (acid reagents such as CH_3COOH and HCl)

ii. Organically complexed metal fraction (chelating agents such as DTPA and EDTA)

iii. Exchangeable (un-buffered mild extractants such as $NaNO_3$, $CaCl_2$, NH_4Cl, NH_4NO_3, $AlCl_3$ and $BaCl_2$) or neutral salt solutions (Bakiricioglu *et al*., 2011; Rao *et al*., 2010).

Exchangeable extractants includes metals that are weakly adsorbed on the solid surface by relatively weak electrostatic forces of interaction. These metals can be released by methods like ion-exchange processes and are considered as bio-available (Rao *et al*., 2008). The un-buffered salt solutions are simple and rapid extractants to extract bio-available metals. But they do not represent metals that are available to plant. The chelating agents can displace metals from insoluble organic or organometallic complexes beside those absorbed on inorganic soil components. Chelating agents dissolve exchangeable elements as well as element fraction fixed on the soil hydroxides. The organic fraction released in the oxidiseable fraction is not considered very mobile or bio-available since it is associated with stable high molecular weight humic material that slowly releases small amounts of metals. Weak organic or strong mineral acids can be used in acid extraction. Silicate matrix does not get dissolve in strong acids. These reagents dissolve metal pollutants which largely enter the soil environment in non-silicate bound forms. Single extractants can also be considered regarding their role of releasing elements from particular soil phases with which they are associated or bound. Such classification of extractants is extremely useful in elucidating the mechanisms of metal transformation, release or binding in soils and in the study of soil chemistry (Rao *et al*., 2008).

Metals that are soluble in water can be analyzed from the saturation paste extract of a soil. This phase contains ions complexed with soluble organic matter and free ions and other constituents. It comprises potentially the most available and the most mobile metalloid and metal species. Trace element nutrients or pollutants concentration in the soil solution is very low. This phase can be separated through ultra filtration, dialysis, displacement, filtration or centrifugation. Unfortunately most the extractants are less specific than desired. The release from the target site is either less than complete or more than one target site are liable to attack. Despite these limitations, single extractant releasing elements associated with a particular soil phase or element species is still a

useful concept and constitutes one of the few tools available for assessing the mobilization and binding of elements in soils (Bakiricioglu *et al.*, 2011; Rao *et al.*, 2008).

1.5   Sequential Extraction Procedure

Determining total metal concentration is not sufficient to estimate biological effects of metals. Toxicity and biogeochemical distribution of an element is dictated by its form and particular species present in a given matrix (Canepari *et al.*, 2006). A laboratory approach mostly used to study the bioavailability, transport and mobility of metals in different types of environmental samples (sludge, sediments, soils, etc.), is the use of chemical fractionation, employing multi-step sequential extraction approaches combined with multi-elemental analysis of each fraction (Cid *et al*, 2001.Canepari *et al.*, 2006). Sequential extraction procedures can be applied to a great variety of materials for example soil, sewage sludge, industrially contaminated soil, sludge amended soil, run-off, wastes and road dust, etc.

Sequential extractions involve treatment of the same sediment or soil sample with a series of reagents. In this way phase specificity is improved as each reagent has a different chemical nature and the steps are performed in order of increasing vigour. Thus the first species to be isolated are those already in the soil solution, possibly with those loosely attached at cation-exchange sites in the matrix. Generally it is followed by stepwise attack on the carbonate phase, manganese and iron oxy-hydroxides and organic matter. Finally, more refractory components, sometimes including primary silicates may be dissolved. The mineralogical phases may be further sub-divided by the use of additional reagents. For instance many procedures involve separate attacks on the more refractory crystalline forms and the more labile amorphous iron oxy-hydroxides etc (Rao *et al.*, 2008).

Initially sequential extraction procedure was described by Crawford and McLaren in 1973 but an extended method was developed by Tessier (Tlustos *et al.*, 2005). A five-step procedure was published by Tessier *et al.* (1979). This scheme distinguishes between exchangeable metal, residual metal, manganese/iron oxides or organic matter and metal bound to carbonates. This method has been modified by varying the extractant concentration (Gomez-Ariza *et al.*, 1999) the extraction time (Maher, 1984) and the soil-extractant ratio (Rauret *et al.*, 1989). Meguellati *et al.* (1983) and Qiang *et al.* (1994) changed the extractants order to leach the metal bound to the organic phase before dissolving the iron oxide and carbonate phases. This provides the destruction of organic

phases which may entrap the mineral materials and thus providing a better extraction for the following phases (Gomez Ariza *et al*., 2000).

The Community Bureau of Reference (BCR) procedure that was proposed by the former Community Bureau of Reference, now Standards Measurements and Testing (SM&T) is rapid and simpler than the methods which were described by the sequential extraction procedure of Tessier (Dundar *et al*., 2012). Initially, BCR sequential extraction scheme was developed for the analysis of heavy metals in sediments. It has been standardized and has been successfully applied to a variety of matrices that include contaminated soils, road side soils, calcareous soils, sewage sludge, sludge amended soils, fly ash and wastes products of mining (Rao *et al*., 2008). The original BCR procedure consisted of three stages of extraction; acid extractable, reducible and oxidiseable (Cappuyns *et al*., 2007). It was tested in inter-laboratory trial on BCR 601on Cu, Cr, Cd, Ni, Zn, and Pb. The results showed that improvements were necessary as only 60% of the extractable metals were certifiable (Ure *et al*., 1993). The rest of the metals could not be certified due to the high variability between the results among different laboratories. It was attributed to small changes in the pH of the hydroxylamine hydrochloride. Davidson *et al*. (1999) reported that when three analysts prepared their own reagent and performed sequential extraction independently and on a different day, results showed significant variability (Oyeyiola *et al*., 2011). During the certification of Reference Materials, lack of reproducibility was observed for the reducing extraction ($NH_2OH.HCl$) (Cappuyns *et al*., 2007).

After a trial of different reaction conditions the $NH_2OH.HCl$ concentration was changed to 0.5 M from the previous value of 0.1 M and the pH of the reagent was adjusted to 1.5 instead of 2 by addition of a fixed volume of HNO_3 (Cappuyns *et al*., 2007). The revised protocol improves reproducibility and involves more efficient dissolution of the reducible fraction of the soil matrix, most probably the iron oxy-hydroxide phase (Mossop and Davidson, 2003). A fourth step of residual extraction was also added to allow comparison with pseudo-total digestion results for the purpose of validation (Oyeyiola *et al*., 2011). Currently the optimized BCR procedure is the only scheme that is standardized and harmonized. For it certified reference samples are also available, CRM 701 being the most recent one. This is a significant advantage and provides for a degree of comparability between researchers that is generally not obtainable for the other procedures presented in the literature (Rao *et al*., 2008).

Modified BCR (mBCR) sequential extraction procedure is summarised below (Zemberyov´a *et al*., 2006):

Extraction Step	Reagent(s)	Nominal target phase(s)
Exchangeable	CH_3COOH (0.11 mol/L)	Soil solutions, carbonates, exchangeable metals
Reducible	$NH_2OH·HCl$ (0.5 mol/L)	Iron/manganese oxy-hydroxides
Oxidiseable	H_2O_2 (8.8 mol/L) then CH_3COONH_4 (1 mol/L), pH 2	Organic matter and sulphides
Residual	Aqua regia	Remaining, non-silicate bound metals

Although sequential extraction procedures are useful in providing valuable information regarding environmental studies, yet these have some drawbacks and limitations (Rao *et al*., 2008). Concerning sequential extraction procedures the major problems are:

❖   Selectivity of reagent

❖   Operative definition of methods

❖   Redistribution or re-adsorption of element

❖   Variable experimental conditions

❖   Difficulty in validation of the existing methods

❖   Precision evaluation

❖   Scarcity of certified reference materials

Sequential extractions provide semi-quantitative information on distribution of elements between operationally defined geochemical fractions of soil. Although sequential extractions are frequently used to infer the speciation of heavy metals, the fractions obtained from sequential extraction do not necessarily reflect the true chemical speciation (Cappuyns *et al*., 2007). It has been emphasized that sequential extraction procedures should always be applied with full consideration of their limitations. The results of sequential extractions procedures depend on the operational conditions of the method, as well as vary from element to element and between different samples types (Rao *et al*., 2008).

As the present sequential extraction schemes were meant for trace metals in sediment, their application to heavily contaminated soils may be suspect where

concentrations of the "trace" metals are no longer trace but major constituents. Also the comparisons of sequential extractions with thermodynamic models and direct instrumental analyses are lacking (Rao *et al.*, 2008). Extraction schemes can be a useful tool in metal fractionation but should always be confirmed by other methods. Sequential extraction procedures that were developed to characterize pollutant species in normal sediments and soil may be unsuitable for industrial contaminated samples which contain encapsulated pollutants, larger pollutant particles and man-made materials e.g., slag, metals and plastics (Rao *et al.*, 2008).

Sometimes there may be reproducibility problem when total metal concentrations are compared with the sum of concentrations associated with the individual phases. This problem is observed in heavily contaminated materials. It is partially because of the material/ sample heterogeneity (Cappuyns *et al.*, 2007). Samples pre-treatment, for example, air drying, oven drying, freeze-drying effects the distribution of the metals. Pre-treatment effects are more important for anoxic materials than for oxic ones (Usero *et al.*, 1998). Thus more standardized sequential chemical extraction procedures are in demand for various types of materials to get more trusted and useful data about the different phases of the toxic metals in wide variety of materials (Rao *et al.*, 2008). Beside these limitations sequential extraction procedures give the most accurate information about transformation and fractionation of elements in the soil, especially when related to different soil physicochemical properties, such as soil pollution and long-term effect of soil amendments (Tlustos *et al.*, 2005).

Sequential extractions give detailed information about mode of occurrence, transport of trace metals, physicochemical properties, soil pollution, biological availability, mobilization and the origin (Tlustos *et al.*, 2005, Dundar *et al.*, 2012). Applications of sequential extraction procedure, although, have been mostly limited to low contamination sites still these are very useful to identify trace element fractionation in the various solid phases of sediment and soil and to determine labile fractions of trace elements (Dundar *et al.*, 2012). Sequential extraction procedures are useful in studies such as; to assess the composition and structure of soil components, to improve understanding of the processes in the soil that control the retention of toxic elements or nutrient and their mobilization, as well as to elucidate soil chemistry and to illuminate their transport mechanisms. Single and sequential extraction methods are also used in radionuclides (Rao *et al.*, 2008).

1.6   Physiochemical Characteristics of Soil

Being a component of land ecosystem, soil fulfils many essential functions for survival of plant growth. The functions it perform include storing plant available water, immobilization of contaminants and suppressing plant pathogens, portioning of the applied water into run-off or/and drainage, provide suitable conditions for establishment of seedling, supply of enough oxygen to plant roots, store nutrients that are essential for plant growth. Organic carbon, cation exchange capacity, soil texture, pH, and depth of soil are the attributes to assess soil quality. Soils differ among these attributes and thus among the productivity potential, as it is limited by these inherited constraints. Therefore, managing and identifying these constraints is essential for sustainable production system. Increased cation exchange capacity (or in other words the ability of soil to retain metals) of the soil is reported with increased sludge application. The metals movement down the soil profile is usually evidenced in heavy metals applications, often in sewage sludge, in soils with low clay and organic matter contents, acidic conditions, and when high irrigation water or rainfall rates have been applied. This movement occurs via soil cracks or macro pores which are referred to as preferential flow (Akan *et al*., 2010).

Total nitrogen (TN) and soil organic carbon (SOC) contents vary between regions and within landscapes. Generally, soil organic carbon is influenced by soil texture, drainage, climate, land management, vegetation and topography. Generally soils lose carbon with conversion from native to agriculture ecosystems. In any given environment the amount of soil organic carbon increases as soil texture becomes finer. It is explained by the ability of clay and silt sized particles to interact with organic matter forming macro- and micro- aggregates that prevent biological mineralization of SOC. Soil tillage leads to losses in macro-aggregates as well as in the associated SOC. In tropical and temperate environments linear, positive correlations between SOC concentrations and silt/clay or soil clay content of surface soils have been found. Better relationships with SOC are obtained with silt/clay, in some cases, and in others with clay that suggest that mineralogy and possibly other factors play a role in processes of SOC stabilization. In a few studies N-soil/SOC texture relationships with depth or bulk density data and combined concentration to calculate N and C stocks have been studied. Estimates of SOC stocks are important for calculating current global, continental and regional stores and potential for soil organic carbon sequestration to offset anthropogenic emissions of CO_2 (Gami *et al*., 2009).

1.7 Aims and Objectives

Based on the deliberations in foregoing sections, present study is designed and executed for;

➢ Determination of the selected metals distribution in soil samples from paddy fields.

➢ Measurement of the metals fractionation using modified BCR sequential extraction procedure and studying their comparative variations in various fractions of the soil.

➢ Determination of the plausible associations among the metals in terms of their correlation coefficients in various fractions.

➢ Monitoring the physicochemical characteristics, soil nature and particle texture of the soil samples.

➢ Exploring the enrichment/contamination of the metals in the soil samples.

➢ Ascertaining the mobility and bioavailability of the metals by fractionation in soil.

➢ Classifying metals by multivariate apportionment employing cluster analysis (CA) and to assess their probable anthropogenic and natural inputs in various fractions.

Chapter 2

EXPERIMENTAL METHODOLOGY

2.1   Site Description

In the present study, soil samples were collected from the paddy fields of a selected irrigated area which is located within the district Sargodha, Pakistan. The district expands over an area of 5,854 km^2 with population of about one million. Although there are a few small hills on the Sargodha-Faisalabad road, it mainly comprise of fertile, flat plains. It is an industrial and agricultural city. Farmers have developed a range of animal breeding programs and crops. Some of the chief crops include rice, wheat, sugar-cane and citrus fruits which are exported internationally as well as locally to other cities. The river Chenab lies on the eastern side of the city while the river Jhelum flows on its western and northern sides. The city has a climate of extreme cold and heat. The minimum temperature recorded in winter is as low as freezing point of water, while the maximum temperature reaches 50°C in the summer. Average rainfall of the area varies from 12.0 to 381.0 mm per month.

2.2   Sample Collection

Sampling of the material to be analyzed is always a matter of immense importance. Generally it is considered a more important procedure than the analysis itself. Its major objective is to get a representative sample for the analysis (Patnik, 2010). In the present study soil samples were collected from the paddy fields using following methodology (Aichberger and Back 2001; Patiram, 2007).

The area of sampling was cleaned with the help of a spade. For taking a single soil sample, spade was used for digging the area into a V shaped pit up to 5-30 cm and a lump of soil was removed. Soil slice was then taken from both the exposed surface of the pit from the top to bottom. Three to five such samples were taken from each site and mixed thoroughly to form one sample. Enough cores were taken to represent the whole sampling area adequately. The core was of the same volume and represented the same cross section of the sampling volume. Soil samples were taken from the uniform areas with identical vegetation (rice). Six to nine sub-samples were combined to a composite sample in contamination free containers and thoroughly mixed. The homogenized composite samples were put in clean polythene bags and labelled. In this way, thirty-nine composite

soil samples were collected at each of the three stages; first sampling was carried out at the sowing stage; second sampling was conducted at the tillering stage; and third sampling was carried out at the maturity stage of the crop. The collected samples were carefully transported to the laboratory for further processing and analysis.

2.3   Sample Processing and Extraction

In the laboratory, large items like stones, wood pieces, grass, straws, etc., in the soil samples were manually removed. In the next step, the samples were dried in an electric oven at 80°C for 48 hours until constant weight was achieved. Dried samples were grinded and stored in desiccators until further processing (Guvenc *et al*., 2003). Precautionary measures were taken to avoid any sort of contamination during sampling, drying, grinding and storage.

Among various extraction procedures reported in the literature, sequential extraction procedures are instrumental in investigating the environmental ecotoxicity and mobility of the metals in complex materials such as, solid wastes, sediments and soils (Sulkowski and Hirner, 2006). Sequential extraction furnishes detailed information about mode of occurrence, origin, transport and mobilization of trace metals in different environmental segments (Zemberyova *et al*., 2006).

2.3.1   Sequential Extraction Procedure

Modified sequential extraction procedure recommended by community bureau of reference (mBCR) was performed on the soil samples in the present study (Zemberyova *et al*., 2006; Rauret *et al*., 2000; Alvarenga *et al*., 2007; Pueyo M.*et al*., 2008). The method is briefly outlined below.

In the first step, the nominal targeted phase was carbonates, exchangeable and weak acid soluble fraction. Aliquot of 40 mL of 0.11 mol/L acetic acid was added to 1.0 g of previously dried soil sample taken in 100 mL Erlenmeyer flask. Then it was subjected to mechanical shaking for 16 hours at 320 vibrations per minute at room temperature. Continuous suspension of the mixture was ensured during shaking. The extract was separated from solid residue by centrifugation for 20 minutes at 3800 rpm. The supernatant liquid was decanted into a second flask and was covered with a watch glass to avoid any contamination. The residue was washed with 20 mL distilled water by shaking for 20 minutes and then centrifuged. The washings were discarded without losing the

residue, which was processed to the next step of extraction procedure.

The second step targeted iron/manganese oxy-hydroxides and reducible fraction. To the residue from step one, 40 mL of hydroxylamine hydrochloride (0.5 mol/L) was added. The pH was adjusted by acidifying with 25 mL of 2 mol/L HNO_3 in one litre volumetric flask containing hydroxylamine hydrochloride dissolved in distilled water. As described earlier, it was then shaken for 16 hours and centrifuged. The extract was separated and the residue was washed.

Third step targeted organic matter, sulphides and oxidiseable fraction. An aliquot of 10 mL 8.8 mol/L hydrogen peroxide was added carefully in small portions to the residue from the second step. The mixture was digested at room temperature for one hour while it was loosely covered with watch glass and occasionally shaken. It was further digested by heating at 85°C in water bath for one hour. The volume was reduced to less than 3 mL keeping the flask uncovered. Again a second aliquot of 10 mL was added, and the digestion procedure was repeated. The volume was reduced to about 1 mL. The residue was extracted with 50 mL of ammonium acetate (1 mol/L, adjusted to pH 2 using nitric acid) by shaking for 16 hours at room temperature. The extract was separated by centrifugation and the residue was washed as in the previous steps.

In the last step the targeted phase is non-silicate bound metals. The residue from the third step was digested with aqua-regia, following the ISO 11466 (ISO, 1995). 2.3 mL of HNO_3 (65%) and 7.0 mL of HCl (37%) were added to the residue drop by drop to avoid foaming. The reaction vessel was allowed to stand for 16 hours at room temperature for the purpose of slow oxidation of organic matter in the soil. The temperature of the reaction vessel was slowly raised up to 80°C and refluxed for two hours. After cooling to room temperature, the digest was filtered into 50 mL volumetric flask. The residue retained on filter paper was washed using 0.5 M HNO_3. The volume was made up to the mark with dilute HNO_3 (0.5 M). Blanks were also prepared with each batch of the samples and subjected to the same procedure as that of samples.

2.3.2   Single Extraction Procedure

In this case 5.0 g of previously dried soil sample was weighed in a 100 mL conical flask and extracted with 50 mL of water (1:10 ratio). The mixture was subjected to mechanical shaking for 24 hours at 320 rpm at room temperature and centrifuged and filtered through fine filter paper to separate the un-dissolved residue. Blanks were prepared following the same procedure (Arain *et al*., 2008).
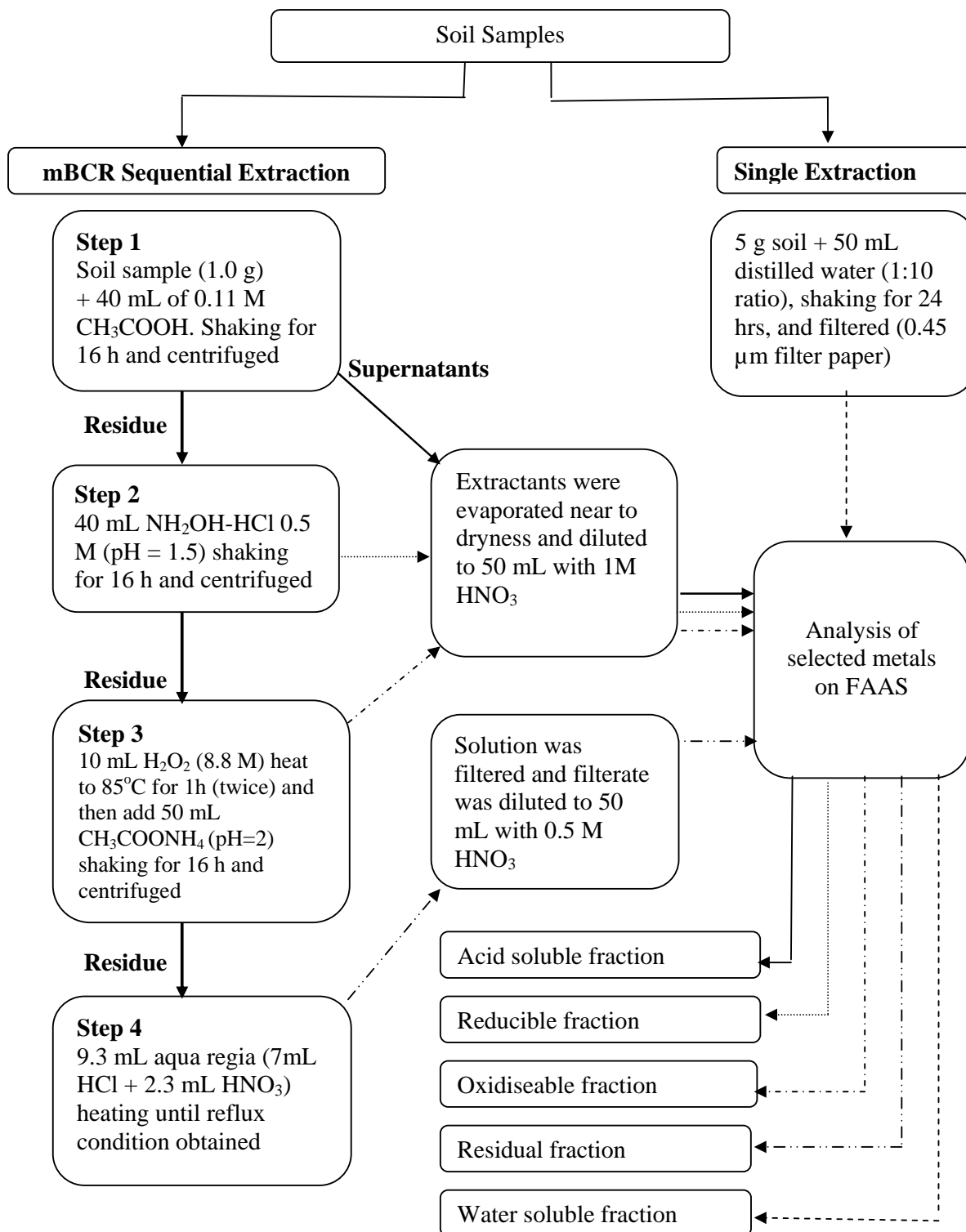
Flow diagram of the modified BCR sequential and single extraction procedure

2.4   Metal Analysis by Atomic Absorption Spectrophotometry

Flame Atomic Absorption Spectrophotometer (Shimadzu AA-670, Japan) was used for the quantitative measurement of selected metals (Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr and Zn) in the soil samples under optimum analytical conditions as mentioned in Table 1. Atomic absorption spectroscopy (AAS) holds the advantage of rapid, convenient and low detection levels for metal analysis (Patnaik, 2010). This technique is widely accepted because of its element selectivity and analytical sensitivities at the parts-per-million level and less. In AAS quantitative analysis is accomplished by measuring the amount of absorbing species at the analytical wavelength. The reason for the popularity of AAS is its ability to determine more than 60 elements in different matrices and in wide range of concentration, its different mode and because it is relatively inexpensive. FAAS is rapid and sufficiently sensitive for determination of most of the transition elements (Reilly, 2002). The increase in absorbance is directly proportional to the concentration of gaseous phase atoms (Beer-Lambert Law). Instrumentation of AAS is similar to that required by other high resolution spectroscopic techniques, differing only in the nature of radiation source used and the use of heat energy to produce the absorbing species (Lagalante, 1999). Atomic absorption spectrophotometer consists of the following major components:

Most frequently used line source of radiation is a hollow cathode lamp (HCL). It provides light of a specific wavelength with width of approximately 0.002 nm. Commercially these are available as both a single element and multi-element sources. Thermal energy generated either by flame or electro thermal method vaporise the analyte and break the chemical bonds within the component molecules. Flame is the most frequently used method for producing free atoms in AAS. Frequently used flame gases include nitrous-oxide (N_2O) or air-acetylene, with operating temperatures of 2800 and 2400 K, respectively. Burner path length in air-acetylene flame is commonly 10 cm while for N_2O-acetylene flame it is shorter, typically 5 cm. The analyte containing solution is converted to an aerosol and passed into the flame using a pneumatic nebulizer. To isolate the wavelength of interest from polychromatic light, monochromator is used, with a typical resolution of 0.02 nm. Grating monochromator is most commonly used in the modern instruments. Photomultiplier tube (PMT) is the most often used detection system. Its function is converting light signal into an electrical signal. Amplifier amplifies the electric signals fed from the photomultiplier detector. Various types of readout devices are

employed in the spectrophotometers. However, in modern instrumentation graphic presentation or digital displays on external computers are most commonly used readout systems.

Calibration line method was used for the quantification of selected metal levels in the soil samples. Working standards of low and high concentration were prepared from the stock solutions (1000 mg/L) of each metal which were procured from Merck or BDH. Standard reference material was used to check the reliability of the instrument and the analytical results. Generally a maximum of ±2% difference was observed in the certified and measured levels. Some of the samples were also subjected for interlaboratory comparison at an independent laboratory and the two results matched within ±2.5%. Doubly distilled water was used throughout this work for the preparation of the samples, standards and reagents.

Table 1.   Optimum analytical conditions for selected metal analysis using air-acetylene flame on Shimadzu AA-670

Metal	Wavelength (nm)	HC lamp current (mA)	Slit width (nm)	Fuel-gas flow rate (L/min.)	1% Absorption concentration (ppm)
Ca	422.7	6.0	0.5	2.0	0.08
Cd	228.8	4.0	0.3	1.8	0.02
Co	240.7	6.0	0.2	2.2	0.20
Cr	357.9	5.0	0.5	2.6	0.09
Cu	324.8	3.0	0.5	1.8	0.09
Fe	248.3	8.0	0.2	2.0	0.10
K	766.5	5.0	0.5	1.9	0.04
Li	670.7	4.0	0.5	1.6	0.05
Mg	285.2	4.0	0.5	1.6	0.007
Mn	279.5	5.0	0.4	1.9	0.05
Na	589.0	6.0	0.5	1.6	0.02
Ni	232.0	4.0	0.15	1.7	0.10
Pb	217.0	7.0	0.3	1.8	0.20
Sr	460.7	4.0	0.5	1.6	0.10
Zn	213.9	4.0	0.5	2.0	0.02

2.5   Analysis of Selected Anion by Ion Chromatography

Ion chromatography is broadly used for the determination of anions that commonly occur in environmental matrices. Various anions, such as, chloride, bromide, iodide, floride, oxyhalides, nitrate, nitrite, sulphate, sulphite, phosphate, and many carboxylates are determined rapidly by ion chromatography. This method involves detection of separated ions via a conductivity detector and the chromatographic separation of water-soluble analytes (Patnaik, 2010). In the current study anions (chloride, nitrate and sulphate) were measured by ion chromatograph (Shimadzu LC-10, Japan). Mobile phase was 1.2 mM potassium hydrogen phthalate (pH 4.2 and flow rate1.5 mL/min). Temperature of the oven during the analysis was kept 40˚C. The column was packed with polymethacyralate (PMA) that was the anion exchange resins, supported with the particle size of 10 µm incorporating a quaternary ammonium based as functional groups, suitable for pH ranges from 2 to 11. The instrument was equipped with thermal conductivity detector (CDD- 10 AVP) which has broad linear range.

2.6   Particle Size Measurement and Soil Texture

Particle size distribution is the quantitative measure of the particle size that comprises the solid fraction, while, soil texture refers to a qualitative measure of particle sizes that is based on feel of the soil material, which may be smooth, fine, coarse or gritty (Lal, 2004). Soil structure is directly related to its particle size distribution and mineral composition which is among fundamental physical characteristics of the soil (Eshel *et al*, 2004). Soil texture effects the mechanical and physical behaviours of the soil and on the properties related to movement of water and the water content. Particle size analysis of soil is sometimes referred as "mechanical analysis". The procedure consists of two-steps: fractionation and dispersion. Fractionation refers to the physical separation of the particles into different size ranges, whereas, dispersion involves separation of soil particles through removal of cementing material to break secondary particles into primary particles or soil separates. The choice of the dispersion agents used depends on the nature of the cementing materials. A wide range of methods of fractionation can be used. The choice of the method depends on the objectives, particle size and the facilities available. Two of the most commonly used procedures are sieving and hydrometer methods (Lal, 2004). Dry sieving technique covers the quantitative estimation of particle size distribution in soil and it is used for determining the distribution of particle sizes larger than 75 µm (retained by the

No. 200 sieve), while a sedimentation process, using a hydrometer is used for determining the distribution of particle sizes smaller than 75 µm.

In the present study, soil particle size was determined by the ASTM 422 method (1998). Dried soil sample (50 g) was mixed with 125 mL of sodiumhexametaphosphate (40 g/L) solution which was used as a dispersing agent. The resulting mixture was stirred until the soil got thoroughly wet. The soil was soaked for 16 hours at room temperature. Similarly, 125 mL of the dispersing agent was added into a control cylinder and diluted with distilled water up to the mark. Reading was taken at the upper meniscus formed by the control solution and the hydrometer stem. The difference between the level of solution in the jar and the top of the meniscus is called the meniscus correction. Control cylinder was shaken to get its contents thoroughly mixed. Temperature and zero correction were noted by inserting thermometer and hydrometer respectively. Soil slurry was thoroughly mixed after 16 hours and transferred to sedimentation cylinder and filled with distilled water up to the mark. Mixture was thoroughly mixed by covering the open end of the cylinder with a stopper. Readings were taken by observing the upper meniscus formed by the hydrometer stem and the suspension. The hydrometer was slowly removed and put back into the control cylinder. Readings were taken after time period of 40, 120, 300, 480, 900, 1800 second 60, 120 and 1440 minutes.

The suspension was transferred to a No. 200 sieve after taking the final hydrometer reading and washed with tap water until the wash water was clear. Residue retained on the No. 200 (75 µm openings) sieve was transferred to a suitable container dried in an oven at 105°C and was passed through a series of sieves of mesh numbers; 10 (1.68 mm), 25 (0.707 mm), 35 (0.420 mm), 48 (0.297 mm), 65 (0.210 mm), 100 (0.149 mm), 120 (0.125 mm), 140 (0.106), 170 (0.088 mm), 200 (0.075). Percentage of each fraction was calculated by determining the mass of each fraction retained on each sieve. Particles diameter was calculated by formula:

$$D = K\sqrt{\dfrac{L}{T}} \tag{01}$$

Where, K is the constant depending on the specific gravity of the soil particles and the temperature of the suspension. The value of K remains constant for a series of readings constituting a test, while values of T and L do vary. L is the effective depth for hydrometer in cm and T is the time in minutes. The percentage of each fraction was calculated by following relationship:

$$P = \left[(100000/W)G/(G - G_1) \right] (R - G_1) \tag{02}$$

where, P is soil percentage in suspension as measured by hydrometer; W is oven-dried mass of soil in a total test sample; G is soil particles specific gravity; G_1 is specific gravity of suspension liquid; R is hydrometer reading with composite correction applied.

For sieve analysis percentages were calculated by:

$$P = \frac{Mass.retained.on.each.sieve}{Initial.mass} \times 100 \tag{03}$$

2.7 Soil Organic Matter

Organic matter is defined as soil material derived from residues of plant and mostly consists of hydrogen, oxygen and carbon. Organic matter when decomposes, the elements are recycled (Evangelou, 1998). Loss on ignition or dry ashing serves as a rough indicator of the amount of organic matter present in the sample (Radojevic and Bashkin, 1999). In the present study, loss on ignition was determined gravimetrically. It includes organic matter, carbonates, water linked to the crystal lattice with a little residual non-structural adsorbed water and volatile salts. To ensure the combustion of the organic matter, use of an oxidizing atmosphere is essential (Pansu, 2006). Loss on ignition was determined using a two-step procedure employing a drying oven and a muffle furnace (ASTM, 2000). For determination of moisture, about 1.0 g of the sample was placed into a pre-weighed crucible and weighed to the nearest of 0.1 mg. The crucible along with the specimen was placed, without a cover, into the preheated drying oven (104-110°C). The oven was closed and the sample was heated for 1 h; afterwards the crucible was removed, covered immediately and allowed to cool to ambient temperature in a desiccators and weight was recorded. For dry ashing, the crucible along with dry sample was placed without cover in a furnace and the temperature was raised at a rate such that it reached 450 to 500°C at the end of first hour and 950°C at the end of second hour. The temperature was maintained for an additional two hours or until the sample reached a constant weight. Then loss on ignition or organic matter was calculated by the weight difference before (m_1) and after (m_2) ashing the sample (Radojevic and Bashkin, 1999).

$$Loss.on.Ignition = \frac{m_1 - m_2}{m1} \times 100 \tag{04}$$

2.8   pH and Electrical Conductivity

pH measurement is widely used to characterize the soil at a given time. Preliminary measurement of pH helps in choosing suitable methods of analysis and extraction that are suitable for neutral, basic or acidic soil. However, soil pH represents only the pH of a solution in equilibrium with the soil (Pansu, 2006). It is useful in determining the mobility of ions in the soil, the solubility of soil minerals and assessing the viability of the soil-plant environment. It controls many of the physical, chemical and biological properties of soil (Brady and Weil, 2002; Li *et al.*, 2007). It affects the chemical reactions in soil and variations in soil pH change the nutrients availability and results in element imbalances in the plants. Availability of macronutrients to plants may improve by increasing the pH of acidic soils; reduction in the elements solubility, such as, Mn and Al would simultaneously occur (Flis *et al.*, 1993; Zhao *et al.*, 2011). In present study, pH of soil samples was measured in their water extracts (5 g soil in 50 mL distilled water), using a pH meter. Precalibrated pH meter was used for measuring the soil pH. Buffer solutions of pH 4, 7 and 9 were used to calibrate the pH meter. After calibration, the electrode was dipped in the water solution and the pH was recorded. In case of drift, the reading was allowed to stabilize before recording (Radojevic and Bashkin, 1999).

Electrical conductivity (EC) or specific conductance is defined as the measure of the ability of a sample to conduct an electrical current. It is related to the concentration of ionized substances in sample. It is used as an approximate measure of the total concentration of inorganic substances in sample. Organic compounds have little influence on the conductivity as these do not dissociate in aqueous solution hence are poor conductors of electricity. Conductivity increases with temperature due to a decrease in viscosity and increasing dissociation. It also increases with increasing mineral contents. (Radojevic and Bashkin, 1999). In the present study, electrical conductivity of the soil was measured in their water extract (5 g of soil in 50 mL distilled water) using conductivity meter which was pre calibrated with 0.1 M and 0.01 M KCl solutions (Arain *et al*, 2008).

2.9   Soil Alkalinity

Alkalinity in the soil arises mainly because of dissolution of calcite ($CaCO_3$) which results in increase of pH. The carbonate and bicarbonate levels increase as soil becomes more alkaline. Alkalinity in the soils can have an important effect on soil processes, which have undesirable effects on plants (Radojevic and Bashkin, 1999). In the present study

alkalinity of the soil samples was measured in their water extract (5 g soil in 50 mL distilled water) by titrating it with the standard 0.0255 M HCl using phenolphthalein as an indicator (if the pH of solution is greater than 8.3). This is called as phenolphthalein alkalinity or carbonate alkalinity. In case of soil extract having pH less than 8.3, only total alkalinity by titrating the soil extract with standard HCl using methyl orange as an indicator was measured. This is called as bicarbonate alkalinity. Final results were expressed in form of mg of $CaCO_3$/L, and mg of HCO_3^-/L using formula (Radojevic and Bashkin, 1999):

$$Alkalinity = \frac{V_t \times M}{V_s} \times 1000 \tag{05}$$

Where, V_s is the volume of sample used (mL); V_t is the volume of standard acid (mL); M is mass (mg of $CaCO_3$ equivalent to 1 mL of titrant).

2.10 Statistical Analysis

Descriptive data analysis included minimum, maximum, mean, median standard deviation (SD), standard error (SE), skewness and kurtosis which showed the individual variations, dispersion and symmetry in the dataset. Correlation study was carried out in order to determine the extent of the relationship between the measured variables (Gong *et al*, 2010). Along with the basic statistical parameters and correlation study, multivariate methods consisting cluster analysis was also performed using the STATISTICA software (StatSoft, 1999). There is a wide range of multivariate statistical methods that take account of, or particularly focus on the variety of relationships between variables. They may be particularly useful when there is a large volume of experimental results and sometimes they provide imminent into the multidimensional patterns in the data that would be overlooked with univariate analysis. The purpose of such a statistical interpretation is to try to make some assumptions on experiential variations in the data and biogeochemical processes controlling the variations in elemental concentrations thus assessing the environmental behaviour of different metals (Shtangeeva *et al*, 2009).

Cluster analysis (CA) is the statistical operation of grouping the variables into a limited number of groups known as clusters which have two properties. On the one hand, they are not define in advance by the analyst but are discovered through the operation. On the other hand, the clusters are combination of variables having similar characteristics, which are separated from the variables having dissimilar characteristics, thus resulting in

internal homogeneity and external heterogeneity. The cluster to which each variable belongs is not known in advance, yet the number of clusters is not fixed because there is no dependent variable. Hence, clustering is descriptive not predictive (Toffery, 2011). There are two general approaches to classify the observations, hierarchial clustering and partitioning. In hierarchial clustering we start with the number of clusters equal to the number of observations and end with one cluster. Partitioning is the reverse of this process. One of the most popular hierarchial clustering methods is Ward's method which is very efficient method based on analysis of variance approach. A significant step in any clustering is to select a distance measures which determine how the similarity of two observations is calculated (Chwiej, 2010). Hierarchical clustering shows the resemblance between the variables with respect to all. Typical output is a dendrogram where the variables are set in a hierarchy reflecting their resemblance (Gottelt *et al*, 1997).

2.11 Contamination Factor of the Metals

The potential soil contamination can be assessed using contamination factor (C_f) in which the mean metal concentration measured in the soil samples is compared to the baseline reference level in earth crust according to the following relationship (Hakanson, 1980; Abrahim and Parker, 2008);

$$C_f = C_n \Big/ C_b \qquad (06)$$

Where, 'C_n' and 'C_b' refer to the mean concentration of a metal in the soil and earth crust, respectively.

2.12 Geoaccumulation Index

The index of geoaccumulation (I_{geo}) enables the assessment of accumulation of the metals in soil by comparing the current and pre-industrial concentrations (Chen *et al*., 2007; Duan *et al*., 2010; Kaushik *et al*., 2009). It is computed using the following relationship:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5B_n}\right) \qquad (07)$$

Where, 'C_n' is the mean metal concentration in the examined soil and 'B_n' is the geochemical background value in the earth crust. The factor 1.5 is introduced to minimize the effect of possible variations in the background values which may be attributed to

lithogenic variations.

2.13 Enrichment Factor of the Metals

The extent of metal pollution due to anthropogenic activities in the soil samples can be assessed using enrichment factor (Duan *et al*., 2010; Sutherland, 2000; Vega *et al*., 2009). Enrichment factor (EF) represents the pollution level in the soil and is a good tool to differentiate between the anthropogenic and natural sources of the metals. EFs can be calculated using the relationship:

$$EF = \frac{[X/M_{ref}]_{sample}}{[X/M_{ref}]_{crust}} \tag{08}$$

Where, $[X/M_{ref}]_{sample}$ and $[X/M_{ref}]_{crust}$ refer, respectively, to the ratios of mean concentrations (mg/kg, dry weight) of the target metal and 'M_{ref}' in the soil and continental crust. In the present study, Fe was used as a reference element.

Chapter 3

RESULTS AND DISCUSSION

3.1 Layout of Data

The analytical data related to the distribution, correlation and apportionment of selected metals in different fractions of the soil samples collected from paddy fields are shown in various Tables and Figures. The first part of this chapter deals with the distribution of selected metals in the water soluble, exchangeable, reducible, oxidiseable and residual fractions of the soil samples. It is followed by the study of statistical distribution related to the physiochemical parameters (pH, electrical conductivity, organic matter, nitrate, chloride and sulphate) and particle size distribution ranging from <1.0 μm to >2000 μm. Soil texture is also evaluated to find out the relative proportion of the clay, silt and sand. Inter-relationships among the metals in various soil fractions, physicochemical parameters and particle size fractions are envisaged in terms of Pearson correlation coefficients.

Second part of this chapter is related to the evaluation of average concentrations of selected metals in different fractions of the soil at three sampling stages; Stage 1 (seedling stage), Stage 2 (tillering stage), and Stage 3 (maturity stage). Particle size gradation is also studied at the three stages along with the physicochemical parameters. Average metal contents in different fractions of the soil were also evaluated on comparative basis. Multivariate apportionment of the measured metals levels forms an important part of the study. Cluster analysis (CA) was used for the apportionment and source identification of the metals by Ward's method in various fractions of the soil. Pollution index of the metals in paddy soil is also evaluated in terms of contamination factor, geoaccumulation index and enrichment factor, which are discussed at the latter part of this chapter. These indices help to identify and quantify the degree of pollution by selected metals in the paddy soil. Predominant chemical nature of the soil samples collected from the paddy fields were also envisaged by Piper diagram. Significant findings emerging from the present study are then presented at the end.

The above outlined layout of the entire data forms the basis of an orderly sequence of discussion which now follows.

3.2   Distribution of Selected Metals in Water Soluble Fraction of Soil

Basic statistical parameters related to the distribution of selected metal levels (mg/kg) in the water soluble fraction of the paddy soil are shown in Table 2. An examination of the Table revealed highest average contribution of Na (333.6 mg/kg), followed by, Ca (133.3. mg/kg) and Mg (67.72 mg/kg). Average concentrations of K, Fe, Sr, Cd, Co, Ni, Pb, Cr, Cu Mn and Zn were recorded at 34.42 mg/kg, 9.681 mg/kg, 2.277 mg/kg, 1.744 mg/kg, 1.483 mg/kg, 1.461 mg/kg, 1.39 mg/kg, 0.962 mg/kg, 0.343 mg/kg, 0.199 mg/kg, and 0.127 mg/kg, respectively. Among the metals, Li was found at the lowest concentration (0.113 mg/kg). On the average basis, following increasing order of the metal concentration was observed in the water extract of the soil; Li < Zn < Mn < Cu < Cr < Pb < Ni < Co < Cd < Sr < Fe < K < Mg < Ca < Na. Almost comparable mean and median levels depicted more or less normal distribution pattern of Cu, Cr, Mn, Sr, Na, Zn and Li in water soluble fraction of the soil.

Table 2.   Statistical distribution parameters for selected metal concentrations (mg/kg) in the water soluble fraction of soil

	Min	Max	Mean	Median	SD	SE	Skew	Kurtosis
Ca	24.77	297.2	133.3	122.6	70.06	11.21	0.488	-0.413
Cd	0.010	50.02	1.744	0.190	8.810	1.557	5.655	31.99
Co	0.060	3.337	1.483	1.359	0.865	0.146	0.490	-0.366
Cr	0.107	2.498	0.962	0.930	0.633	0.108	0.563	-0.237
Cu	0.010	0.940	0.343	0.360	0.227	0.038	0.467	-0.128
Fe	1.309	37.98	9.681	5.610	9.736	1.559	1.713	1.982
Mg	17.67	158.5	67.72	60.13	31.83	5.098	0.924	0.762
Mn	0.020	0.530	0.199	0.160	0.136	0.023	0.844	0.014
Ni	0.140	3.828	1.461	1.258	0.962	0.158	0.907	0.243
Pb	0.040	3.446	1.390	1.155	0.956	0.159	0.513	-0.682
Sr	0.350	9.988	2.277	2.000	1.634	0.269	2.988	13.42
Zn	0.010	0.350	0.127	0.120	0.086	0.015	0.744	-0.002
K	15.43	76.97	34.42	28.56	14.10	2.258	1.267	1.359
Li	0.020	0.450	0.113	0.100	0.083	0.015	2.248	7.934
Na	49.33	748.7	333.6	325.6	173.8	27.83	0.273	-0.509

Considerably higher SD and SE values for Ca, Fe, Cd, K, Mg and Na indicated their higher dispersion and non-Gaussian distribution in the soil samples. Relatively higher skewness and kurtosis values for Cd, Sr, Li, K and Fe indicated their asymmetric distribution in the water soluble fraction of the soil. Overall, most of the metals revealed random variations and significantly elevated dispersion in this fraction of the soil. Box and whisker plot showing the quartile distribution of selected metals in the water soluble fraction of soil is portrayed in Figure 1, which revealed broad range and predominantly unsystematic distribution for Cd, Cu, Co, Pb, Ni, Zn and Na. However, relatively symmetric distribution pattern was shown by Sr, Li, Mn and Fe in the soil samples while K, Ca and Mg showed somewhat narrow but asymmetric distribution.
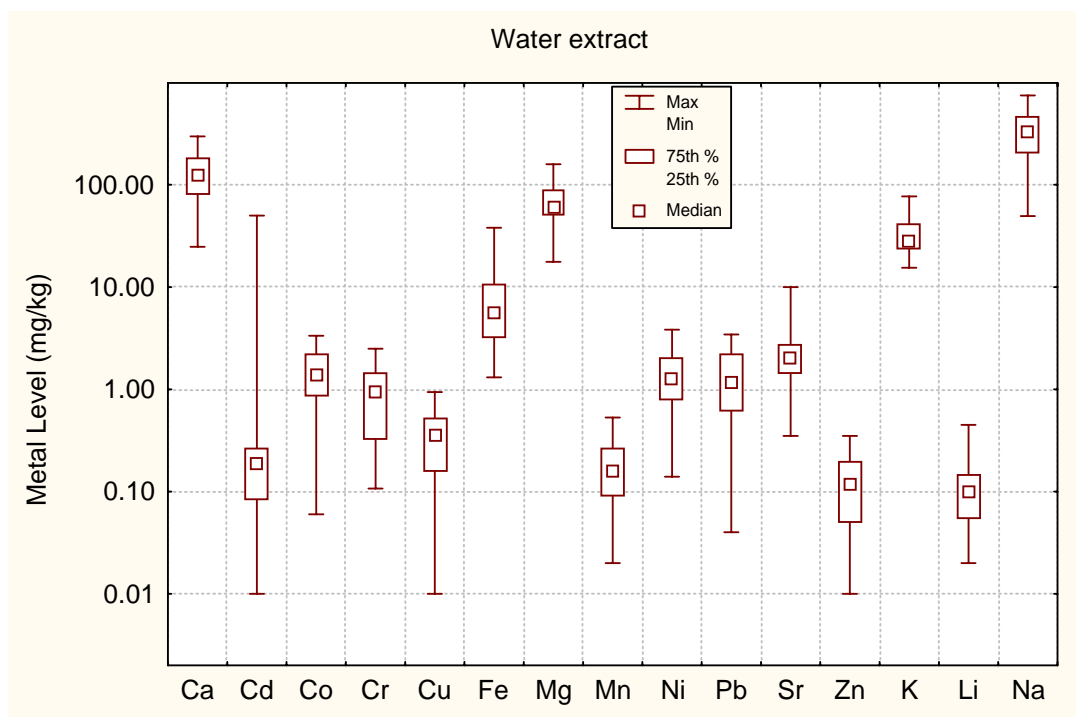
Figure 1. Quartile distribution of selected metals in the water soluble fraction of soil

3.3   Distribution of Selected Metals in Exchangeable Fraction of Soil

Basic statistical distribution parameters for selected metal concentrations (mg/kg) in the exchangeable or weak acid soluble fraction of paddy soil are shown in Table 3. Among the selected metals, highest mean level was shown by Ca (58865 mg/kg), followed by Mg (1870 mg/kg), Mn (185.7 mg/kg), Sr (116.4 mg/kg) and Fe (98.37 mg/kg). Average levels of Co, Pb, Ni, Cr, Cu and Zn were found at 10.55 mg/kg, 8.058 mg/kg, 7.527

mg/kg, 5.529 mg/kg, 3.86 mg/kg and 3.226 mg/kg, respectively. The lowest concentration was noted for Cd (0.582 mg/kg). Overall, the mean levels of the metals decreases in the following order: Ca > Mg > Mn > Sr > Fe > Co > Pb > Ni > Cr > Cu > Zn > Cd. Among these metals Cd, Cr, Cu, Ni, Pb and Zn showed relatively normal distribution pattern as manifested by lower SD and SE values, nonetheless, Ca, Co, Fe, Mn, Mg and Sr showed predominantly random distribution pattern as reflected by their large range and considerably higher SD and SE values. Comparatively symmetric distribution was noted for Cr, Mg and Pb as shown by somewhat lower skewness values while higher skewness and kurtosis values of Zn, Mn and Co indicated their asymmetrical distribution in the exchangeable fraction of the soil.

Quartile distribution of the selected metals in the exchangeable fraction of paddy soils is represented in Figure 2. Very broad range was noted for Ni, Cd, Pb and Zn with noticeable asymmetry, however, Co showed fairly broad but symmetric distribution. In the cases of Sr, Mg and Ca very narrow range was observed as shown by nearly merging upper and lower quartiles. Relatively symmetrical distribution was observed for Cu in the exchangeable fraction of soil.

Table 3.   Statistical distribution parameters for selected metal concentrations (mg/kg) in the exchangeable fraction of soil

|	Min	Max	Mean	Median	SD	SE	Skew	Kurtosis
Ca	40062	85482	58865	57897	10852	1738	0.710	0.299
Cd	0.050	1.748	0.582	0.350	0.542	0.088	0.932	-0.588
Co	1.099	76.10	10.55	8.338	11.93	1.989	4.971	27.65
Cr	1.450	9.350	5.529	5.600	2.133	0.342	-0.198	-1.016
Cu	1.347	8.741	3.860	3.643	1.804	0.289	0.973	0.971
Fe	12.65	219.0	98.37	85.22	47.76	7.647	0.623	0.090
Mg	1387	2543	1870	1891	254.4	40.74	0.051	0.212
Mn	17.14	248.5	185.7	189.4	40.63	6.506	-1.757	6.863
Ni	0.500	20.95	7.527	6.250	4.580	0.733	0.843	0.762
Pb	0.400	18.15	8.058	8.092	4.830	0.841	0.126	-1.098
Sr	79.15	183.8	116.4	110.6	23.04	3.690	0.871	0.544
Zn	0.050	36.63	3.226	1.350	6.151	0.985	4.693	24.25
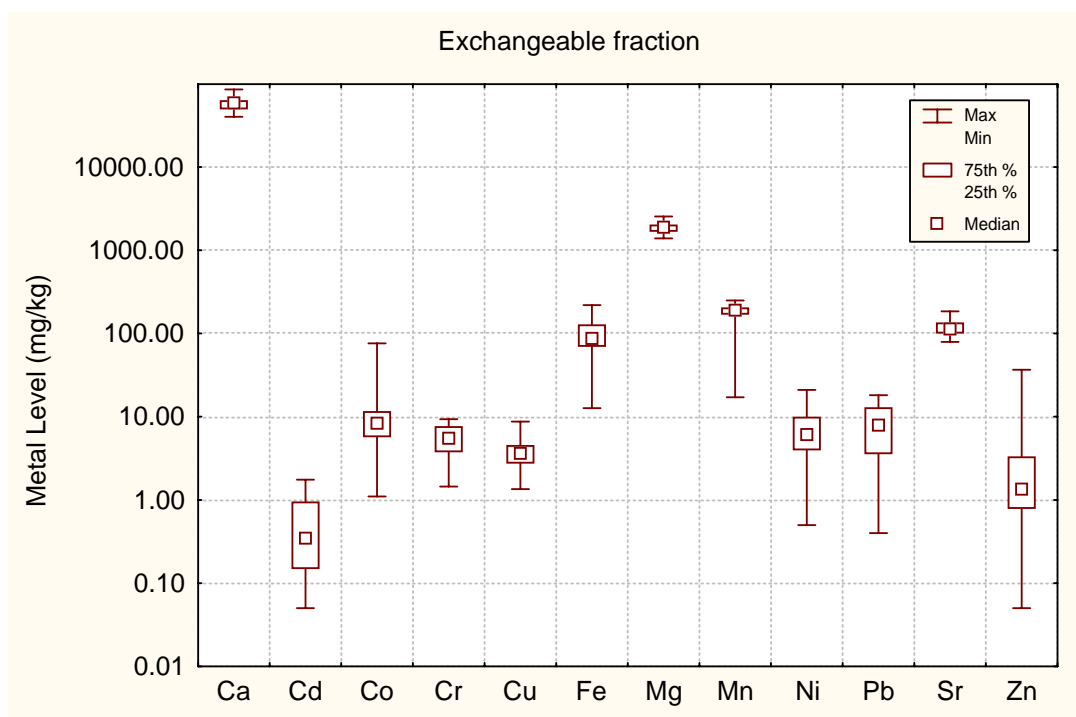
Figure 2. Quartile distribution of selected metals in the exchangeable fraction of soil

3.4 Distribution of Selected Metals in Reducible Fraction of Soil

Table 4 shows the concentrations (mg/kg) of selected metal in term of their statistical distribution parameters in the reducible fraction of soil. Dominant mean levels were shown by Ca (4658 mg/kg), followed by Fe (1381 mg/kg), Mg (781.9 mg/kg) and Mn (56.83 mg/kg). Mean levels of Co, Sr, Ni, Zn, Pb, Cu, Cr and Cd were found to 9.875 mg/kg, 9.136 mg/kg, 7.875 mg/kg, 6.677 mg/kg, 5.984 mg/kg, 2.396 mg/kg, 1.836 mg/kg and 0.982 mg/kg, respectively. On the average basis, following trend in the metal levels was observed; Ca > Fe > Mg> Mn > Co > Sr > Ni > Zn > Pb > Cu > Cr > Cd. More or less normal distribution pattern was shown by Cd, Cr, Cu, Co, Ni, and Pb as evidenced by very small SD and SE values, whereas, Ca, Fe, and Mg showed dominantly random distribution pattern as manifested by considerably higher SD and SE values. Fairly symmetric distribution was noted for Cd, Cr, Cu, Fe, Ni, Mg, Mn, Sr and Pb, supported by relatively lower skewness and kurtosis values. On the other hand, higher skewness and kurtosis values depicted asymmetrical distribution for Ca, Co and Zn in the reducible fraction of soil. The quartile distribution of the metals in the reducible fraction of soils is shown in Figure 3 which revealed broad distribution pattern for Cd, Cr, Mn, Zn and Sr, while Ca, Co, Ni, Mg and Pb exhibited somewhat broad and asymmetric variations.

Table 4.   Statistical distribution parameters for selected metal concentrations (mg/kg)
in the reducible fraction of soil

|	Min	Max	Mean	Median	SD	SE	Skew	Kurtosis
Ca	2060	11671	4658	4204	2046	327.6	1.708	3.493
Cd	0.050	2.050	0.982	1.050	0.563	0.095	0.172	-1.024
Co	1.950	22.23	9.875	8.550	4.966	0.816	0.523	-0.375
Cr	0.050	4.800	1.836	1.700	1.139	0.182	0.843	0.515
Cu	0.700	5.200	2.396	2.100	1.291	0.207	0.612	-0.730
Fe	648.9	2077	1381	1411	374.4	59.95	-0.055	-1.078
Mg	170.5	1786	781.9	703.3	397.4	63.63	0.763	-0.125
Mn	2.581	117.8	56.83	54.19	27.32	4.376	0.477	-0.307
Ni	0.848	22.66	7.875	7.500	4.816	0.792	0.853	0.975
Pb	0.650	14.97	5.984	5.847	3.651	0.626	0.401	-0.362
Sr	0.848	26.60	9.136	7.435	6.271	1.004	0.921	0.282
Zn	2.300	45.11	6.677	5.300	7.030	1.140	4.780	25.46
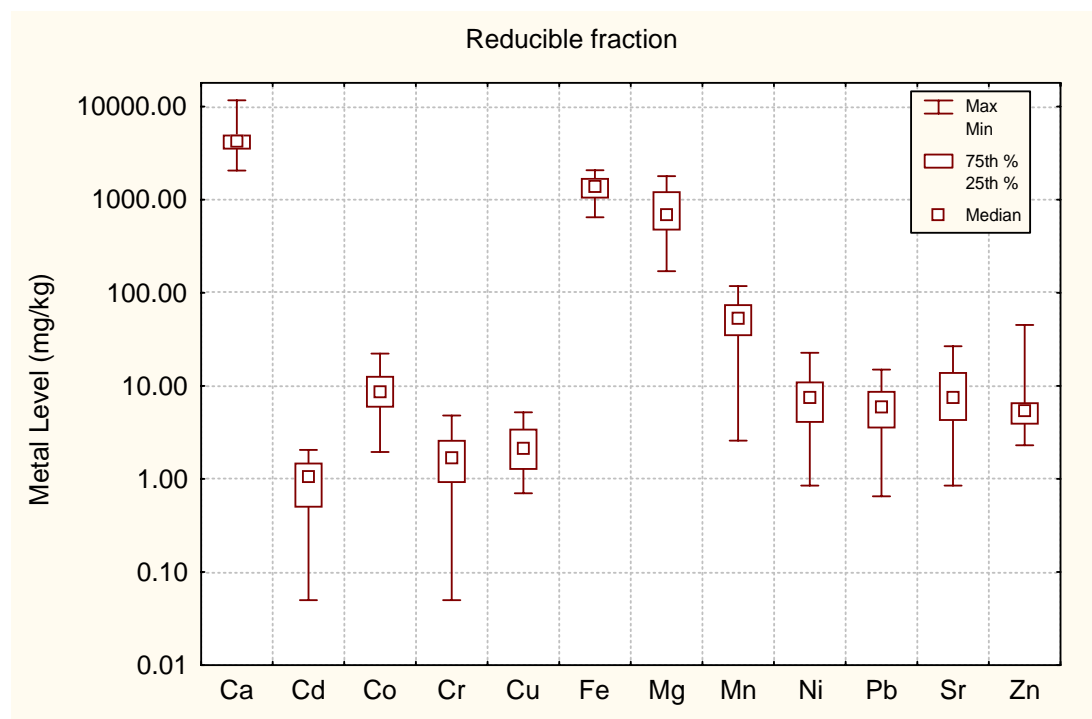
Figure 3. Quartile distribution of selected metals in the reducible fraction of soil

3.5   Distribution of Selected Metals in Oxidiseable Fraction of Soil

The statistical parameters corresponding to selected metals distribution in the oxidiseable fraction of paddy soil are illustrated in Table 5. Average concentration of Mg was observed at the highest value (1151 mg/kg), followed by Ca (512.9 mg/kg) and Fe (693.9 mg/kg). Mean levels of Mn, Ni, Pb, Zn, Co, Cr, Sr and Cu were found at 28.98 mg/kg, 12.45 mg/kg, 6.133 mg/kg, 4.436 mg/kg, 3.425 mg/kg, 3.409 mg/kg, 2.585 mg/kg and 2.323 mg/kg, respectively. Lowest concentration of 0.491 mg/kg was observed for Cd. The increasing order of average metal concentrations followed the order: Cd < Cu < Sr < Cr < Co < Zn < Pb < Ni < Mn< Fe < Ca < Mg. Among the selected metals, Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, and Sr showed almost comparable median and mean levels indicating more or less normal distribution in this fraction. However, Ca, Mg and Fe showed relatively large spread as indicated by their maximum and minimum levels and higher dispersion as indicated by their large SD and SE values. However, Co, Cr, Mn, Ni, Fe, Mg, Pb, Sr and Cu showed fairly symmetric distribution supported by lower skewness and kurtosis values while, Ca, Cd and Zn showed asymmetric distribution as indicated by relatively larger skewness and kurtosis values.

Table 5.   Statistical distribution parameters for selected metal concentrations (mg/kg) in the oxidiseable fraction of soil

	Min	Max	Mean	Median	SD	SE	Skew	Kurtosis
Ca	0.050	4142	512.9	197.5	857.4	144.9	3.168	10.82
Cd	0.012	1.950	0.491	0.449	0.380	0.063	1.672	4.890
Co	0.250	8.650	3.425	3.147	2.213	0.398	0.638	-0.247
Cr	0.150	6.350	3.409	3.343	1.639	0.269	0.001	-0.670
Cu	0.050	5.045	2.323	2.098	1.268	0.206	0.302	-0.736
Fe	9.850	2617	693.9	73.00	923.7	147.9	0.841	-1.183
Mg	52.87	2248	1151	1130	451.9	72.38	0.109	0.368
Mn	15.20	40.70	28.98	28.92	5.805	0.929	0.088	-0.288
Ni	1.100	28.02	12.45	11.57	6.650	1.079	0.398	-0.536
Pb	0.649	16.27	6.133	6.066	4.340	0.767	0.819	0.193
Sr	0.050	6.200	2.585	2.650	1.477	0.257	0.460	0.147
Zn	0.200	14.77	4.436	3.796	3.265	0.537	1.061	1.384

Box and whisker plot showing the quartile distribution of selected metals in the oxidiseable fraction of paddy soil is shown in Figure 4 which revealed broad range and predominantly asymmetric distribution of Ca, Fe, Sr, Mg, Cu, Co, Cr and Zn. Among the selected metals, Mn and Cd exhibited very narrow range with overlapping lower and upper quartiles in oxidiseable fraction of the soil. Relatively symmetric distribution in this fraction of the soil was noted for Ni and Pb, while rest of the metals showed asymmetric and random variations in their concentrations.
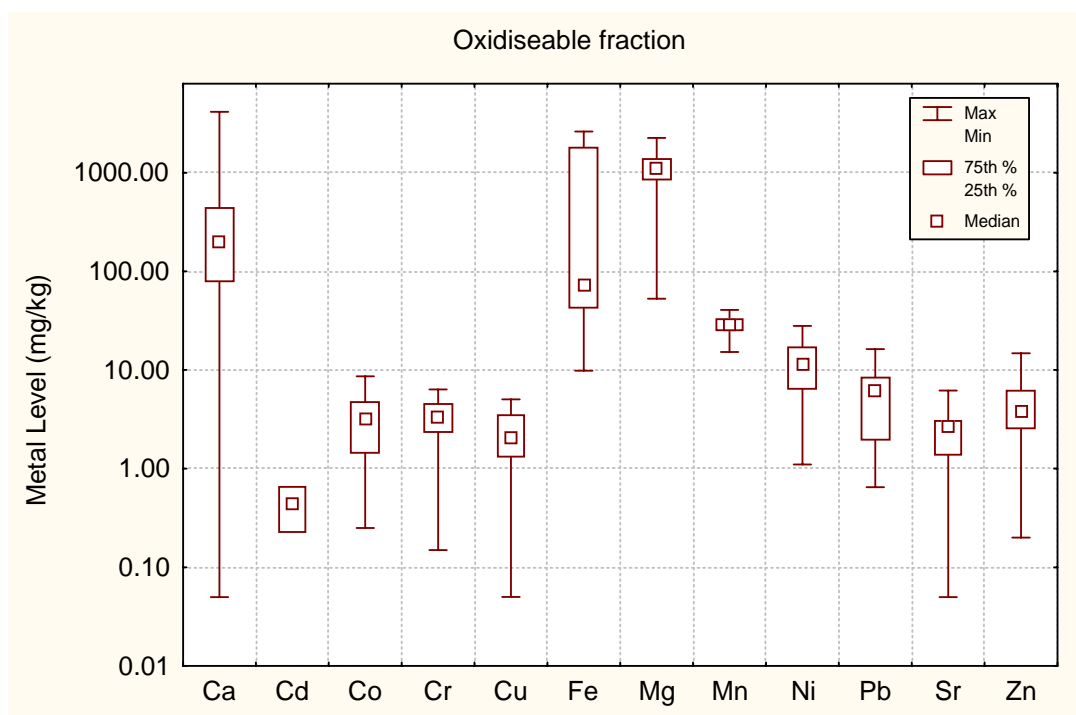
Figure 4. Quartile distribution of selected metals in the oxidiseable fraction of soil

3.6   Distribution of Selected Metals in Residual Fraction of Soil

The statistical parameters pertaining to selected metals distribution in the residual fraction of paddy soils are shown in Table 6. Mean concentration of Fe was found at 7724 mg/kg, which was the highest among the selected metals. It was followed by Mg (7211 mg/kg), Mn (189.0 mg/kg) and Ca (80.75 mg/kg). Average levels of Zn, Ni, Cu, Cr, Co, Pb and Sr were found at 46.65 mg/kg, 35.19 mg/kg, 19.51 mg/kg, 18.27 mg/kg, 9.792 mg/kg, 9.071 mg/kg and 4.735 mg/kg, respectively. Lowest concentration at 1.311 mg/kg was observed for Cd in the residual fraction of soil. Mean levels of the metals showed following trend in their concentrations in this soil fraction; Fe > Mg > Mn > Ca > Zn > Ni

> Cu > Cr> Co> Pb> Sr> Cd. Relatively normal distribution as manifested by comparable mean and median levels was noted for Cu, Cr, Co, Mn, Mg, Fe, Ni, Sr and Zn in the soil samples. Significantly higher SD and SE values for Cd and Pb indicated their higher dispersion in residual fraction of the soil. Relatively lower skewness and kurtosis values for Cr, Co, Mn, Mg, Fe, Ni, Sr and Zn reflected their symmetrical variations in this particular fraction. However, Cd, Ca, Cu and Pb showed asymmetric distribution as evidenced by their higher skewness and kurtosis values in the soil. Overall, most of the metals showed lower dispersion and asymmetry in this fraction of soil compared with other fractions of the soil.

Table 6.   Statistical distribution parameters for selected metal concentrations (mg/kg) in the residual fraction of soil

	Min	Max	Mean	Median	SD	SE	Skew	Kurtosis
Ca	0.750	439.7	80.75	42.31	107.5	26.07	2.636	7.942
Cd	0.050	8.783	1.311	1.100	1.619	0.282	3.448	14.55
Co	1.347	20.11	9.792	9.100	4.866	0.779	0.401	-0.627
Cr	10.20	23.95	18.27	17.93	3.149	0.504	-0.272	-0.036
Cu	9.900	49.30	19.51	18.80	6.127	0.981	3.008	14.51
Fe	6869	8436	7724	7799	391.4	62.67	-0.219	-0.769
Mg	4581	9584	7211	7256	1033	165.4	0.240	0.536
Mn	140.2	225.7	189.0	188.2	17.65	2.827	-0.109	0.886
Ni	9.900	61.36	35.19	35.25	10.90	1.746	-0.435	0.687
Pb	0.200	86.83	9.071	4.668	15.82	2.638	4.101	18.17
Sr	0.350	12.89	4.735	4.375	3.503	0.640	0.622	-0.647
Zn	30.30	67.45	46.65	45.80	8.152	1.305	0.367	-0.043

Figure 5 illustrates the box and whisker plot showing the quartile distribution of selected metals in the residual fraction of soil. Among the selected metals, Cd, Ca, Pb and Sr showed broad range and relatively higher dispersion, therefore indicating comparatively higher variation in most of the soil samples. On the other hand, Fe, Cr, Mg, Zn and Mn revealed very narrow range with small variations in their median values, minimum, maximum, 25th percentile, and 75th percentile. Moderate variations were observed for Co, Cu and Ni levels in the residual fraction of soil.
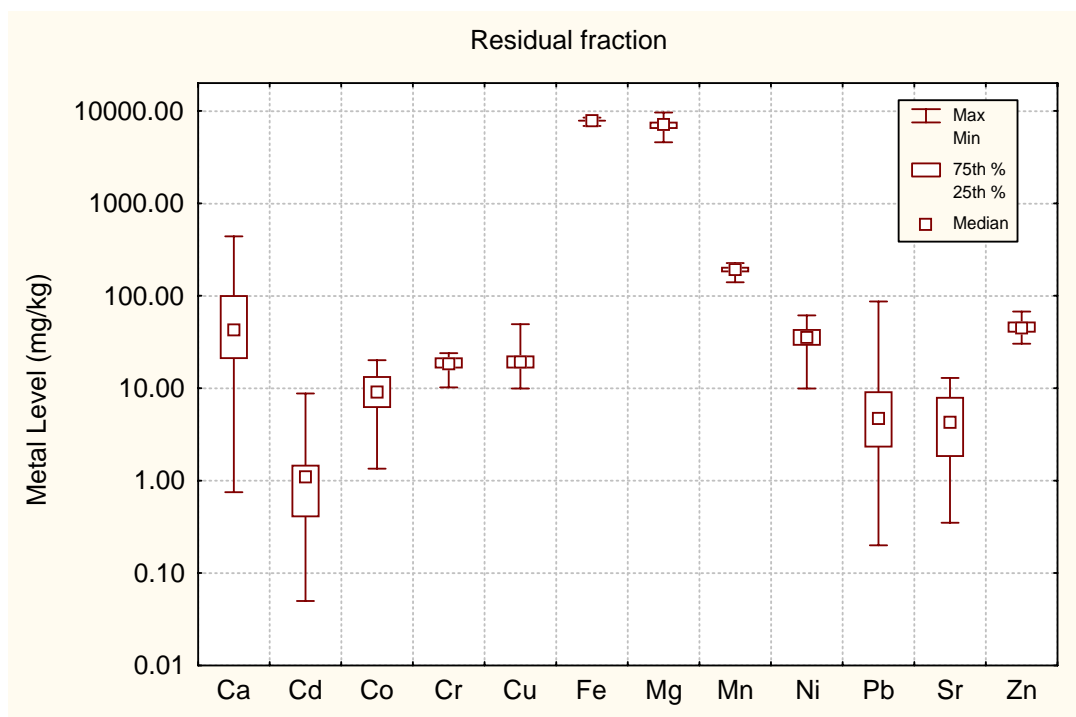
Figure 5. Quartile distribution of selected metals in the residual fraction of soil

3.7   Physicochemical Characteristics of the Soil

The physicochemical characteristics of the soil, including pH, electrical conductivity (EC), nitrate, chloride and sulphate were evaluated in the water-extract of the soils. Organic matter (OM) was determined in the dried samples. The statistical summery of these physicochemical parameters are shown in Table 7. The mean value for pH was found at 7.775 and remained in the range of 7.260-8.630 during the entire study, which showed predominantly alkaline nature of soil in the study area. Median and mean levels of pH were comparable indicating more or less normal distribution in the soil samples. Lower skewness and kurtosis values reflected their symmetrical variations. Electrical conductivity (EC) values ranged from 77.9 - 624.7 μS/cm with average value of 295.5 which were within the safe range for the agricultural soil (<1000 μS/cm). Lower kurtosis and skewness values depicted their symmetrical variations. On the average basis, nitrates, chlorides and sulphates were found at 161.2 mg/kg, 190.3 mg/kg and 276.3 mg/kg, respectively in the soil samples. Among the physicochemical parameters, chlorides, sulphates and nitrates showed random distribution pattern as manifested by considerably higher SD and SE values. On the other hand, lower skewness and kurtosis values reflected

their symmetric distribution. Organic matter ranged from 6.384 to 11.271% with mean value of 8.378%. Relatively higher values of organic contents were associated with the use of fertilizer in the form of organic manure in the paddy soil.

Table 7.　　Statistical summery of the physicochemical parameters of soil

	pH	EC (µS/cm)	OM (%)	NO_3^- (mg/kg)	Cl^- (mg/kg)	SO_4^{2-} (mg/kg)
Min	7.260	77.90	6.384	3.040	4.620	19.68
Max	8.630	624.7	11.27	739.9	787.6	891.9
Mean	7.775	295.5	8.378	161.2	190.3	276.3
Median	7.740	271.5	8.461	47.86	123.6	201.4
SD	0.265	129.5	0.914	228.5	179.0	230.9
SE	0.043	20.74	0.146	36.59	28.67	36.97
Skewness	1.158	0.591	0.477	1.552	1.403	1.125
Kurtosis	2.456	0.159	1.658	0.944	1.843	0.498

3.8   Soil Texture and Particle Size Gradation

Particle size distribution was examined in the soil for various size fractions expanding over the range of less than 1 µm to greater than 2000 µm as shown in Table 8. Mean contribution of colloids (< 1 µm) was 15.11% and of those lying in the range of 1-5 µm (fine fraction) were 15.75%. These two particulate fractions constitute the clay contents of the soil. Particles with diameter in the range of 5-75 µm belonged to silt fraction of the soil. Table 8 shows their percentages with highest contribution for particles in the range of 15-30 µm. Particles with diameter in the range of 75 to 4000 µm are classified as sand particles. This class is further categorised as coarse, medium and fine. Fine sand (75-400 µm) showed the contribution of 22.46%, while medium sand (400-2000 µm) was found at 1.957% and that of coarse sand (>2000 µm) was 0.421% in the paddy soil. Overall, relatively higher contribution was noted for the fine fractions.

The ternary diagram showing the soil texture is depicted in Figure 6. Present study showed predominantly silty loam texture of the soil in the study area, where silt constituted about 44% of the soil, clay approximately 30% and sand about 25%. Moisture, nutrients and humus content of silty loam are generally higher having better infiltration

and drainage of water and air. Thus, the soil from study area was considered as good quality soil for agricultural purposes.

Table 8.   Statistical summery of the particle size distribution in soil

|	Min	Max	Mean	Median	SD	SE	Skew	Kurtosis
< 1 μm	6.660	20.00	15.11	16.66	2.963	0.474	-0.592	0.629
1 - 5 μm	10.00	26.66	15.75	16.66	3.653	0.585	0.711	0.950
5-10 μm	6.670	20.00	12.74	13.33	2.654	0.425	0.340	0.493
10-15 μm	3.000	10.00	6.376	6.670	1.949	0.312	0.012	0.144
15-30 μm	10.00	20.00	14.16	13.34	2.997	0.480	0.356	-0.431
30-50 μm	6.660	16.01	9.411	10.00	2.323	0.372	0.630	0.467
50-75 μm	0.287	2.875	1.171	1.000	0.530	0.085	1.208	1.637
75-100 μm	0.643	3.794	2.163	2.058	0.805	0.129	0.212	-0.928
100-200 μm	4.608	19.58	11.37	11.11	4.327	0.693	0.132	-1.126
200-400 μm	3.919	15.03	8.948	7.891	3.342	0.535	0.577	-0.928
400-2000 μm	0.579	4.059	1.957	1.736	0.897	0.144	0.643	-0.329
>2000 μm	0.000	1.682	0.421	0.183	0.453	0.075	1.330	1.120
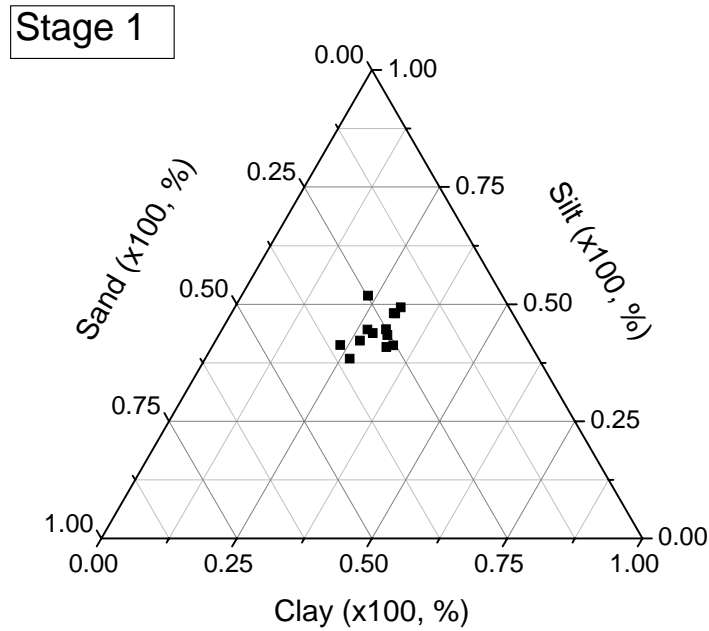
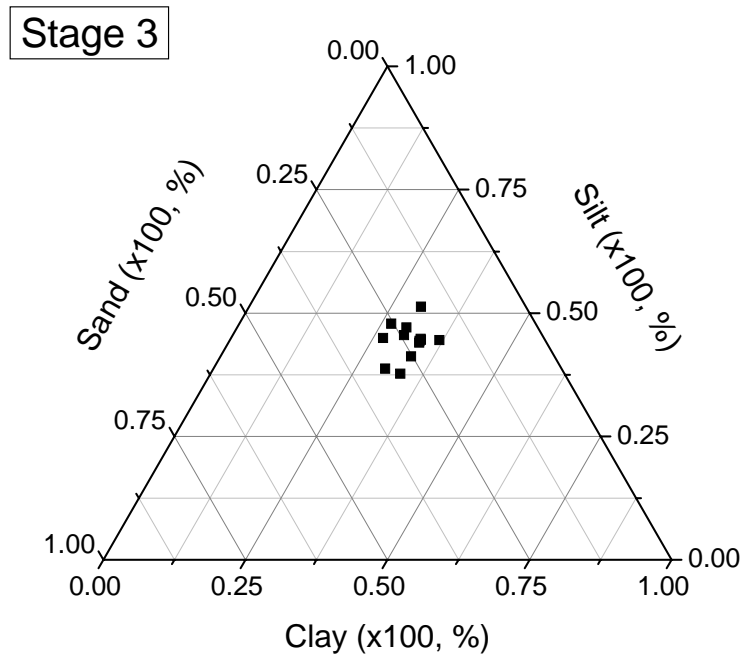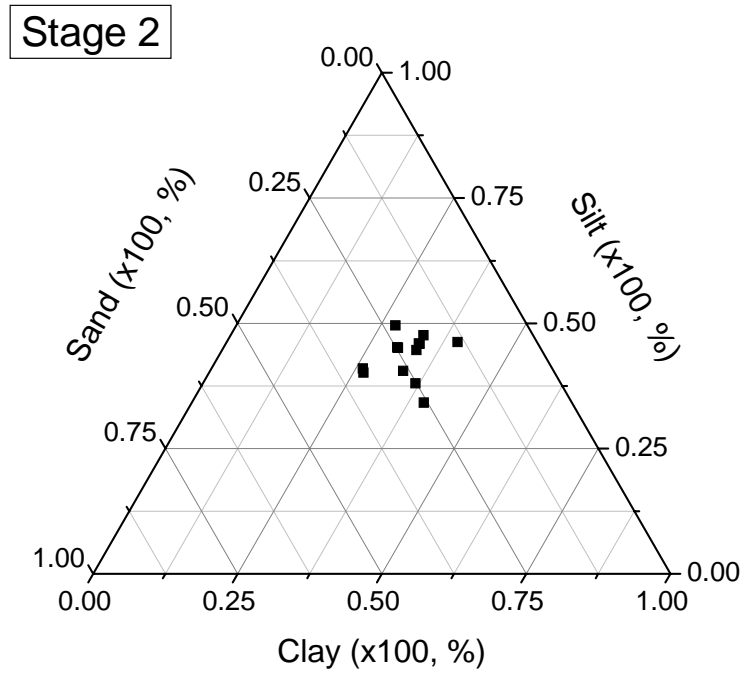Figure 6.   (Contd…..)

Figure 6. Ternary diagram showing the texture of the soil

3.9 Correlation Study of Selected Metals in Various Soil Fractions

Pearson correlation coefficients (r) between the selected metals were calculated to investigate their inter-relationships in various soil fractions. Correlation coefficient matrix for selected metals in the water soluble fraction of the soil is shown in Table 9, wherein significant r-values are shown in bold at p<0.05. Strong positive correlations were noted between Mg-Ca (r = 0.575), Mn-Fe (r = 0.767) and Fe-K (0.529) in water soluble fraction of the soil manifesting their mutual variations in this soil fraction. Some of the metals also manifested strong inverse relationships; Ca-Fe, Ca-Mn, Mg-Fe and Mg-Mn thus showing their opposing variations in the water-extracts of the soil. Some other significant relationships were also noted but most of the metal pairs revealed insignificant relationships in this soil fraction. Correlation coefficient matrix for selected metals in the exchangeable fraction of the soil is shown in Table 10, wherein significant r-values are shown in bold (p<0.05). Strong correlation was only observed between Sr-Ca (r = 0.583) while rest of the metals revealed either weak or insignificant relationships in the exchangeable soil fraction. Similarly, correlation coefficient matrix for selected metals in the reducible fraction of the soil is shown in Table 11, wherein significant r-values are shown in bold at p<0.05. An examination of the Table showed strong association between Cu-Cd (r = 0.607) in the reducible fraction while some other metal pairs (Co-Mn, Co-Sr, Fe-Mg) also exhibited significant positive relationships. Significant negative correlation was noted between Fe and Sr thus reflecting their opposing variations in this fraction of soil.

Correlation coefficient matrix for selected metals in the oxidiseable fraction of the soil is shown in Table 12, wherein significant r-values are shown in bold at p<0.05. Among the selected metals, Mg-Ca (r = 0.704), Mn-Mg (r = 0.755) exhibited very strong correlations between them while Fe showed strong significant and strong inverse correlations with Ca, Mg and Mn in the oxidiseable fraction. The correlation study pointed out mutual occurrence of Ca, Mg and Mn with organic matter of the soil, most probably originating from the organic fertilizers. Correlation coefficient matrix for selected metals in the residual fraction of the soil is shown in Table 13. Significantly strong correlations were observed among Sr-Ca (r =0.678), Pb-Cd (r = 0.711), Mg-Cu (r = 0.540), Mn-Cu (r = 0.502), Cu-Zn (r = 0.614), Mn-Mg (r = 0.663), Mg-Zn (r = 0.561), and Mn-Zn (r = 0.616) which indicated mutual variations of these metals in the earth crust. Overall, correlation study indicated mutual variation of the metals in the residual fraction of soil.

Table 9.　　Correlation coefficient (r) matrix of selected metals in the water soluble fraction of soil

	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn	K	Li
Cd	-0.162													
Co	-0.131	0.254												
Cr	0.104	0.023	0.181											
Cu	-0.190	0.129	-0.206	0.084										
Fe	**-0.685**	0.021	0.056	-0.062	0.212									
Mg	**0.575**	-0.037	-0.262	0.002	-0.085	**-0.679**								
Mn	**-0.587**	0.062	0.255	0.032	0.026	**0.767**	**-0.605**							
Ni	-0.042	0.314	0.134	-0.162	0.238	-0.108	0.152	-0.157						
Pb	-0.276	0.022	**0.392**	0.170	0.118	0.152	**-0.363**	0.135	-0.080					
Sr	0.008	-0.097	0.079	0.189	-0.113	0.166	-0.010	0.053	0.265	0.050				
Zn	-0.143	0.240	0.317	0.236	-0.246	**0.367**	**-0.475**	0.238	-0.276	0.110	-0.074			
K	-0.276	0.064	0.019	0.047	0.142	**0.529**	-0.096	**0.454**	-0.077	-0.026	0.175	0.112		
Li	-0.001	-0.313	-0.127	**0.402**	0.036	-0.044	0.145	-0.167	**0.370**	-0.104	0.272	0.053	0.043	
Na	-0.270	0.108	-0.004	0.095	-0.026	0.137	0.323	0.029	0.108	-0.067	0.238	-0.068	**0.376**	0.248

Significant r-values are shown in bold (p<0.05)

Table 10.　　Correlation coefficient (r) matrix of selected metals in the exchangeable fraction of soil

	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn
Ca	1.000											
Cd	0.214	1.000										
Co	-0.008	-0.156	1.000									
Cr	0.163	0.255	**0.337**	1.000								
Cu	0.181	0.038	0.054	**0.393**	1.000							
Fe	**0.481**	0.273	0.050	**0.399**	0.165	1.000						
Mg	0.209	-0.230	-0.081	-0.165	0.045	-0.093	1.000					
Mn	-0.014	0.135	-0.143	-0.109	-0.069	-0.012	0.312	1.000				
Ni	0.251	0.220	0.028	**0.447**	**0.457**	**0.425**	0.038	-0.040	1.000			
Pb	-0.017	0.024	-0.092	0.058	0.286	0.121	0.072	0.290	**0.380**	1.000		
Sr	**0.583**	0.272	0.155	**0.352**	0.107	0.257	0.243	-0.083	0.310	-0.185	1.000	
Zn	-0.239	-0.031	-0.037	0.200	0.329	-0.080	-0.058	-0.077	0.056	-0.169	0.004	1.000

Significant r-values are shown in bold (p<0.05)

Table 11.   Correlation coefficient (r) matrix of selected metals in the reducible fraction of soil

	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn
Ca	1.000											
Cd	0.094	1.000										
Co	0.046	0.169	1.000									
Cr	-0.156	0.140	-0.028	1.000								
Cu	0.255	**0.607**	0.194	0.201	1.000							
Fe	-0.305	-0.178	-0.366	0.193	-0.282	1.000						
Mg	0.077	0.074	-0.194	-0.004	-0.143	**0.392**	1.000					
Mn	0.082	0.236	**0.334**	0.069	0.205	0.013	-0.074	1.000				
Ni	-0.143	-0.267	-0.010	-0.248	-0.044	-0.208	-0.138	-0.243	1.000			
Pb	0.092	0.126	0.278	-0.041	0.183	-0.184	0.210	-0.174	-0.201	1.000		
Sr	0.309	0.171	**0.338**	-0.125	0.211	**-0.408**	-0.125	-0.193	0.118	0.041	1.000	
Zn	0.182	0.162	0.284	0.081	0.214	-0.052	-0.021	0.038	-0.216	0.291	-0.143	1.000

Significant r-values are shown in bold ($p < 0.05$)

Table 12.   Correlation coefficient (r) matrix of selected metals in the oxidiseable fraction of soil

	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn
Ca	1.000											
Cd	-0.128	1.000										
Co	-0.164	-0.012	1.000									
Cr	0.027	-0.272	0.155	1.000								
Cu	-0.111	0.099	0.203	-0.168	1.000							
Fe	**-0.331**	-0.007	0.241	-0.044	**0.366**	1.000						
Mg	**0.704**	0.034	-0.290	-0.035	-0.010	**-0.680**	1.000					
Mn	**0.491**	-0.082	-0.140	0.071	**0.372**	**-0.457**	**0.755**	1.000				
Ni	-0.042	-0.078	0.120	**0.393**	-0.052	-0.102	0.137	0.314	1.000			
Pb	0.215	-0.049	-0.100	-0.106	0.168	-0.091	0.155	0.266	-0.223	1.000		
Sr	0.161	0.208	-0.264	0.105	-0.020	-0.008	0.162	0.168	0.136	0.091	1.000	
Zn	-0.003	-0.086	-0.004	-0.085	**0.435**	0.158	0.119	**0.351**	0.110	**0.449**	-0.062	1.000

Significant r-values are shown in bold ($p < 0.05$)

Table 13.   Correlation coefficient (r) matrix of selected metals in the residual fraction of soil

	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Sr	Zn
Ca	1.000											
Cd	-0.095	1.000										
Co	-0.187	0.048	1.000									
Cr	-0.240	-0.145	**-0.389**	1.000								
Cu	0.255	-0.039	-0.054	0.227	1.000							
Fe	0.207	0.298	-0.034	-0.110	0.147	1.000						
Mg	**0.454**	0.093	0.027	0.304	**0.540**	**0.474**	1.000					
Mn	0.152	-0.071	-0.053	**0.484**	**0.502**	0.223	**0.663**	1.000				
Ni	-0.037	0.106	0.256	-0.031	0.150	0.294	0.247	0.156	1.000			
Pb	-0.218	**0.711**	-0.119	-0.229	-0.060	0.307	-0.003	-0.059	-0.018	1.000		
Sr	**0.678**	-0.042	-0.257	0.036	0.017	**0.426**	**0.350**	0.045	0.117	-0.114	1.000	
Zn	0.269	0.070	-0.248	0.440	**0.614**	0.224	**0.561**	**0.616**	0.223	0.135	0.052	1.000

Significant r-values are shown in bold ($p < 0.05$)

3.10   Correlation Study of Physicochemical Parameters in Soil

Correlation coefficient matrix for physicochemical parameters in the soil is shown in Table 14, wherein significant r-values are shown in bold at $p < 0.05$. Organic matter showed negative correlation with electrical conductivity (r = -0.576), sulphate (r = -0.588), and chlorides (r = -0.471). Strong positive correlations were noted for EC with chlorides (r = 0.576) and sulphates (r = 0.621), while nitrates and sulphates also revealed strong association in the soil samples. The plausible association among the physicochemical parameters and selected metals levels in the water soluble fraction of the soil were also investigated in this study. Correlation coefficient matrix for physicochemical parameters and selected metal contents in the soil is shown in Table 15. Strong positive correlation was observed for pH with Cd (r = 0.569), while electrical conductivity exhibited strong relationships with Mg and Na with respective "r" values of 0.573 and 0.730. Strong positive correlations were also observed for nitrates with Mg (r = 0.658), chlorides with Na (r = 0. 805), and sulphates with Mg (r = 0.713). pH revealed significant inverse relationships with Ca and Sr while organic matter showed negative associations with Li and Na levels in the soil.

Table 14.   Correlation coefficient (r) matrix of physicochemical parameters of soil

	pH	EC	OM	NO_3^-	Cl^-	SO_4^{2-}
pH	1.000					
EC	-0.052	1.000				
OM	-0.044	**-0.576**	1.000			
NO_3^-	-0.255	**0.410**	-0.293	1.000		
Cl^-	0.009	**0.576**	**-0.471**	0.104	1.000	
SO_4^{2-}	-0.087	**0.621**	**-0.588**	**0.512**	**0.331**	1.000

Significant r-values are shown in bold ($p < 0.05$)

Table 15.   Correlation coefficient (r) matrix of physicochemical parameters and selected metals in water soluble fraction of soil

	pH	EC	OM	NO_3^-	Cl^-	SO_4^{2-}
Ca	**-0.348**	0.090	0.148	**0.345**	-0.216	0.126
Cd	**0.569**	0.042	0.016	-0.126	0.057	-0.047
Co	0.004	-0.281	0.133	-0.302	0.027	**-0.416**
Cr	-0.127	0.122	0.051	0.152	0.073	-0.170
Cu	0.206	0.000	0.027	-0.002	-0.048	0.183
Fe	0.278	-0.173	0.002	**-0.368**	0.170	-0.327
Mg	-0.265	**0.573**	-0.308	**0.658**	0.138	**0.713**
Mn	0.255	-0.212	0.015	**-0.456**	0.077	**-0.386**
Ni	0.018	-0.001	-0.262	0.062	-0.044	0.228
Pb	0.013	-0.177	0.029	-0.323	0.118	-0.297
Sr	**-0.438**	0.189	-0.048	0.139	0.188	-0.005
Zn	0.104	-0.100	0.111	-0.278	0.102	**-0.437**
K	0.273	0.238	-0.180	-0.148	**0.462**	0.061
Li	-0.051	0.304	**-0.417**	0.236	0.112	0.237
Na	0.074	**0.730**	**-0.642**	0.319	**0.805**	**0.488**

Significant r-values are shown in bold ($p < 0.05$)

3.11 Comparative Evaluation of the Metals at Three Stages

Mean concentrations of selected metals in each soil fraction were compared at three stages of sample collection in order to assess the temporal variations of the metal levels in paddy soil. Average metal levels in the water extract of the soil at three stages are shown in Figure 7, which revealed that decreasing order of metal concentration (Na > Ca > Mg > K > Fe) was the same in the three stages. Rest of the metals showed the decreasing order; Cd > Sr > Ni > Co > Pb > Cr > Cu > Mn > Zn > Li at stage 1, Sr > Pb ~ Co > Cr > Ni > Cu > Mn > Cd > Zn > Li at stage 2 and Sr > Ni > Co > Pb > Cr > Cu > Cd > Li > Zn > Mn at stage 3. Average levels of Cd, Co, Cu, Fe, Mn, Ni and K were found to be higher at stage 1 while mean concentrations of Cr, and Zn were elevated at stage 2 and those of Ca, Mg, Sr, Li and Na were noted at stage 3.
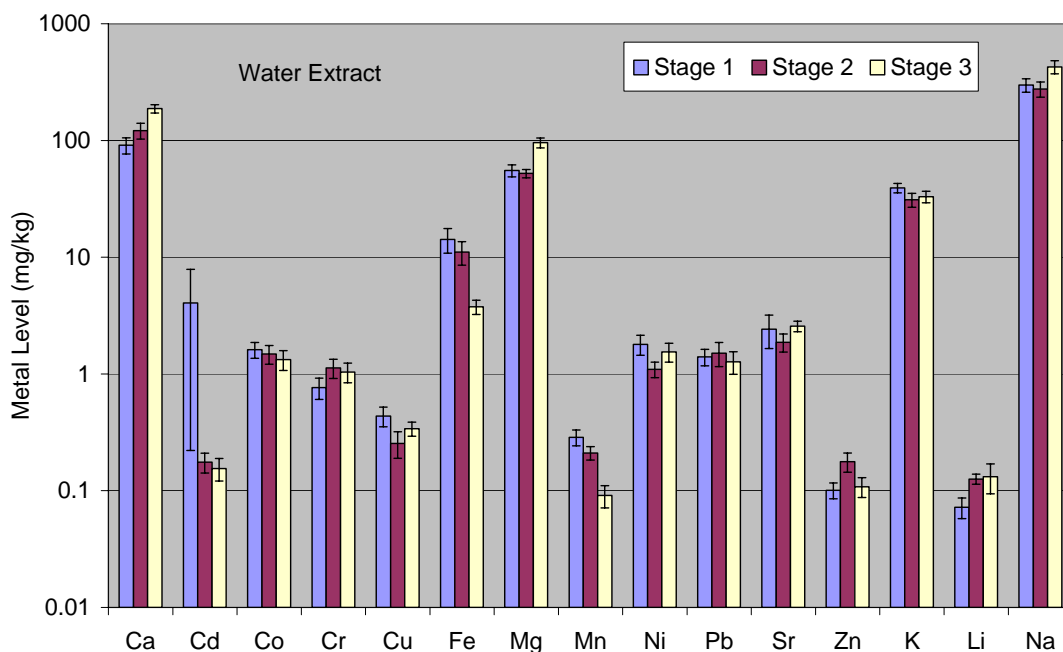
Figure 7. Comparison of the metal levels (mg/kg, ±SE) in water soluble fractions of soil

Comparison of average metal levels in the exchangeable fraction of the soil at three stages is shown in Figure 8. Among the selected metals, Ca > Mg > Mn concentration order was same for all of the three stages. However, at stage 1, it was followed by > Fe > Sr > Co > Ni > Pb > Cr > Cu > Zn > Cd; at stage 2, the remaining order was > Sr >Fe > Pb > Co > Ni > Cr > Zn > Cu > Cd and for stage 3, the following order was > Sr > Fe > Co > Ni > Pb > Cr > Cu > Zn > Cd. Overall, more or less comparable levels at the three stages

were observed for Ca, Mg, Mn and Sr. However, elevated metal levels at stage 1 were observed for Fe in the exchangeable soil fraction, while Cd, and Pb showed higher concentrations at stage 2 and Co, Cr, Cu, Ni and Zn revealed relatively higher contribution at stage 3 in the exchangeable fraction of soil.

Comparative evaluation of the mean metal levels in reducible fractions of soil at three stages is shown in Figure 9. Similar order of decreasing metal concentrations at the three stages were noted for Ca > Fe > Mg > Mn. Rest of the metals revealed the order; Co > Sr > Ni > Zn > Pb > Cr > Cu > Cd at first stage, Co > Ni > Sr > Pb > Zn > Cu >Cr > Cd at second stage and Co ~ Sr > Zn > Pb ~ Ni > Cu >Cr > Cd at third stage. Most of the metals (Ca, Cd, Cu, Mn, Sr and Zn) exhibited relatively higher concentrations at the third stage, while mean levels of Co were comparable at the three stages. Higher contents of Cr, Mg, Ni and Pb were noted at the second stage whereas, Fe exhibited higher concentration at the first stage in the reducible fraction of soil.

Comparison of average metal levels in the oxidiseable fraction of the soil at three stages is shown in Figure 10. For the first stage the metal concentrations showed the decreasing order; Fe > Mg > Ca > Mn > Ni > Pb > Zn > Co > Cr > Sr > Cu > Cd. For the second stage it was; Mg > Fe ~ Ca > Mn > Ni > Pb > Zn > Co ~ Cr > Cu > Sr > Cd. For the third stage it was, Mg > Ca >Fe > Mn > Ni > Pb > Zn > Co ~ Cr ~ Sr > Cu > Cd. On the average basis, elevated levels of Ca, and Fe were noted at stage 1, while mean concentrations of Cd, Co, Cr, Mn, Ni, Pb and Zn were relatively higher at stage 2 and those of Mg, and Sr were higher at stage 3.

Comparative evaluation of the average metal levels in residual fractions of soil at three stages is shown in Figure 11. Similar decreasing order of Fe ~ Mg > Mn concentration was observed at three stages, which was followed by Ca > Zn > Ni > Cu > Cr ~ Pb > Co > Sr > Cd at first stage, Ca > Zn > Ni > Cu ~ Cr > Co > Sr > Pb > Cd at second stage and Zn > Ca > Ni > Cu ~ Cr > Co > Pb > Sr > Cd at third stage. More or less comparable average concentrations at the three stages were observed for most of the metals (Fe, Mg, Mn, Zn, Cu, Cr and Co) in the residual fraction of soil. However, elevated levels at the first stage were noted for Cd, Pb and Sr while Ca and Ni exhibited relatively higher concentration at the second stage in residual soil fraction. Overall, the measured metal levels were found to be relatively consistent with minimal variations in their average contents in the residual fraction of the soil compared with the other soil fractions.
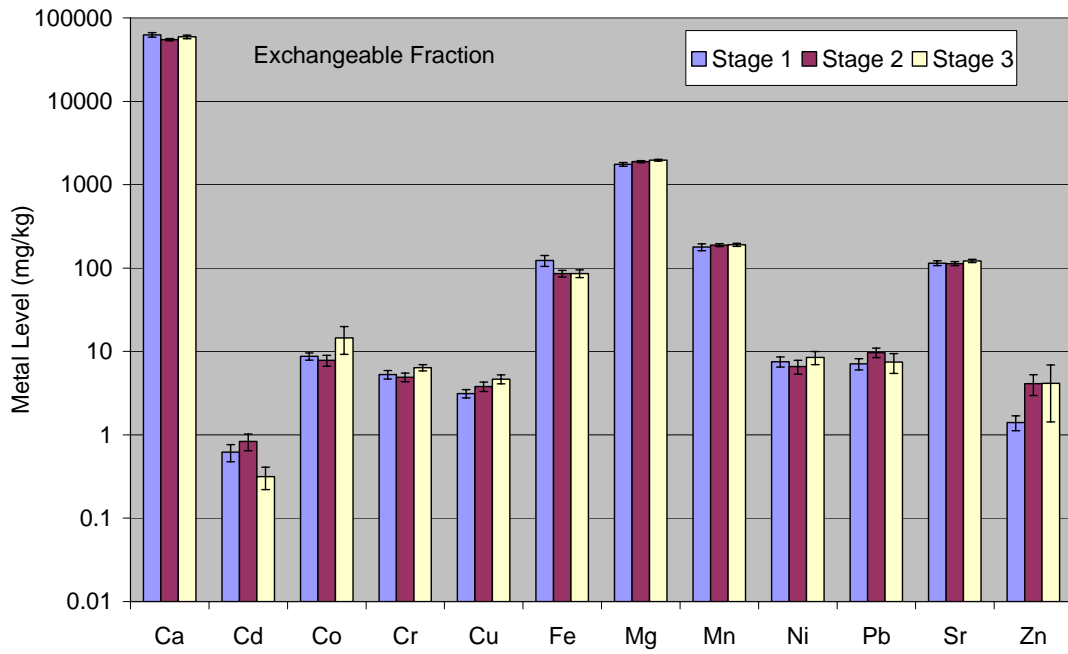
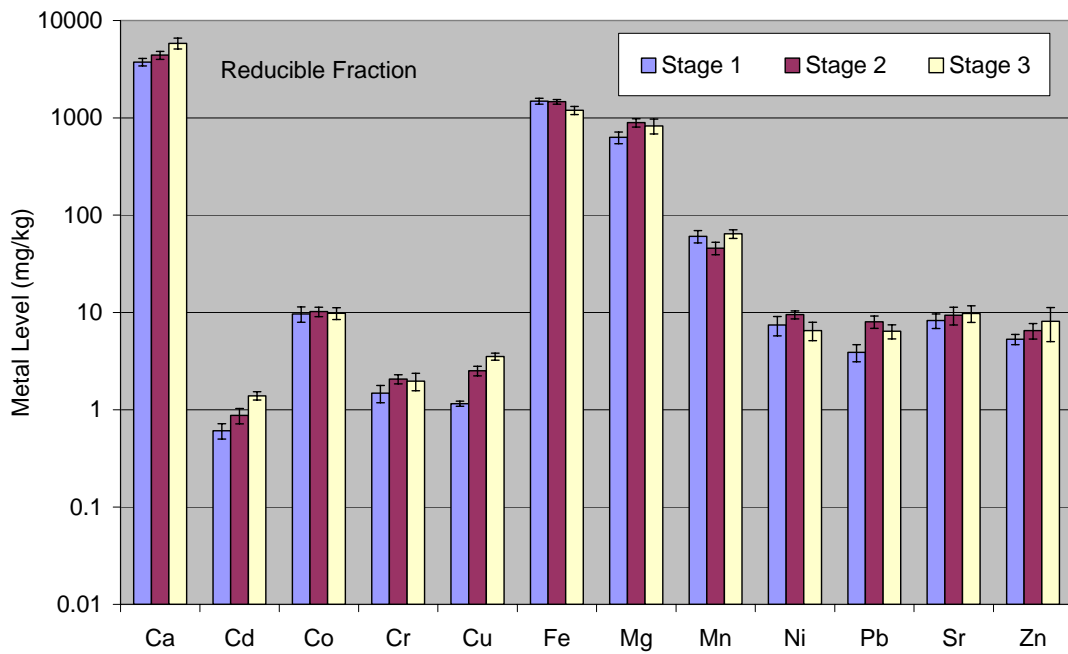Figure 8. Comparison of the metal levels (mg/kg, ±SE) in exchangeable fractions of soil

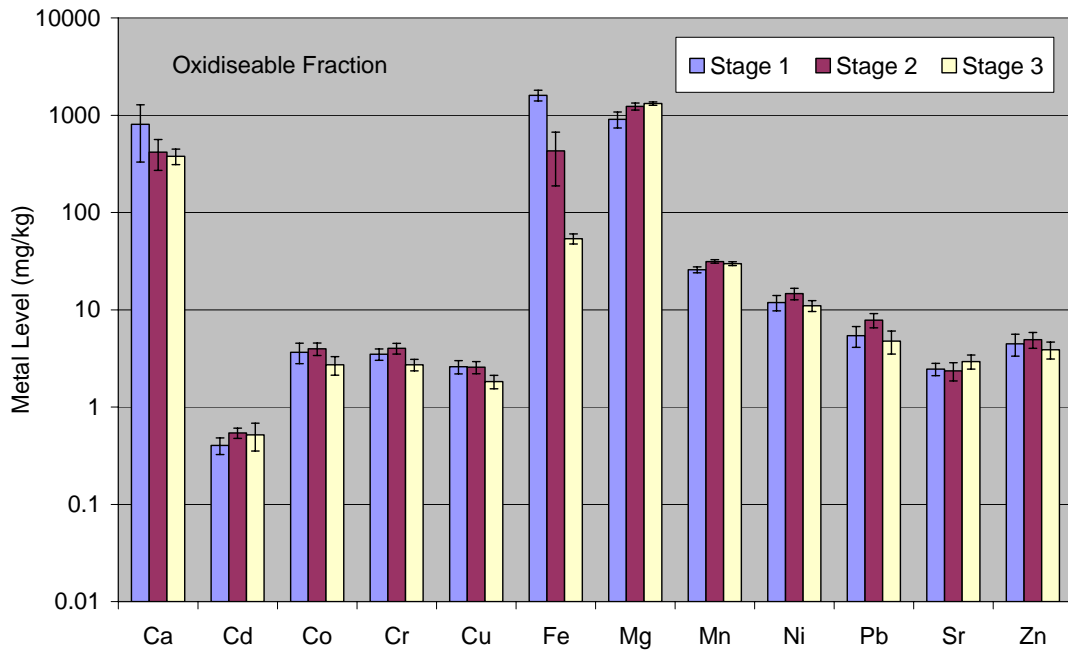Figure 9. Comparison of the metal levels (mg/kg, ±SE) in reducible fractions of soil

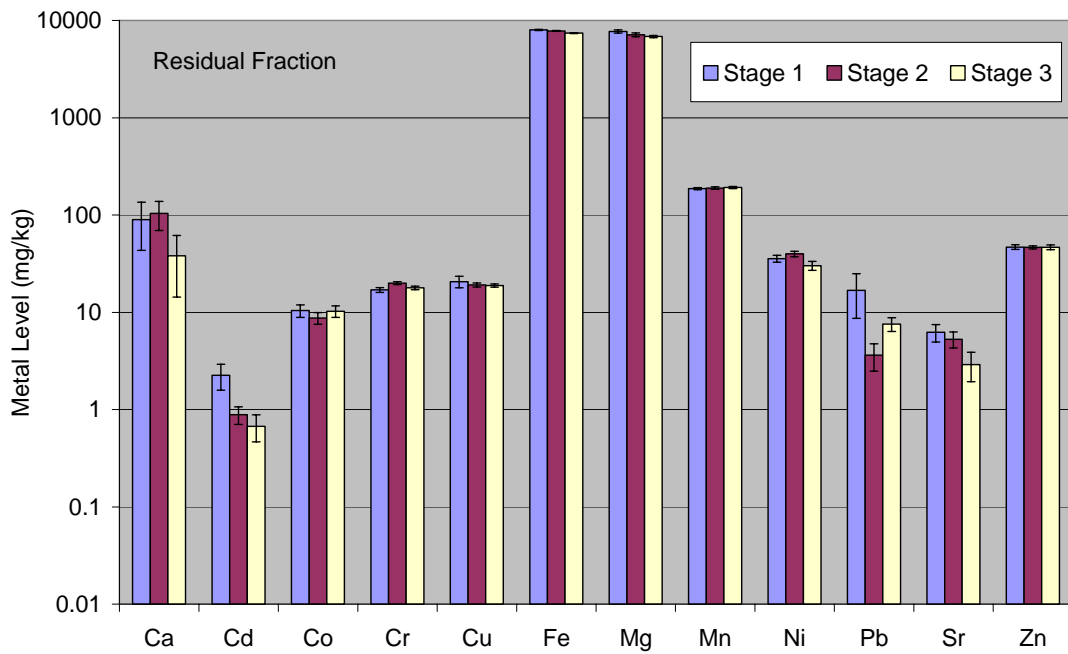Figure 10. Comparison of the metal levels (mg/kg, ±SE) in oxidiseable fractions of soil

Figure 11. Comparison of the metal levels (mg/kg, ±SE) in residual fractions of soil

Total metal levels in the soil (except silicates) at the three stages were also evaluated as shown in Figure 12. Generally, the pseudo-total metal levels in soil exhibited higher contribution of Ca (64117 mg/kg), followed by Mg (11014 mg/kg) and Fe (9897 mg/kg). Significant contribution of Mn (460.5 mg/kg), Sr (132.8 mg/kg), Ni (63.03 mg/kg), Zn (60.98 mg/kg), Pb (29.25 mg/kg), Co (33.64 mg/kg), Cr (29.04 mg/kg), Cu (28.09 mg/kg), and Cd (3.366 mg/kg) were also noted in the soil. Almost all metals showed insignificant variations on overall basis at the three stages, nevertheless, minor variations were observed for Cd, Co, Ni and Pb for their total contents in the soil.
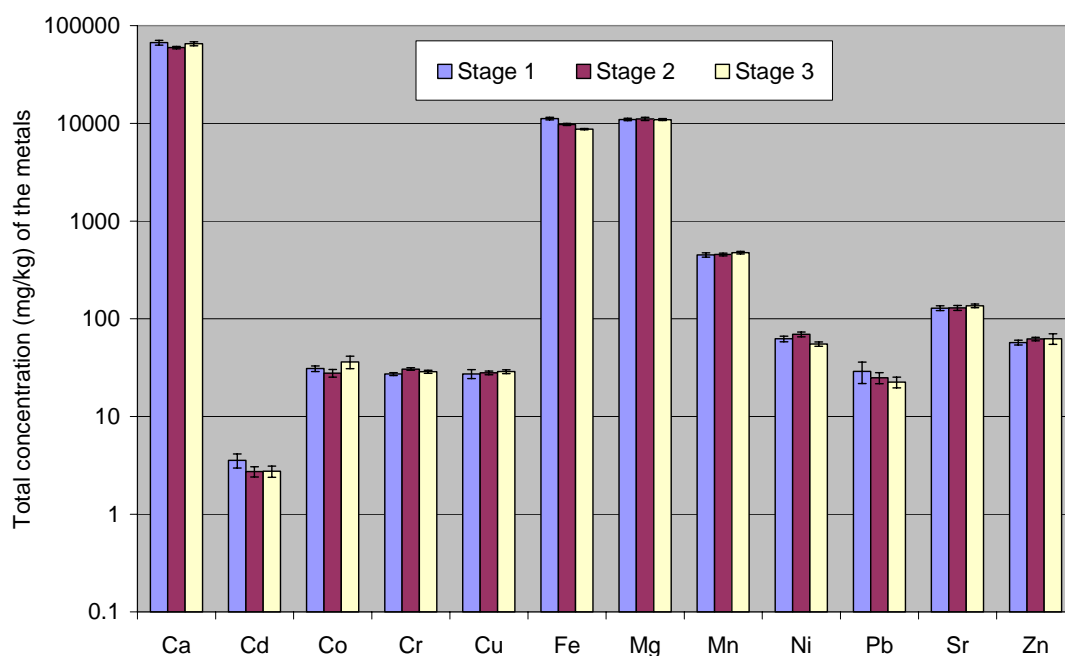
Figure 12. Comparison of the total metal levels (mg/kg, ±SE) in soil

3.12 Comparative Evaluation of the Physicochemical Parameters at Three Stages

Comparison of the average values of the physiochemical parameters at three stages is shown in Figure 13. More or less comparable levels of organic matter and pH were noted at three stages in the soil samples. The comparative evaluation showed highest levels of sulphates and nitrates at stage 3, followed by stage 1 while lower levels were observed at stage 1. Chloride contents showed gradual increase from stage 1 to stage 3 in the soil samples. Relatively higher EC was noted at stage 3, followed by almost comparable levels at the first two stages.
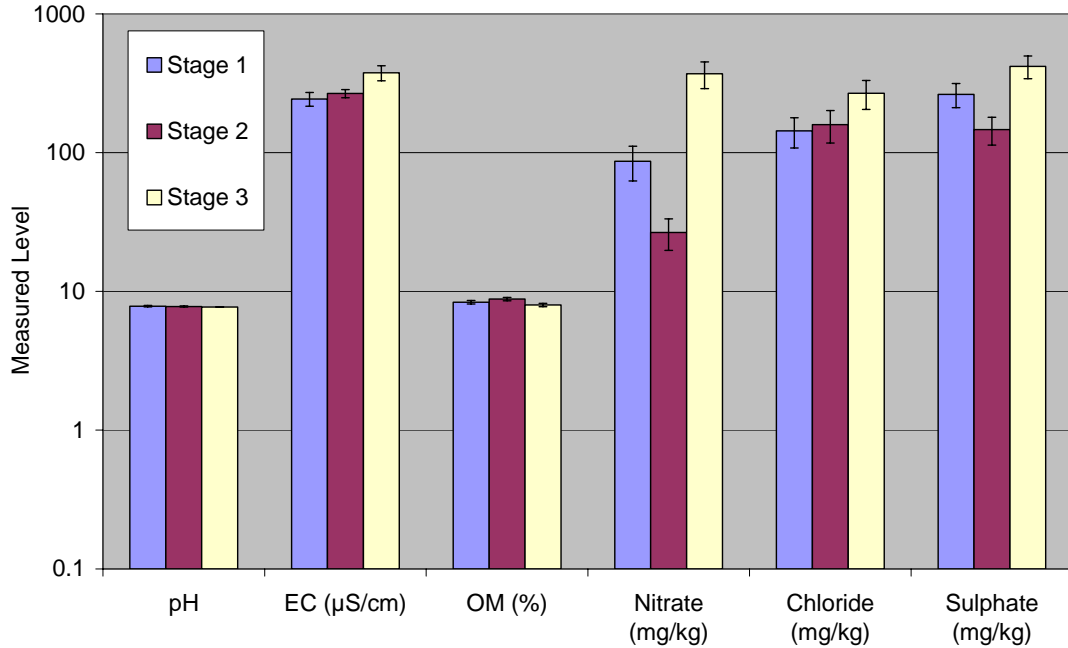
Figure 13. Comparison of the physicochemical parameters of soil

3.13   Comparative Evaluation of Particle Size Fractions at Three Stages

Various particle size fractions in the soil samples were also compared at the three stages as shown in Figure 14. Generally, fine particulate fractions showed higher contributions than the coarse fraction at all three stages. Highest contribution was noted for particles with diameter 1-5 μm, followed by <1 μm particles. Among various particle fractions of silt, the particles lying in the range of 15-30 μm showed relatively higher contribution. Collectively, silt contents were the highest than the clay and sand contents. Among the sand particles, the contribution of fine sand was greater than medium, which was greater than coarse sand. Relatively higher contribution of the particulate fraction 30-50 μm, 50-75 μm, 75-100 μm, 100-200 μm and >2000 μm were noted at stage 1 while higher contributions at stage 2 were observed for 1-5 μm, 5-10 μm, and 10-15 μm. Comparatively elevated contributions at stage 3 were found for <1.0 μm, 15-30 μm and 200-400 μm. On the average basis, majority of the particulate fractions showed higher contribution at stage 2 in the soil samples.
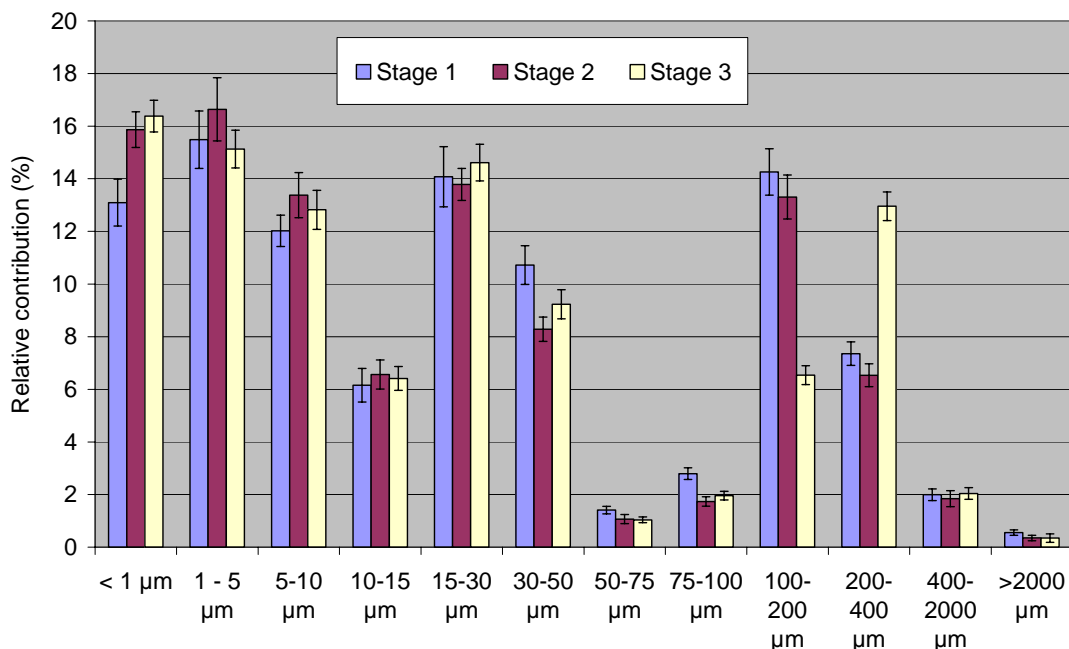
Figure 14. Comparison of the particle size fractions of soil

3.14 Comparative Evaluation of the Metal Levels in Various Soil Fractions

Mean concentrations of selected metals in each fraction of sequential extraction and water extract of the soil were compared as shown in Figure 15. Average level of Ca was 133.3 mg/kg in water extract, 58865 mg/kg in exchangeable fraction, 4658 mg/kg in reducible fraction, 512.9 mg/kg in oxidiseable fraction and 80.75 mg/kg in residual fraction. The distribution pattern of Ca in various fractions of paddy soil followed the order; exchangeable fraction (91.61%) > reducible fraction (7.24%) > oxidiseable fraction (0.79 %) > water extract > residual fraction (0.12%). Mean level of Cd was observed at 1.744 mg/kg in water extract, 0.582 mg/kg (11.38%) in exchangeable fraction, 0.982 mg/kg (19.21%) in reducible fraction, 0.491 mg/kg (9.60%) in oxidiseable fraction and 1.311 mg/kg (25.65%) in residual fraction of the soil. Thus the relative concentration order of Cd in paddy soil was; water extract > residual fraction > reducible fraction > exchangeable fraction > oxidiseable fraction. Similarly, mean level of Co was found at 1.483 mg/kg in water extract, 10.55 mg/kg in exchangeable fraction, 9.875 mg/kg in reducible fraction, 3.425 mg/kg in oxidiseable fraction and 9.792 mg/kg in residual fraction of the soil. Therefore, the relative contribution of Co was observed as

exchangeable fraction (30.03 %) > reducible fraction (28.11 %) > residual fraction (27.87%) > oxidiseable fraction (9.75 %) > water extract.

Mean concentrations of Cr in various fractions of soil showed the contributions of 0.962 mg/kg in water extract, 5.529 mg/kg in exchangeable fraction, 1.836 mg/kg in reducible fraction, 3.409 mg/kg in oxidiseable fraction and 18.27 mg/kg in residual fraction. In terms of percentage the contribution of Cr was; residual fraction (60.88%) > exchangeable fraction (18.42%) > oxidiseable fraction (11.36%) > reducible fraction (6.11%) > water extract. Mean concentrations of Cu were noted as 0.343 mg/kg in water extract, 3.860 mg/kg (13.58%) in exchangeable fraction, 2.396 mg/kg (8.42%) in reducible fraction, 2.323 mg/kg (8.17%) in oxidiseable fraction and 19.51 mg/kg (68.62%) in residual fraction of the soil collected from the paddy fields. The decreasing order of metal concentration was; residual fraction > exchangeable fraction > water extract > reducible fraction > oxidiseable fraction.

Average concentration of Fe was observed at 9.681 mg/kg in water extract, 98.37 mg/kg (0.99%) in exchangeable fraction, 1381 mg/kg (13.94%) in reducible fraction, 693.8 mg/kg (7.00%) in oxidiseable fraction, 7724 mg/kg (77.97%) in residual fraction of the soil. The decreasing order of concentration in various fractions was; residual fraction > reducible fraction > oxidiseable fraction > exchangeable fraction > water extract. Mean concentrations of Mg observed was 67.72 mg/kg in water extract, 1870 mg/kg (16.87%) in exchangeable fraction, 781.9 mg/kg (7.05%) in reducible fraction, 1151 mg/kg (10.39%) in oxidiseable fraction and 7211 mg/kg (65.06%) in residual fraction. The decreasing order of Mg concentration in the soil was; residual fraction > exchangeable fraction > water extract > reducible fraction > oxidiseable fraction. Mean concentration of Mn in various fractions was noted at 0.199 mg/kg in water extract, 185.7 mg/kg in exchangeable fraction, 56.83 mg/kg in reducible fraction, 28.98 mg/kg in oxidiseable fraction and 189.0 mg/kg in residual fraction. In terms of percentage the relative distribution pattern of Mn was; residual fraction (41.02%) > exchangeable fraction (40.30%) > reducible fraction (12.33%) > oxidiseable fraction (6.29%) > water extract. Average level of Ni was measured at 1.461 mg/kg in water extract, 7.527 mg/kg in exchangeable fraction, 7.875 mg/kg in reducible fraction, 12.45 mg/kg in oxidiseable fraction and 35.19 mg/kg in residual fraction of the soil. The distribution pattern of Ni in various fractions of paddy soils followed the order; residual fraction (54.56%) > oxidiseable fraction (19.29%) > reducible fraction (12.21%) > exchangeable fraction (11.67%) > water extract.

Mean concentration of Pb extracted in various fractions of the soil was noted at 1.39 mg/kg in water extract, 8.058 mg/kg (26.30%) in exchangeable fraction, 5.984 mg/kg (19.53%) in reducible fraction, 6.133 mg/kg (20.02%) in oxidiseable fraction and 9.071 mg/kg (29.61%) in residual fraction. The decreasing order of metal concentration was in the order of; residual fraction > exchangeable fraction > oxidiseable fraction > reducible fraction > water extract. Mean concentration of Sr was noted at 2.277 mg/kg in water extract, 116.4 mg/kg (86.13%) in exchangeable fraction, 9.136 mg/kg (6.76%) in reducible fraction, 2.585 mg/kg (1.91%) in oxidiseable fraction, 4.735 mg/kg (3.50%) in residual fraction. The decreasing order of concentration in various fractions was; exchangeable fraction > reducible fraction > water extract > residual fraction > oxidiseable fraction. Similarly, mean level of Zn was observed at 0.127 mg/kg in water extract, 3.226 mg/kg in exchangeable fraction, 6.677 mg/kg in reducible fraction, 4.436 mg/kg in oxidiseable fraction and 46.65 mg/kg in residual fraction. Therefore, the relative abundance of Zn was noted as; residual fraction (76.33%) > reducible fraction (10.92%) > oxidiseable fraction (7.25%) > exchangeable fraction (5.27%) > water extract. Overall, most of the metals (Zn, Pb, Ni, Mn, Mg, Fe, Cu and Cr) showed higher contribution in the residual fraction of the soil; nonetheless, elevated levels of Ca, Sr, Mn, and Co were noted in the exchangeable fraction of the soil.
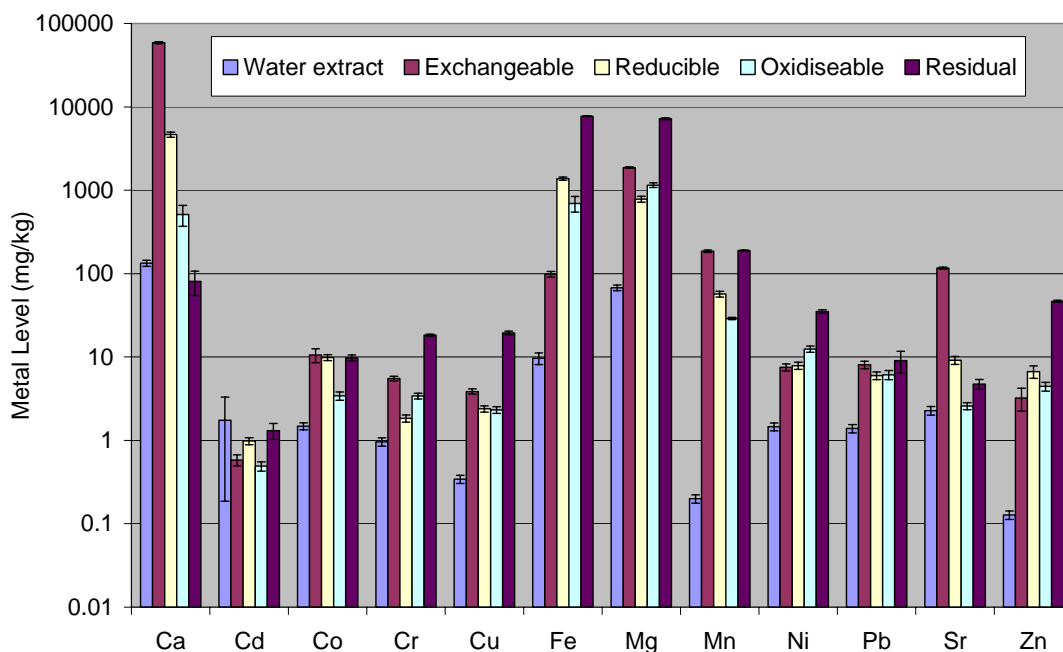

Figure 15. Comparison of the metal levels (mg/kg, ±SE) in various fractions of soil

3.15   Multivariate Analysis of Selected Metals in Various Soil Fractions

Multivariate cluster analysis was used for the apportionment and source identification of selected metals in water extract, exchangeable, reducible, oxidiseable and residual fractions of the soil. The resulting dendrograms using Ward's method are shown in Figures 16-20. The linkage distance represents the degree of association among the metals; the smaller value of the linkage distance for a specific cluster revealed strong/significant association among the metals. Figure 16 represents the cluster analysis of selected metals in the water soluble fraction of the soil. The strongest cluster was observed among Fe-Mn-K which were mostly contributed by the excessive use of fertilizers in the paddy fields. Another closely resembling secondary cluster was noted for Pb-Co-Cu-Cr. These metals in the water extract of the soil were mostly originating from the agricultural sprays. Another cluster was observed among Li and Zn which were contributed by mixed sources. These clusters were mostly anthropogenic in origin in the soil. Another major cluster was formed by Ca-Sr-Mg-Na-Cd-Ni which is actually combination of three sub-clusters. These metals in the water soluble fraction of the soil were mostly natural in origin predominantly contributed by lithogenic activities.
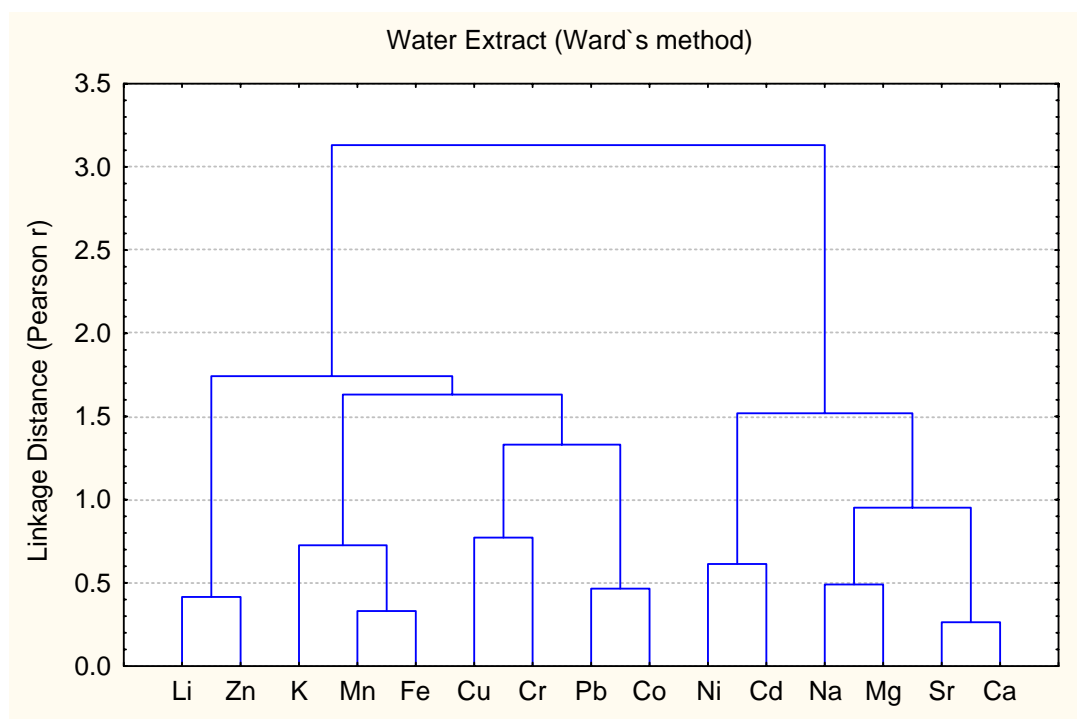

Figure 16. Cluster analysis of selected metals in the water soluble fraction of soil

Cluster analysis of selected metals in the exchangeable fraction of soil is shown in Figure 17. A strong mutual cluster was formed by Ca-Sr-Fe-Cd which were mostly contributed by the natural processes. Another joint cluster noted among Ni-Cu-Cr-Zn-Co and these metals in the exchangeable fraction of the soil were mostly contributed by the anthropogenic activities. Similarly, Mg-Mn-Pb constituted another cluster which was mostly contributed by mixed sources with significant natural and anthropogenic contributions.
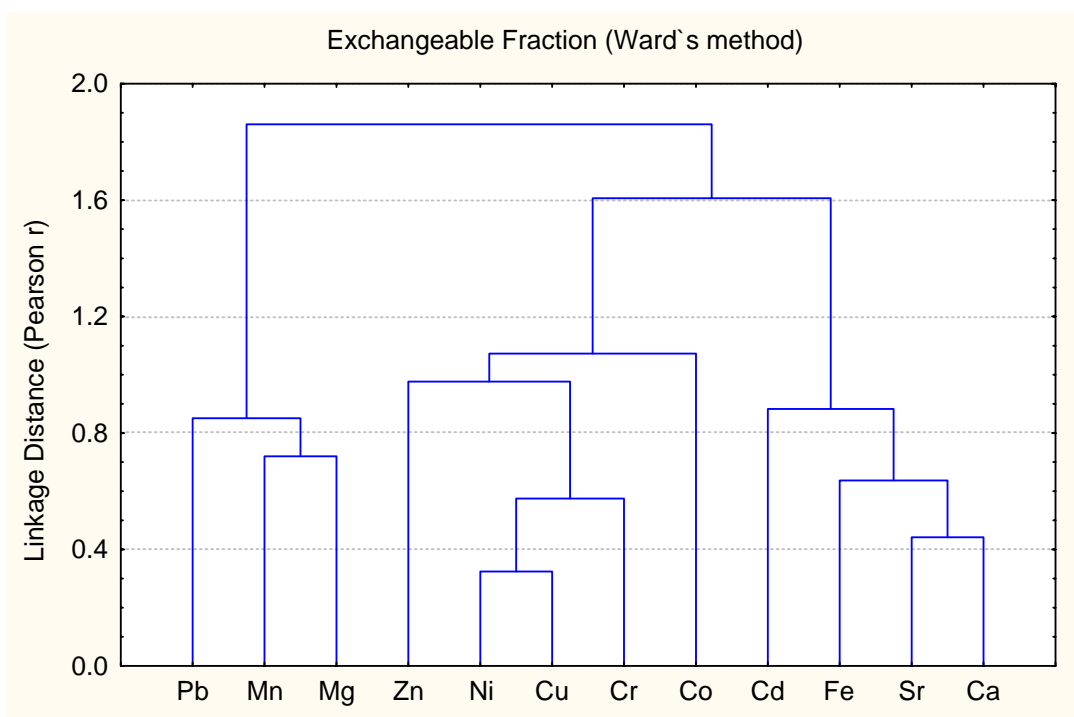

Figure 17. Cluster analysis of selected metals in the exchangeable fraction of soil

Cluster analysis of selected metals in the reducible fraction of soil is shown in Figure 18. One of the significant clusters was observed for Zn-Pb-Cr which shared another secondary cluster composed of Cd-Mn-Cu-Ca and together these two clusters showed predominantly anthropogenic contributions particularly related with the agricultural activities. A separate cluster was formed by Fe and Mg which were mostly derived by natural sources. Contribution of the mixed sources was shown by a mutual cluster of Co-Sr-Ni which were partially contributed by lithogenic activities and partially by the combustion processes.
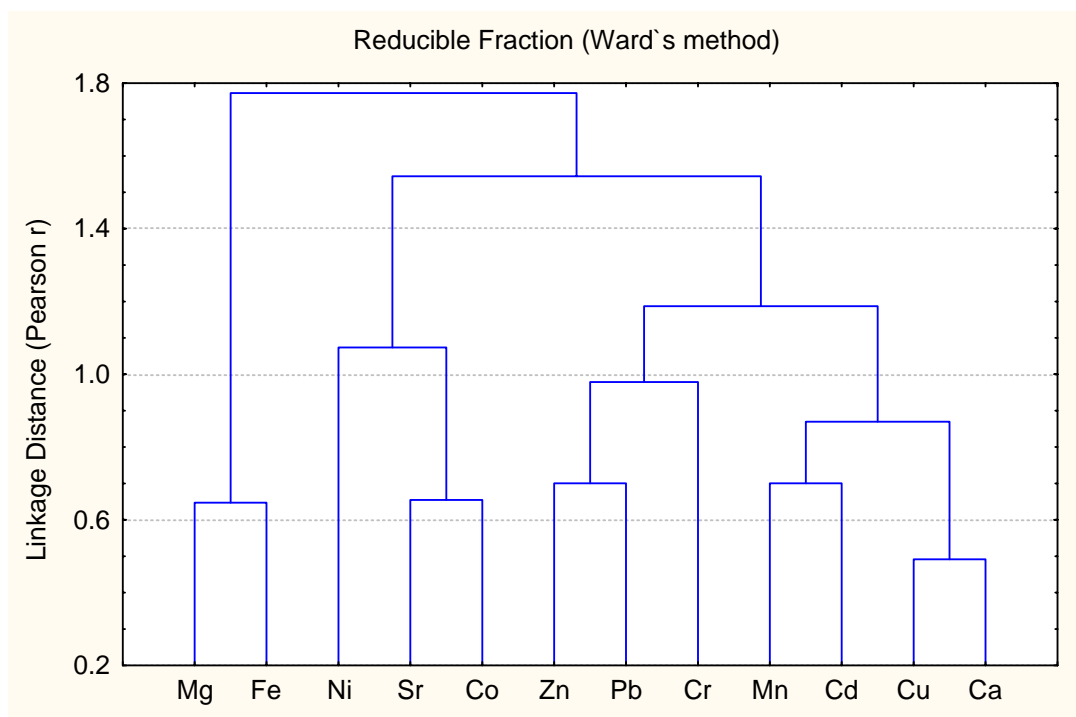
Figure 18. Cluster analysis of selected metals in the reducible fraction of soil

Figure 19 shows the cluster analysis of selected metals in oxidiseable fraction of the soil. The strongest cluster in oxidiseable fraction of the soil was noted for Ca-Mg-Mn-Pb and these metals were mostly anthropogenic in origin particularly emanating from the organic fertilizers in the paddy fields. Another strong cluster for Cu-Zn-Co-Fe showed the lithogenic contributions in this fraction of soil. Two more clusters were observed among Ni-Cr and Sr-Cd which were mostly contributed by the mixed sources.

Cluster analysis of selected metals in the residual fraction of the soil is shown in Figure 20. This fraction of the soil is mostly associated with the earth crust and hence lithogenic in origin. Similar results were obtained in the present study. Strongest cluster was observed for Mn-Mg-Fe-Zn-Ni showing the similar geochemical behaviour of these metals in the soil. Likewise, Cu-Co and Ca-Sr revealed their similar nature in the soil. All these metals were believed to be natural/lithogenic in origin in the residual fraction of soil. Nevertheless, anthropogenic activities may affect the crustal abundance of the metals in soil and this was observed in the present study as Pb-Cd showed a strong and separate cluster compared to other metals and hence showing the effect of anthropogenic activities on the agricultural soil.
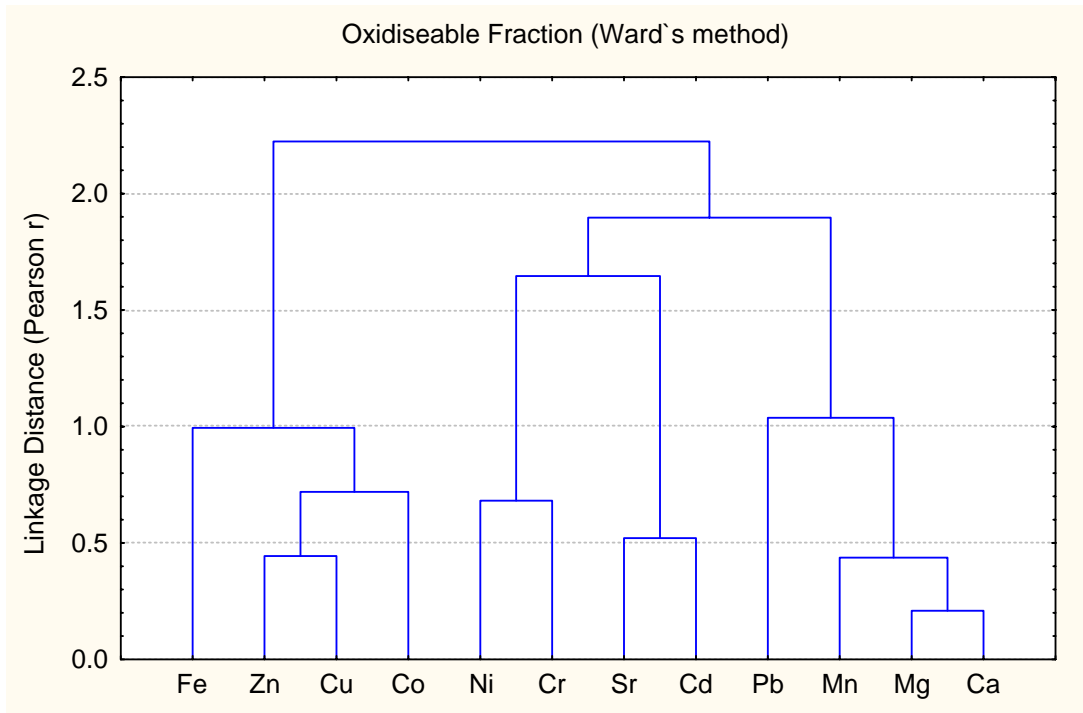
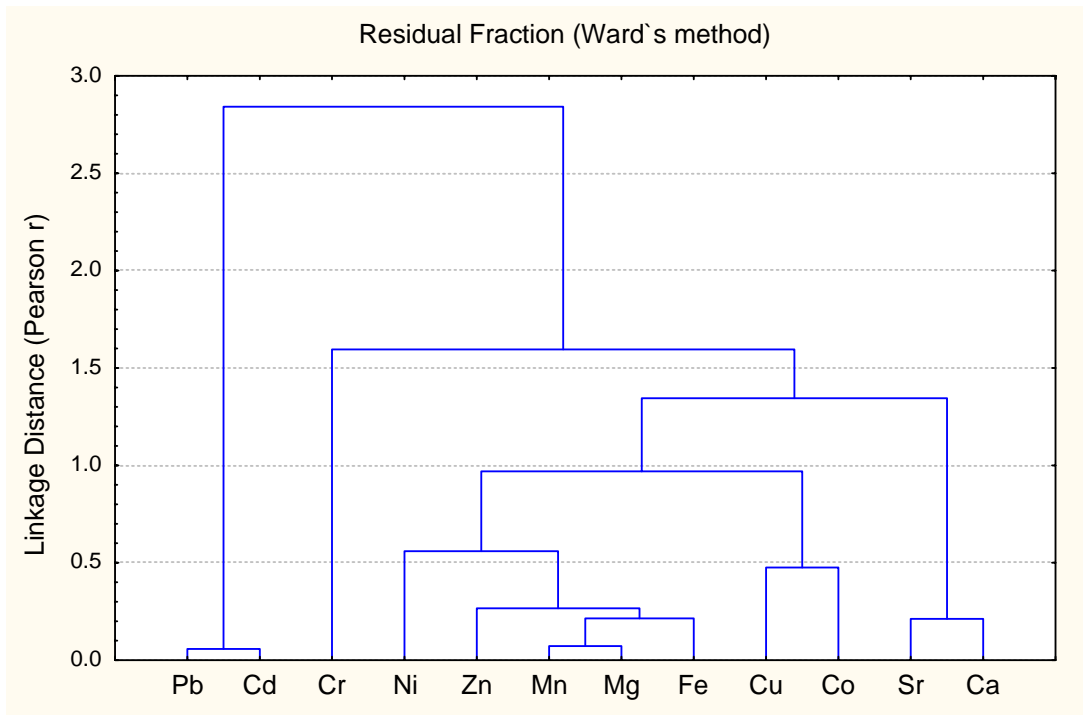Figure 19. Cluster analysis of selected metals in the oxidiseable fraction of soil

Figure 20. Cluster analysis of selected metals in the residual fraction of soil

3.16 Pollution Index of Selected Metals in the Soil

The effects of anthropogenic activities on the metal contents of the soil were also investigated in terms of various pollution indices including contamination factor (C_f), geoaccumulation index (I_{geo}) and enrichment factor (EF). These indices helped to quantify the effect of elevated metal levels on the local environment. Contamination factors (mean ± SE) of selected metals in the soil are shown in Figure 21 while their description is shown in Table 16. Low/minor contamination was noted for Fe, Cr, Sr, Cu, Mg, Mn, Ni and Zn contents in the soil as their C_f values were less than 1. Moderate contamination in the soil was observed for Ca, Co and Pb with values greater than 1 but less than or equal to 3. However, C_f values for Cd was greater than 6; showing very high contamination of the metal in paddy soil.

Figure 22 shows the average values of geoaccumulation index for selected metals in the soil. The I_{geo} values are not readily comparable to the other indices of metal pollution because of the nature of the I_{geo} calculation, which involves a background multiplication factor of 1.5 and a log function. Average values of I_{geo} for Fe was found to be more negative than any other metal, followed by Cr, Sr, Cu, Mg, Mn, Ni, Zn and Co. The negative values of these metals showed that soil was not polluted or contaminated by these metals. However, for Ca and Pb the average value was a slightly greater than zero, indicting slight but insignificant contamination. Nonetheless, I_{geo} values for Cd were the greatest among all the metals that showed moderate to heavy contamination in the paddy soil by this metal.

Concentration alone does not provide information about the extent of modification in soil composition but enrichment factor (EF) can be used as a convenient tool for this purpose. To assess the anthropogenic input of metals in the soil EFs is a useful statistical parameter. Utilizing the mean levels of the metals in soil, EFs were calculated in the present study. Fe was used as the normalizing element owing to high concentration in earth crust and least variability, therefore, least affected by small fluctuations in the anthropogenic sources. If the EF value was close to one, it suggested that the anthropogenic sources contributed only a minor/negligible fraction of the metal or in other words the metal is predominantly from the crustal materials. The values much greater than 1 revealed anthropogenic source, which include combustion, automobile/industrial emissions, agricultural activities, etc. Figure 23 shows the mean values of EFs for selected metals in the soil. Among the metals, relatively lower EF values for Cr, Sr, Cu, Mg and

Mn revealed minimal to moderate enrichment in the soil while Ni, Zn, Ca, Co and Pb exhibited moderate to significant enrichment in the paddy soil. Nevertheless, highest EF values were noted for Cd which showed extremely severe enrichment in the paddy soil.
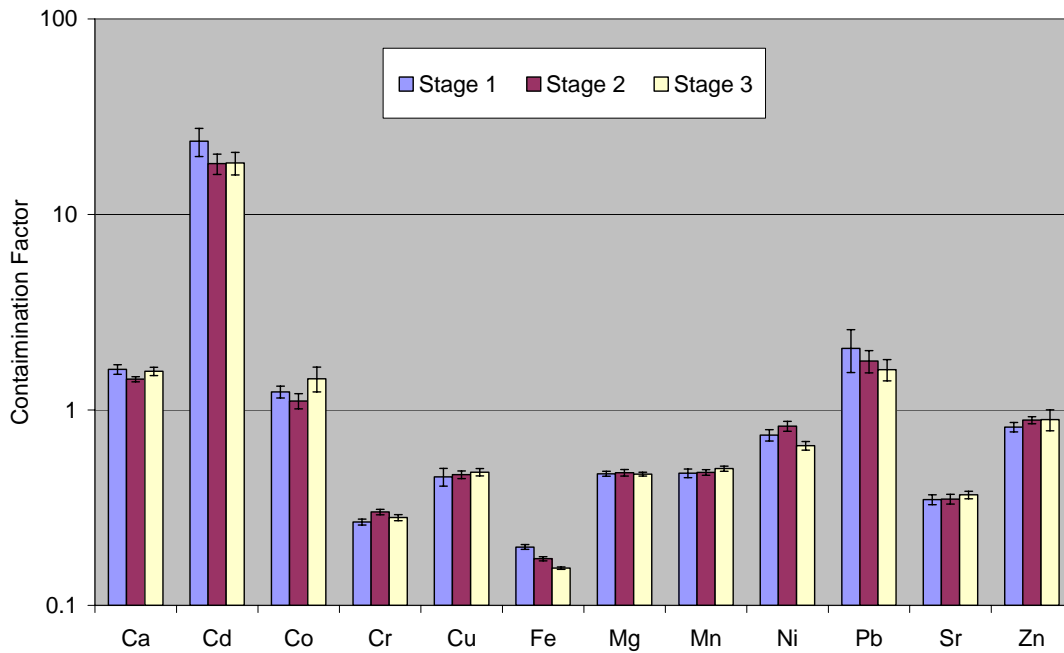

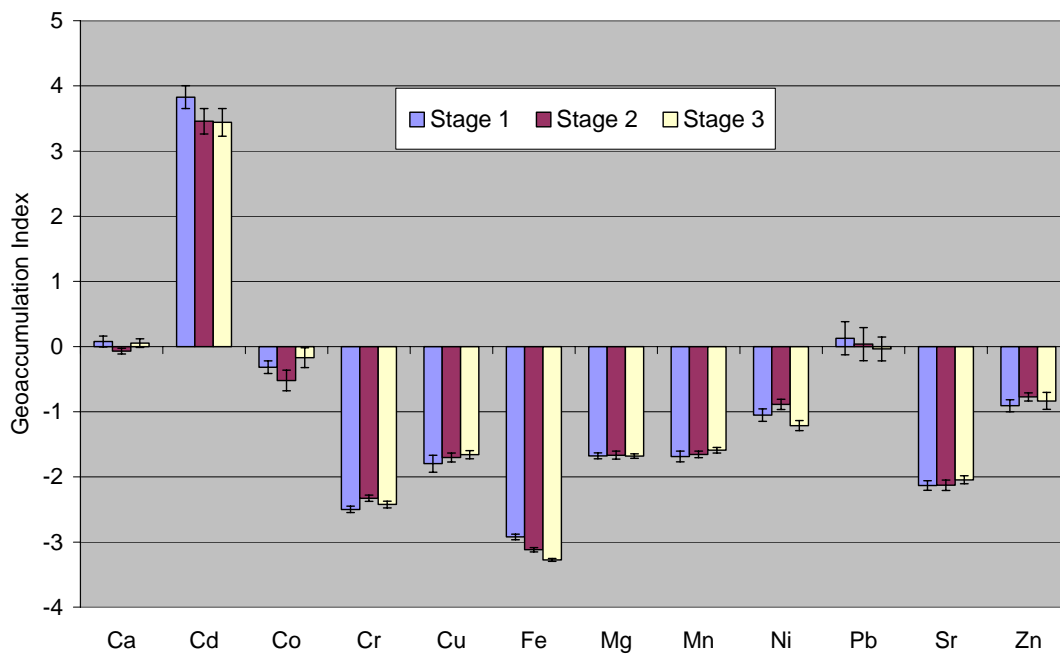Figure 21. Contamination factors (mean ±SE) of the metals in soil

Figure 22. Geoaccumulation index (mean ±SE) of the metals in soil.

Table 16.   Description of the pollution indices

| Class	Value	Soil quality
1	$C_f < 1$	low contamination
2	$1 \leq C_f < 3$	moderate contamination factor
3	$3 \leq C_f < 6$	considerable contamination factor
4	$6 \leq C_f$	very high contamination factor
0	$I_{geo} \leq 0$	practically uncontaminated
1	$0 < I_{geo} < 1$	uncontaminated to moderately contaminated
2	$1 < I_{geo} < 2$	moderately contaminated
3	$2 < I_{geo} < 3$	moderately to heavily contaminated
4	$3 < I_{geo} < 4$	heavily contaminated
5	$4 < I_{geo} < 5$	heavily to extremely contaminated
6	$5 < I_{geo}$	extremely contaminated
1	$EF < 2$	deficiency to minimal enrichment
2	$EF = 2 - 5$	moderate enrichment
3	$EF = 5 - 20$	significant enrichment
4	$EF = 20 - 40$	very high enrichment
5	$EF > 40$	extremely high enrichment
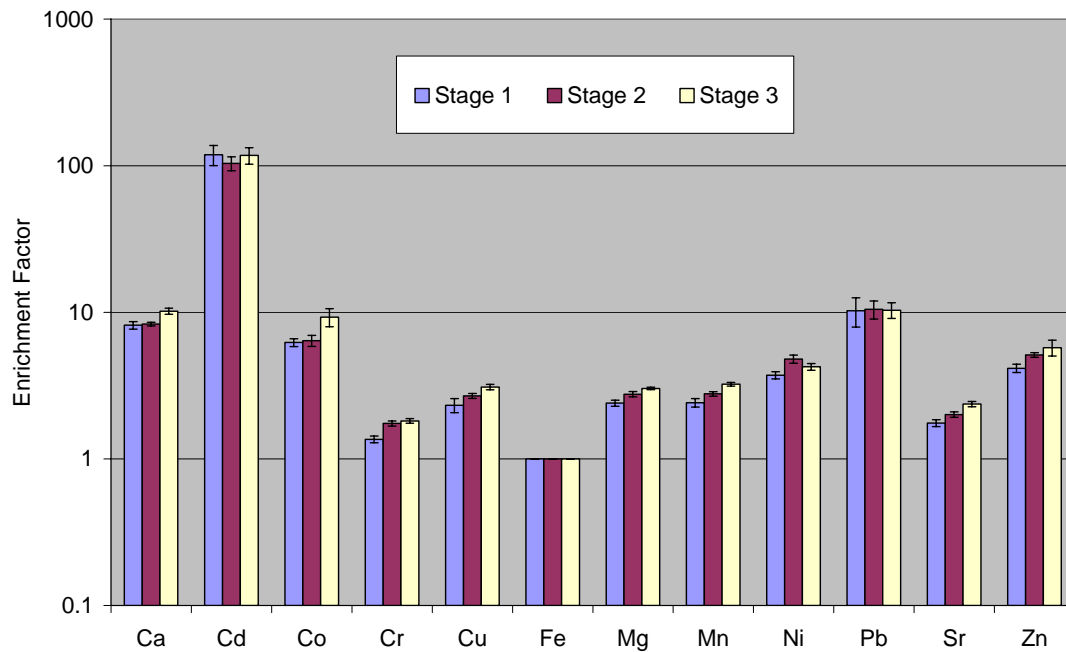
Figure 23. Enrichment factors (mean ±SE) of the metals in soil

3.17 Chemical Nature of Soil

Piper diagram is used for assessing major composition of the soil and its chemical nature. The results are shown in Figure 24. It is a graphical presentation of soil quality, in which a single point on the left triangle represents cations, with their concentration expressed in terms of mg/kg, while in the right triangle anions are expressed as a single point in units of mg/kg. The two set of data are then combined into a central diamond-shaped parallel to the upper edges of the central area, where single point shows the total ionic distribution. Soil samples having similar qualities tend to plot together in a group. An examination of the figure revealed that mostly the soil samples in present study were high in Na and K and low in Ca and Mg. However, anions showed almost equivalent contributions; slightly higher chloride contents were observed. Overall, the central diamond showed that the soil samples were higher in Na and K along with sulphate and chloride while low in Ca and Mg with smaller carbonate and bicarbonate contents.
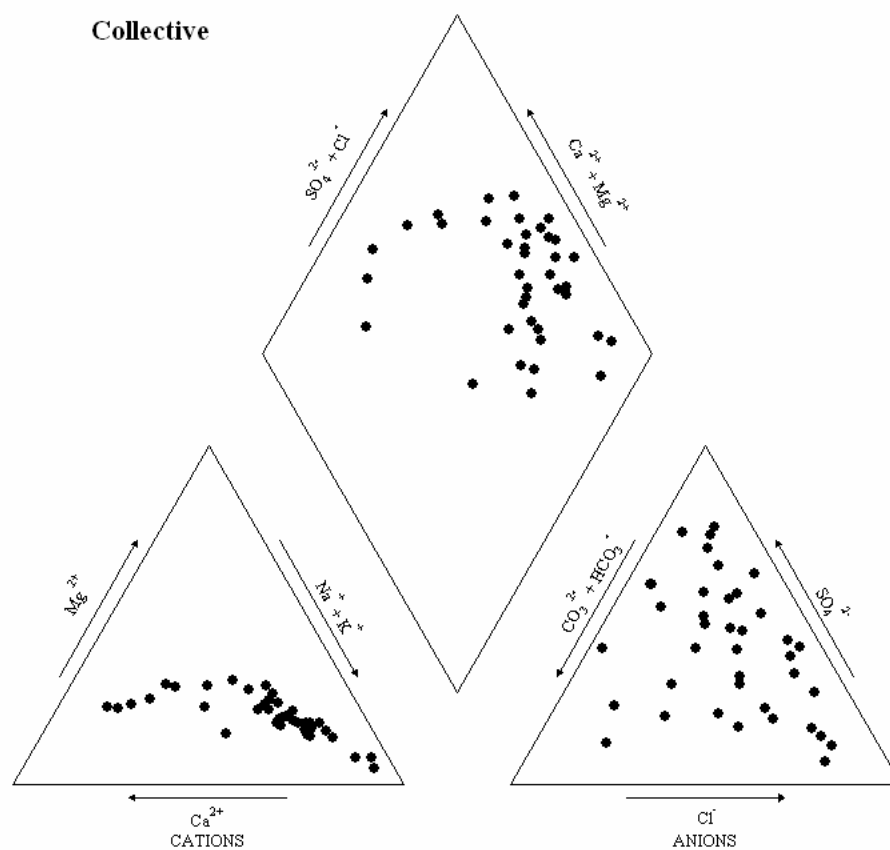

Figure 24. Piper diagram showing the predominant nature of soil

3.18   Salient Findings of the Study

Based on the deliberations put forward in foregoing sections, following major findings emerged from the present study.

1.   Relative distribution of selected metals (Ca, Cd, Cr, Co, Cu, Fe, Mg, Mn, Ni, Sr, Pb and Zn,) in various soil fractions was mostly diverse and random.

2.   On the average basis, major contribution was shown by Fe, Mg, Ca and Mn in various soil fractions.

3.   Most of the soil samples revealed slightly alkaline nature. The measured values of EC and pH were within the safe limits for agricultural soils.

4.   Organic matter in the soil was found at 8.37%, on the average basis.

5.   The soil texture of the studied area was found to be silty loam in its nature.

6.   The correlation study showed some significant/strong relationships among the metal pairs which were quite diverse in various soil fractions.

7.   Significant relationships were noted for the metal contents and anions in the soil.

8.   Comparative variations of selected metals in various soil fractions showed relatively higher bioavailability of Ca, Sr and Mn, while predominantly higher concentrations of most of the metals were found in residual fraction of the soil.

9.   Multivariate cluster analysis manifested divergent apportionment of selected metals in various soil fractions; nonetheless, significant anthropogenic contributions were noted.

10.   Among the selected metals, Cd showed significantly higher contamination and enrichment in the soil samples; followed by Pb, Ca and Co. Rest of the metals showed insignificant or minimal contamination in the soil.

11.   Piper diagram showed higher Na and K along with chlorides and sulphates in most of the soil samples.

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