Dissertation for Doctor

ACCUMULATION OF TRACE AND RARE EARTH ELEMENTS IN BROWN SOIL AND SOYBEAN AFTER LONG TERM APPLICATION OF FERTILIZERS

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Major: Plant Nutrition

Specialty: Agricultural Resources and Environment

Research Field: Fertilization and Environmental Protection

A thesis submitted for the partial fulfillment of the requirements
for the degree of Doctor of Philosophy in
Environmental Sciences

College of Land and Environment

Shenyang Agricultural University, Liaoning, China

December，2017

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This thesis by **Muhammad Imran Ashraf** is hereby approved for submission to the Shenyang Agricultural University, Liaoning, China, in the partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Nutrition.

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Contents

ACCUMULATION OF TRACE AND RARE EARTH ELEMENTS IN BROWN SOIL AND SOYBEAN AFTER LONG TERM APPLICATION OF FERTILIZERS

ABSTRACT

Purpose

In agricultural production, specific elements in soil and plants are very important for soil quality and plant productivities. However, the information of trance and rare earth elements in soils and plants after long-term fertilizations is limited. This study was focused on temporal changes in accumulation of trace elements/micronutrients (cobalt (Co), nickel (Ni), molybdenum (Mo), selenium (Se)) and rare earth elements (erbium (Er), gadolinium (Gd), lanthanum (La), lutetium (Lu), neodymium (Nd), praseodymium (Pr), terbium (Tb), Yttrium (Y) and ytterbium (Yb)) in brown soil and soybean after long term application of different chemical fertilization and pig manure in corn-corn-soybean rotation in brown soil since 1979. The main subject in different ways, the usage of manure along with chemical fertilizers for a reasonable improvement and optimization of soil physical and chemical properties, improve soil C / N ratio and soil beneficial protein and enzyme activity in the low-yield farmland. Based upon past work and literature review, we hypothesized that manure would affect availability of micronutrients in soil and accumulation in plants.

Method

The soil samples of the years 1979, 1989, 1992, 2002 and 2014 and plant samples (stem and seed) of the years 1989, 2002 and 2014 were selected for this study. Atomic emission spectrometry with inductively coupled plasma (ICP-OES) technique was applied to determine the concentrations of trace elements and rare earth elements. Microwave assisted digestion was used for preparation of soil and plant samples to run for element analysis. For soil samples, a mixture of suprapure HNO_3 (65%), HCl (37%) and HF (GR) at the ratio of 6:2:2 ml was selected and for plant sample only 10 ml of suprapure HNO_3 (65%) was used.

Results

The overall accumulation of trace elements (TEs) concentrations level pattern in soil was Ni > Co > Se > Mo and rare earth elements (REEs) were in the pattern La > Nd > Tb > Y > Pr > Gd > Er > Yb > Lu. In soybean stem the TEs and REEs concentrations level patterns were Se > Ni > Co > Mo and Pr > Gd > Er > Yb > Tb > Nd > Lu > La > Y respectively. However, Ni > Se > Mo > Co and Pr > Gd > Er > Yb > Tb > Nd > Lu > Y > La pattern was seen in soybean seeds. The ability of Co, Mo, Ni, Se, Gd, Nd, Pr, Er, Lu, Tb, Y and Yb to accumulate in soil and plant parts was ranked as soil > soybean seed > soybean stem and that of La was soil > soybean stem. However, the Se, Nd, Y and Yb showed soil > soybean stem > soybean seed. Higher concentrations of Se, Nd, Y, and Yb were found in soil. These elements were higher in soybean stem followed by the seed. However these elements are within safe toxic level and light pollution level.

Conclusion

The result based conclusion drawn from this study is, the higher concentrations of Se, Nd, Y, and Yb were found in soil. These elements were higher in soybean stem followed by the seed. However these elements are within safe toxic level and light pollution level. It is also suggested that this data can be analyzed through multivariable statistics sufficiently to determine the difference between treatments regarding the contribution of fertilizers to the accumulation of related soil elements to improve the biological effectiveness.

Keywords: Elements, Fertilizers, Accumulation, ICP-OES.

CHAPTER ONE: HIGHLIGHTS THE OBJECTIVES OF THE PROJECT

1.1 Introduction to trace and rare earth elements

The world’s agriculture is challenged by the increasing global population and global warming. The availability of assessable and qualitative food adequately without adverse environmental effects on ecosystem is demanding advanced agricultural management practices demonstrated by the development of new soil additives.

In agriculture, specific concentration and distribution of elements in soil are very important factors. These factors reflect the environmental conditions in that particular area. The trace elements have significant importance in research to exploit environmental quality of soil and practical development and exploitation of land resources (Tao et al., 2001; Wang et al., 2014). It is basic to determine the concentrations of mineral elements in soil and produce to evaluate the needs of minerals for ruminants. The improvements of mineral elements in agricultural system are influential for nutrition because, in human body, the micronutrients are derived from food chain. Suitable mineral concentrations with a main role in several metabolic processes are essential for development and growth of all animals.

Some trace elements like heavy metals in higher concentration pose threats to vegetation and water resources and the persistent soil contaminants are difficult to remove (Agrawal et al., 2011; Alloway, 2013a; Beesley and Dickinson, 2011; Beesley et al., 2011). Agricultural practices are the main source of these inadvertent trace elements through the application of contaminated organic fertilizers or inorganic fertilizers repeatedly (Alloway, 2013b; Kloss et al., 2014; Mench et al., 2010; Nriagu and Pacyna, 1988). To overcome these threats, different techniques of stabilization comprising the addition of manure, composts and sludge have been examined to immobilize the trace elements (Adriano et al., 2004; Agrawal et al., 2011; Walker et al., 2004). Furthermore, some of the trace elements identified as vital nutrients for the plants metabolism and growth (Cu, Mn, Mo, Zn) as well as for human (Mn, Mo, Se, Zn) and are enlisted as micronutrients (Alloway, 2013c; Kloss et al., 2014). Some of these elements such as Co and Se are not necessary for plant growth but essential for animals and Cd, Pb, Cr, Ni, Hg and As have not been recognized as required by both plants and animals. Hence some studies exploit that Co, Ni, Cu and Cr are essential in biological structures up to a certain limits(Fraga, 2005b; Karadaş and Kara, 2012; Khan et al., 2014) while the deficiency of Se creates implications in many serious diseases in human body (Chen and Berry, 2003; Rayman, 2000; Tapiero et al., 2003; Wei et al., 2014b; Yu et al., 2008).

In agro-ecosystem, the sources of trace and rare earth elements (REEs) are geological distribution in parent soil, atmospheric deposition from industrial air emissions (Adriano, 2001; Cui et al., 2007; Tang et al., 2015) through rain water, agricultural amendments like organic/inorganic fertilizers and application of pesticides (Adriano, 2001; Avci and Deveci, 2013; Fairbrother et al., 2007; Yaman, 2006). Industrialization has also increased the concentration of trace elements and rare earth elements in different compartments of the environment where most of these elements are generated from anthropogenic activities. The REEs enter in food chain through accumulation in soil and bioaccumulation in agricultural produce and cause adverse environmental effects (d'Aquino et al., 2009; Dołęgowska and Migaszewski, 2013; Li et al., 2013a; Šmuc et al., 2012) in China especially (Guo et al., 2013; Wang et al., 2008a).

Approximately 89% of the world’s REEs are present in China and 97% are produced and used in industrial activities as well as in agricultural practices as micronutrient fertilizers (U.S.Geological-Survey, 2011). The application is resulted in accumulation of REE in agro-ecosystem. The research related to trace elements and rare earth elements accumulation has been increased assisted with high quality determination techniques. The concentration of rare earth elements varies among different plant species (Fu et al., 2001) and proven to promote agricultural production in an adequate concentration (Ding et al., 2006).

1.2 Objectives of the study

This study was focused on temporal changes in accumulation of trace elements/micronutrients (Ni, Mo, Co, Se) and rare earth elements (Er, Gd, La, Lu, Nd, Pr, Tb, Y and Yb) in brown soil and soybean after long term application of different chemical fertilization and pig manure. The main subject in different ways, the usage of manure along with chemical fertilizers for a reasonable improvement and optimization of soil physical and chemical properties, improve soil C / N ratio and soil beneficial protein and enzyme activity in the low-yield farmland. Based upon past work and literature review, we hypothesized that manure would affect availability of micronutrients in soil and accumulation in plants.

The aims of the present study was to determine (a) the concentration of Ni, Mo, Co and Se in soil and soybean taking into account other soil parameters such as pH and soil organic carbon (SOC) and nitrogen, and (b) to determine whether there was a relation between the micronutrient concentrations in crops and in corresponding soil. This study was focused on the long term application of fertilizers and pig manure on corn-corn-soybean rotation.

1.3 Significance

Soybean (Glycine max L. Merr) is considered as one of the most important crop in the world with high nutritional values containing 40% protein, 32% carbohydrates, 20% oil, 20% fat 5% minerals and 3% fibers, and trace substances including protein-enriched fodder and feed for animals. In the year 2014, 15 million metric tons of soybean was produced in china (about 5% of the world’s total production). The soybean oil is used in numerous industrial applications. The soybean contains several organic compounds like alpha-linolenic acid, phytic acid and isoflavones in significant amounts. The traditional foods of soybean are soy milk, tofu, soy sauce, bean paste, natto and tempeh.

For China’s large population relative shortage of arable land conditions, the use of manure and straw along with chemical fertilizers only help to improve crop yields, while also reducing the large number of inputs of fertilizer, as well as conducive to high yield of crops and promote efficient use of nutrient resources, reduce greenhouse gas emissions to combat environmental pollution and destruction, and promote sustainable development in harmony.

1.4 Layout of thesis

Chapter 1 throws light on the rationale of study, introduces trace and rare earth elements and highlights the objectives of the project. Chapter 2 is comprised of an extensive review of literature on availability and accumulation of trace and rare earth elements in soil and plants. Chapter 3 deals with methodology adopted to analyze the trace elements and rare earth elements using atomic emission spectrometry with inductively coupled plasma (ICP-OES/AES) assisted with microwave digestion. Chapter 4 illustrates results and discussions. Chapter 5 covers the conclusions and recommendations.

CHAPTER TWO: LITERATURE REVIEW

2.1 Trace elements

Trace elements pose important health impacts on human and other mammals and involve in physiochemical activities in body when they are present in accessible forms and are toxic at concentration levels exceeding the limits in uninterrupted environments as a result of accumulation due to pedogenic, biogenic and geogenic processes. Generally, these elements are placed in micronutrient category and cobalt, selenium, zinc, manganese chromium and vanadium are essential for human body in a quantity of less than 100 mg/day (Karadaş and Kara, 2012). In nutrition, micronutrients are considered to be involved in the formation of metalloproteins, polyphenols, phosphates, chelates and phytates compounds. These compounds take part in enzymatic activities with specific functions or transport proteins from manufacturing site to target site (Fraga, 2005a). Beyond to the quantitative concentration limits of their functions, cobalt, nickel, chromium and copper are toxic (Khan et al., 2014). Even at low concentration, due to non-biodegradable nature with long half-life, some of the trace elements are very toxic and accumulate potentially inside the body (Behbahaninia et al., 2009). Their availability in the soil affects the safety and food quality by plant uptake at elevated levels (Muchuweti et al., 2006).

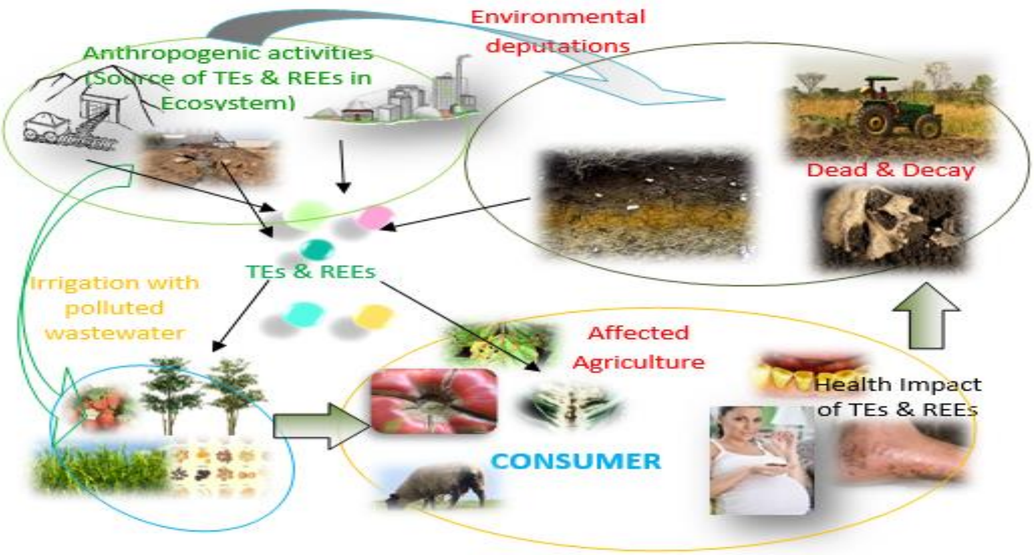

Fig 2.1: Fat of trace and rare earth elements in environment.

The plants cultivated in soil take up both the essential and non-essential elements through roots without any discrimination following law of natural gradient obeyed by the elements present in soil (Brady and Weil, 1999; Epstein, 1994; Peralta-Videa et al., 2009). The nutritional elements uptake by plants is regulated by availability of the required elements and elements accumulation ability of the plants (Kabata-Pendias and Pendias, 2010). The absorption of elements by root is affected by the element interactions like synergistic, incompatible or multiplicative (Peralta-Videa et al., 2003; Peralta-Videa et al., 2014). Under ideal irrigation and cultivation conditions the absorption of elements by plants through roots is affected by some factors like soil type, pH of soil, plant species and soil organic matter (Brady and Weil, 1999; Lavado et al., 2001; Maathuis, 2009) and these factors determine the uptake of the elements and bio-accumulation in the plant tissues. In the literature it is reported that soybean accumulate more cationic elements in stem tissue and in leaves if cultivated in acid soil as compare to cultivated in neutral soil (Wang et al., 2000).

2.1.1 Cobalt

The contamination of soil and water with cobalt is a result of extensive use in electroplating, excessive use of fertilizers, sewage sludge spreading, petrochemicals, smelting and mining industries in the vicinity in many countries (Dantu, 2009; Hamilton, 1994; Hamilton, 2000; Kiran et al., 2006; Krishna and Govil, 2007; Nair et al., 2012; Sočo and Kalembkiewicz, 2007; Williams et al., 1985). From the industrial activity cobalt can easily enter into the food chain causing toxicity (Denkhaus and Salnikow, 2002; Gál et al., 2008; Nair et al., 2012). Numerous studies reflect that the exposure time and entry path of cobalt affect animal health adversely (Haga et al., 1996; Lison et al., 2001; Migliori et al., 1994; Nair et al., 2012). High concentration of cobalt in plants causes many diseases like stomata closure of premature leaf, inhibition of greening, reduced shoot weight and leaf fall (Nair et al., 2012; Palit et al., 1994). The removal of cobalt pollutant in the low contamination area is not economically feasible. So, non-biodegradable inorganic pollutants are very difficult to remove from contaminated soil (Nair et al., 2012; Pilon-Smits, 2005). Therefore, it is essential to adapt effective remedial measures for waste management to control the contamination level. Cobalt contaminants have been removed from the environment by biosorption techniques (Egila et al., 2010; Nair et al., 2012; Pal et al., 2006). The economically feasible green technology termed as phytoremediation is also used for the removal of heavy metal pollutants with low concentration over large areas (Eapen and D'souza, 2005; Nair et al., 2012).

Cobalt is absorbed by passive transportation from soil by higher plants while the cortical cells transport cobalt by passive diffusion and active uptake. Transpiration plays and important role in cobalt transportation through xylem. Transportation of cobalt from stem to leaves is affected by its low mobility (Nair et al., 2012; Palit et al., 1994). Hyper accumulation of cobalt is also observed in plants grown in serpentine soil (Ensley et al., 2000; Nair et al., 2012), however, the growth rate of these plants is slow due to terrestrial distribution and low biomass (Doty, 2008; Eapen and D'souza, 2005; Eapen et al., 2007; Kotrba et al., 2009; Nair et al., 2012).

Some studies have been conducted to assess the toxicity of cobalt to soil biota and limited material is cited about its risk and toxicity to higher plants. According to Dekock, Chatterjee and Chatterjee, and Osman et al., at high doses exposure, relatively the cobalt is toxic to plants (Chatterjee and Chatterjee, 2003; DeKock, 1956; Li et al., 2009; Osman et al., 2004). Cobalt is accumulated in plants from soil in small amounts as it is reflected from our results also. The different mechanisms control the cobalt uptake and its distribution and it vary amongst species. Plant root absorb Co^{2+} by active transport trough cell membrane however, its molecular mechanisms are still unknown (Li et al., 2009). Cobalt may be distributed in the form of complex organic compounds and its transportation from roots to shoots is affected by its low mobility (Bakkaus et al., 2005; Barysas et al., 2002; Li et al., 2009; Palit et al., 1994). Cobalt availability is also influenced greatly by physical and chemical properties of soil. For the quantification of the effects of soil properties to express the toxicity of cobalt in plants biodiversity, a little information is available and difficult to compare (Li et al., 2009).

2.1.2 Molybdenum

Molybdenum is also placed in trace element group required by the living organisms in small amount leading to toxicity in high concentration (Mendel and Kruse, 2012; Turnlund, 2002). Molybdenum is vital for life on earth due to the key role in redox reactions involved metabolism of organisms and in biogeochemical cycles of carbon, nitrogen and sulfur (Mendel and Kruse, 2012; Stiefel, 2002). Molybdenum is present in a variety of metalloenzymes in animals, plants, fungi, algae and bacteria to facilitate enzymatic activities by forming active sites. Thus, molybdenum forms a prosthetic group called molybdenum cofactor (Moco) to perform its biological activities by coordinating with pyranopterin (an enzyme). This biological activity of molybdenum includes the redox reactions of sulfite, xanthine and aldehyde oxidase. Molybdenum is present in the form of molybdate anion in marine ecosystem abundantly and this is the only available form for bacteria, fungi and plants in soil. About more the 50 known enzymes are associated with molybdenum out of which only seven enzymes have been recognized in plants and animals (Hille et al., 2011; Mendel and Kruse, 2012).

The detailed studies about the deficiency of molybdenum in plants have been conducted by some researchers. Molybdenum bioavailability in the soil influences the molybdenum contents of plant directly. Low pH of the soil decreases the molybdenum available for plants causing alteration in leaf morphology that is actually a phenotypic characteristic development (Mengel et al., 2001). Mutation in uptake system of molybdenum can also cause Mo deficiency (Gasber et al., 2011). Defect in biosynthesis of molybdenum cofactor can cause deficiency of molybdenum resulting in suppressed or lost in enzymatic activities in the cell. The animals and human beings cannot loss in synthesis of molybdenum cofactor because their necessary metabolic functions depend upon molybdenum. Deficiency of molybdenum cofactor is a recessive hereditary disorder rarely found in human (1:100,000) ultimately leads to death of affected individual (Mendel and Kruse, 2012). After birth of a baby when metabolism is started to operate the accumulation of toxic metabolites in the body is also started and the symptoms appears in very short time. In this disorder the clinically observed symptoms is the lack of sulfite oxidase activity which degrade the lipids and amino acids containing sulfur results in elevation of sulfite level to toxicity particularly in the brain. Duran (Researcher) firstly described the Mo-enzymes deficiency in human and more than 100 cases have been diagnosed up till now (Duran et al., 1978; Mendel and Kruse, 2012).

Molybdenum also plays a significant role to control several environmental stresses like cold, drought and salt stress (Al-Issawi et al., 2013; El-Samad et al., 2005; Ghafarian et al., 2013; Sun et al., 2009; Wu et al., 2014; Zhang et al., 2012). In the earlier studies it is reflected that under cold stress the biosynthesis of chlorophyll is inhibited by molybdenum deficiency results in decreased chlorophyll in winter wheat (Wu et al., 2014; YU and Wang, 2006). Under low temperature stress, the molybdenum application to winter wheat show positive effect on decreased transpiration rate, stomatal performance and photosynthesis rate (Sun et al., 2005).

Though, excess concentration of molybdenum in chickpea decrease the quality of produce, seed yield and biomass (Nautiyal and Chatterjee, 2004). In environmental stresses, drought is a well-known stress that worldwide affects both the yield and growth of the crop plants and activates the biochemical and physiological responses in plants (Yue et al., 2012). Instead of main characteristics including closure of stomata, inhibited photosynthesis, decreased chlorophyll contents reduced transpiration rate, the biochemical and physiological responses are also involved in osmotic adjustment ability and antioxidant capacity to cope with drought conditions by increasing resistance in plants (Nezhadahmadi et al., 2013). Several researchers have reported that the production of superoxides, superoxide radicals and hydroxyl radicals is increased (i.e. active oxygen species) under drought stress leads to accumulation of malonaldehyde, damage of cell membrane and death of cell. The degree of oxidative stress is calculated by accumulated malonaldehyde concentration (Farooq et al., 2009; Gill and Tuteja, 2010; Li et al., 2013c). Plants vanish the effects of active oxygen species by increasing the antioxidant enzymatic activities of the enzymes including catalase, superoxide dismutase, ascorbate peroxidase and peroxidase or by increasing the level of glutathione, carotenoid and ascorbic acid (Jiang and Zhang, 2002; Yordanov et al., 2000). Another important technique adopted by the plants to resist drought stress is osmotic adjustment. The osmoregulatory substances like soluble proteins, soluble sugar and proline plays key role to maintain osmotic equilibrium and make the cell membrane reliable under drought conditions (Mahajan and Tuteja, 2005).

Molybdenum is also an essential trace element and plays a vital role in biological nitrogen fixation (Shah et al., 1984) and metabolism of nitrogen and sulphur (Jacobson et al., 1986; Kaiser et al., 2005; Price et al., 1972) and under field conditions its deficiency limits biological fixation of atmospheric nitrogen frequently (Becking, 1961; BRODRICK and GILLER, 1991; Campo et al., 2000; Chatt et al., 1978). Molybdenum takes part in the synthesis of nitrogenase as cofactor of proteins involved in transfer of electrons to convert nitrogen (N_2) into ammonium (NH_3) (Campo et al., 2009; Martens and Westermann, 1991; Shah et al., 1984).

The necessary macro and micro nutrients are easy available to soybean crop in naturally highly fertile soil however molybdenum availability is affected by acidification, reduction in organic matter, soil erosion and continuous cropping (Balík et al., 2006).

Various factors including contents of molybdenum in seed influence the response of soybean to molybdenum fertilization. It is experienced that the soybean seed from the soil with 22.4 µg/g molybdenum contents are adequate to fulfill the crop's need (Campo et al., 2009; Harris et al., 1965). To maintain a high level of biological nitrogen fixation, yield and protein contents in grain, Mo-enriched soybean seed are generally introduced in Brazil.

In the nature molybdenum does not exist in native form and is extracted from ores like ferrimolybdate, wulfenite, jordicite and molybdenite. By the combustion of fossil fuel, the molybdenum is emitted in the atmosphere along with particulate matter at relatively higher concentrations.

According to the literature review the molybdenum concentrations in soil range between 0.2–6 g/kg and 10–100 g/kg in metal enriched soil. Cong et al., in 2009 reported that the concentrations of molybdenum in an agricultural field in the vicinity of mining industrial area were measured as 0.305 µg/g (Geng et al., 2014). Many researchers have studied the molybdenum adsorption in detail and reported that the maximum adsorption rate is at soil pH 3–5 and at pH up to 8 the adsorption rate is significantly lower (Geng et al., 2014; Goldberg et al., 1998; Goldberg and Forster, 1998; Goldberg et al., 1996; Manning and Goldberg, 1996; O'Connor et al., 2001). In the soil solution at low pH, the molybdate anion is present as predominant species of molybdenum on which the molybdenum bioavailability is strongly dependent (Bibak, 1994; Buekers et al., 2010; van Gestel et al., 2011; Zimmer and Mendel, 1999).

2.1.3 Nickel

Nickel plays an active role as a catalyst in several enzymatic activities in the body and remains nontoxic up to a certain limits. The release of nickel into the agro-environment is a concern of human health as a result of groundwater contamination and polluted particulate matter (metal-enriched dust) (Cempel and Nikel, 2006; Chardot et al., 2007; Oller et al., 2008; Rajapaksha et al., 2012; Wesolowski, 2003). In crops, the nickel concentrations were affected by the plant uptake from soil and aerial deposition on crops. These factors influence the exposure of nickel for leaf crops, however, the aerial deposition is not considered for root crops.

Understanding about nickel interaction and assimilation processes in soil is important to calculate the uptake and availability of nickel. Ion exchange reactions are involved in nickel interaction mostly and nickel in soil is allied with parent material (Uren, 1993) including clay mineral (Reddy and Dunn, 1986; Vandenhove et al., 2009), oxides of manganese and iron (Anderson and Christensen, 1988; Vandenhove et al., 2009) and organic matter in the soil (Anderson and Christensen, 1988; Sauve et al., 2000; Vandenhove et al., 2009). Just after entrance in the soil about 90% nickel is sorbed rapidly. Soil pH considerably affects the availability of nickel. Increase in soil pH decreases Ni availability (Anderson and Christensen, 1988; Sauve et al., 2000; Staunton, 2004; Staunton et al., 2002; Tiller et al., 1984; Vandenhove et al., 2009; Weng et al., 2003; Weng et al., 2001).

For higher plants (vascular plants), nickel is also an essential nutritional element. These plants are hyperaccumulators and show 0.1% nickel concentration for shoot dry mass (Alves et al., 2011; Mengoni et al., 2003; Reeves and Baker, 2000). It is suggested that in transport and storage of nickel, histidine amino acid can be involved in chelating nickel. The researches elaborate that the export of histidine amino acid from root to shoot influences the entrance of nickel into xylem (Alves et al., 2011; Kerkeb and Krämer, 2003). A study conducted on some plants (hyperaccumulator) belongs to family Brassicaceae using X-ray spectroscopy showed that translocation of nickel is linked with carboxylic acid compounds however, histidine amino acid was not detected in shoots and roots of the investigated plants (Alves et al., 2011; Montarges-Pelletier et al., 2008). The detoxification of the metals in plants is also governed by the mechanism involved in attachment if these metals to the carboxylic acids and proteins (Alves et al., 2011; Callahan et al., 2006; Haydon and Cobbett, 2007; Sharma and Dietz, 2006; Verbruggen et al., 2009). These plant favorably uptake radioactive Ni (Brown et al., 1987; Sengar et al., 2008; Vandenhove et al., 2009) however, above 50 µg/g concentration of metals in tissue adversely affect the growth rate by their phytotoxic effects in most of the plant species (Sengar et al., 2008; Vandenhove et al., 2009). Some previous studies reported that the plants grown in serpentine soils, nickel is accumulated at the concentration of 30 – 40 µg/g (dry weight) in shoot and leave tissues (Alves et al., 2011; Freitas et al., 2004; Kidd et al., 2007; Lazaro et al., 2006). The nickel accumulation in soil at higher concentration impedes the chemical processes like carbon mineralization, nitrification and enzymatic activities of phosphatases and arylsulphatase (Al-Khafaji and Tabatabai, 1979; Bhuiya and Cornfield, 1972; Cornfield, 1977; Juma and Tabatabai, 1977; Liang and Tabatabai, 1978; Zaidi et al., 2006).

In soil with low pH, the root uptake of nickel is higher under suitable nickel interaction pattern. In plants, Nickel is highly mobile and distributed homogeneously (Coughtrey and Thorne, 1983; Vandenhove et al., 2009). In the literature reported that the average transfer factor values of ^{63}Ni from soil-to-plant for legumes is 0.5 and for other plant species is 0.1 (Coughtrey and Thorne, 1983; Vandenhove et al., 2009).

2.1.4 Selenium

One of the important trace elements is Se with key role in nutrition (De Temmerman et al., 2014a; Hartikainen, 2005). In human and animal, the antioxidant protection is provided by the selenoproteins and it also takes part in metabolism of thyroid hormone (Rayman, 2000; Wei et al., 2014a). For animals, the main source of selenium is selenomethionine (SeMet) and is bioavailable easily. Selenium is bio-accumulated at high concentration in some plants and ascribed to non-protein selenoamino acid synthesis like selenomethylselenocysteine (SeMeSeCys) and c-glutamyl-selenomethyl-selenocysteine (c-glu-SeMeSeCys) (Terry et al., 2000). Oral intake is the main integrating way of selenium into the body. The selenium concentration and chemical form varies depending upon the quality, quantity, ingestion frequency and nature of food.

In spite of benefits linked with selenium supplements, the risk assessment of cancer associated with selenium species is in progress (Vinceti et al., 2014a; Vinceti et al., 2015a). The literature reveals that medical complications like metabolic and cardiovascular diseases, prostate and skin cancer and cognitive and neurological disorders have emerged (Albanes et al., 2014; Colangelo et al., 2014; Duffield-Lillico et al., 2003; Martinez et al., 2014; Ode et al., 2015; Rees et al., 2013; Vinceti et al., 2015a; Vinceti et al., 2015b; Vinceti et al., 2014b). Long term exposure of selenium acquaintance in term of its influence on human health retained serious position and attraction of researchers to identify indicators like its concentration in nail, hair, urine and in blood and its oral intake (Fairweather-Tait et al., 2011; Harvey et al., 2009; Vinceti et al., 2013; Vinceti et al., 2014a; Vinceti et al., 2015a; Vinceti et al., 2009)

Several techniques have been adapted to evaluate its bio-accessibility in vegetables (Dumont et al., 2006a; Dumont et al., 2006b; Thiry et al., 2012). The physiologically-based extraction test method is widely used (Funes-Collado et al., 2015; Ruby et al., 1996). Primarily this method is applied to soil samples to calculate the metal releasing potential in to digestion zone and its absorption into blood stream (Funes-Collado et al., 2011; Funes-Collado et al., 2015). Various studies prove that in human nutrition, the consumption of vegetables, cereals, mushrooms meet and sea food is an important selenium source which reduces the cancer risk (Dias, 2012; Funes-Collado et al., 2015).

Numerous researchers have been elaborated both the bioavailability and bioaccessibility of selenium in vegetable, nuts, cereals, yeast and fish products (raw and cooked) (Cabañero et al., 2007; Cabañero et al., 2004; Crews et al., 1996; Dumont et al., 2006a; Dumont et al., 2006b; Dumont et al., 2004; Funes-Collado et al., 2015; Govasmark et al., 2010; Reyes et al., 2006). However, after gastrointestinal digestion in human/animal body, the bioaccessibility of released selenium species and their identification is needed to be evaluate (Funes-Collado et al., 2015).

2.2 Rare earth elements

Even it is reported that the quality of crops is improved and yield is boosted by the application of rare earth elements. The usage of rare earth element fertilizers is also increasing their concentration in environment. The rare earth elements enter into the environment from anthropogenic activities are present in bioavailable form that may disturb the biogeochemical cycle balance of these species (Zhang and Shan, 2001a).

The rare earth elements in extreme elevated concentration in several urban, industrial and agricultural soils have been reported (Hong et al., 2000; Hu et al., 2006; Shi-Ming et al., 2006; Šmuc et al., 2012; Yoshida et al., 1998) that may cause damage to ecosystem significantly (Barry and Meehan, 2000; Fang et al., 2007). While assessing the environmental impacts, mobility characteristics of rare earth elements present in soils are the most decisive factors. Though, the rare earth element species like chelated, soluble and exchangeable species in soil are the only fractions fiducially available to plants (Kabata-Pendias and Pendias, 2001; Šmuc et al., 2012). Being non-essential for living organisms, in some studies, positive effects of rare earth elements have been reported on the growth of several plant species and farming animals (Hu et al., 2004; Pang et al., 2001; Wang and Liang, 2014). In china, the rare earth elements are being used as fertilizers and food additives at low concentration for the last few decades (Wang et al., 2008b).

The established evidence points to rare earth elements related soil pollution along with their bioaccumulation in agro-ecosystem, however, the toxicological health effects related to rare earth elements have not been unfolded in spite of sharp rise in manufacturing and extraction of REEs and increased environmental as well as human exposure.

The population residing in the vicinity of mining sites of rare earth elements showed higher bioaccumulation of REEs as compared to distance from the mining sites (Bustamante and Miramand, 2005; Censi et al., 2013; Fu et al., 2014; Hu et al., 2002; Kulaksız and Bau, 2011; Liang et al., 2014; Moermond et al., 2001; Pagano et al., 2015; Peng et al., 2003; Qiang et al., 1994; Tong et al., 2004; Tranchida et al., 2011).

The data available for rare earth elements associated with human health effects is scarce especially for heavy rare earth elements except cerium and lanthanum, while very limited information is cited for gadolinium and neodymium. The patients exposed to gadolinium were reported with nephrogenic systemic fibrosis (Bernstein et al., 2012; Chang et al., 2013; Chien et al., 2011; Pagano et al., 2015; Thomsen, 2006). It is also reported that the cerium, lanthanum and neodymium are significantly cytotoxic and their fumes are considered as potentially fibrogenic to lung tissues (Pagano et al., 2015; Palmer et al., 1987).

Some researchers investigated oxidative stress caused by several rare earth elements through redox imbalance in plants and animal models and suggested that toxicity induced by rare earth elements is triggered by oxidative stress. Nonetheless, some investigators reported antioxidant effects of clinical application of cerium oxides (CeO_2) (Pagano et al., 2015; Wong and McGinnis, 2014).

The hormetic effects associated with several rare earth elements have been appeared in some available studies. Jenkins et al., conducted an extensive study to evaluate the effects of rare earth elements including La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu on keratinocytes in human these elements showed hormetic effects at concentration 1–100 µM (Jenkins et al., 2011; Pagano et al., 2015).

Yttrium exposure to aquatic plants at the concentration level of 1–5 mg/L induced enzymatic activities and significant decrease in catalase activity was observed (Fu et al., 2014). The change in mitotic activity is also one of the adverse effects of rare earth elements. A few studies were reported on the variation in toxicity of rare earth elements induced by change in pH by some processes mentioning acid rain and variety of acidic ligands (Liang and Wang, 2013; Ould-Moussa et al., 2014).

The effects of lanthanide and actinide elements (i.e. rare earth elements) in plants have been cited in few studies. The rare earth elements are present in the form group and almost show same properties. The soil pH, organic contents and clay mineralogy control the bioavailability of rare earth elements (Cao et al., 2001; Diatloff et al., 1996; Li et al., 2007; Li et al., 2013b; Tyler, 2004; Xinde et al., 2000). Most of these studies reflect that lanthanum affects some of the growth parameters in plants negatively. The lanthanum addition at the rate 0.5-25 mg/L in hydroponic media inhibited the elongation in primary root, reduction in dry weight of root and shoot and Ca, Cu, K, Mg and Zn contents in wheat (Hu et al., 2002). It also reduced the growth of fine root, chlorophyll contents, rate of photosynthesis and Ca, Mg, Ni and P concentrations in shoots and root tissues in Juglans nigra (Nicodemus et al., 2009). In soybean seedlings grown hydroponically, the leaf area, the main root length, plant weight and the dry weight of leaves, stem and root is decreased by the application of lanthanum (Wen et al., 2011). Conversely, the effects of oxides of rare earth elements (nanoparticles) on uptake of macro and micro elements and their accumulation in crop plants have not been reported.

The analysts are still facing challenge to develop suitable technologies to determine multi-elements at trace level in environmental and biological samples. For the multi-element detection methods being used comprise complicated sample preparation procedure. Highly pure reagents are also required in large quantity for a conventional wet digestion process (Matusiewicz and Ślachciński, 2015).

Several techniques have been applied to determine the macro and micro elements in soil and plant samples including atomic absorption spectrometry (AAS) (Amorim Filho et al., 2007; Ashu and Chandravanshi, 2011; Barbosa et al., 2014; dos Santos et al., 2009; Duran et al., 2010; Krivan et al., 1993), inductively coupled plasma mass spectrometry (ICP-MS) (Santato et al., 2012) and atomic emission spectrometry with inductively coupled plasma (ICP-OES/AES) (Barbosa et al., 2014; dos Santos et al., 2009; Krejčová and Černohorský, 2003; Saint’Pierre et al., 2006; Yin et al., 2005). However, the technique with multi-elements detection capability provided with much lower detection limits and wider linear dynamic range is ICP based technique (Barbosa et al., 2014; Chudzinska and Baralkiewicz, 2011; Nardi et al., 2009; Parsons and Barbosa Jr, 2007).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Experimental Plots Location and Design

The experimental plots were located between 40°48´ N and 123°33´ E at Shenyang Agricultural University in Shenyang, Shenhe district of Liaoning Province, China. The area is semi-humid with the mean annual precipitation and temperature 574-684mm and 7.0-8.1°C, respectively. Geology of the experimental field is 23% clay, 48% sand and 29% silt (clay brown loam) at 20cm deep and classified as Alfisols dominated with hydromica clay mineral. This experimental area was established in 1979. It is rainwater irrigated area. The total experimental area is divided into 18 different treatment plots, 160m^2 each, with maize-maize-soybean rotation. Tillage is being done up to 20cm deep by machine generally at the time of planting or the day before. The treatments were N_1P (I-1), N_1PK (I-2), N_1 (I-3), CK (I-5), M_2N_1P (III-1), M_2N_1PK (III-2), M_2N_1 (III-3) and M_2 (III-5). The notation N, P, K and M means nitrogen, phosphorus and potassium inorganic fertilizers and pig manure respectively. The detail of fertilizers applied were, for soybean, nitrogen fertilizer = 22.5 kg/hm^2, phosphorus fertilizer (P_2O_5) = 90 kg/hm^2, potassium fertilizer (K_2O) = 90 kg/hm^2 and pig manure = 0 kg/hm^2.

3.2 Sampling

The eight soil profiles were sampled to 20 cm depth with appropriate auger. The S shaped pattern was used during sampling randomly and all samples of individual treatment were mixed to generate a composite sample of each treatment plot. The same procedure was adopted for plant (stem and seed) samples. These soil and plant samples were sealed in plastic bags and transferred to laboratory. The collected soil samples were dried at room temperature and homogenized by grinding with wooden mortar and pestle, passed through 63 µm nylon sieve and stored at 25 °C until elemental analysis.

3.3 Materials and Equipment

A Milestone ETHOS One (High Performance Microwave Digestion System) equipped with MPR-600/12 HT high pressure segmented rotor used for soils, sediments, sludge, wastewater, plastics, food and feed samples and DRN-41 rotor used for easy organics like vegetables, biological tissues and foods containing less than 20% fat contents. All Teflon vessels had been pre-cleaned with 5% HNO_3 in a hot acid bath and then rinsed with Milli-Q water. Ultrapure (Milli-Q) water obtained (18.2 MΩ cm TC, 25 °C) from Milli-Q (Milipore, Billerica, MA) water purification system was used in the experiments. The pH meter (phs-3e, made in China) was used to determine the pH of the soil samples.

Suprapure concentrated nitric acid (HNO_3) (65%), concentrated hydrochloric acid (HCl) (37%), concentrated hydrofluoric acid (HF) (GR), potassium dichromate salt ($K_2Cr_2O_7$), concentrated sulphuric acid (H_2SO_4), ferrous sulphate salt ($FeSO_4 \cdot 7H_2O$) and O-phenanthroline monohydrate supplied by Sinopharm Chemical Reagent Co. Ltd. China, were used. Standard solutions for external calibration were prepared from solutions of 100-1000 µg/ml concentrations supplied by "analytical measurement center of national nonferrous metals and electronic material, china".

The soil reference material (GBW07403) and plant reference material (GBW10012) supplied by National Center for Reference Materials, china were used in these experiments.

Trace elements (Co, Mo, Ni and Se) and rare earth elements (Er, Gd, Ge, La, Lu, Nd, Pr, Tb, Y and Yb) were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) VISTA-MPX, VARIAN, GERMANY.

3.4 Sample preparation and element determinations

The soil and plant samples were prepared for trace and rare earth elements analysis according to the US EPA3051A method. The acid mixture or an acid for microwave digestion is chosen according to the nature of analytes. For 100 mg of soil sample, a mixture of suprapure HNO_3 (65%), HCl (37%) and HF (GR) at the ratio of 6:2:2 ml was selected and for 250 mg of plant sample only 10 ml of suprapure HNO_3 (65%) was used. Blank digests for soil and plant samples were also prepared following the same method.

All the vessels used for digestion were immersed with 5% HNO_3 for at least two hours. These vessels were rinsed with Milli-Q water and dried in clean environment.

For soil samples analysis, 100 mg of already prepared each sample according to the procedure mentioned above were accurately weighed using an analytical balanced and transferred into digestion vessels. Then 6 ml of concentrated nitric acid was added in each vessel followed by 2 ml of concentrated hydrofluoric acid and 2 ml of concentrated hydrochloric acid under a fume ventilation system operated properly because after addition of acids the digestion is started resulting in the production of chlorine fumes and toxic nitrogen oxide(s). Before starting microwave digestion the samples were predigested for about 12 hours with vessels loosely capped in a hood to let gases to escape.

Then, the vessels were sealed and placed in the microwave digestion unit according to the directions and specifications of the manufacturer. The turntable was rotated at the speed of 3 rpm to ensure the consistent distribution of microwave radiations inside the unit. The heating program used for microwave digestion as followed: 5 min ramp from room temperature to 80 °C; 5 min ramp from 80 to 120 °C; 10 min ramp at 120 °C; 10 min ramp from 120 to 160 °C; 10 min ramp at 160 °C. At the end of heating program the vessels were allowed to cool to room temperature for 60 minutes and then removed from the microwave digestion unit. To check the maintenance of the seals of vessels throughout digestion, the sealed vessels were weighed before and after digestion, the weight loss of sample did not exceed 1% (more than 1% considered as compromised). The vessels were uncapped in a fume hood according to the manufacturer’s recommendations after cooling sufficiently to handle. The acid was evaporated at 120 °C in a heating system and the residue after cooling was dissolved in 1ml of 2% HNO_3 (GR) and transferred in 25 ml volumetric flasks to make the volume with ultrapure water. These prepared samples were filtered into a cleaned container through Whatman 40 filter paper and stored at 4 °C until element analysis.

For plant samples analysis, 250 mg of already prepared each sample according to the procedure mentioned above were accurately weighed using an analytical balanced and transferred into digestion vessels. Then 10 ml of concentrated nitric acid was added in each vessel under a fume ventilation system operated properly because after addition of acids the digestion is started resulting in the production of toxic nitrogen oxide(s). Before starting microwave digestion the samples were predigested for about 12 hours with vessels loosely capped in a hood to let gases to escape. Then, the vessels were sealed and placed in the microwave digestion unit according to the directions and specifications of the manufacturer. The turntable was rotated at the speed of 3 rpm to ensure the consistent distribution of microwave radiations inside the unit. The heating program used for microwave digestion as followed: 5 min ramp from room temperature to 80 °C; 5 min ramp from 80 to 120 °C; 10 min ramp at 120 °C; 10 min ramp from 120 to 160 °C; 10 min ramp at 160 °C. At the end of heating program the vessels were allowed to cool to room temperature for 60 minutes and then removed from the microwave digestion unit. The vessels were uncapped in a fume hood according to the manufacturer's recommendations after cooling sufficiently to handle.

The digested samples were transferred in 25 ml volumetric flasks to make the volume with ultrapure water. These prepared samples were filtered into a cleaned container through Whatman 42 filter paper and stored at 4 °C until element analysis.

For the evaluation of the performance of all analytical processes, the reference samples were handled exactly in the same way as actual samples.

These soil and plant samples were run over inductively coupled plasma optical emission spectroscopy (ICP-OES/AES) VISTA-MPX, VARIAN, GERMANY for the analysis of cobalt (Co), nickel (Ni), molybdenum (Mo), selenium (Se), erbium (Er), gadolinium (Gd), lanthanum (La), lutetium (Lu), neodymium (Nd), praseodymium (Pr), terbium (Tb), Yttrium (Y) and ytterbium (Yb).

ETHOS One (High Performance Microwave Digestion System)

ICP-OES/AES, VISTA-MPX, VARIAN, GERMANY

3.5 Determination of Soil pH

3.5.1 Apparatus used

- Analytical balance

- pH meter
- Measure cylinder (20 ml)
- Beakers (100 ml)
- Magnetic stirrer

3.5.2 Procedure

To determine the pH of the soil in laboratory, accurately weighed soil samples were dissolved in reagent water at the ratio of 1 gram of soil into 2.5 milliliter of water and stir for one minute to disperse soil well. The pH was determined at room temperature by a pH meter model phs-3e made in china. Before using the pH meter was standardized according to instructions of the manufacturer. The electrode was rinsed with distilled water repeatedly before every measurement.

3.6 Determination of soil organic carbon (SOC)

Soil organic carbon was determined by using the method adopted by McLeod, S (McLeod, 1973).

3.6.1 Apparatus used

- Analytical balance
- Electric hot plate
- Heat resistant sheet
- Fume cupboard
- Mohr pipette (10 ml)
- Rubber sucker
- Burette (50 ml)
- Measuring cylinder (20 ml)
- Erlenmeyer (conical) flasks (250 ml)
- Volumetric flask (1000 ml)

- Volumetric flask (100 ml)
- Thermometer (200 °C)

3.6.2 Chemicals and Reagents

- 1N Potassium Dichromate: prepared by accurately weighing and dissolving 49.04 grams of $K_2Cr_2O_7$ in deionized water in volumetric flask to make volume 1 L.
- Sulphuric Acid (98% w/w): commercially available (satisfactory until the strength is < 96%)
- 0.4N Ferrous Sulphate: prepared by accurately weighing 112 grams of $FeSO_4 \cdot 7H_2O$ and dissolved in deionized water containing 15 ml of concentrated H_2SO_4 to make the volume 1000 ml and stored in dark.
- Ferroin indicator: prepared by accurately weighing 1.485 grams of O-phenanthroline monohydrate and 0.695 grams of ferrous sulphate and dissolved in deionized water then dilute to 100 ml. stored in dark colored bottle.

3.6.3 Procedure

500 mg of prepared soil samples were weighed accurately and transferred into a tarred dry flask. 10 ml of 1N potassium dichromate ($K_2Cr_2O_7$) was added with pipette accurately. The flask was swirled gently to disperse the soil in solution and 20 ml of concentrated sulphuric acid (H_2SO_4) was added into suspension. The flask was swirled immediately to mix the soil in the reagent. After that the suspension was heated to 135 °C for less the 1 minute. After cooling for 20-30 minutes over heat resistant sheet under fume cupboard it was diluted to 200 ml with deionized water and titrated with 0.4N ferrous sulphate ($FeSO_4$) using 3-4 drops of ferroin as indicator. The endpoint of the titration was the turning of the color of the solution from blue-green to reddish-grey.

In each experiment, two blank titrations were also carried out to standardize the $FeSO_4$ Solution.

The percentage of organic carbon was determined by the equation:-

$$\text{Organic Carbon (\%)} = \frac{0.003\text{g} \times N \times 10\text{ml} \times (1 - T/S) \times 100}{W}$$

1 ml of 1N potassium dichromate solution is equivalent to 3 mg of carbon.

Where:-

N = Normality of $K_2Cr_2O_7$ solution

T = Volume of $FeSO_4$ solution used in sample titration (ml)

S = Volume of $FeSO_4$ solution used in blank titration (ml)

W = Sample weight (g)

3.7 Statistical Analysis

Mean values of all treatments are reflected here in the tables below. Data were analyzed at the significant differences ($p < 0.05$) using one-way analysis of variance (ANOVA) following Tukey’s post-hoc test (HSD) and Duncan test, in the Statistical Package for Social Sciences (SPSS) version 22 (IBM Corp). The one-way ANOVA is performed in inter-treatments only.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.1 Results

The soil pH and soil organic carbon (SOC) was determined and results are displayed below in the table 4.1.

Table 4.1: The soil pH and soil organic carbon (SOC).

Sr. #.	Treatments	1989		1992		2002		2014	
		pH	SOC	pH	SOC	pH	SOC	pH	SOC
1	N_1P	6.98	8.06	6.69	8.47	6.48	8.87	5.43	8.93
2	N_1PK	6.90	8.18	6.83	8.00	6.39	8.58	5.33	8.82
3	N_1	7.28	7.25	6.91	7.66	6.52	7.71	5.43	8.64
4	CK	7.09	8.41	6.51	8.41	6.26	8.06	5.92	8.29
5	M_2N_1P	6.96	11.77	6.21	9.51	6.14	11.14	5.69	10.61
6	M_2N_1PK	6.67	11.31	6.13	10.96	5.95	11.19	5.87	12.06
7	M_2N_1	6.87	10.90	5.98	10.56	5.99	9.98	5.90	11.83
8	M_2	7.29	12.70	5.99	10.73	5.97	10.03	5.90	11.77

Note:

- The Soil Organic Carbon (SOC) was calculated in grams/kilograms (g/Kg).
- The pH and SOC of the only CK treatment of the year 1979 was calculated as 6.5, 9.22 g/kg respectively.

The concentrations of trace elements in soil samples were displayed in figures 4.1 – 4.8 under the description of each treatment. The concentrations of trace elements and rare earth elements of the harvested soil samples of the years 2013 and 2014 were displayed in figures 4.9 – 4.16. The rare earth elements concentrations in soil samples are mentioned in the table 4.3. The concentrations of trace elements in plant (stem and seed) samples are cited in table 4.2. Table 4.4 reflects the results of rare earth elements concentrations in stem samples. However, table 4.5 shows the rare earth elements concentrations in seed samples. All trace and rare earth elements are determined in µg/g.

4.1.1 The concentration of TEs and REEs in Nitrogen-Phosphorous amended treatment (N_1P)

The concentration of cobalt (Co) is increasing gradually and significantly higher after the latest application of fertilizer in the treatment as compare to the start of the experiment field. Cobalt is accumulated in soil mostly. A very small concentration is bio-accumulated in soybean plant (both in stem and seed) in the year 1989 but in 2002 only in stem. Molybdenum (Mo) was present in significantly higher concentration in 2014 soil sample and its concentration 0.617 µg/g and 0.466 µg/g was determined in stem and seed in the years 1989 and 2014 respectively. However, the concentration of Nickel (Ni) is increasing gradually and accumulates in soil on application of fertilizers and bio-accumulated in plant but at higher concentration rate in seeds as compare to stem. The selenium (Se) element is determined significantly lower in concentration in soil samples of the years 1992 and 2002. In the plant samples of the years 1989 and 2002, the Se is detected both in stem and seed samples in the order of stem > seed but is significantly higher in the 1989 samples.

The concentration of all rare earth elements except Y in soil is gradually increasing and significantly higher in the year 2014. Table 5 shows that, in stem, the concentration of Gd, La, Nd, Tb and Y is almost same and no change was observed during the whole period of long term fertilization while the concentration of Pr, Er, Lu and Yb is significantly decreased in 2014. In seed, the concentration of Gd, Pr and Y show the same pattern as in stem samples. Nd was observed only in seed samples of the year 1989 while Er, Lu, and Yb concentration show no significant difference in seed. La is bio-accumulated only in stem.

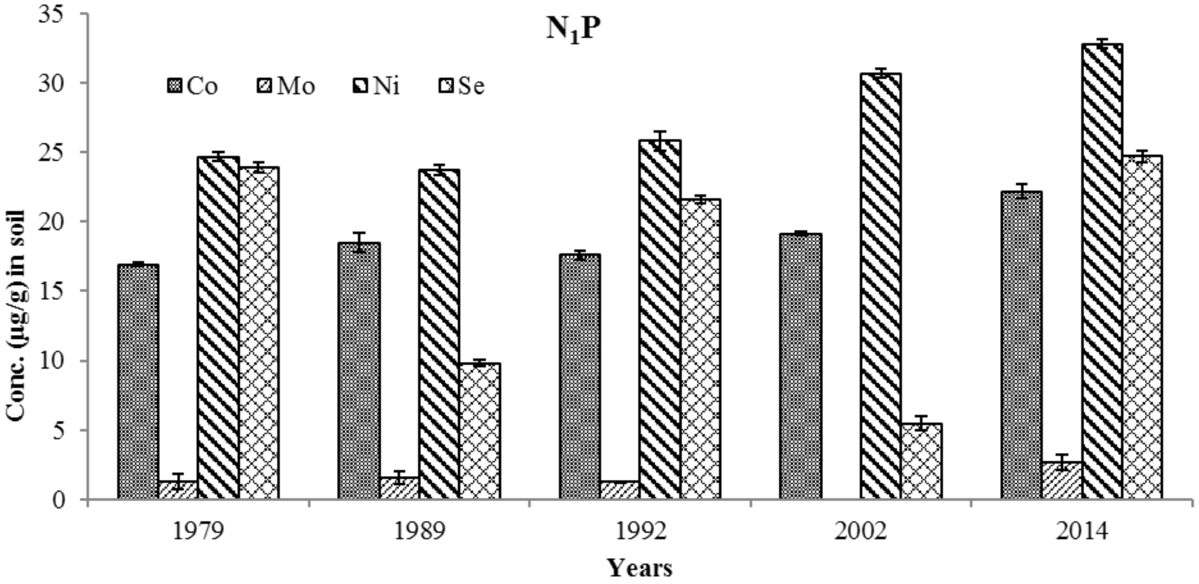

Figure 4.1: Trace element concentrations in N_1P-Treatment in soil.

4.1.2 The concentration of TEs and REEs in Nitrogen-Phosphorous-Potassium amended treatment (N_1PK)

Cobalt concentration was significantly higher in soil and stem samples of the year 1989, 1992 and 2002 respectively, however, in seeds only present in sample of the year 1989. Mo was determined in the three soil samples in the order 1.38 < 2.56 < 3.10 µg/g in the years 1979, 1992 and 2014 respectively and in plant samples (stem + seed) of the year 2014 only. Ni concentration was significantly higher in soil and seed sample of the year 2014 and in stem in the sample of the year 2002. Se was significantly higher in the soil sample of the year 2014 and was at the same level as in the year 1979. It is detected in stem samples of the years 2002 and 2014 at almost same concentration level. In seed Se was bio-accumulated in the samples of the years 1989 and 2002 but significantly higher in 1989.

In treatment N_1PK, the REEs in soil shows almost same pattern as was observed in N_1P except Lu. No significant change in concentration of Lu was observed in this treatment. In stem, no significant difference was observed in the concentration of Gd, Lu and Yb during the long term application of fertilizers. However, the concentration of Nd, Pr, Er, Tb and Y were decreased yearly except La. In seed, Gd, Pr, Lu and Yb concentration is decreasing in the pattern 1989 > 2002 > 2014. The concentration of Nd and Er were significantly higher in the year 2002.

Tb and Y concentration were almost same except Tb concentration in 2002 which was significantly lower. Nd and Y were not detected in the seed samples of the year 2014. La was absent in seed samples of all the tree years.

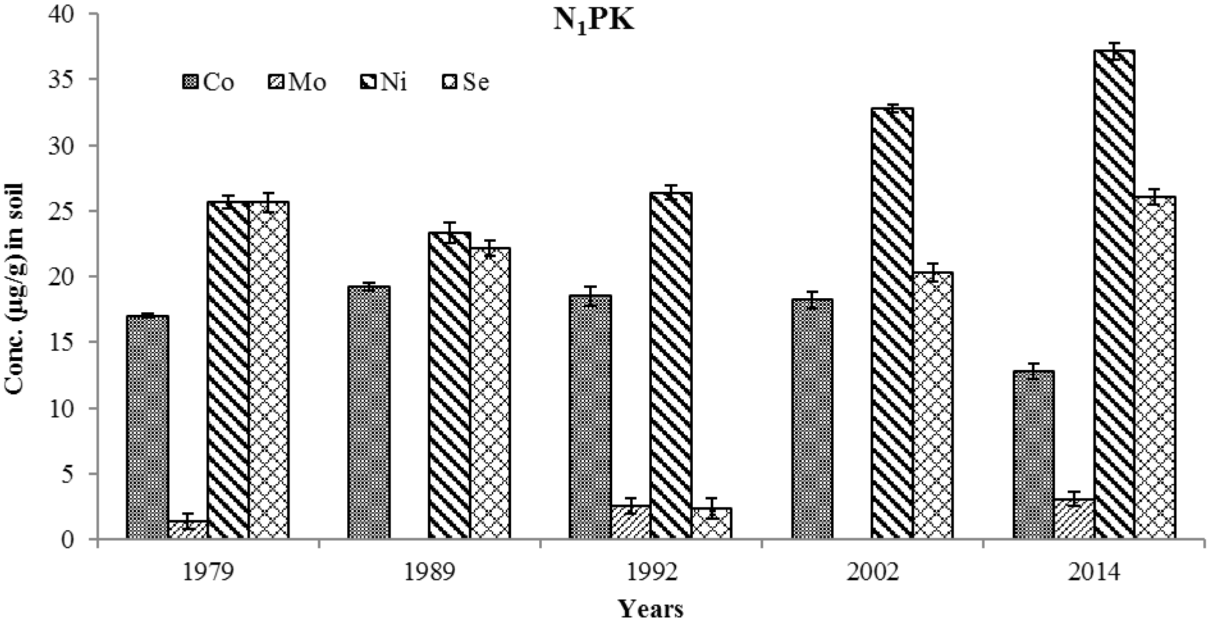

Figure 4.2: Trace elements concentrations in N_1PK-Treatment in soil.

4.1.3 The concentration of TEs and REEs in Nitrogen-Phosphorous-Potassium amended treatment (N_1)

The Co concentration in this treatment was significantly higher in soil sample of the year 1989 as compare to the sample of the year 2014. In stem, it is present only in the sample of the year 2002. In seed, it is not present in the sample of the year 2014 and bio-accumulated at higher rate in 2002 than 1989. Mo was significantly higher in soil sample of the year 1992, however, detected only in stem sample of the year 2014 and in seeds of the year 1989. Ni concentration was significantly higher in soil and seed sample of the year 2014 while no significant change in concentration was observed in stem. Se was significantly higher in the soil samples of the years 1979 and 1992. In stem and seed sample it is significantly higher in the sample of the year 1989 and was absent in the 2014.

In N_1-treatment, the concentrations of Gd, Pr, Er, Lu Tb and Yb in soil were significantly higher in the samples of the year 2014 than rest of the three years. No significant difference in La and Nd concentration in soil were detected, however, the concentration of Y was significantly higher in the year 1979 and 1989 than 2002 and approximately equal in the year 2014. In stem, the concentrations of Gd, Nd, Pr, Tb and Yb were significantly higher in the sample of the year 1989 as compare to 2014. The concentration of La and Y were significantly higher in the sample of the year 2014 while the concentration of Er and Lu in the sample of the year 2002. In seed sample of the year 1989 the concentrations of Gd, Nd, Pr and Y were significantly higher than 2002 and 2014. However, the concentrations of Er, Lu, and Yb were significantly lower in 2014 than other two years. The concentration of Tb was significantly lower in the sample of the year 2002.

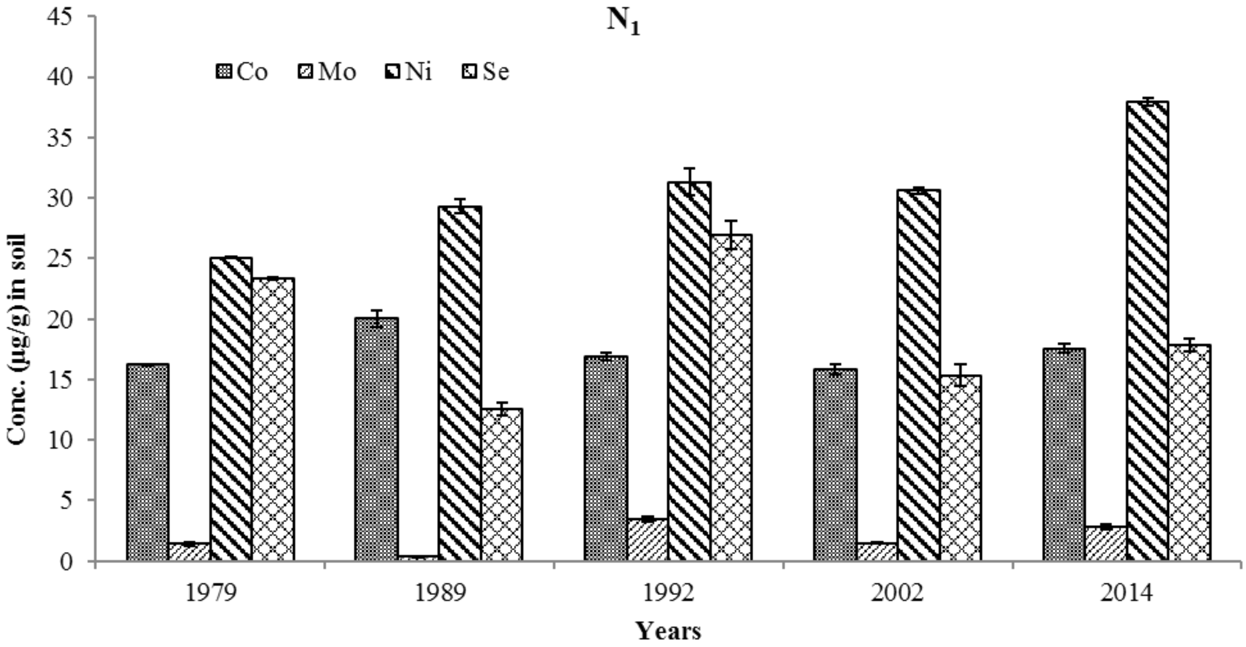

Figure 4.3: Trace elements concentrations in N_1-Treatment in soil.

4.1.4 The concentration of TEs and REEs in treatment without fertilizer (CK)

Co concentration was significantly higher in soil samples of the years 2014 and 1992 in this treatment. In stem, it is higher in sample of the year 2002 than 2014. In seed, it is significantly higher in the sample of the year 2014. Mo was present only in the soil samples of the year 1992 and 2014 at the rate of 2.88 and 1.61 respectively.

It was totally absent in stem samples of all the three year in this treatment and was detected in seed sample of the year 2014. Ni concentration was significantly higher in the soil and seed samples of the year 2014, however, in stem it was higher in 1989 than 2002 and was absent in 2014. Se, after 1989 shows almost same level of concentration that was significantly higher than 1979 and was detected only in stem and seed samples of the year 1989 only.

In CK-treatment the concentrations of Gd, La, Pr, Er, Lu and Tb in the soil samples were significantly higher in the samples of the year 2014 then other three years while the concentrations of Nd, Y and Yb were not significantly different. In stem samples, the concentrations of Gd, Pr, Lu, Tb and Y were significantly higher in the sample of the year 2002 and the concentration of Nd and Er in the sample of the year 1989. The concentration of La was observed in an increasing pattern as 1989 < 2002 < 2014 while the concentration of Yb remains almost same during the whole period of long term application of fertilizers. In seed samples the concentrations of the Gd, Pr, Tb, Y and Nd, Er significantly higher in the years 1989 and 2002 respectively while the concentration of Lu and Yb were significantly lower in the sample of the year 2014. In seed sample of the year 2014, Nd and Y were not detected.

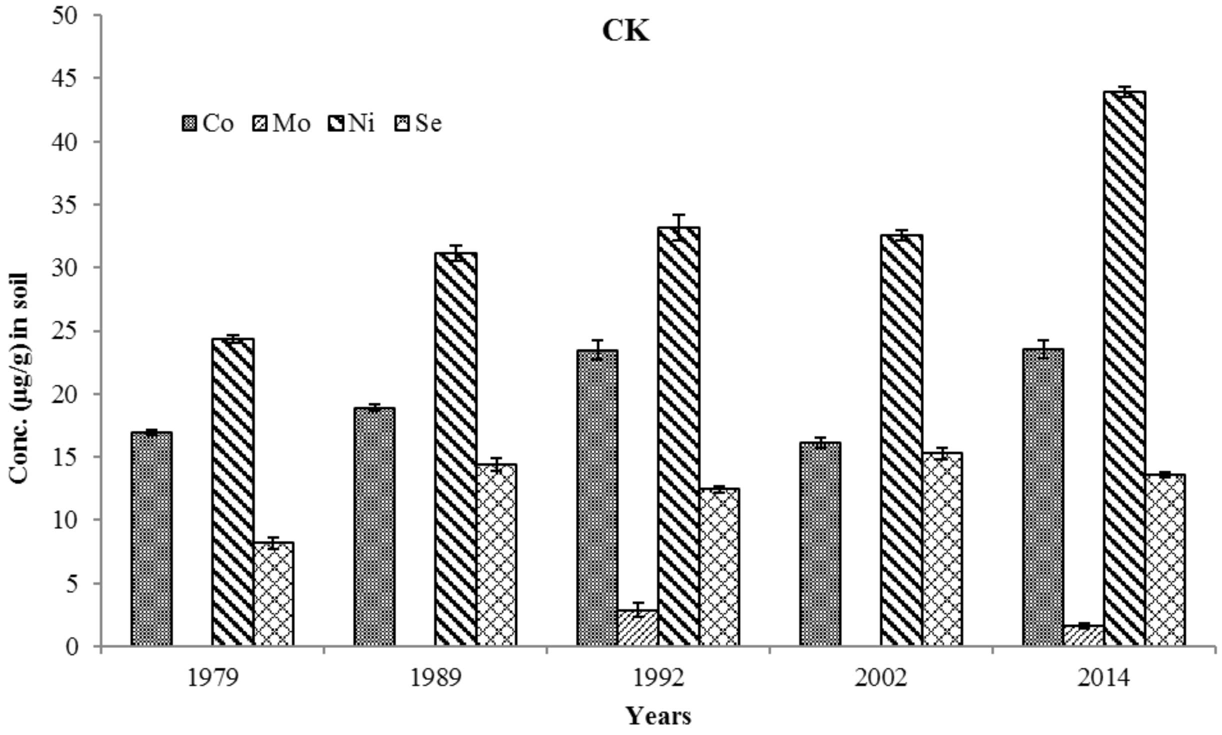

Figure 4.4: Trace elements concentrations in CK-Treatment in soil.

Table 4.2: Concentrations (µg/g) of TEs in soybean plant.

Treatments		Stem				Seed			
	/Year	Co	Mo	Ni	Se	Co	Mo	Ni	Se
N_1P	1989	0.386a	0.617	0.368c	5.252a	0.311	--	9.524b	4.453a
	2002	0.289b	--	2.144a	3.705b	--	--	8.036c	3.373b
	2014	--	--	0.568b	--	--	0.466	13.090a	--
N_1PK	1989	0.061b	--	1.468ab	--	0.050	--	8.879b	9.515a
	2002	0.320a	--	1.670a	2.245	--	--	8.049b	1.699b
	2014	--	0.636	1.375b	2.242	--	1.539	11.852a	--
N_1	1989	--	--	1.118	7.530a	0.169b	0.887	10.193a	7.250a
	2002	0.393	--	1.676	3.329b	0.232a	--	6.941b	1.828b
	2014	--	0.134	1.270	--	--	--	11.872a	--
CK	1989	--	--	1.679a	0.555	0.323b	--	5.965b	9.437
	2002	0.643a	--	0.883b	--	0.297c	--	5.182c	--
	2014	0.45b	--	--	--	0.349a	0.975	8.992a	--
M_2N_1P	1989	--	--	--	--	--	1.548b	7.282b	--
	2002	0.392	--	0.198b	--	--	--	7.049b	--
	2014	--	--	0.426a	--	--	2.300a	9.107a	--
M_2N_1PK	1989	--	--	--	2.833	0.491a	0.978b	5.3000c	4.377a
	2002	0.179	--	0.661	--	0.117c	0.630c	7.067b	2.255b
	2014	0.224	--	1.152	--	0.275b	1.628a	10.300a	--
M_2N_1	1989	--	--	--	2.808b	0.243	0.844b	5.723c	--
	2002	0.279a	--	0.962b	8.354a	0.384	0.656c	6.797b	--
	2014	0.114b	--	1.290a	--	--	1.960a	9.767a	--
M_2	1989	0.154	--	0.332b	5.777	0.255a	2.662b	4.991c	3.241a
	2002	--	--	0.252c	--	0.172b	2.771b	5.930b	2.437b
	2014	--	0.074	0.472a	--	0.278a	3.509a	6.541a	--

4.1.5 The concentration of TEs and REEs in Manure-Nitrogen-Phosphorous amended treatment (M_2N_1P)

In this treatment Co concentration in soil was significantly higher in the years 1989 and 1992. In plant, it was detected only on stem sample of the year 2002. Mo was present in soil samples of the year 1989 and 2014 at almost same concentration level, however, in seed samples of the year 1989 and 2014 at the rate of 1.548 µg/g and 2.30 µg/g respectively. Ni concentration in soil was significantly higher in the sample of the year 1992. In stem, its concentration was determined at the rate of 0.661 µg/g in 2002 and 1.152 µg/g in 2014. In seed, Ni was significantly higher in sample of the year 2014. Se was only determined in soil showing the pattern 1979 > 2002 > 2014.

In the M_2N_1P – treatment, the concentrations of Gd, Pr and Lu were significantly higher in the soil sample of the year 2014 while Tb, Y and Er in 1979, 1989 and 2002 respectively. No significant change in concentrations of La, Nd and Yb in soil was observed. In stem samples, a significant increase in concentration of La and Yb was observed in sample of the year 2002 while the concentration of Gd and Tb was significantly higher in the samples of the year 2002 and 2014 as compare to 1989. No significant change in concentrations of Pr, Lu and Y in stem was observed in the samples of the years 1989, 2002 and 2014. Nd was only detected in stem sample of the year 1989. However, La was not confirmed in the seeds in this treatment also. In seed, the concentration of Gd and Y was significantly higher in the sample of the year 1989 and a significant decrease in concentration of Lu and Yb was observed in the year 2014 while in the concentration of Pr and Tb in the year 2002. Nd and Er accumulated in seeds almost at the same rate. Nd and Y was not detected in the sample of the year 2014, however, La was not detected in seed samples of all the three years.

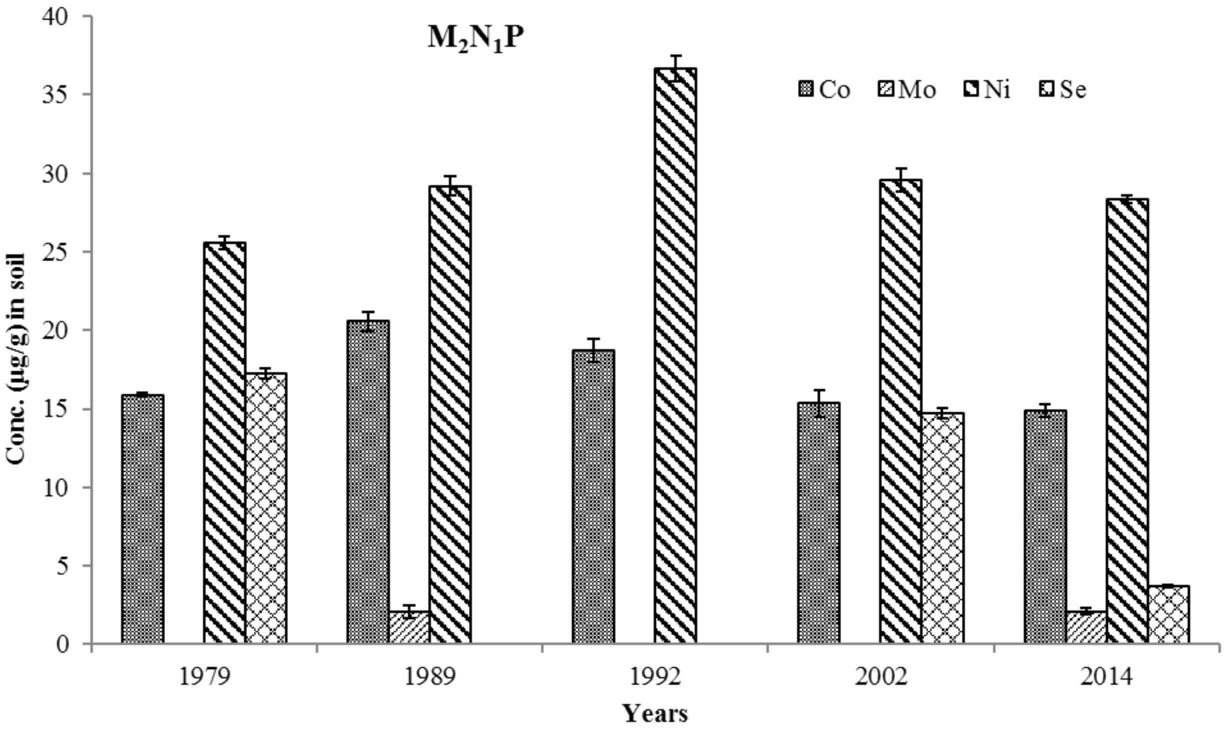

Figure 4.5: Trace elements concentrations in M_2N_1P-Treatment in soil.

Table 4.3: Concentrations (µg/g) of REEs in soil.

Treatments /	Year	Gd	La	Nd	Pr	Er	Lu	Tb	Y	Yb
N_1P	1979	5.04c	20.38b	19.09	7.12b	2.41b	0.58c	10.24b	15.12	1.98b
	1989	4.98c	21.15ab	19.08	5.81c	2.41b	0.62c	7.58c	15.1	1.95b
	1992	5.40c	21.16ab	19.01	7.81b	2.85b	0.59c	9.42b	15.16	2.35ab
	2002	6.04b	21.46a	18.5	7.26b	2.87b	0.78b	10.79b	15.37	2.25ab
	2014	11.81a	21.99a	22.85a	11.23a	3.73a	1.45a	21.66a	15.29	2.49a
N_1PK	1979	5.50b	20.34b	18.74b	6.88ab	2.33b	0.71	10.39b	15.08bc	2.07ab
	1989	5.02b	21.22a	18.92a	6.23b	2.51b	0.66	8.38c	15.22ab	1.97b
	1992	5.47b	20.71b	17.44b	7.26ab	2.56b	0.66	11.65a	14.89c	2.14ab
	2002	5.79b	21.46a	18.88a	7.72ab	2.68b	0.76	11.71a	15.33a	2.28a

	2014	7.33a	21.42a	19.06a	8.21a	3.36a	0.8	12.81a	15.17ab	2.25a
N_1	1979	4.71b	21.51	19.65	5.65c	2.31b	0.58b	10.13b	15.14a	1.93b
	1989	5.42b	21.26	19.16	6.40bc	2.64b	0.69b	9.95b	15.11a	2.02b
	1992	5.27b	21.36	19.41	8.17ab	2.78b	0.55b	10.11b	14.98ab	2.01b
	2002	5.14b	21.19	19.92	7.00abc	2.56b	0.57b	9.47b	14.90b	2.01b
	2014	8.27a	21.4	19.16	8.49a	3.47a	0.86a	12.80a	15.03ab	2.28a
CK	1979	5.84bc	20.48b	19.13	6.78c	2.91b	0.78bc	10.58d	15.2	2.17
	1989	5.12c	21.54ab	19.69	6.78c	2.87b	0.65c	9.64e	15.46	2.16
	1992	6.44b	21.68ab	19.87	8.25b	3.17b	0.93b	12.95b	15.53	2.33
	2002	5.62bc	21.27b	21.1	8.00bc	2.95b	0.67c	11.36c	15.52	2.19
	2014	10.20a	22.59a	20.59	11.66a	4.37a	1.71a	19.63a	15.68	2.87
M_2N_1P	1979	4.70bc	21.35	18.23	7.01ab	2.05c	0.58c	10.32a	14.92b	1.96
	1989	4.62c	21.34	18.95	6.63ab	3.01ab	0.58c	9.65b	15.70a	2.05
	1992	5.18abc	21.81	20.13	6.90ab	2.79b	0.68bc	9.33b	15.58ab	2.17
	2002	6.12ab	21.33	20.22	6.15b	3.44a	0.77ab	9.09bc	15.45ab	2.26
	2014	6.29a	22.23	20.04	7.71a	3.02ab	0.88a	8.51c	15.60ab	2.48
M_2N_1PK	1979	5.35	21.00b	18.63b	6.13bc	2.37	0.63	10.67b	14.93b	2.07ab
	1989	5.26	21.26ab	19.84a	6.60bc	2.83	0.71	9.34c	15.41a	2.18ab
	1992	5.9	21.54a	19.56a	7.48ab	2.94	0.76	12.53a	15.25a	2.22a
	2002	5.25	21.10ab	19.69a	5.39c	2.77	0.67	9.26c	15.24ab	2.03b
	2014	5.47	20.75b	17.22c	8.23a	2.77	0.75	9.71bc	15.12ab	2.06ab
M_2N_1	1979	5.32b	21.32	19.04bc	6.40bc	2.74bc	0.66b	10.42c	15.24ab	2.04b
	1989	4.66b	21.06	19.61ab	5.04c	2.35c	0.85b	6.58e	15.02ab	1.94b
	1992	5.75b	20.97	20.11a	7.23b	3.15b	0.70b	11.14b	15.26a	2.23ab
	2002	5.12b	21.06	18.50c	6.08bc	2.49c	0.71b	9.47d	14.94b	2.06b
	2014	10.85a	21.42	20.33a	12.61a	5.03a	1.59a	23.58a	15.27a	2.47a
M_2	1979	5.68	21.35a	18.94bc	6.59ab	2.75a	0.71	10.43a	15.1	2.14
	1989	5.19	21.53a	20.18ab	5.61b	2.57a	0.58	7.98b	15.47	2.08
	1992	5.45	20.54b	20.54a	7.60a	2.83a	0.73	10.41a	15.08	2.17
	2002	4.79	21.15a	18.58c	6.78ab	1.61b	0.65	8.79b	15.1	2.08
	2014	5.42	21.17a	18.23c	7.55a	2.72a	0.71	8.60b	15.11	2.05

4.1.6 The concentration of TEs and REEs in Manure-Nitrogen-Phosphorous-Potassium amended treatment (M_2N_1PK)

Here the concentration of Co in soil showed same pattern as in M_2N_1P-treatment. It was determined in stem samples of the years 2002 and 2014 but no significant difference was observed. In seed, the concentration of Co was significantly higher in 1989. Mo was determined in the soil sample of the years 1989, 2002 and 2014 and significantly lower in 2002. In plant, Mo was determined only in seeds with significantly higher concentration in 2014. Ni showed almost the same pattern of accumulation in both soil and plant samples as in M_2N_1P-treatment. Se concentration was significantly higher in the soil sample of the year 2002. It was not detected both in soil and plant samples of the years 1992 and 2014, however, in stem sample of the year 1989 at the rate of 2.822µg/g only. In seed, it was at the rate of 4.377 µg/g and 2.255 µg/g in 1989 and 2002 respectively.

In the M_2N_1PK-treatment the concentration of Gd, Er, and Lu was not significantly changed, however the concentration of Nd, Pr, Tb, Y and Yb were significantly higher in the soil sample of the year 1992. In stem samples, all REEs showed the same behavior as in M_2N_1P-treatment except La and Nd. La was present only in stem sample of the year 2014, however, Nd was detected also in the sample of the year 2014 but significantly lower in concentration as compare to sample of the year 1989. In seed samples, all REEs showed the same behavior as in M_2N_1P-treatment except Nd, and Er. Nd was detected only in the seed sample of the year 2002 while Er concentration was significantly increased in this year. However, La was only detected in stem sample of the year 2014.

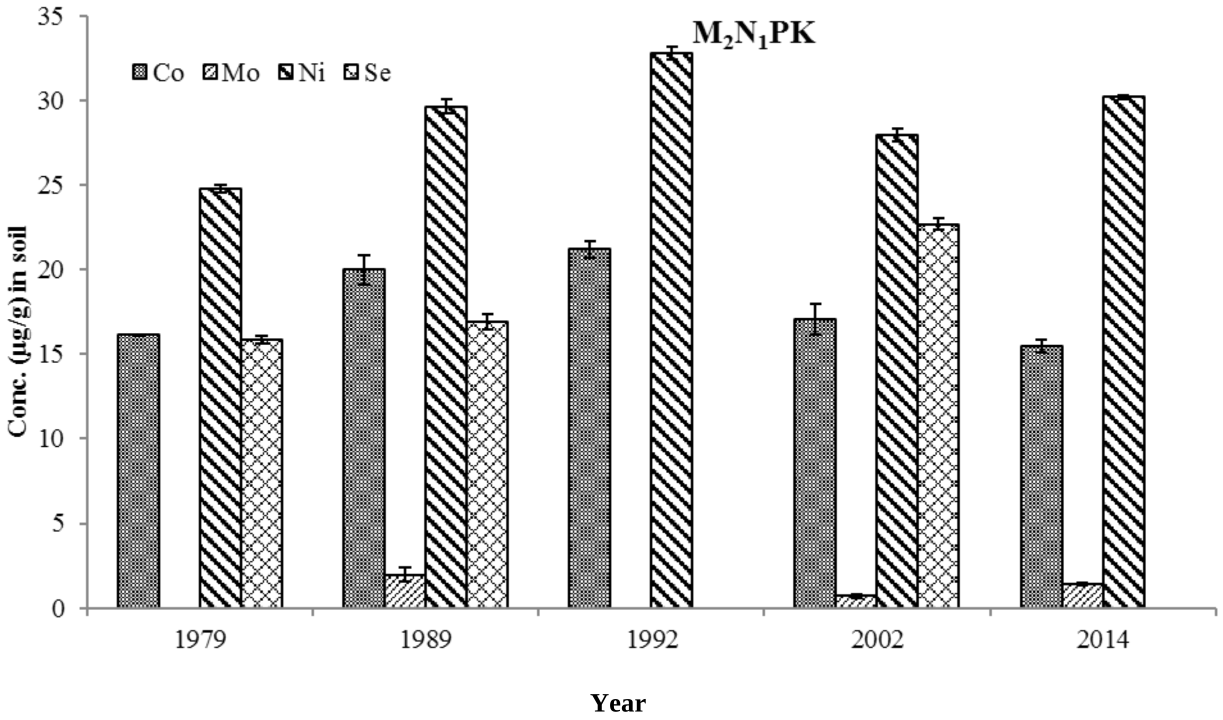

Figure 4.6: Trace elements concentrations in M$_2$N$_1$PK-Treatment in soil.

Table 4.4: Concentrations (µg/g) of REEs in soybean stem

Treatments /Year		Gd	La	Nd	Pr	Er	Lu	Tb	Y	Yb
N$_1$P	1989	4.547	0.285	--	7.839a	1.822a	0.223b	1.118	0.034	1.613b
	2002	4.454	0.145	0.378	6.669b	1.972a	0.251a	1.417	0.037	1.637a
	2014	4.446	0.169	0.514	6.362b	1.591b	0.129c	1.256	0.035	1.526c
N$_1$PK	1989	4.511	0.119b	0.837a	7.695a	1.972a	0.281	1.831a	0.148a	1.650
	2002	4.576	0.119b	0.127c	6.935b	1.939a	0.237	1.190b	0.053c	1.645
	2014	4.542	0.241a	0.664b	7.7229ab	1.633b	0.250	1.147c	0.138b	1.649
N$_1$	1989	4.630a	0.239b	1.390a	7.804a	1.770b	0.233b	1.297a	0.118b	1.618a
	2002	4.504b	0.173c	0.430b	6.670b	1.852a	0.258a	1.468a	0.119b	1.621a
	2014	4.458b	0.430a	0.498b	6.436c	1.631c	0.229b	0.868b	0.162a	1.590b
CK	1989	4.219c	0.208c	1.265a	7.557a	1.877a	0.228ab	0.341c	0.092c	1.618

	2002	4.565a	0.239b	0.475b	7.644a	1.765a	0.247a	1.551a	0.119a	1.627
	2014	4.445b	0.287a	0.109c	6.494b	1.542b	0.218b	0.935b	0.108b	1.591
M_2N_1P	1989	4.259b	0.049b	1.263	7.067	1.890a	0.246	0.263b	0.456	1.626ab
	2002	4.378a	0.181a	--	7.097	1.609b	0.260	0.871a	0.437	1.658a
	2014	4.409a	0.040c	--	6.846	1.520b	0.206	1.046a	0.663	1.578b
M_2N_1PK	1989	4.196b	--	1.219a	6.535	1.924a	0.223	0.259c	0.264	1.623a
	2002	4.464a	--	--	6.482	1.595b	0.220	0.692b	0.024	1.625a
	2014	4.457a	0.134	0.603b	6.456	1.645b	0.214	0.948a	0.055	1.585b
M_2N_1	1989	4.238b	0.312a	0.949a	6.843	1.952a	0.265a	0.509c	0.067	1.622a
	2002	4.421a	0.215b	0.164b	6.917	1.462b	0.225b	0.647b	0.052	1.618a
	2014	4.417a	--	0.123c	6.833	1.505b	0.199b	0.861a	0.040	1.567b
M_2	1989	4.218b	--	1.495a	6.397	1.892a	0.254	0.273c	0.036a	1.621a
	2002	4.526a	0.192a	0.544b	6.806	1.582b	0.243	0.550b	0.013c	1.632a
	2014	4.427a	0.060b	0.420b	6.877	1.623b	0.215	0.846a	0.028b	1.580b

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4.1.7 The concentration of TEs and REEs in Manure-Nitrogen amended treatment (M_2N_1)

Cobalt concentration was significantly higher in the soil sample of the year 2014. Its concentration in stem was 0.279 µg/g in 2002 and 0.114 µg/g in 2014. However, in seed it was 0.243 µg/g in 1989 and 0.384 µg/g in 2002. Mo was absent in the sample of the year 1979, however, no significant difference was seen in other soil samples. In plant, Mo was determined only in seeds with significantly higher concentration in 2014. Ni concentration was significantly higher in soil and Plant (stem and seed) samples of the year 2014. Se was significantly higher in 1979 after that no significant difference was observed in soil. It was detected only in stem samples of the years 1989 and 2002 at the concentration level of 2.808 µg/g and 8.35 µg/g respectively.

In M_2N_1-treatment, the concentrations of all REEs were significantly higher in the soil sample of the year 2014 except La. In stem, the concentration of La, Nd, Er, Lu and Yb was significantly higher in the sample of the year 1989. The concentration of Gd and Tb was significantly higher in the sample of the year 2014. In seed, the Gd, Pr, Lu, Tb, Y and Yb concentrations were significantly high in the sample of the year 1989.

However, Nd and Er were bio-accumulated in seed at significantly higher concentration rate in sample of the year 2002. Nd and Y were not detected in sample of the year 2014.

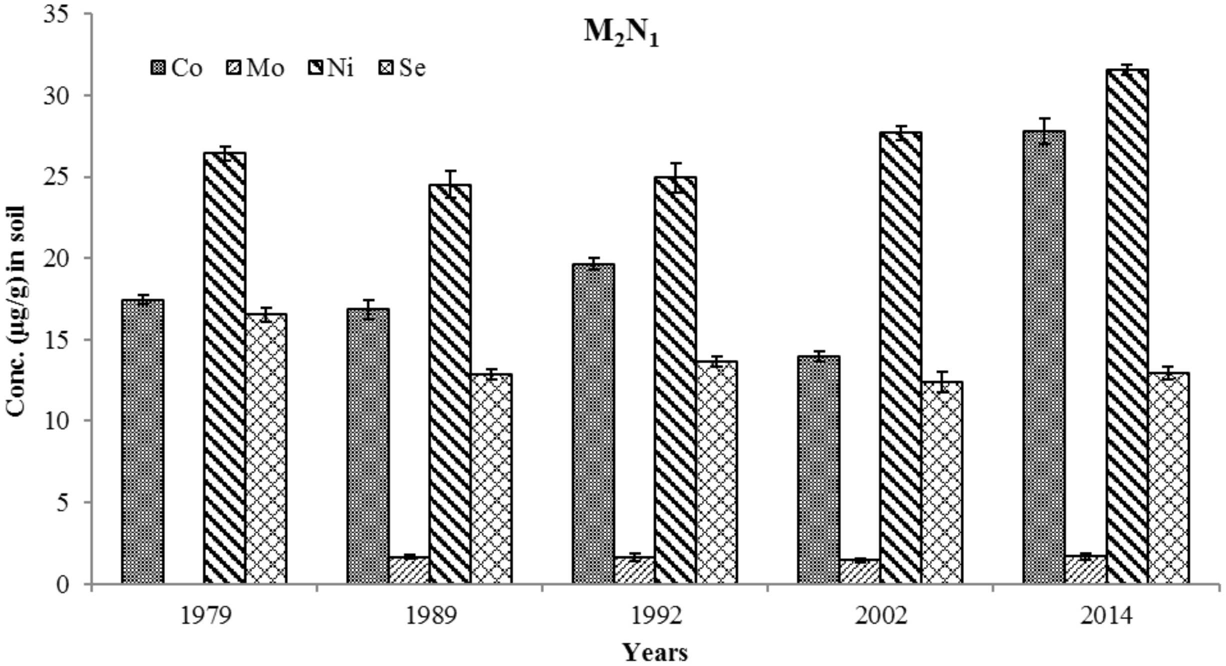

Figure 4.7: Trace elements concentrations in M_2N_1-Treatment in soil.

4.1.8 The concentration of TEs and REEs in Manure amended treatment (M_2)

In M_2-treatment the Co concentration was significantly higher in soil sample of the year 1989 and only detected in the same year's stem sample. In seed, it was in the pattern of 0.255 µg/g, 0.172 µg/g and 0.278 µg/g in the 1989, 2002 and 2014 respectively. Mo was detected in the soil samples of the year 1989, 2002 and 2014 and significantly higher in 2014. In stem sample of the year 2014, 0.074 µg/g of Mo was measured only. However, it was significantly higher in the seed sample of the year 2014. Ni showed same pattern of accumulation in both soil and plant samples like in M_2N_1-treatment. Se was significantly higher in the soil samples of the years 1989 and 2002, however, in stem sample of the year 1989 at the rate of 5.777 µg/g only. In seed, it was at the rate of 3.241 µg/g and 2.437 µg/g in 1989 and 2002 respectively.

In M_2-treatment, no significant difference in concentration of the Gd Lu, Y and Yb in soil was observed in the whole period of long term application of fertilizers. The conc, of Nd and Tb was significantly higher in the soil sample of the years 1992. However the concentration of La, Pr and Er was significantly decreased in the soil samples of the years 1992, 1989 and 2002 respectively.

The Gd, Nd, Pr, Er, Tb and Yb showed same behavior as in M_2N_1-treatment in stem samples of all the three years. La and Y concentration were significantly higher in the stem samples of the years 2002 and 1989 respectively. The concentrations of all REEs were significantly higher in the seed sample of the year 1989 and decreased gradually over the years except Er.

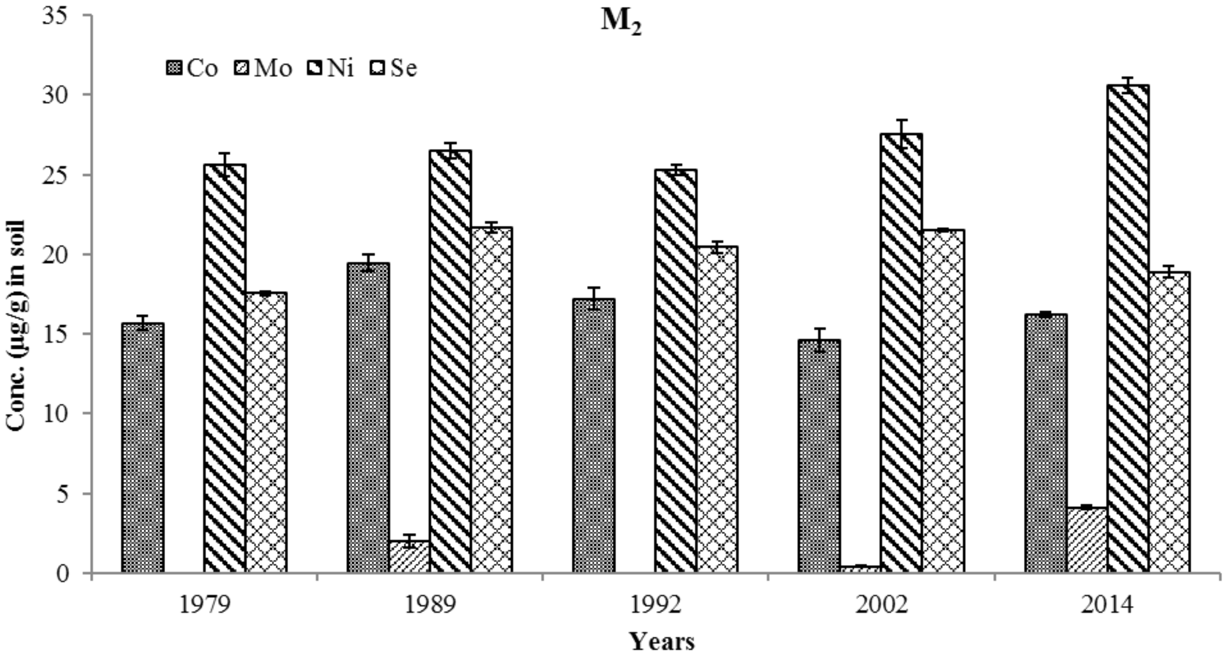

Figure 4.8: Trace elements concentrations in M_2-Treatment in soil.

Table 4.5: Concentrations (µg/g) of REEs in soybean seed.

Treatments /	Year	Gd	La	Nd	Pr	Er	Lu	Tb	Y	Yb
N_1P	1989	4.703a	--	1.553	7.482a	1.895	0.279	1.370a	0.077a	1.661
	2002	4.292b	--	--	6.807b	1.908	0.233	0.394b	0.002b	1.611
	2014	4.379b	--	--	7.095b	1.892	0.200	1.263a	0.001b	1.566
N_1PK	1989	4.553a	--	0.208b	7.841a	1.749b	0.218a	1.452a	0.027a	1.616a

	2002	4.270b	--	1.316a	6.787b	1.847a	0.226a	1.269b	0.024a	1.618a
	2014	4.290b	--	--	6.830b	1.755b	0.139b	1.527a	--	1.514b
N_1	1989	4.621a	--	0.667a	7.652a	1.681c	0.230a	1.346a	0.063a	1.609ab
	2002	4.177c	--	0.153b	6.353b	1.942a	0.232a	0.532b	0.044b	1.659a
	2014	4.351b	--	0.162b	6.414b	1.868b	0.142b	1.186a	--	1.517b
CK	1989	4.582a	--	0.253b	7.827a	1.791b	0.209a	1.410a	0.098a	1.609a
	2002	4.300b	0.027	0.636a	6.424c	2.034a	0.213a	1.037c	0.034b	1.612a
	2014	4.359b	--	--	6.573b	1.830b	0.144b	1.242b	--	1.527b
M_2N_1P	1989	4.610a	--	0.231	7.927a	1.754	0.209a	1.423a	0.080a	1.613a
	2002	4.345b	--	0.228	6.302b	1.907	0.210a	0.704b	0.023b	1.604a
	2014	4.409b	--	--	7.262a	1.859	0.144b	1.267a	--	1.525b
M_2N_1PK	1989	4.517a	--	--	7.543a	1.800b	0.227a	1.389a	0.076	1.604b
	2002	4.204b	--	0.405	6.041c	2.116a	0.205a	0.711b	0.023	1.624a
	2014	4.291b	--	--	7.034b	1.703b	0.146b	1.175a	--	1.522c
M_2N_1	1989	4.557a	--	0.015b	7.517a	1.872b	0.228a	1.421a	0.084a	1.606a
	2002	4.388b	--	0.252a	6.376c	2.052a	0.212a	1.291b	0.006b	1.610a
	2014	4.393b	--	--	6.573b	1.710c	0.148b	1.317b	--	1.523b
M_2	1989	4.513a	--	0.459a	7.549a	1.809	0.225a	1.376a	0.057a	1.614a
	2002	4.279b	--	0.248b	6.647b	1.867	0.227a	1.251b	0.017b	1.608a
	2014	4.375ab	--	0.239b	6.682b	1.745	0.149b	1.366a	--	1.523b

The harvested soil samples of the years 2013 and 2014 were analyzed to determine the concentrations of trace elements and rare earth element and the results were: 1) In N_1P – treatment the concentrations of all the measured elements were increased except selenium that was decreased. The significant increase in the concentrations of Co, Ni, Gd, Nd, Pr, Er and Tb was observed. Before planting soybean, the Mo was not detected in soil sample however, in harvested soil sample about 2.69 μg/g was calculated. 2) In N_1PK- treatment, almost the same pattern of accumulation of these elements was observed as in N_1P- treatment. However, Co and

Tb were accumulated in lower quantity in this treatment. 3) In N_1-treatment the accumulation pattern of trace elements and rare earth elements was same as observed in N_1PK- treatment except Se which was significantly decreased. 4) In CK-treatment almost more than 100% increase in concentrations of Co, Ni, Gd, Pr, Er, Lu and Tb was observed and the concentrations of Mo,La, Nd, Y and Yb were also slightly increased Except Se that was decreased.

5) In M_2N_1P- treatment, the Ni concentration was significantly increased and the concentration of Se was significantly decreased. A slight increase in concentrations of the other elements is observed. 6) In M_2N_1PK- treatment, the concentrations of all the elements were decreased slightly except Ni that was increased. The Se was totally absent in the harvested soil sample. 7) In M_2N_1-treatment, the concentrations of Co, Ni, Gd, Pr, Er, Lu and Tb were significantly increased while, the La, Nd, Y and Yb increased slightly. The Se concentration was significantly decreased however, Mo concentration was little decreased. 8) In M_2- treatment, the concentrations of Co, Mo, Gd, La, Pr, Er, Lu, Tb and Y were increased, however, Ni, Se, Nd and Yb concentrations were decreased.

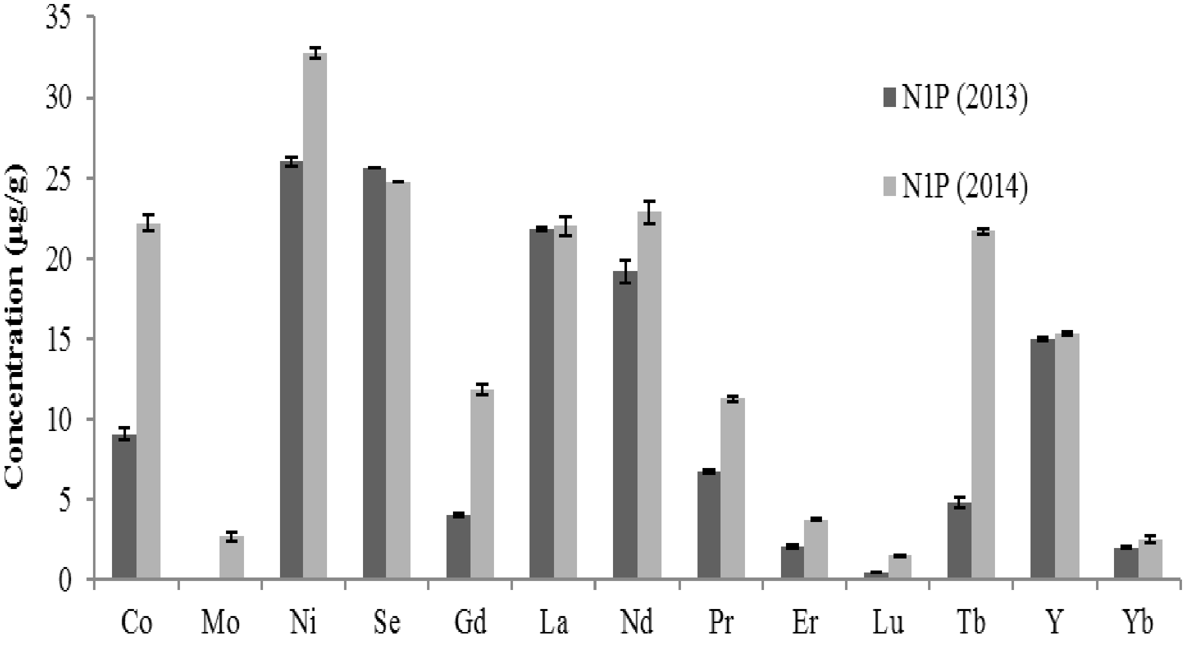

Figure 4.9: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of N_1P-treatment.

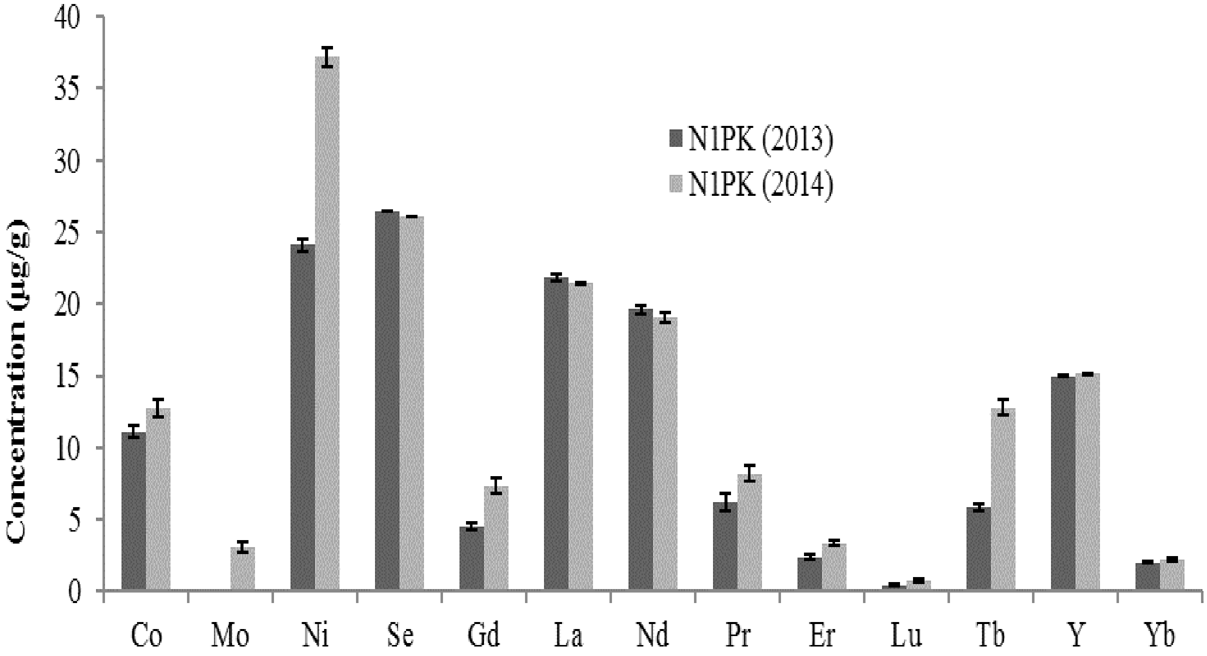

Figure 4.10: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of N_1PK-treatment.

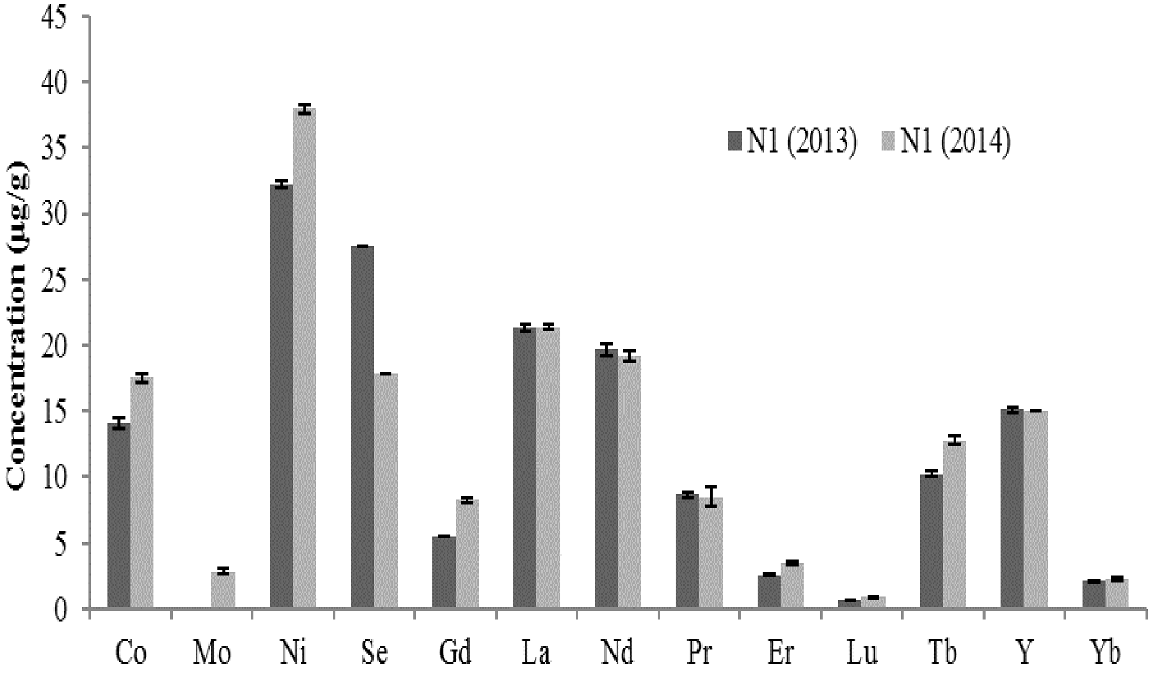

Figure 4.11: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of N_1-treatment.

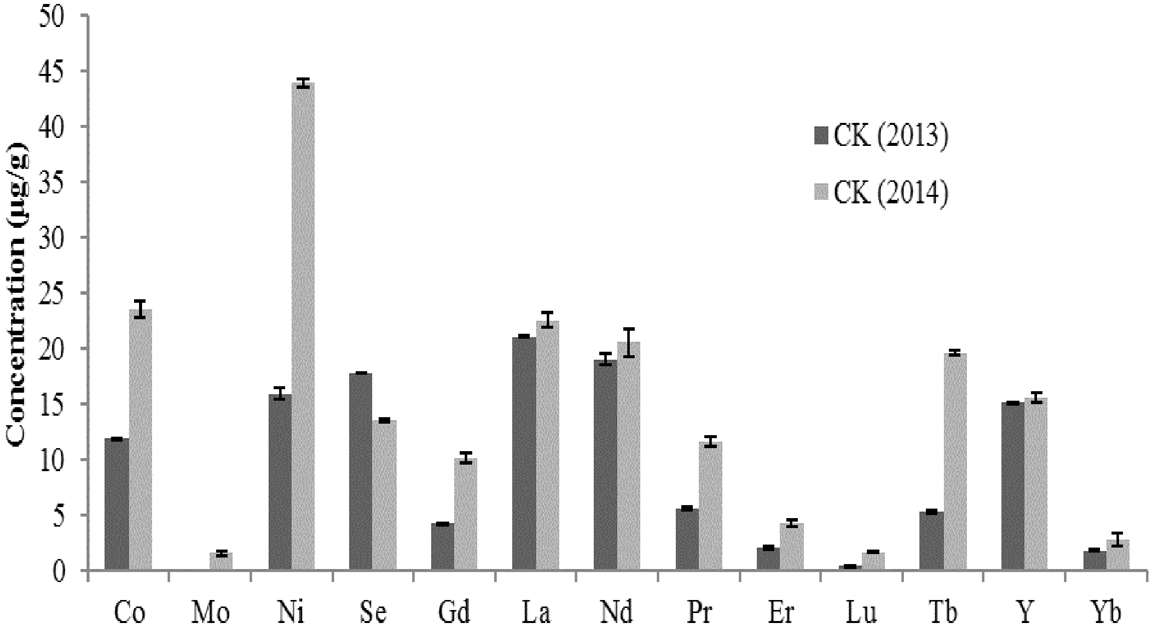

Figure 4.12: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of CK- treatment

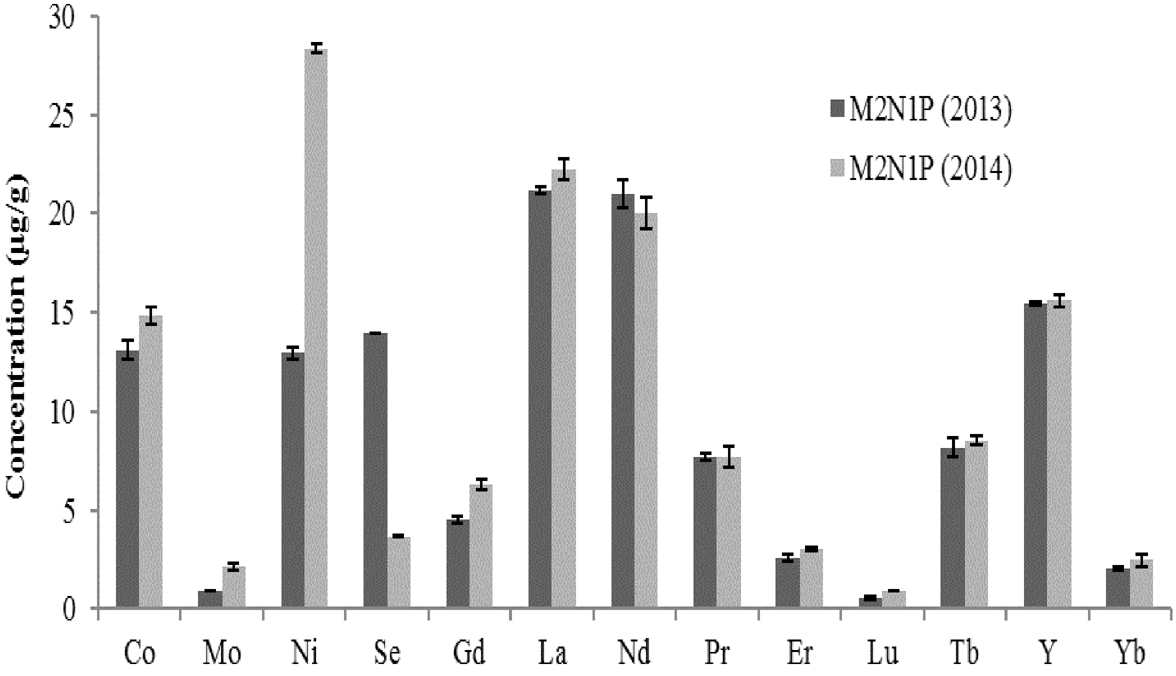

Figure 4.13: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of M_2N_1P-treatment.

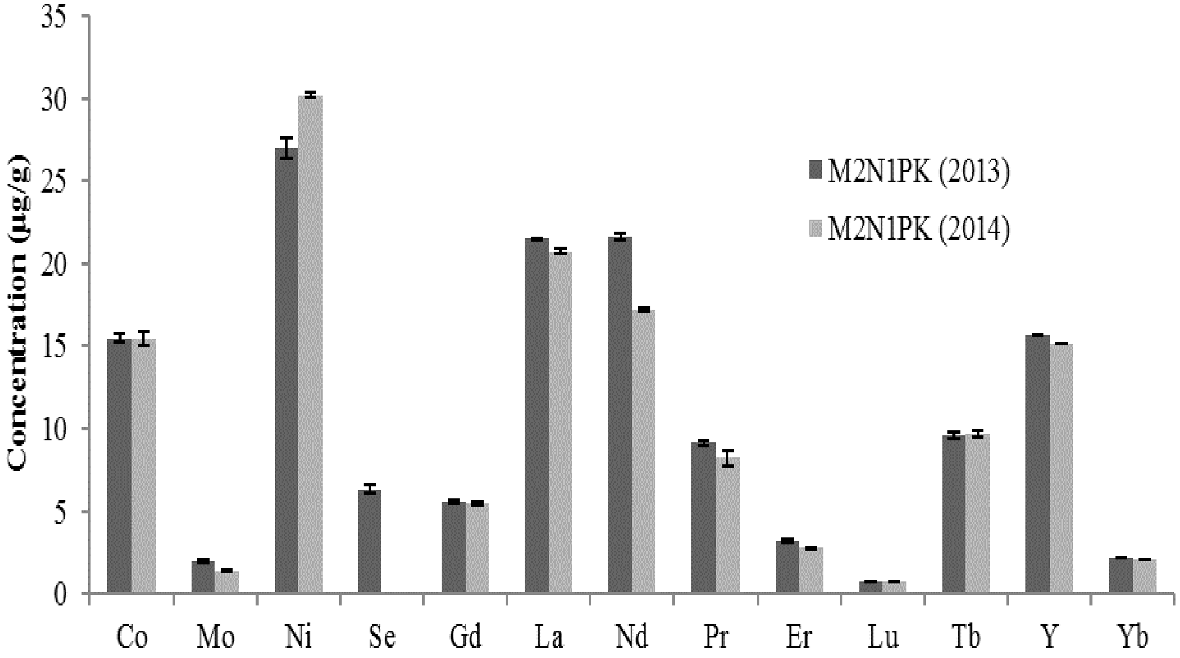

Figure 4.14: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of M_2N_1PK-treatment.

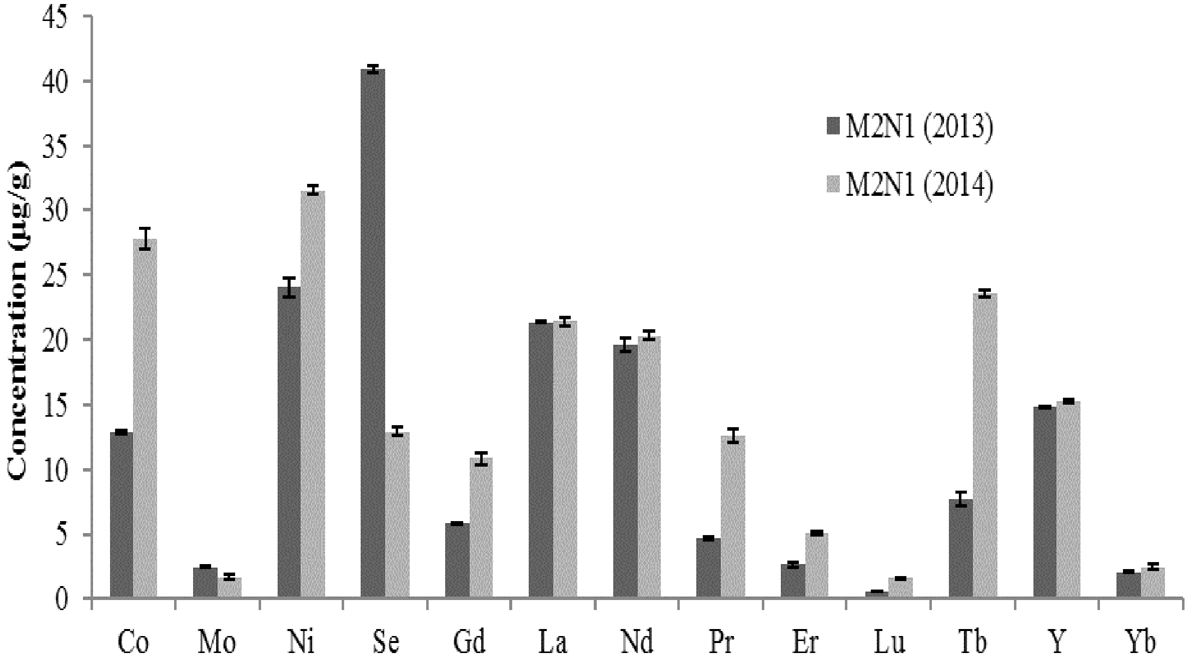

Figure 4.15: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of M_2N_1-treatment.

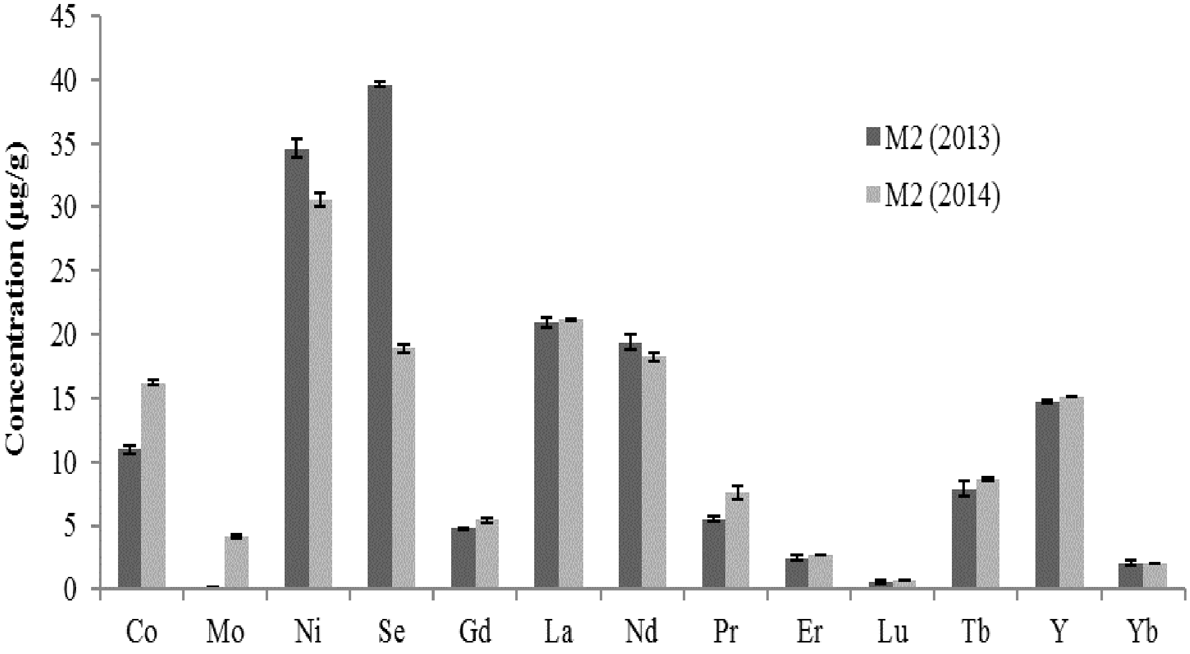

Figure 4.16: TEs and REEs concentrations in soil samples of the years 2013 and 2014 of M_2-treatment.

4.2 Discussions

Industrialization and urban development elevate the level of TEs and REEs in environments (Adriano, 2001). Accumulation of trace and rare earth elements in soil and uptake and bio-accumulation in plant is influenced by soil pH, CaCO3 contents, crop rotation, plant species, cultivar and fertilization approaches like use of compost and manure instead of inorganic fertilizers and avoid using heavy metal enriched pesticides. Bioavailability, solubility, transportation and concentration level of TEs and REEs is affected by the factors mentioned above (Abdel-Haleem et al., 2001; Adriano, 2001; Avci and Deveci, 2013; He et al., 2005; Otero et al., 2005; Smith, 2009). It is also reported by some researchers that root development and growth of plant is restricted by the high concentrations of the trace elements (Gisbert et al., 2006; Parra et al., 2014; Shi et al., 2011; Zheljazkov et al., 2006).

In our study, it is reflected from the result that the overall accumulation of trace elements concentrations level pattern was in soil as Ni > Co > Se > Mo and rare earth elements were in the pattern La > Nd > Tb > Y > Pr > Gd > Er > Yb > Lu.

In stem the TEs and REEs concentrations level patterns were Se > Ni > Co > Mo and Pr > Gd > Er > Yb > Tb > Nd > Lu > La > Y respectively. However, Ni > Se > Mo > Co and Pr > Gd > Er > Yb > Tb > Nd > Lu > Y > La pattern was seen in seeds. The ability of Co, Mo, Ni, Se, Gd, Nd, Pr, Er, Lu, Tb, Y and Yb to accumulate in soil and plant parts in our study was ranked as Soil > Seed > stem and that of La was Soil > stem. However, the Se, Nd, Y and Yb showed soil > stem > seed coinciding with literature reported by (Ding et al., 2006; Wang et al., 2001). The organic labile compounds present in pig manure form soluble organic ligands. The increments in accumulation of some trace elements in plants maybe as a result of the formation of these organic ligands (Parra et al., 2014).

Cobalt plays and important in Nitrogen fixation especially in legume cultivar (Adriano, 2001), whereas its concentration is lower in cereals and grasses as compare to leafy plants like spinach, cabbage and lettuce. Mo plays a critical role to reduce nitrogen to nitrate during absorption by plants and its average concentration in food plants 0.04 to 1.0 mg/kg is reported. The relative higher Mo concentration in plant resulted in its foliar application also. Ni is available in abundance in environment. It is naturally bio-accumulate at higher concentration level in many plants to their tissues. Ni concentration in plant tissues depends upon the available form and soil properties (Avci and Deveci, 2013; Yusuf et al., 2011). It is reported that SOC affect the trace elements availability and speciation and uptake by plant (Cloutier-Hurteau et al., 2014). In our study the measured Ni concentration was lined with the range reported by the Lorenz and Lal. (2009) (Lorenz and Lal, 2009), Ajmone-Marsan and Biasioli (2010) (Ajmone-Marsan and Biasioli, 2010) and Vasenev et al. (2013) (Vasenev et al., 2013) in soil and higher in concentration level than the WHO/EU (1983) (WHO/EU, 1983) and FAO/WHO (2001) (FAO/WHO, 2001; Wu et al., 2014) limits (0.2 mg/kg) in plant samples, may be due to the different uptake characteristics (Kabata-Pendias and Mukherjee, 2007). Se is present in environment in Se^{-2}, Se^{0}, Se^{4+} and Se^{6+} oxidation states. It is present in one of the volatile organic compound formed in plants escaped through leafs. Selenite (SeO_3^{2-}) has strong adsorption affinity then selenite to different soil components and reduce the bioavailability of Se^{4+}, so more Se accumulate in plant from selenate compounds (Kamei-Ishikawa et al., 2007; Songshan et al., 2010; Su and Suarez, 2000; Wang et al., 2012; Zhu et al., 2009).

Plants uptake selenite as such through root and transport to stem while selenite is converted into organic compound and bio-accumulated in roots (Li et al., 2008; Terry et al., 2000; Zayed et al., 1998). Se transportation within plant is also affected by climate conditions like humidity, temperature and soil moisture indirectly by affecting the soil chemical and physical properties that determined it concentration in plants (De Temmerman et al., 2014b; Spadoni et al., 2007).

The rare earth elements are almost evenly distributed in soybean plant except La which is accumulated only in stem of the soybean plant. In literature, the pH dependent accumulation behavior of REEs has been elaborated. The cell wall structure is changed due to change in osmotic pressure in plant cells with soil pH. The REEs could barely cross the cell wall to enter in the plant cells in acidic soil resulting in lower accumulation in stem and seed (Zhang and Shan, 2001b).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

The result based conclusion drawn from this study is, the higher concentrations of Se, Nd, Y, and Yb were found in soil. These elements were higher in soybean stem followed by the seed. However these elements are within safe toxic level and light pollution level. It is also suggested that this data can be analyzed through multivariable statistics sufficiently to determine the difference between treatments regarding the contribution of fertilizers to the accumulation of related soil elements to improve the biological effectiveness.

It is also concluded that long-term annual application of mineral and organic fertilizers changes the pH, SOC and concentrations of trace and rare earth elements in agricultural soil. Mineral and organic fertilizers increase the micronutrients concentration in plants and yields. The combined use of mineral and organic fertilizer is an applied technique for green environment in China (Ming-gang et al., 2008). However, the mechanism is still unclear.

The literature showed that Se bio-accumulation in plants and its concentration in soil has a relationship but contrary results were observed in our study which is due to the pH, SOC and cultivar factors. The Se uptake is increased at higher pH. It also varies among plant species and SOC concentration levels. Mo concentration in plant can be increased in soybean seed by foliar application at different plant stages with a suitable spraying interval (Campo et al., 2009). This study reveals that the REEs were present more abundantly in soil as compare to bio-accumulated in plants reflecting that these are not essential for living organisms. Despite of that at low concentration, they speed up metabolism.

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ACKNOWLEDGEMENT

All praise and thanks to Almighty Allah Who is the source of all knowledge and wisdom. The most Gracious, The Merciful and Beneficent. I also send Darood to the Holy Prophet Muhammad (PBUH) who is Allah’s messenger to guide us in affairs of life by his examples and percepts.

I express my thanks and gratitude to my research advisor, Prof. Dr. Han Xiaori, for his valuable suggestions and friendly attitude. His constructive criticism helped me to improve understanding towards the research project and developed confidence to take such initiative.

I would also like to pay sincere thanks to honorable teachers of college of Land and Environment including Liu Xiaohu，Yang Junfeng, Zhan Xiumei, Liu Houjun, Wang Yue, Li Na, Liu Yifei, Luo Peiyu, Dang Xiuli, and colleagues especially Fan Shumao, Peng Jing, Zhao Enfeng, Zhang Junwen, Li Xueping, and sincere friends for their cooperation, coordination, continuous encouragement and moral support.

My heartfelt thanks to my family, my brother Dr. Muhammad Irfan, my country mates Mr. Tasawar Ahsan and Mr. Jamil Shafi and international colleagues and batch fellows Miss Roseline Love Macarthur, John Farmer, Mohammad Ali Abdullah Hamad ElNeil and Jamilu Ya’u for helping supporting me during my study and stay in China.

Muhammad Imran Ashraf

PUBLICATIONS

1. **Muhammad Imran Ashraf**, Li Na, Han Xiaori, Yang Jinfeng, Wang Yue, Fan Shumao, Muhammad Irshad, Qaisar Mahmood, (2017). *Temporal Changes in trace elements in brown soil and soybean after long term fertilization.* Arabian journal of geosciences, 10:289.
2. **Muhammad Imran Ashraf**, Muhammad Ateeb, Muhammad Hussnain Khan, Naseer Ahmed, Qaisar Mahmood, Zahidullah, 2016. *Integrated treatment of pharmaceutical effluents by chemical coagulation and ozonation.* Separation and Purification Technology, 158: 383-386.
3. Ning Liu, Chongjun Zhou, Shifeng Fu, **Muhammad Imran Ashraf**, Enfeng zhao, Hui Shi, Xiaori Han, Hongzhibai, 2013. *Study on Characteristics of Ammonium Nitrogen Adsorption by Biochar Prepared in Different Temperature.* Advanced Materials Research Vols. 724-725. pp 452-456.

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