REMOVAL OF SOME HEAVY METALS FROM CONTAMINATED SOILS USING TWO COMPLEXING AGENTS

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BY

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NOVEMBER, 2016

DECLARATION

I declare that the work in this dissertation entitled **'Removal of some heavy metals from contaminated soils using two complexing agents'** has been performed by me in the department of chemistry, under the supervision of Professor E.B. Agbaji and Professor V.O. Ajibola. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this research was previously presented for another degree or diploma at any university or institution.

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Name of Student	Signature	Date

CERTIFICATION

This project dissertation entitled **'Removal of some heavy metals from contaminated soils using two complexing agents'** by Sani Yakubu meets the regulation governing the award of the degree of MSc. in analytical chemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the Almighty GOD, members of my family and all my friends.

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ABSTRACT

A study on the removal of heavy metals from polluted site where metal-works activities are carried out was studied. The soil samples were treated separately with aqueous solution of two complexing agents – namely, tiron and glutamic acid to determine the removal efficiency of heavy metals. Before treatment with the complexing agents, the soils showed remarkably high levels of heavy metals above background concentrations. The distribution pattern were in the following order: Pb> Zn> Cr> Cd> Ni. Across all the sampling locations, Pb showed the highest (199.97 mg Kg^{-1}) mean concentration and Cd (1.35 mg Kg^{-1}) the least mean concentration respectively. Key factors that determine the absorption of heavy metal by complexing agents such as pH, concentration, amount of soil, and contact time were considered and the process optimized by the application of 2^4 factorial design of experiment. Results indicated optimum removal of Cr 98.81%, Ni 86.00%, Pb 76.18%, Zn 62.63% and Cd 83.95% using tiron while the optimum absorption for glutamic acid was 37.68% Cd, 56.41% Cr, 37.68% Ni, 1.81% Pb, and 63.23% Zn respectively. Optimum removal Cr 98.81% was obtained at pH 3, 25 mg dm^{-3}, 10g amount of soil and 60 minutes contact time. The optimum removal of Ni 86.00% obtained at pH 5, 25 mg dm^{-3}, 10g amount of soil and 60 minutes contact time. Optimum removal of Pb 76.16% was obtained at pH 5, 10 mg dm^{-3}, 5 g amount of soil and 60 minutes contact time. Zn 62.63% was obtained at pH 3, 5g amount of soil and 60 minutes contact time. Cd 83.95% was obtained at pH 5, 25 mg dm^{-3}, 10 g amount of soil and 30 minutes contact time using tiron. This indicates a good yield when compared to other used chelating agents like EDTA, EDDS, and NTA. Optimum removal of glutamic acid was Cr 99.98%, Ni 53.26%, Pb 99.99%, Zn 99.98% and Cd 99.98%.Cd 99.98% obtained at pH 5, concentration of 10 mg dm^{-3}, 10g amount of soil, and 60

minutes contact time. Cr 99.98% obtained at pH 5, concentration of 25 mg dm^{-3}, 5 g amount of soil, and 60 minutes contact time. Ni 53.26% obtained at 25 mg dm^{-3} concentration, 10 g of soil, at pH 3, and 60 minutes contact time. The optimum removal of Pb was 99.99% at pH 3, 10 mg dm^{-3}, 10 g amount of soil, and 60 minutes contact time. Optimum removal of Zn was 99.98% achieved at 25 mg dm^{-3} concentration of glutamic acid, 10 g of soil, 30 minutes contact time, and pH 5. The results of cube plots to show the interaction of the absorption process by factorial design of experiment indicate 83.22% Cr, 9.16% Cd, 61.03% Ni, 69.86% Pb and 59.32% Zn removal by tiron. In conclusion, the results from this study suggested that tiron and glutamic acid have high removal efficiency. However, glutamic acid is more promising extracting agent than tiron.

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ABBREVIATIONS AND SYMBOLS

C.V	-	Coefficient of variation
CL	-	Confidence Limit
DPTA	-	Diethylenetriamine penta acetic acid
EDTA	-	Ethylenediammine tetra acetic acid
EDDS	-	N, N'- Ethylenediamine disuccinic acid
GPS	-	Global Positioning System
IDSA	-	Iminodisuccinic acid
MGDA	-	Methylglycinediacetic acid
NTA	-	Nitrilotriacetic acid
Prob.	-	Probability
R^2	-	Regression Correlation
R^2adj	-	Adjusted Regression Correlation

CHAPTER ONE

1.0 INTRODUCTION

Industrial development and urbanization have led to increase in the amount of solid wastes frequently discharged into the natural environment. This has increased the amount of many chemical substances in the environment. The chemical substances which are of great environmental interest are the heavy metals. Heavy metals are of special interest because of the major risks they contribute to the environment. They are toxic to humans as well as other organisms; they are released by metal-bearing soil constituents and migrate through the soil solution downward to the water table (Van Oort *et al.*, 2006; Shina and Alok, 2010). The contamination by these metals is a threat to the quality of groundwater, if these metals are not properly treated. Unlike organic compounds that can be biodegraded with time or can be incinerated, metals are robust and remain a potential threat to the environment and human health for a long time (Hong *et al*., 2002). Soil and sediments are rich in these heavy metals since they are not subject to degradation phenomena. They can easily be suspended or dissolved by surface water, hence become available to plankton, nekton and deposit feeders. The consequence of this is that, they can enter the food chain and become concentrated in fish and other edible organisms (Di Palma and Mecozzi, 2007).

The environmental impact of soil contamination depends not only on the total amount of metals in the soil but mainly on their mobility and availability. This is influenced by leaching and interactions with other components of the ecosystem such as air and water. Soil washing remediation technology is used to remove undesirable contaminants in soil and sediments by

dissolving or suspending them in a washing solution (Freeman and Harris, 1995; Moutsatsou *et al.*, 2006; Weihua *et al.*, 2010), and also by concentrating contaminants in small volume of soil through particle size separation (Detzner *et al*., 1998; McCready *et al*., 2003). This is based on findings that contaminants tend to bind either physically or chemically to clay, silt or organic soil particles.

Liberated metal ions are variously trapped by a wide range of reactive soil constituents that is organic matter, iron, manganese oxides, hydroxides, silicates, phosphates, carbonates. A fraction of these metals can thereafter be remobilized, either in dissolved or colloidal form before migrating downward. Mobilization is defined here, as the potential capacity of metals to be transferred from the solid phase into the soil solution. It depends on the various links between metals and reactive sites of solid phase surfaces. (McGrath, 1996; Gleyzes *et al*., 2002; Krishnamurti *et al*., 2002; Chaignon *et al*., 2003; Feng *et al*., 2005;). Metals readily extracted, were called ‘‘labile’’ by (Fangueiro *et al*., 2005), and more slowly extracted metals, called ‘‘less labile’’, which might be reasonably attributed to potentially ‘‘mobile’’ and/ or ‘‘bioavailable’’ metal fractions (Bermond *et al*., 2005). Degryse *et al*., (2006) showed that the proportion of labile metals was well-correlated with metal uptake in plants.

Chelating agents are most effective extractants, which can be introduced in the soil washing to enhance heavy metal extraction from contaminated soils. The advantages of chelating agent include high efficiency of metal extraction, high thermodynamic stabilities of the metal complexes, and low absorption of the chelating agents to a catalyst (Jerome *et al*., 2007). In

addition, the chelating agents cause only minor impact on the physical and chemical properties of the solid matrix as compared to acids (Lee and Marshall, 2002).

1.1 Statement of Research Problem

Metal contaminated soils are a serious environmental problem with implications for human health. The presence of metals in the soil has two main origins: the alteration of the bedrock and human activities, the latter being the major cause of high level of metals in the soil. The risks are related to the mobility and bioavailability of the metals and consequently to their speciation in soil. Chelating agents have been widely investigated as efficient extracting agent to enhance the performance of soil washing (Peters, 1999).

Naidu and Harter (1998) stated that metals extracted by a mixture of organic acids are well-correlated with the mobile metal fraction in the soil solution. Low molecular weight organic acids, naturally exuded by plant roots or produced by microbial activity (Fox and Comerford, 1990), have been hypothesized to influence nutrient mobilization (Van Hees *et al*., 2002) or translocation of metals in soil profiles (Van Hees and Lundstrom, 2000; Li *et al*., 2006). Ethylene diaminetetraacetic acid (EDTA) is a well-known strong chelating agent and has been widely used in agronomy for estimating the total extractable metal pool (Alvarez *et al*., 2006; Manouchehri *et al*., 2006).

1.2 Justification of Research

Soil washing process, amongst other remediation technology gives high removal efficiency for remediating sites contaminated with heavy metals and organics using suitable chelating agents, surfactant, acids alkalis and complexing agents because it can be applied to

large contaminated areas due to its operational easiness and economic efficiency (US-EPA, 2001). Several types of extractants can be used to extract heavy metals and metalloids from contaminated soil for soil washing technology. The extractants can be acids, chelating agents, electrolytes, oxidizing agents and surfactants (Reddy and Chinthamreddy, 2000; and Sun *et al.*, 2001), out of which acids and chelating agents are the most used extractive reagents for heavy metal decontamination. Acid washing leads to decreased soil productivity and adverse changes in the chemical and physical structures of soil due to mineral dissolution (Reed *et al*., 1996). Chelating agents are regarded as more attractive alternatives to acids because they can form strong metal-ligand complexes and are thus highly effective in remediating heavy metal contaminated soil (Kim and Ong, 1998 and Wei *et al*., 2011). EDTA continues to be explored extensively for soil remediation because of its ability to mobilize metal cations efficiently coupled with only a minor impact on the physical and chemical properties of the soil matrix (Lee and Marshall, 2003). However, of the use of dominant complexing agents (tiron and glutamic acid) could be employed as alternatives to EDTA in soil washing and for chelant-enhanced phytoremediation. The use of nontoxic and biodegradable biopolymer for wastewater and soil treatment is becoming increasingly recognized (No and Meyers, 2000).

1.3 Aim and Objectives

This study was aimed at determining the pollution level of the soils of some metal-work sites and estimating the percentage removal of different heavy metal (Cd, Cr, Ni, Pb and Zn) by washing the most polluted soil with complexing agents (tiron and glutamic acid).The specific objectives include:

i. Determination of some physicochemical parameters of the contaminated soil, such as pH, carbonate content, particle size and cation exchange capacity.

ii. Determination of the concentrations of cadmium, chromium, nickel, lead and zinc in parent soil.

iii. Assessing the metal removal efficiency of the used chemical extractant through batch extraction using solutions of the two complexing agents at different contact times, pH, masses and concentrations.

iv. Applying factorial design of experiment to determine the optimum operational condition for the process.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil and Its Properties

Nicholas *et al.*, (2004) stated that the soil is a key component of terrestrial ecosystem, being essential for the growth of plants and the degradation and recycling of dead biomass. It is a complex heterogeneous medium comprising mineral, organic solids, aqueous and gaseous components. Brady and Weil, (2002) stated that the solid components are usually clustered together in the form of aggregates, thus creating a system of interconnected bids (pores) of various sizes filled with either water or air. The solid components have the ability to absorb ions, but this differs between materials and is strongly influenced by the prevailing pH, redox conditions and the relative concentrations of the ions present in the aqueous soil solution. The structured heterogeneous mixture of organic and mineral components is the habitat for many organisms as well as the medium in which plant roots grow, extracting water, oxygen and ions. Roots also release carbon dioxide and exude organic compounds which are responsible for the intense microbial activity in the interracial zone between the root and the soil called the 'rhizosphere'. Plant roots modify the chemical and physical properties of the soil around them and thus influence the bioavailability of some chemical elements. The soil is a dynamic system subject to short-term fluctuation such as variation in moisture status, pH and redox conditions and also undergoing gradual alterations in response to changes in management and environmental factors (Ann and Clain, 2005).

These changes in soil properties could affect the form and bioavailability of metals and need to be considered in decision on the management of polluted soil or the use of soil for disposal of waste materials (Gardiner and Miller, 2004). Soil can show marked spatial variability in physical and chemical properties at the macro and micro scales, thus emphasizing the need for the thorough sampling to include the range of variance in parameters at any site of investigation.

2.1.1 Soil texture

According to Basu, (2011) soil texture is the proportion of three mineral particles, sand, silt and clay in the soil. These particles are distinguished by size and make up the fine mineral fraction, particles over 2mm in diameter (the coarse mineral fraction) are not considered in texture. The relative amount of various particle sizes in a soil defines its texture, i.e. whether it is clay, loam, sandy-loam or other textural category. Gurevitch *et al.*, (2002) stated that texture is the result of weathering, the physical and chemical breakdown of rocks and minerals, weathering is a relatively slow process, hence texture remains fairly constant and is not altered by management practice.

2.1.2 Soil pH

Brady and Weil, (2002) defined the pH of a soil as the hydrogen ion concentration in the solution present in soil pores which is in dynamic equilibrium with the predominant negatively charged surfaces of the soil particles, hydrogen ion is strongly attracted to the surface negative charges and it has the power to replace most other cations. The diffuse layer close to the negatively charged surface has a higher concentration of hydrogen ion than the bulk soil solution. Smith and Doran, (1996) stated that when the soil solution is diluted, the diffused layer

expands, causing the pH of the bulk solution to increase. The dilution effect is usually overcome by measuring the pH in a suspension of soil in a solution of a neutral salt such as calcium chloride or potassium chloride. Reducing conditions generally cause a pH increase and oxidation brings about a decrease. Soils have several mechanisms which serve to buffer pH to varying extents including hydroxyl aluminium ion, carbon dioxide, carbonates and cation exchange reactions. However, even with these buffering mechanisms, soil pH differs significantly due to localized variations within the soil. Soils generally have pH values within the range of 4.0 – 8.5, owing to the buffering by aluminium at the lower end and by calcium carbonate at the upper end of the range. The normal pH is 5.0 – 7.0 in soil of humid regions and 7.0 – 9.0 in the soil of arid region (Brady and Weil, 2002).

2.1.3 Organic Matter

According to Sparks, (2003), the main feature which distinguishes soil from regolith (decomposed rock) is the presence of living organisms, organic debris and humus. All soils contain organic matter, although the amount and type may vary considerably. Colloidal soil organic matter has a major influence on the chemical properties of soil and can be divided into humic and non-humic substances. The non-humic substances comprise of unaltered biochemicals such as amino acids, carbohydrates, organic acids, fats and waxes that have not changed from the form in which they were synthesized by living organisms. Humic substances are a series of acidic coloured polyelectrolytes of moderately high molecular weight. They are formed by secondary synthesis reactions involving micro-organisms and have characteristics which are not similar to any compounds in living organisms. They have a wide variety of functional groups

including carboxyl, phenolic, hydroxyl, carbonyl, ester and possibly quinine and methoxy groups, which are predominantly composed of humic substances (Huang and Germida, 2002; Sparks, 2003). Soil humus also contains some biochemical bound to the humic polymers. Methods used to determine the organic matter content of the soil include the percentage loss in weight after ignition in a furnace at 375^{o}C for 16 hours and oxidation of carbon by acid dichromate followed by the titration of excess dichromate.

Cultivated soils have organic matter content less than 10%. General mineral soils contain 3 – 5% organic matter. Within the soil profile, the organic matter content is always highest in the surface horizon (Ann and Clain, 2005).

2.1.4 Cation Exchange Capacity

Quirine *et al.,* (2007) defined ion exchange as the exchange between the counter ions balancing the surface charge on the colloids and the ions in the solution.

Casagrande and Alleoni, (2001) stated that the negative charge in the soil constituents are of two types:

(i) Permanent charges due to isomorphous substitution of major element ions within the structures of silicate mineral layer and are independent of the pH value, the valence of the counter-ion and the electrolyte level or composition of the bulk solution.

(ii) pH dependent charges on humus polymers and hydrous oxides: these are due to broken bonds at mineral edges and external surfaces, dissociation of acidic functional groups in organic compounds and the preferential absorption (by chemical reaction)of

certain ions on the particle surface. All of these vary in magnitude depending on the pH value, electrolyte level, and valence of the counter-ion, dielectric constant of the medium and nature of the anion in the solution phase.

Another source of variable charge in acid soils is the neutralization of permanent negative charge by strongly absorbed aluminium hydroxyl polymers that carry positive charge. As pH value rises, these polymers are precipitated as $Al(OH)_3$, thereby freeing the negative sites of participation in normal cation exchange reactions. Negative sites can be similarly neutralized by the absorption of positively charged mineral particles such as iron oxides.

Another type of neutralization of permanent charge is that caused by highly selective absorption associated with the mica silicate minerals, which contain potassium ion and ammonium ion between the contracted platelets. These inter-layer cations are not readily exchangeable, although they can be dissolved with certain chemical treatments and through weathering. Cation exchange capacity is a soil property that is dependent of the conditions under which it is measured. The soil's capacity to absorb cations from an aqueous solution varies with pH, ionic strength and dielectric constant (Donald and Quirine, 2011).

2.2 Heavy Metals

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc) and atomic number >20. The most common heavy metal contaminants are: Cd, Cr, Cu, Hg, Pb and Zn. Metals are natural components in soil. (Kabata-Pendias and Pendias, 1992). The metals are classified as "heavy metals" if in their standard state, they have a specific gravity of more than 5 g cm^{-3}. There are

sixty known heavy metals. Heavy metals can accumulate overtime in soils and plants and could have a negative influence on physiological activities of plants (e.g photosynthesis, gaseous exchange, and nutrient absorption), causing reduction in plant growth, dry matter accumulation and yield (Devkota and Schmidt, 2000). There are many terms used to describe and categorize metals, including trace metals, transition metals, micronutrients, toxic metals and heavy metals. Many of them have been used loosely in the literature to include elements that do not strictly meet the definition of the term. Metals are defined as any element that has a silvery luster and is a good conductor of heat and electricity (McLean and Bledsoe, 1992).

2.2.1 Cadmium

Cadmium (atomic number 48; relative atomic mass 112.40) is a metallic element belonging to group II b of the periodic table. Cadmium derives its toxicological properties from its chemical similarity to zinc, and essential micro nutrients for plants and animals. Cadmium is bio-persistent and once absorbed by an organism, remains resident for many years (over-decades for humans) although it is eventually excreted. The average daily intake for humans is estimated as 0.15 μg from air and 1 μg from water. Smoking a packet of twenty cigarettes can lead to the inhalation of 2-4 μg cadmium but levels may vary widely (Sindhu, 2005). In humans, long term exposure to cadmium is associated with renal dysfunction. High exposure can lead to lung cancer. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in humans and animals. In addition, the metal can be linked to increased blood pressure and affects the myocardium in animals (Sindhu, 2005). Other toxic effects of cadmium include dry and scaly skin, loss of sense of smell, hair loss, pain in the back and legs and yellow teeth in humans.

People exposed to low level of cadmium overtime may suffer from kidney, liver and lung damages. Cardiovascular and reproductive systems are also affected (Hrudey *et al*., 1995).

2.2.2 Zinc

Zinc (atomic number 30; relatively atomic mass 65.38) is a metallic element belonging to group II b and the fourth period of the periodic table. Zinc is chalcophilic element like copper and Lead, and a trace constituent in most rocks. Zinc rarely occurs naturally in its metallic state, but many minerals contain zinc as a major component from which the metal may be economically recovered. Sphalerite (ZnS) is the most important ore mineral and the principal source for zinc production (WHO, 2001a). For non-contaminated soils worldwide, Adriano reported average zinc concentrations of 40-90 mg kg^{-1}, with a minimum of 1 mg kg^{-1} and a maximum of 2000 mg kg^{-1}. Low levels are found in sandy soils (10-30 mg kg^{-1}) (Adriano, 1986). The fate and transport of zinc (Zn^{2+}) in the environment is dependent on cation exchange capacity, pH, organic matter content, nature of complexing ligands, and the concentration of the metal in the soil. As pH increases, there is an increase in negatively charged binding sites on soil particles, which facilitates the adsorption of zinc in anaerobic soils for the insoluble zinc sulfide. Therefore, mobility is limited in anaerobic conditions. Zinc mobility increases with low pH under oxidizing conditions and low cation exchange capacity. The presence of competing metal ions and organic ions such as humic material may cause the adsorption of Zn^{2+} ions to the soil, particularly in soils with an elevated pH, via ligand exchange reactions. These reactions reduce the solubility of zinc in the soil solution and, therefore, reducing its mobility and limit its bioavalability.

2.2.3 Chromium

Workers who breathe hexavalent chromium compounds at their jobs for many years may be at increased risk of developing lung cancer. Breathing high levels of hexavalent chromium can irritate or damage the nose, throat and lungs. Irritation or damage to the eyes and skin can occur if hexavalent chromium contacts these organs in high concentration or for a prolonged period of time (Ottaway, 1978). Dietary allowance of chromium per day is fixed at 50-200 μg per day, even though it is not allowed to be added to food stuffs and medicines (Ottaway, 1978). Moreover, long term exposure to chromium causes damage to the kidney, liver and nerve tissues. Studies on workers in chromate production, chromate pigment and chrome electroplating, industries employed before 1980's show increase rates of lung cancer mortality. Chromium is highly toxic (chromium unit in water is 0.05 mg dm^{-3}) and a possible carcinogen. If continuously exposed to it, humans may develop nasal perforation and bronchogenic carcinomas (Sindhu, 2005).

2.2.4 Nickel

Nickel is an essential trace element in animals, although the functional importance of nickel has not been clearly demonstrated. However, there is evidence of uptake and accumulation in certain plants. Nickel deficiency is manifested primarily in the liver. Its effects include abnormal cellular morphology, oxidative metabolism and increase and decrease in lipid levels. The essentiality of nickel in humans has not been established and nickel dietary recommendations have not been established for humans (ATSDR, 2003).

Nickel compounds are known carcinogens in both human and animal models (Feder *et al*., 1996). There is evidence that the geno-toxic effects of nickel compounds may be indirect through the inhibition of DNA repair systems. As a result of this inhibition it has been suggested that accumulation of nickel in breast tissue may be closely related to malignant growth process (ATSDR, 2005).

2.2.5 Lead

Lead is one of the four metals that have the most damaging effect on human health. The metal arises from both natural and anthropogenic sources, exposure can occur through drinking water (65%), air (20%), and dust (15%) from old painting containing lead. High levels of exposure may result in toxic biochemical effects in human which in turn can interfere with the synthesis of haemoglobin, can affect the kidneys, gastrointestinal tract, joint and reproductive system, and acute or chronic damage to the nervous system (http://www.lenntech.com/heavy-metal.htm). Excessive lead intake can cause mental retardation, abortion, brain damage, memory difficulties, weakness, miscarriage and decrease in man's fertility through sperm damage. Lead also diminishes intelligent quotient (IQ) and impaired learning in children (http://www.lenntech.com/heavy-metals.htm). The risk of young children suffering neuro-psychological effect because of excessive lead ingestion appear to be more serious than had been previously recognized (Needleman *et al.,* 1990). In humans lead causes anaemia, kidney disease, brain damage and impaired function of peripheral nervous system.

2.3 Soil Remediation

Soil remediation is the removal of pollutants or contaminants from the soil. Remediation technologies are many and vary but can be categorized into ex-situ and in situ methods. Ex-situ methods involve excavation of affected soil and subsequent treatment at the surface while in-situ methods treats the contamination without removing the soil. It basically consists of two fundamental principles, the first is to completely remove contaminations from polluted sites and the second is to transform these pollutants to harmless forms by using one or more engineering technologies, which mainly include excavation, separation, extraction, electrokinetics, washing, oxidation, reduction, phytoextraction, phytovolatilization or solidification, vitrification and so on. (Mulligan *et al*., 2001; Zhou and Song, 2004; Aboulroos *et al.*, 2006; Chen *et al*., 2006).

2.4 Washing Technology

Soil washing is the most commonly used treatment technology for remediation of metal contaminated soils. The technique is an ex-situ treatment method and is less expensive compare to other treatment technologies (Krishna and Supraja, 2000).

There are two main types of remediation for metal-contaminated soil; (i) technologies that leave the metal in the soil and technologies that remove the heavy metals from the soil (Rampley and Ogden, 1998). Technologies such as solidification/stabilization and vitrification immobilize contaminants, thereby minimizing their migration. Techniques such as soil washing and in-situ soil flushing transfer the contaminants to a liquid phase by desorption and solubilization (Reed *et al.*, 1996). Soil washing can be a physical and/or chemical process that results in the separation, segregation and volume reduction of hazardous materials and/or the

chemical contaminants to non-hazardous materials (Semer and Reddy, 1996). Generally, in-situ technologies are more economical and safer than the ex-situ technologies because excavation is not required. Cation exchange and specific absorption are two mechanisms that control metal absorption (Cline and Reed, 1995). Heavy metals can also be retained by other mechanisms other than absorption (e.g. solid state diffusion and precipitation reactions) especially when lead exists as $PbCO_3$, $PbSO_4$, or as an organic lead form.

Soil washing involves the separation of contaminants from soil solids by solubilizing them in a washing solution. The technology is generally an ex situ method, it usually employs wash solution that contain acids, bases, chelating agents, alcohols or other additives. A chelant is a ligand that contains two or more electron-donor groups so that more than one bond is formed between the metal ion and the ligand. EDTA forms 1:1 molar ratio complexes with several metal ions. Acids and chelating agents are generally used to remove heavy metals from the soils but the particular reagent needed can depend not only on the heavy metal involved but also on the specific metal compound or species involved. Pickering (1986), identified four ways in which metals are mobilized in soils; (i) changes in the acidity; (ii) changes in solution ionic strength; (iii) changes in the redox potential; (iv) formation of complexes. In practice, acid washing and chelator soil washing are the two most prevalent removal methods. The most common chelating agent studied in the literature is EDTA. (Cline and Reed, 1995; Griffiths, 1995; Reed *et al*., 1996; Ramphey and Ogden, 1998; Steele and Pichtel, 1998).

Metal removal efficiencies during soil washing depend on the soil characteristics, metal characteristics, extractant chemistry and processing conditions. pH plays a significant role in the

extractability of heavy metals from soils (Atanassova and Okazaki, 1997). Heavy metals that are less soluble in water often require chelating agents or other extractants for successful soil washing. The ability to form stable metal complexes makes chelating agents such as EDTA and NTA effective extractants for heavy metal-contaminated soil (Davis and Singh, 1995; Pichtel and Pichtel, 1997; Polettini *et al*., 2006).

There are many factors to consider when comparing studies of chelating agent assisted soil washing in order to decide whether the chelating agent is suitable for field-scale decontamination of polluted sites. The ratio of chelating agent to toxic metals, pH, quantity of major cations extracted, and source of contamination (artificial or anthropogenic) are the most important ones. Most studies of chelant-assisted soil washing have found that a ratio >1 between chelant and toxic metal is required to give good toxic metal extraction (Pichtel and Pichtel, 1997; Steele and Pichtel, 1998; Pichtel *et al*., 2001; Kim *et al*., 2003.,).

2.5 Chelant Extraction Technology Description

Contaminants absorbed into the soil particles are separated from soil in an aqueous-based soil washing system. The wash water may be augmented with a basic leaching agent, acids, surfactant, pH adjustment or chelating agents to help remove organics and heavy metals. Factors affecting soil washing/soil flushing processes include clay content, complex waste mixtures, high humic content, metal concentrations, mineralogy, particle size distribution/soil texture, separation coefficient and wash solution. Given that metals are not like organics and cannot be destroyed or degraded away, the metals and radionuclide can only be transformed or transferred (Peters, 1999).

2.6 Chemistry of Metal Extraction Using Chelating Agents

2.6.1 Acid-base equilibrium of chelating agents: Effective chelating agents typically have multiple coordination sites (i.e. ligand atom) available for complexation with a metal center. They are often multi-protic acids capable of undergoing acid-base equilibrium reactions in the aqueous phase, metal chelate extraction is usually investigated under equilibrium conditions.

2.6.2 Metal complexation with chelating agents: Each conjugate acid/base of the chelating agent may form a strong complex with the metal, resulting in the formation of various complexes. The general equation for extraction of a simple metal chelate is

$$M^{n+} + nHR \rightarrow MR_n + nH^+ \qquad (2.1)$$

HR represents the chelating agent used in the form of solution, R is its anion, M^{n+} is the hydrated cation of the metal.

2.6.3 Interaction of soil with metals and complexes: When the amount of heavy metals of interest exceeds the solubilities of their corresponding hydroxides, carbonates and /or hydroxy-carbonate mineral phases at a given pH value, the metals will precipitate as solids. Hence these solid minerals will be entrapped in the soil or sediments matrix. In addition, soils contain mineral and humic constituents which carry hydroxyl and carboxylic groups. The acid-base characteristics of these functional groups contribute to the formation, at the soil surface of electrically charged groups important for the retention of metal ions and complexes. Thus solution pH can influence acid-base equilibrium reactions of the surface groups; this in turn can influence the soil's retention of metals by absorption and complexation with metal ions and complexes to different

degrees depending on the pH of the zero point charge of the soil. Hence, in addition to physical entrapment of metal hydroxide or carbonate solids, the soil can accommodate metals through more direct interactions, including surface complexation and surface precipitation mechanisms.

2.6.4 Chelating agent selectivity towards target heavy metals: For target heavy metals extraction application, the chelating agents should satisfy the following criteria;

(i) The chelating agents shall be stable with the foam and display no adverse effects on the stability of the foam.

(ii) The ligands possess high metal complexing abilities towards heavy and transition metals as opposed to hard sphere cations such as Ca and Mg. the relative magnitude of the equilibrium complexation constant towards heavy metal and towards alkali metals are an indicator.

(iii) The ligands containing sulphur and nitrogen as donor atoms are generally preferred for higher selectivity towards metals of interest, which are transition metals and soft sphere cations. Ligands containing sulfur or nitrogen as donor atoms generally form more stable complexes with soft sphere metals, whereas ligands containing oxygen as the donor atom prefer hard sphere cations.

(iii) Multidentate ligands are preferable because they contain multiple coordinating sites capable of forming more stable complexes with metals.

2.7 Studies on Glutamic Acid

The discovery of a potent glutamic acid producing bacterium by Kinoshita *et al.*,(1957) was the start of the subsequent development of amino acid production by regulation of biosynthetic

metabolism. At that time, a two-step L-glutamic acid production process was used. The glutamic acid producing bacterium first discovered was reported as *Micrococcus glutamicus*in 1958, and other glutamic acid producing coryneform species, all patent strains, were subsequently isolated by many other workers.

Wild strains of glutamic acid bacteria are able to produce only a few amino acids extracellularly such as glutamic acid, valine, alanine, proline and glutamine. For the extracellular production of any amino acid, changes in cellular metabolism and/or regulatory control, are required. It has been shown that biotin promotes the glutamate excretion by bacteria in both. Under biotin excessive growth conditions, synthesized glutamic acid does not permeate through the cell membrane and it accumulates internally. In order to overcome this problem, the sub-lethal concentrations of penicillin or a detergent is added in early exponential growth phase (Somerson and Phillips, 1962).

Kinoshita *et al.,* (1957) introduced the first fermentation process for industrial production of amino acids by microorganisms. Bacterial cultures were used for glutamic acid production due to the increasing demand for monosodium glutamate as a flavoring agent. He discovered a bacterium *Micrococcus glutamicus* through, which he introduced a new approach for the screening of microorganisms capable of accumulating glutamic acid in the medium.

Shiio *et al.,* (1963) reported the microorganisms that could produce glutamic acid from hydrocarbons. Nakayama (1966) claimed that some of the glutamic acid secreting microorganisms can be easily found in nature, however, this is not true for microorganisms that produce other amino acids.

A variety of bacteria as fermentative agents have been used for the production of L-glutamic acid. Shisoo *et al.,* (1974) produced L-glutamic acid by fermentation process in which carbon source (glucose) and nitrogen source (NHs) were automatically supplied in order to maintain carbon as well as nitrogen concentration. Ammonium hydroxide (NH_4OH) was used to control pH as well as nitrogen source in the medium. In this way, 6.48 mg ml^{-1} of L-glutamiic acid was obtained.

Yoshiro *et al.,* (1974) carried out L-glutamic acid production in biotin-rich media by fermentation without controlling the microbial growth by adding antibiotics. *Micrococasglutamicus* was cultured at 30°C and pH 7.0-8.0 for 48 hours in a medium containing adenine, biotin, glucose, KH_2PO_4, K_2HPO_4, $FeSO_4$, $MnSO_4$, $(NH_4)_2SO_4$ and urea.

Ryochi and Kenichiro (1980) produced L-glutamic acid by *Corynebacterium* and *Brevibacterium* sensitive to lysozyme and noted that the glutamic acid production was not inhibited by the excessive biotin, the observation which is in contrast to the study conducted by Somenon and Phillips (1961). Kinoshita *et al.,* 2004 carried out screening tests for glutamate producing strains, with the media containing carbohydrate and ammonia source as chief ingredients. Accumulation of L-glutamic in a significant amount has been demonstrated by various strain of bacteria, streptomycetes, yeasts and fungi. The highest level of glutamate production has been obtained by a new species of Micrococcus.

Jyothi *et al.,* 2005 studied conditions of optimization of glutamic acid production from cassava starch factory residues using *Brevibacterium divaricatum.* With 0.7% ammonium nitrate in the medium, highest glutamic acid yield of 3.86% based on the weight of the residue was obtained at 30°C at a pH of 7.0. The highest glutamic production was recorded at 5% inoculum

size at an agitation speed of 180 rpm. The study showed that the solid residues from cassava starch factories could serve as a low cost substrate for glutamic acid production.

2.8 Studies on Tiron

Kim *et al*., (2003) carried out a study on the effects of tiron on human promyelotic HL-60 leukemia cell differentiation and death. Tiron is known to be a widely used antioxidant to rescue Ros-evoked cell death and a non-toxic chelator to alleviate an acute metal overload. In this study we showed that tiron is a potent inducer of cell differentiation and apoptotic cell death in human promyelotic HL-60 leukemia cell, from the results, they suggested that tiron-mediated differentiation and cell death result from the disturbance of iron metabolism.

Sharma *et al*., (2002) carried out a study on comparative effectiveness of tiron and $CaNa_2$ EDTA with time after beryllium poisoning. In their study they compared the efficacy of two chelating agents (Tiron and Calcium disodium EDTA) in the treatment of beryllium induced blood biochemistry and hepatic histopathological alteration was investigated at different duration in female albino rats. They found tiron to be more effort than $CaNa_2$EDTA in reducing the beryllium induced haematological alterations and histopathological lesions in liver.

Srivastava *et al*., (2007) carried out an investigation on the effect of tiron with and without supplement against vanadylsulphate intoxication. They planned to evaluate the therapeutic effectiveness of tiron with and without supplement against intoxication. They discovered that treatment of tiron along with various supplements LA, vitamin E, Se showed recovery but the effect was most pronounced with T + Se.

The influence of organic-hexavaelnt-uranium u (vi) complexzation on u (vi) reduction by a sulfate-reducing bacterium (shewanella alga) was evaluated by Raja *et al*., (1997). Four ligands (acetic, molonate, oxalate, and citratrate) and an aromatic ligand (tiron [4,5-dihydroxy -1,3 - benzene disulforic acid]) were used to study complex uranium bioavailability. The trends in uranium reduction varied with the nature and the amount of u (vi) – organic complex formed and the type of bacteria present. D. desulfuricans rapidly reduced uranium from a monodentate aliphatic (acetate) complex. However, reduction from multidentate aliphatic complexes (malonate, axalate, and citrate) was slower. A decrease in the uranium more rapidly from multidentatealiphate complexes then from monodentate aliphatic complexes. The rate of reduction decreased with a decrease in the amount of multidentate complexes present. Uranium from an aromatic (tiron) complex was readily available for reduction by D. desulfuricans, while an in significant level of u (vi) from the tiron complex was reduced by S. alga. The result indicate that selection of bacterial for rapid uranium reduction will depend on the organic composition on waste streams.

To evaluate efficacy of chelating agents tiron (sodium - 4,5 - dihyroxy -1, 3 - benzene disulphonate) and induced toxicity, adult female albino rats were exposed to beryllium nitrate for 28 days followed by therapy with tiron (471 mg kg^{-1}, ip) and $CaNa_3DTPA$ (35 mg kg^{-1}, ip) alone and in combination with α–tocopheral (25 mg kg^{-1}, po). Results revealed non-significant fall in heamoglobin and total serum protein content while significant fall in blood sugar level of activity of serum transminases and LDH was noticed after beryllium administration. Significant increase in total and esterified cholesterol was found in liver and kidney after toxicity. Significant increase in lipid peroxidation and decrease level of reduced glutathione in both the organs

showed oxidative stress due to beryllium exposure. Histopathological and ultra structural observation of liver and kidney revealed lesions due to beryllium toxicity followed by recovery due to combined therapy. $CaNa_3DTPA$ showed moderate therapeutic efficacy, however, its effectiveness was enhanced with α-tocopheral to some extent. Tiron in combination with α-tocopheral exerted statistically more beneficial effects in reversal of beryllium induced biochemical, histopathological and ultra structural alternations. Nirala *et al.*, (2009).

2.9 Properties of glutamic acid

Systematic IUPAC name is 2- Amino pentanedioic acid, other name is 2 - Amino glutaric acid, Chemical formula of glutamic acid is $C_5H_9NO_4$.It has molecular weight of 147.13 $gmo1^{-1}$and is a white crystalline powder.

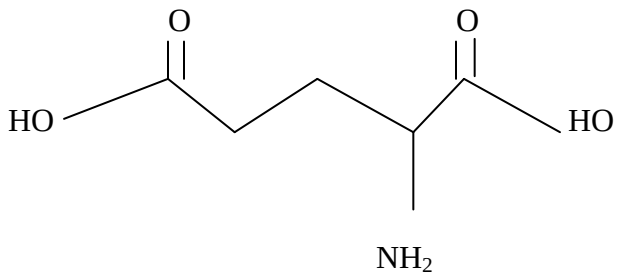


Figure 2.1: Structure of glutamic acid

2.10 Properties of Tiron

Disodium 4,5 - dihydroxy - 1,3 - benzenedisulfonate is a chemical compound used for its ability to form strong complexes with titanium and iron. It is a colorimetric reagent from iron, manganese, titanium, molybdenum, and complexes of zirconium. Other names catecolsulfate;

149-45-1; 3,5-disulfocatecho 1 disodium salt. Chemical formula of tiron is $C_6H_4Na_2O_8S_2$with molecular weight of 314.19 gmo1^{-1}. Its appearance is light tan powder.

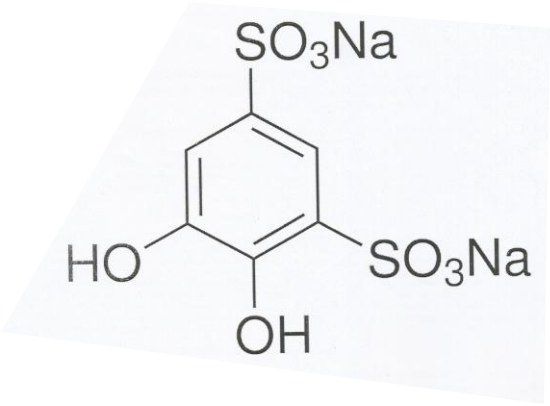


Figure 2.2: Structure of tiron

Principle of Atomic Absorption Spectroscopy

Atomic absorption spectroscopy measures the concentration of chemical elements present in environmental samples by measuring the absorbed radiation of the chemical elements of interest. This is done by reading the spectra produced when the sample is excited by radiation. The atoms absorb ultraviolet or visible light and make transitions to higher energy levels. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelength of light transmitted by the sample, and compares them to the wavelengths which originally passed through the sample. A single processor then integrates the changes in wavelength absorbed, which appear in the readout as peaks of energy absorption at discrete wavelengths. The energy required for an electron to leave an atom is known as ionization energy and specific to each chemical element. When an electron moves from one energy level to another within the atom, a photon is emitted with energy. Atoms

of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelength at which it will absorb energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample. To measure how much of a given element is present in a sample, a basis for comparison must first be established using known quantities of that element to produce a calibration curve. To generate this curve, a specific wavelength is selected, and the detector is set to measure only the energy transmitted at that wavelength. As the concentration of the target atom in the sample increases, the absorption will also increase proportionally. A series of samples containing known concentrations of the compound of interest are analyzed, and the corresponding absorbance, which is the inverse percentage of light transmitted is recorded. The measured absorption at each concentration is then plotted, so that a straight line is drawn between the resulting points. From this line, the concentration of the substance under investigation is extrapolated from the substance's absorbance. The use of special light sources and the selection of specific wavelengths allow for the quantitative determination of individual components in a multi element mixture. (Garcia and Baez, 2012).

2.11 Factorial Design of Experiment

The conventional method of studying a process by changing one variable at a time and keeping the other variables at a constant level does not depict the combined effects of all the factors involved in process optimization. Moreover, univariate method is time-consuming and also requires a large number of experiments to determine optimum levels, which may or may not be reliable. These limitations of the conventional method can be eliminated by simultaneously varying all the parameters affecting the process by using statistical factorial experimental design

such as the response surface methodology (RSM) (Elibol, 2002). RSM, initially described by Box and Wilson (1951), is an experimental approach to identify the optimum conditions for a multivariate system. RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes that can be used to evaluate the relative significance of all factors involved in the process even in the presence of complex interactions. Current literature includes a number of studies that are related to the applications of factorial design in adsorption of dyes from aqueous solution (Mestari *et al*., 2008; Auta and Hameed), 2011; Muthukumar *et al.,* 2004). The main objective of RSM is to determine the optimum operational condition for a system or process (Myers *et al*., 2009). The application of statistical experimental design techniques in adsorption process development can result in improved product yields, reduced process variability, and reduced development time and overall cost (Annaadurai *et al*., 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

Soil samples were collected in Sabon-gari, Samaru, and Tudun-wada areas, Zaria, Kaduna state, Nigeria (figure 3.1) where blacksmith activities (metal-works) were taking place, in March 2015.

Table 3.1: The sampling position of each sample site showing latitude and longitude

Soil	Place	Latitude	Longitude
1	Samaru	$112^{o}12$□ N□	$07^{o}37$□ E
2	Sabon-gari	$10^{o}28$□ N	07^{o} 26□ E
3	Tudun-wada	$11^{o}11$□ N	$07^{o}29$□ E

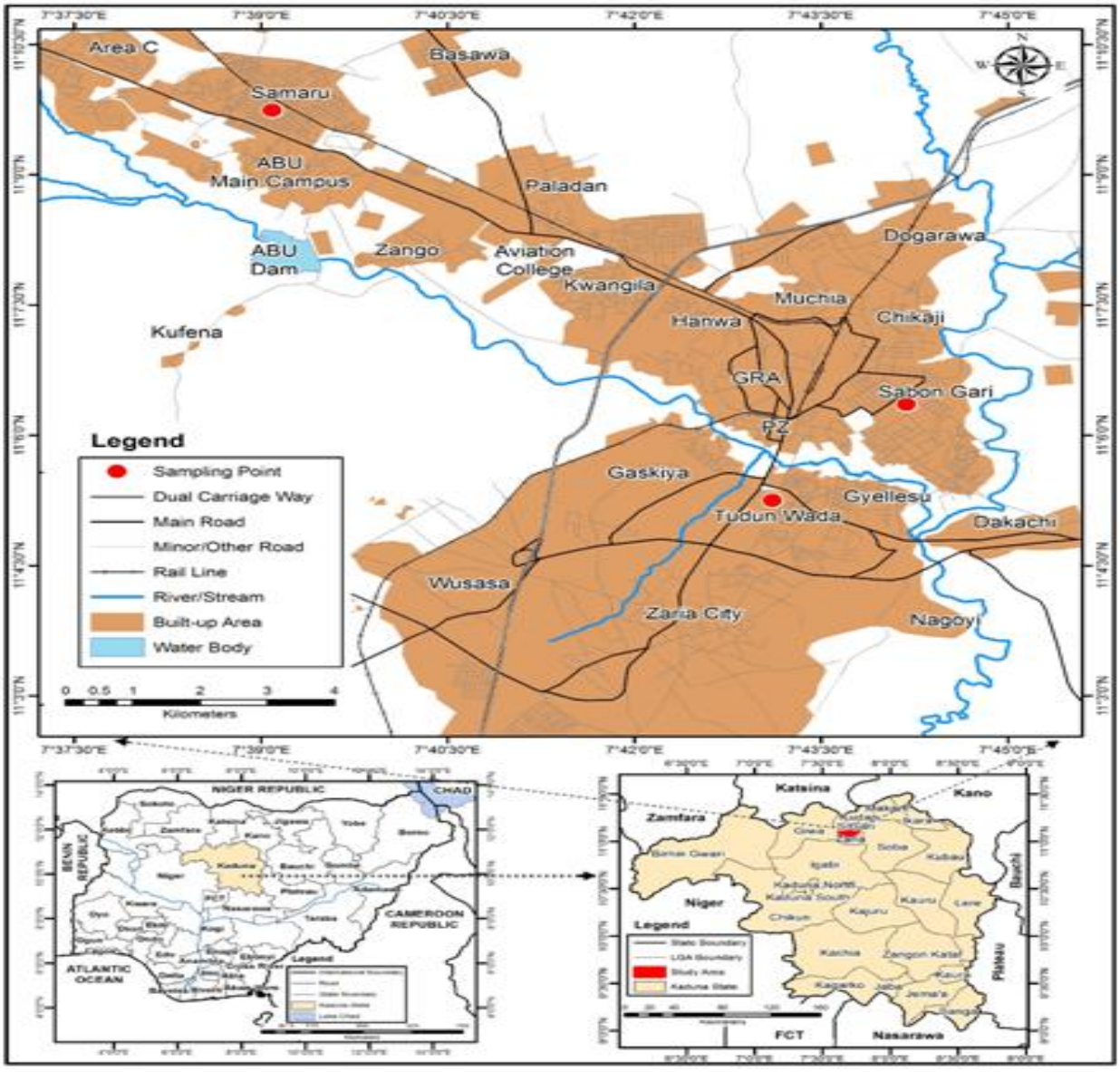


Figure 3.1: Map of Zaria showing sampling locations

3.2 Sample Collection and Treatment

Three different soils samples were collected for this study. Each soil sample was collected from a blacksmith site from a depth (0- 15 cm) layer of the soil profile at five different points and pooled together to give a composite sample. The soil samples were packaged in clean polythene bags and transported to the laboratory. The soil samples were air- dried. The chemical analysis of the soil samples was done in the laboratory of the faculty of Agriculture, department of soil science, Ahmadu Bello University, Zaria, Nigeria. The mean concentration of heavy metals in parent the was determined by AAS. The soil with the highest level of concentration (Tudun-wada) was used in the experimental runs. The other soil samples from Samaru and Sabon-gari were discarded. Each soil samples was washed with solutions of the complexing agents (tiron and glutamic acid) separately to determine the percentage removal of cadmium, chromium, lead, nickel and zinc.

3.3 Chemicals and Reagents

Glutamic acid (99.0 w/w %); tiron (98.0 w/w %) were used to prepare the ligand solution meant for soil washing. Hydrochloric acid (37.0 w/w %) Sodium hydroxide (99.0 w/w %) were used as buffers to maintain pH. All chemicals used in this study were purchased as analytical grade and need no further purification before use. All reagents were of Fluka and Riedel-de Haën patent Sigma-Aldrich.

3.4 Soil Analysis

Characteristic physicochemical parameters of the soil were determined. The properties determined include; particle size, pH, $CaCO_3$ content, HCO_3 and cation exchange capacity (CEC). Total metal concentrations in the soil sample were determined using the flame atomic absorption spectrophotometer after $HF/HClO_4$ acid digestion in a ratio of 1:1.

3.4.1 Particle Size and Texture Determination (Bouyoucos Hydrometer Method, 1951)

Preparation of Reagents

Sodium hexametaphosphate(calgon) (2 mol dm^{-3}): Fifty grams of calgon was weighed and dissolved in deionized water, the solution was made up to 1000cm^3 in a volumetric flask with deionized water.

Procedure

A fifty grams mass of the sieved metal-work soil sample was placed in a 250 cm^3 plastic beaker, 100 cm^3 of 50% sodium hexametaphosphate (calgon) solution was then added to the soil mixture and stirred with a glass rod. After which 100 cm^3 of deionized water was then added to the mixture, stirred and allowed to stand for 30 minutes with occasional stirring. This was then transferred to a 250 cm^3 plastic container and shaken on mechanical shaker for 10 minutes and then transferred into 1 dm^3 measuring cylinder and made up to mark with hand plunger and hydrometer. The temperature readings were taken after 40 seconds and 8 hours respectively. A blank consisting of 0% calgon in 100 cm^3 of deionized water was made, this was diluted to 1

dm^3 in a measuring cylinder and the hydrometer and temperature readings were taken (Gee and Or, 2002).

3.4.2 Soil pH Measurement

Preparation of Reagents

Calcium chloride (0.01 mol dm^{-3}): 1.1g of calcium chloride was dissolved in deionized water and made it up to 1000 cm^3 in a volumetric flask with deionized water.

Procedure

Ten grams of air-dried soil was weighed into a 1000 cm^3 beaker, 10 cm^3 of deionized water was added and the suspension was allowed to stand for 15 minutes with frequent stirring with a glass rod. Prior to use the pH meter was calibrated with pH 4.0 and 7.0 buffers. The electrode of the pH meter was then immersed into the partly settled suspension and the pH was measured. The procedure was repeated using 0.01 $moldm^{-3}$ calcium chloride solution and the pH was measured

3.4.3 pH Measurement (Black, 1965)

Twenty grams of air-dried soil was weighed into a 50 cm^3 beaker, 20 cm^3 of deionized water was added and allowed to stand for 30 minutes with occasional stirring using a glass rod. Using a pH meter, an electrode was inserted into the partly settled suspension and the reading was taken. The suspension was not stirred during the measurement.

3.4.4 Determination of Cation Exchange Capacity (Hendershot *et al*., 1993)

Preparation of Reagents

Ammonium Acetate (1.0 mol dm^{-3}): 57 cm^3 of glacial acetic acid (100% w/w, sp. gr.1.05) was dissolved in 800 cm^3 of deionized water in a 1000 cm^3 volumetric flask, 68 cm^3 of concentrated ammonium hydroxide was added mixed thoroughly and allowed to cool. The pH was adjusted to 7.0 with ammonium hydroxide and diluted with deionized water to 1000 cm^3

Ammonium chloride (1.0 mol dm^{-3}): 53.5 g of ammonium chloride was dissolved in deionized water and made up to 1000 cm^3 volumetric flask.

Silver nitrate (0.1 mol dm^{-3}): 17.0 g of silver nitrate was dissolved in deionized water and made up to mark in a 1000 cm^3 volumetric flask.

Boric acid (0.1 mol dm^{-3}): 2 g of boric acid was dissolved in deionized water and made up to 1000 cm^3 in a volumetric flask.

Sodium hydroxide (1.0 mol dm^{-3}): 40 g of sodium hydroxide pelletes was dissolved in deionized water and made up to mark in a 1000 cm^3 volumetric flask.

Bromocresol green - methyl red mixed indicator: 0.099 g of bromocresol green and 0.066 g of methyl red were dissolved in 100 cm^3 99% ethanol.

Sulphuric acid (0.01 mol dm^{-3}): 0.56 cm^3 of concentrated H_2SO_4 (96% w/w, sp. gr. 1.80) was added to 200 cm^3 of deionized water in a 1000 cm^3 volumetric flask. After thorough shaking, the solution was made up to mark with more deionized water.

Procedure

Ten grams of the soil sample was weighed into a 500 cm^3 Erlenmeyer flask and 40 cm^3 of 1.0 mol dm^{-3} ammonium acetate was added. The flask was shaken thoroughly and allowed to stand overnight. The mixture was filtered under suction using a Buchner funnel. The soil was leached with more ammonium acetate until test for calcium was negative in the filtrate. The soil was leached further with four portions (25 cm^3 of 1.0 mol dm^{-3}) of NH_4Cl. The leachate was tested for chloride using 0.1 mol dm^{-3} $AgNO_3$ and when the concentration was found to be negligible, the soil was allowed to drain thoroughly.

The NH_4Cl leachate was transferred into 500 cm^3 distillation flask and 10 cm^3 of 1.0 mol dm^{-3}NaOH was added then distilled. 60cm^3 of the distillate was collected into 50 cm^3 of 2% boric acid solution in a 250 cm^3 Erlenmeyer flask and 10 drops of bromocresol green-methyl red indicator were added and then titrated with 0.01 mol dm^{-3} H_2SO_4. The end point was indicated by colour change from bluish green to pink.

A blank distillation was carried out; volume of 1.0 mol dm^{-3} NH_4Cl was measured into a 500 cm^3 distillation flask containing 250 cm^3 distilled water and 10 cm^3 of 1.0 mol dm^{-3}NaOH was added and then distilled. 60 cm^3 of the distillate was collected into 50 cm^3 of 2% boric acid solution in a 250 cm^3 Erlenmeyer flask and 10 drops of bromocresol green- methyl red indicator were added and then titrated with 0.01 mol dm^{-3} H_2SO_4 (Jackson, 1962).

Calculation:

$$\text{CEC (meg/100g)} = \frac{(T-B) \times C \times 100}{W} \qquad (3.4)$$

Where: T is the titre value for sample, B is the titre value for blank, C is the concentration of acid used (mol dm^{-3}), W is the weight of soil sample used.

3.4.5 Determination of Carbonate and Bicarbonate

Preparation of Reagents

Phenolphthalein (indicator): 1.0 g of phenolphthalein was dissolved in 500 cm^3 ethanol and 500 cm^3 distilled water.

Methyl orange (indicator): 1.0 g of methyl orange was dissolved in 1.5 $dm^3$of boiling deionized water and filtered.

Sulphuric acid (0.01 mol dm^{-3}): 0.56 cm^3 of sulphuric acid (17% w/w sp. gr. 1.80) was added to 200 cm^3 of deionized water in a 1000 cm^3 volumetric flask, it was mixed thoroughly and made up to the mark with deionized water.

Procedure

Accurately measured volume 20 cm^3 of the soil extract was transferred into a 250 cm^3 conical flask and few drops of phenolphthalein indicator was added. A pink coloured solution which indicated the presence of carbonate was titrated slowly with 0.01 mol dm^{-3} H_2SO_4 until the solution became colourless (phenolphthalein end point). This is called half neutralization stage. The burette reading (volume used) was taken and designated as P. Three drops of methyl orange was then added to the colourless solution and the titration continued until a colour change was observed and the final reading (volume used) was taken and designated as T.

Calculation:

$$\text{Carbonate} = \frac{2P \times \text{concentration}}{\text{weight of soil taken}} \times 100 \quad (3.5)$$

$$\text{Bicarbonate} = \frac{T - 2P \times \text{concetration of } H_2SO_4}{\text{weight of soil taken}} \times 100 \quad (3.6)$$

3.5 Metal Analysis

Reagents

Hydrofluoric acid (1 mol dm^{-3}): was prepared by dissolving 48 cm^3 of hydroflouric acid (48% w/w, sp. gr 1.167) in 52 cm^3 of deionized water in a 100 cm^3 volumetric flask.

Perchloric acid (2 mol dm^{-3}): was prepared by dissolving 70 cm^3 of perchloric acid (70% w/w, sp.1.70) in 30 cm^3 of deionized water in a 100 cm^3 volumetric flask.

Hydrochloric acid (6.0 mol dm^{-3}): was prepared by dissolving 50 cm^3 of hydrochloric acid (36%w/w, sp. gr 1.18) in little volume of deionized water in a 1000 cm^3 volumetric flask and made up to mark with more deionized water.

Procedure

One gram of ground soil sample (wet with deionized water) was placed in a Teflon crucible and 5 cm^3 of 1 mol dm^{-3} hydrofluoric acid and 5 cm^3 of 2 mol dm^{-3} perchloric acid was added. The crucible with its content was placed on a sand bath and heated at about 200^oC in a fume cupboard to dryness. The crucible was removed and allowed to cool. Measured 5 cm^3 of deionized water was added followed by 5 cm^3 of 6.0 mol dm^{-3} HCl and the mixture heated to boiling.

The soil sample dissolved and was transferred to a 100 cm^3 volumetric flask and diluted to mark with deionized water. The concentration of metals (Cd, Cr, Ni, Pb and Zn) were determined using the flame atomic absorption spectrophotometer.

3.6 Preparation of Element Standard Solutions

Lead nitrate solution (1000 mg dm^{-3}): 1.5980 g of lead nitrate, ($Pb(NO_3)_2$) was dissolved in 100 cm^3 of deionized water and diluted to 1 dm^3 in a volumetric flask. It was stored in a polythene bottle where various concentrations (2 mg dm^{-3}, 4 mg dm^{-3}, 6 mg dm^{-3}, 8 mg dm^{-3} and 10 mg dm^{-3}) used for the calibration curve were prepared.

Cadmium chloride solution (1000 mg dm^{-3}): 2.0360 g of cadmium chloride, ($CdCl_2$) was dissolved in 250 cm^3 of deionized water and dilute to 1 dm^3 in a volumetric flask and stored in a polythene bottle where various concentrations (1 mg dm^{-3}, 2 mg dm^{-3}, 3 mg dm^{-3}, 4 mg dm^{-3} and 5 mg dm^{-3}) used for the calibration curve were prepared.

Zinc oxide solution (1000 mg dm^{-3}): 1.2450 g of Zinc oxide, (ZnO) was dissolved in 15 cm^3 deionized water followed by 25 cm^3 of 5 mol dm^{-3} HCl and diluted to 1000 cm^3 in a volumetric flask and stored in a polythene bottle where various concentrations (2 mg dm^{-3}, 4 mg dm^{-3}, 6 mg dm^{-3}, 8 mg dm^{-3} and 10 mg dm^{-3}) used for the calibration curve was prepared.

Nickel nitrate solution (1000 mg dm^{-3}): 4.9530 g of cadmium nitrate, (Ni $(NO_3)_2.6H_2O$) was dissolved in 250 cm^3 of deionized water and dilute to 1 dm^3 in a volumetric flask and stored in a polythene bottle where various concentrations (1 mg dm^{-3}, 2 mg dm^{-3}, 3 mg dm^{-3}, 4 mg dm^{-3} and 5 mg dm^{-3}) used for the calibration curve were prepared.

Chromium nitrate solution (1000 mg dm^{-3}): 7.6960 g of cadmium nitrate, (Cr $(NO_3)_3$. $9H_2O$) was dissolved in 250 cm^3 of deionized water and dilute to 1 dm^3 in a volumetric flask and stored in a polythene bottle where various concentrations (1mg dm^{-3}, 2 mg dm^{-3}, 3 mg dm^{-3}, 4 mg dm^{-3} and 5 mg dm^{-3}) used for the calibration curve were prepared.

3.7 Preparation of Ligand Solutions (1000 mg dm^{-3})

Stock solutions of the tiron and glutamic acid solution were each prepared in separate 1000 cm^3 capacity volumetric flasks by dissolving 1.0 g each of the ligand in small amount of deionized water in the flasks and made up to the mark with deionized water where the various concentrations of 10 mg dm^{-3}, 17.5 mg dm^{-3} and 25 mg dm^{-3} used for the experimental runs were pipetted.

3.8 Full Factorial Design of Experiment For Absorption of Cd, Cr, Pb, Ni and Zn by Tiron and Glutamic acid.

Four factors which are important variables in the absorption process were selected to study the absorption of cadmium, chromium, lead, and nickel from polluted soils by tiron and glutamic acid. The variables were: pH (A), concentration of complexing ligands (B), amount of soil (C), and contact time (D). The absorption process was investigated by varying these factors at two levels (2^4), high (+1) and low (-1) with two central points (0) in the experimental runs (table 3.2). Design softwares (6.0 version) was used to generate the template used in the experimental runs and for statistical analysis of percentage sorbed (table 3.3).Interactions between these factors was studied using cube plotsgenerated by the designsoftwares.

Table 3.2: Factors and Levels Used in the Factorial Design for Absorption of Cadmium, Chromium, Lead, Nickel and Zinc

Factors	Symbol	Low (-)	Centre(0)	High(+)
pH	A	3	4	5
Concentration of ligands (mg dm^{-3})	B	10	17.5	25
Amount of soil (g)	C	5	7.5	10
Contact time (min)	D	30	45	60

Table 3.3: Design matrix and response for removal of cadmium, chromium, lead, nickel and zinc using Tiron and glutamic acid

RUN	Factor 1 A: pH	Factor 2 B:Time (min)	Factor 3 C: Concentration	Factor 4 D:Amount of Soil	Response 1 Pb Removal %	Response 2 Zn Removal %	Response 3 Cd Removal %	Response 4 Ni Removal %	Response 5 Cr Removal
1	3	60	10	10					
2	3	30	10	5					
3	3	60	10	5					
4	3	30	25	10					
5	5	60	10	5					
6	3	60	25	5					
7	5	30	25	10					
8	4	45	17.5	7.5					
9	5	30	10	10					
10	3	60	25	5					
11	5	60	25	5					
12	4	45	17.5	7.5					
13	5	30	25	5					
14	5	30	10	5					
15	5	60	10	10					
16	3	30	10	10					
17	5	60	25	10					
18	3	60	25	10					

3.9 Design Matrix and Response for Extraction of Cd, Cr, Ni, Pb and Zn By Complexing Agents

The concentrations of cadmium, chromium, lead, nickel and zinc of the soils across the Sampling locations was determined by Atomic Absorption Spectrophotometry (AAS). The soil sample (from Tudun-wada) with the highest level of metal concentration was then treated with the aqueous solutions of chelating ligands separately. The concentrations were measured at 10 mg dm^{-3}, 17.5 mg dm^{-3}, 25 mg dm^{-3} (Table 3.3). The masses of the contaminated soil used in each run of experiment was measured at: 5 g, 10 g, 20 g and 25 g. The pH was adjusted to the given concentrations with hydrochloric acid and sodium hydroxide as buffers and kept at (pH 3 – 5). The suspension was shaken with a multipurpose flask shaker at 30 minutes, 45 minutes and 60 minutes. After shaking, the supernatants were carefully filtered, residue washed thoroughly until it is no longer acidic. This was air dried and kept as washed soil (Nolan *et al.*, 2005).

Calculations

The amount of heavy metals absorbed per kilogram of soil in contact with an aqueous chelating agent solution was calculated using an equation similar to the one reported by Reddy and Chinthamreddy (2000) as:

$$S_i = \frac{(C_i - C_f)V}{W} \quad (3.7)$$

Where S_i (mg/kg) is the amount of the species i sorbed per unit weight of soil; C_i (mg/l) is the initial solution concentration of the species; C_f (mg/l) is concentration remaining or final solution concentration of the species V(l) is solution volume; and W (kg) is dry soil weight.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Parameters of Soil

The result of the physiocochemical properties of the soil samples are presented in Table 4.1.Preliminary visual inspection of the soils from polluted metal work sites from Samaru, Sabon-gari and Tudun-wada showed that the soils were dark grey in colour indicating a low amount of humus. Colour is one of the characteristics of soil, which tells much about the origin of the soil and its composition. The value of pH (water) and pH ($CaCl_2$) obtained for soil from Tudun-wada are 7.50 and 9.67 respectively; while the values of pH (water) and pH ($CaCl_2$) obtained for Sabon-gari was 8.43, and 9.03 respectively, indicating the soils are alkaline. The value of pH (water) and pH ($CaCl_2$) obtained for soil from Samaru are 6.47 and 6.60, indicating the soil is moderately acidic.

The CEC values (in $Cmo1^{-1}kg$) was 11.00 at Samaru, 16.33 at Sabon-gari, and 19.67 at Tudun-Wada. Particle size distribution for Samaru was 20.00.% clay, 19.67% silt and 67.33% sand. The particle size distributed for Sabon-gari was 17.00% clay, 17.00% silt and 69.67% silt while the particle size distribution of the soil from Tudun-wada was 12.67% clay, 12.67% silt and 80.00% sand.

4.2 Level of Metal Concentration

The histogram showing the metal concenrtation across all sampling locations is presented in Figure 4.1.The result indicates that the soil sample from Tudun-wada has the highest concentration of heavy metals among the sites under study, this implied it was the most polluted,

and therefore, was the only soil sample used for the washing experiments with the solutions of complexing agents (tiron and glutamic acid).

Table 4.1: Physicochemical Properties of Soil Across All Sampling Locations

Location	pH	pH 0.01M	CO_2	HCO_3	Particle-size Distribution Corrected to 20°C (%)				
	H_2O	$CaCl_2$			CEC	Clay	Silt	Sand	T.class
Samaru	6.47±3.05	6.60±1.71	9.00±10.0	6.00±2.00	11.00±2.00	20.00±2.65	19.67±2.52	67.33±3.06	Sandy Loam
Sabon-gari	8.43±2.45	9.03±1.12	7.67±ND	2.53±0.81	16.33±1.53	17.00±2.00	17.00±2.00	69.67±2.08	Sandy Loam
Tudun-wada	7.50±1.50	9.67±1.53	ND	3.33±1.53	19.67 ±3.2	12.67±3.53	12.67±3.53	80.00±10.00	Sandy Loam

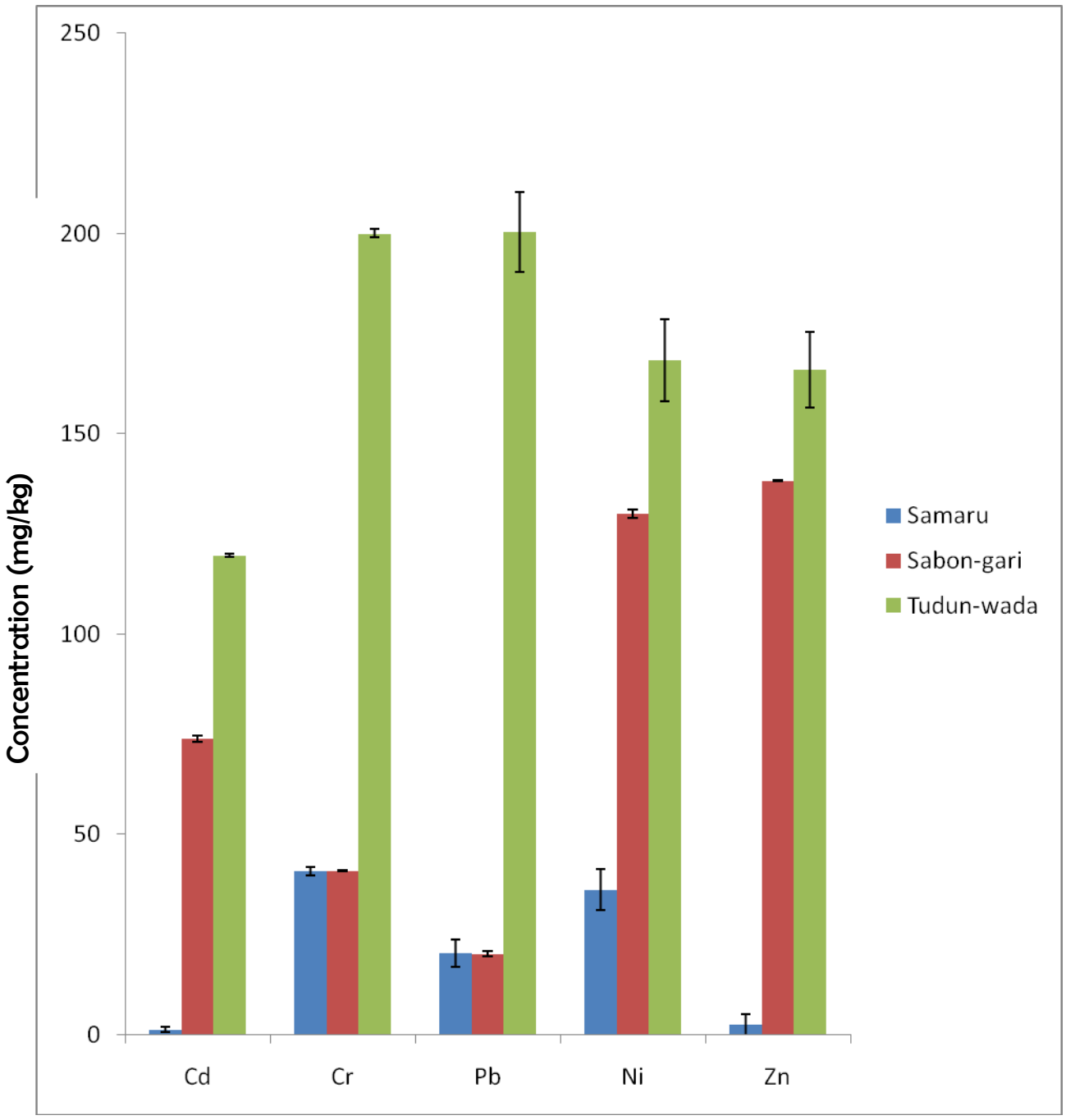


Figure 4.1: Heavy metal concentration (mg/kg) in Samaru, Sabon-gari and Tudun-wada metal workshop soil samples.

4.3 Responses for The Absorption of Heavy Metals By Tiron

The responses or percentage absorbed for the removal of heavy metals by tiron is presented in table 4.2. From the tables, the result indicate good percentage absorbed for all metals under study for the removal of heavy metals using tiron.

The results show that experimental runs (7) and (9) respectively gave the highest removal efficiency value of 83.95%and the least value of 29% for the first run for cadmium. This suggest that for the absorption with tiron, the optimum value of 83.95% percentage sorbed for the interaction of these factors was obtained at pH 5, 30 minutes, 25 mg dm^{-3}concentration with 10 g amount of soil.

The result for chromium shows that the experimental run (18) gave the highest removal value of 98.81% and the least value of 21.43% for the first run. Result infers that optimum removal for the interaction of these factors was obtained at pH 3, 60 minutes contact time, 25 mg dm^{-3} concentration of tiron with 10 gram of soil.

The result for lead shows that experimental run (5) gave the highest removal efficiency value of 76.16% and the least value of 16.6% for the (17) run. The conditions which gave the best percentage sorbed of 76.16% Pb was pH 5, 60 minutes contact time, 10 mg dm^{-3} with 5 g amount of soil.

The result for nickel shows that experimental run (17) gave the highest percentage sorbed of 86.00% and the least value 8.49% for run (1). Best removal efficiency of 86.00% was obtained at pH 5, 60 minutes contact time, 25 mg dm^{-3} and 10 g amount of soil.

The result for zinc shows that experimental run (9) gave the highest removal efficiency of 66.19% and the least value of 7.33% for run (1). The conditions that gave the highest percentage sorbed was of 66.19% was achieved at pH 5, 30 minutes contact time, 10 mg dm^{-3} concentration of tiron and 10 g amount of soil.

Table 4.2: Design Matrix and Response for Removal of cadmium, chromium, lead, nickel and zinc using Tiron

RUN	Factor 1 A: pH	Factor 2 B:Time (min)	Factor 3 C: Concentration (mg dm^{-3})	Factor 4 D:Amount of Soil(g)	Response 1 Pb Removal %	Response 2 Zn Removal %	Response 3 Cd Removal %	Response 4 Ni Removal %	Response 5 Cr Removal
1	3	60	10	10	**22.59**	**7.33**	**29.00**	**8.49**	**21.43**
2	3	30	10	5	**28.72**	**18.35**	**54.74**	**17.13**	**31.90**
3	3	60	10	5	**57.85**	**32.00**	**58.39**	**21.45**	**39.84**
4	3	30	25	10	**61.26**	**24.41**	**47.44**	**19.59**	**47.46**
5	5	60	10	5	**76.16**	**37.98**	**83.94**	**24.36**	**49.44**
6	3	60	25	5	**55.69**	**41.54**	**58.39**	**35.12**	**58.57**
7	5	30	25	10	**63.81**	**53.08**	**83.95**	**48.37**	**61.19**
8	4	45	17.5	7.5	**24.41**	**11.42**	**40.05**	**25.12**	**61.83**
9	5	30	10	10	**63.53**	**66.19**	**83.95**	**36.05**	**63.57**
10	3	60	25	5	**50.00**	**62.63**	**72.99**	**76.59**	**67.04**
11	5	60	25	5	**50.14**	**62.52**	**72.99**	**76.59**	**67.04**
12	4	45	17.5	7.5	**43.04**	**36.58**	**65.99**	**33.78**	**67.62**
13	5	30	25	5	**22.90**	**33.51**	**47.44**	**54.30**	**70.24**
14	5	30	10	5	**38.82**	**52.44**	**58.39**	**25.17**	**70.56**
15	5	60	10	10	**30.20**	**39.86**	**72.99**	**36.05**	**76.27**
16	3	30	10	10	**43.72**	**53.05**	**51.09**	**48.37**	**76.85**
17	5	60	25	10	**16.60**	**23.50**	**72.99**	**86.00**	**90.00**
18	3	60	25	10	**43.39**	**51.43**	**65.69**	**41.63**	**98.81**

4.4 Responses for the Absorption of Heavy Metals by Glutamic Acid

Table 4.3 is the variation of the selected factors affecting absorption process and the corresponding removal efficiency (response) in the percentage obtained after each experimental run was performed for the removal of heavy metals using glutamic acid. The result for chromium shows that experimental run (11) gave the highest removal efficiency of 99.98% and the least value of 35.00% for run (14). Result suggests that the conditions which gave the best compromise was pH5, 60 minutes contact time, 25 mg dm^{-3} and 5g amount of soil.

The results for cadmium shows that the experimental run (15) gave the highest removal efficiency of 99.98% and the least value of 29.00% for run (12). The response which gave the best percentage yield of 99.98% was obtained at pH 5, 60 minutes contact time, 10 mg dm^{-3} concentration and 10 g amount of soil.

The result for lead shows that the run (1) gave the highest removal of 99.99% and the least value of 31.79% for run (16). Result suggest that the interaction of these factors which gave the optimum removal of 99.99% was obtained at pH 3, 60 minutes, 10 mg dm^{-3} concentration and 10 g amount of soil.

The result for chromium shows that experimental run (11) gave the highest removal efficiency of 99.98% and the least value of 35.00% for run (14). The conditions which gave the best percentage sorbed was achieved at pH 5, 60 minutes contact time, 25 mg dm^{-3} and 5 g amount of soil.

The result for zinc shows that run (7) gave the highest removal efficiency of 99.98% and the least value of 17.45% for run (1). Optimum percentage absorbed of 99.98% for the interaction of these factors was obtained at pH 5, 30 minutes contact time, 25 mg dm^{-3} and 10 g amount of soil.

Table 4.3: Design Matrix and Response for Removal of cadmium, chromium, lead, nickel and zinc using glutamic acid

RUN	Factor 1 A: pH	Factor 2 B:Time (min)	Factor 3 C: Concentration (mg dm^{-3})	Factor 4 D: Amount of Soil(g)	Response 1 Pb Removal %	Response 2 Zn Removal %	Response 3 Cd Removal %	Response 4 Nickel Removal %	Response 5 Cr Removal %
1	3	60	10	10	**99.99**	**17.45**	**40.15**	**17.74**	**36.90**
2	3	30	10	5	**65.55**	**24.61**	**43.80**	**37.50**	**51.51**
3	3	60	10	5	**74.56**	**50.03**	**58.39**	**34.94**	**56.67**
4	3	30	25	10	**84.82**	**66.81**	**34.89**	**53.26**	**58.25**
5	5	60	10	5	**59.45**	**48.78**	**40.15**	**28.66**	**51.51**
6	3	60	25	5	**62.16**	**19.31**	**40.15**	**40.70**	**51.83**
7	5	30	25	10	**63.77**	**99.98**	**98.54**	**49.01**	**55.92**
8	4	45	17.5	7.5	**52.24**	**66.81**	**80.29**	**38.20**	**46.67**
9	5	30	10	10	**61.17**	**61.06**	**87.59**	**45.69**	**61.51**
10	3	60	25	5	**57.73**	**50.03**	**51.09**	**50.23**	**61.51**
11	5	60	25	5	**70.24**	**51.14**	**29.00**	**33.60**	**99.98**
12	4	45	17.5	7.5	**99.98**	**38.91**	**72.99**	**37.38**	**45.71**
13	5	30	25	5	**66.64**	**26.48**	**62.04**	**31.22**	**45.48**
14	5	30	10	5	**65.13**	**39.48**	**62.04**	**26.63**	**35.00**
15	5	60	10	10	**65.52**	**65.03**	**99.98**	**51.34**	**48.73**
16	3	30	10	10	**31.79**	**40.12**	**54.74**	**32.73**	**56.00**
17	5	60	25	10	**69.90**	**48.76**	**62.04**	**28.66**	**38.97**
18	3	60	25	10	**62.95**	**59.56**	**58.39**	**41.00**	**92.86**

4.5 The Effect of Interaction of Three Variables on The Removal of Tiron and Glutamic Acid

The cube plots to show the effects of the interaction of three variables on the removal of Cd, Cr, Ni, Pb and Zn using tiron are presented in figures 4.3 – 4.7. The values on each plot represent the percentage absorbed. From each cube plot, the interractions which give the optimum percentage removal can also be deduced. The result show that optimum removal efficiency was 9.16%, 83.22%, 61.03%, 69.86%, 59.32% for Cd, Cr, Ni, Pb and Zn respectively. This means removal efficiency is of the following the order: Cr > Pb > Ni > Zn> Cd. Optimum removal of Cr 69.92% was obtained at pH 4, concetration of 25 mg dm^{-3}, 5 g amount of soil and 60 minutes contact time. Optimum removal of cadmium was obtained at pH 5, 30 minutes contact time, 25 mg dm^{-3} and 10 g of soil. Optimum removal of Pb was obtained at pH 5, 60 minutes contact time, 10 mg dm^{-3} and 5 g amount of soil. Best percentage removal of nickel was obtained at pH 5, 60 minutes contact time, 25 mg dm^{-3} and 10 g amount of soil.

Figures 4.8 – 4.12, are the cubeplots to show the effect of interaction of concetration of glutamic acid, contact time, amount of soil and pH on the removal of Cd, Cr, Ni, Pb and Zn. The results show optimum removal values of 61.43%, 56.41%, 37.68%, 1.81% 63.23% for Cd, Cr, Ni, Pb and Zn respectively. The removal efficiency is of the following order: Zn > Cd > Cr > Ni > Pb.The removal of Cr 56.41% was obtained at pH 5, 10 mg dm^{-3} concetration of glutamic acid, 7.5 g amount of soil and 60 minutes contact time. The response which gave the best percentage removal of Cd 61.43%was pH 5, 60 minutes contact time, 10 mg dm^{-3} and 10 g amount of soil. Result suggest that the condition which gave the best removal of Ni 37.68% was pH 5, 60 minutes contact time, 25 mg dm^{-3} and 5 g amount of soil. The interraction of factors

gave the best removal for Pb 1.81% at pH 3, 60 minutes 10 mg dm^{-3} with 10 g amount of soil. The conditions which gave the optimum percentage sorbed of Zn 63.23% was pH 5, concentration of 25 mg dm^{-3} , 30 minutes contact time with 7.5 g amount of soil.

Removal efficiency (%)

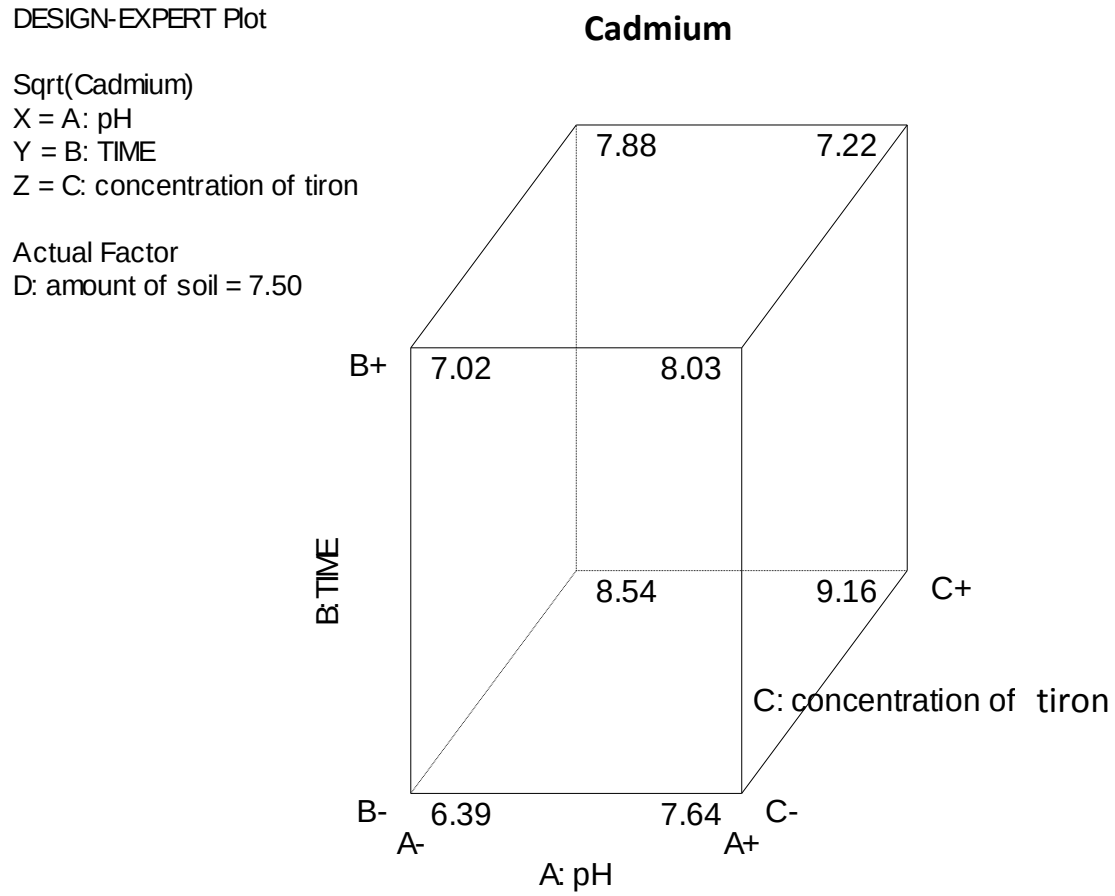


Figure 4.3: Cube plot for the absorption of cadmium using tiron and interaction between pH, time and concetration.

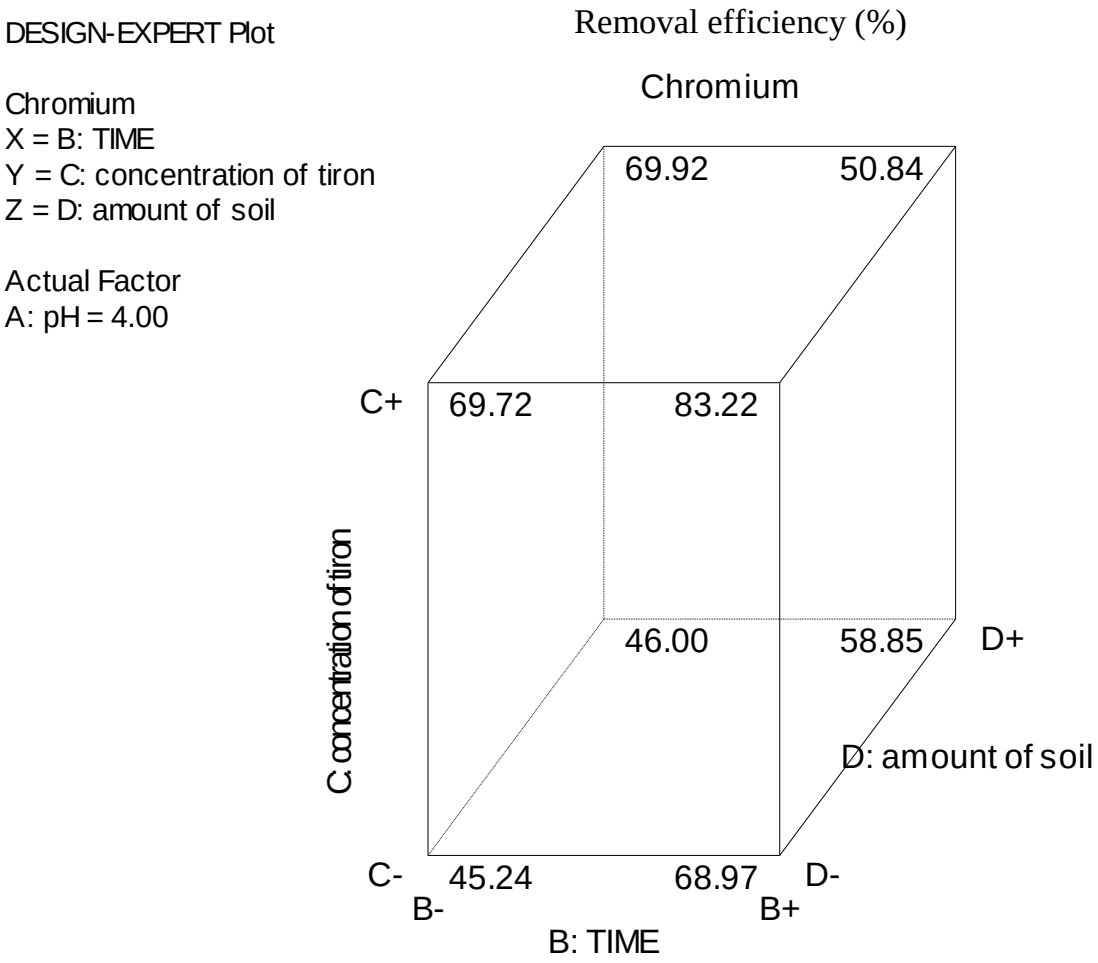


Figure 4.4: Cube plot for the absorption of Chromium using tiron and interaction between amount of soil, time and concetration.

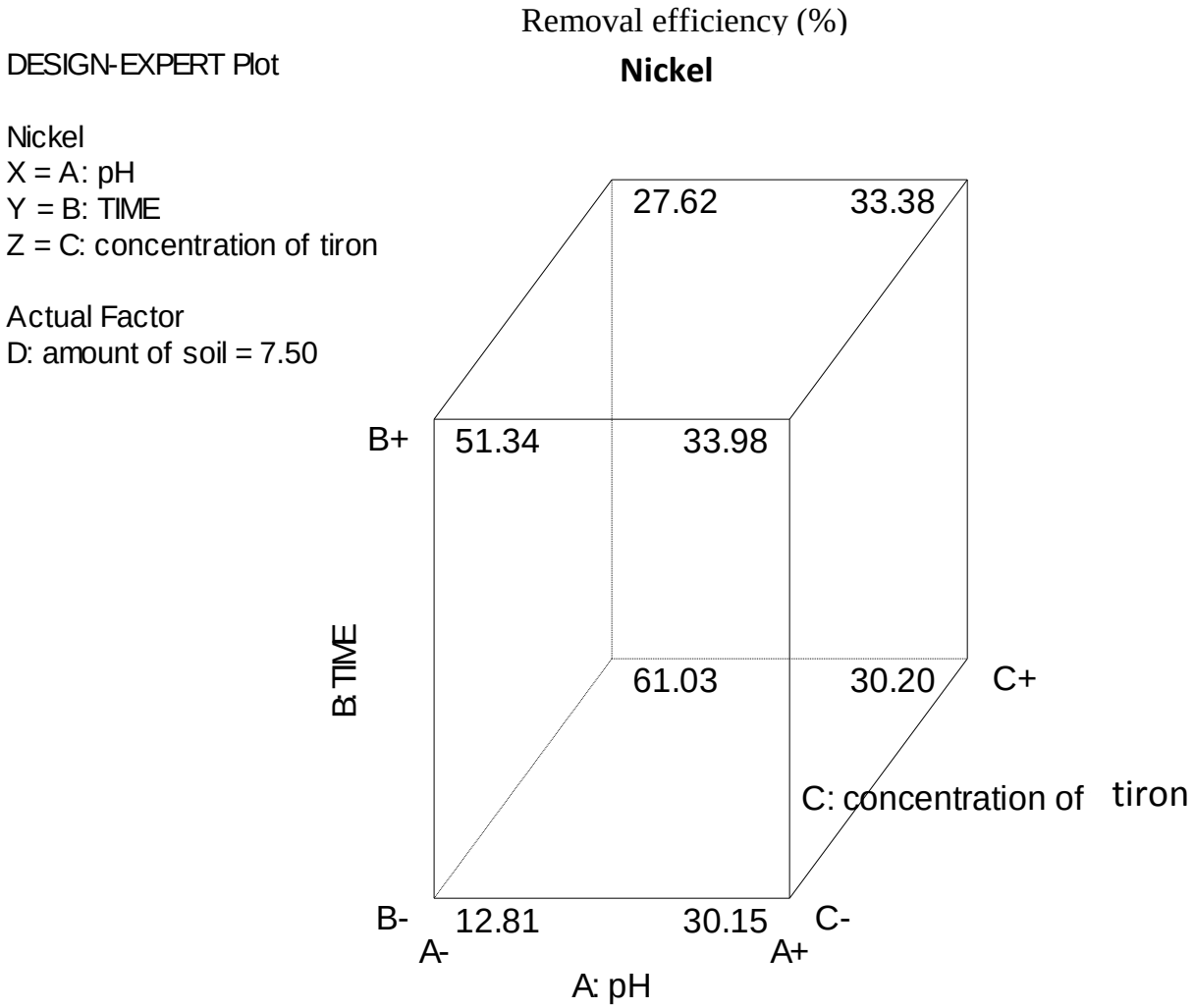


Figure 4.5: Cube plot for the absorption of nickel using tiron and interaction between pH, time and concetration.

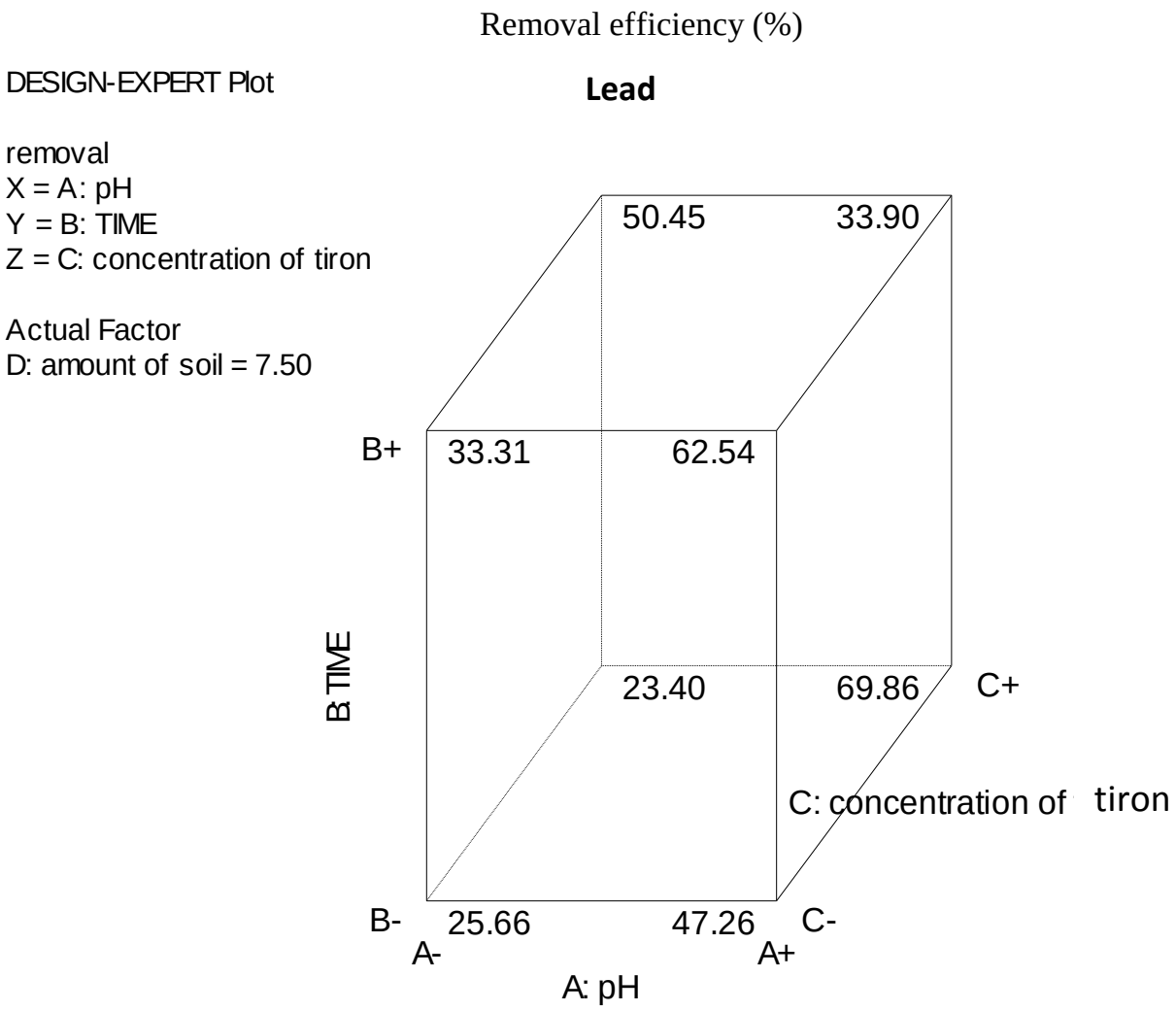


Figure 4.6: Cube plot for the absorption of lead using tiron and interaction between pH, time and concetration.

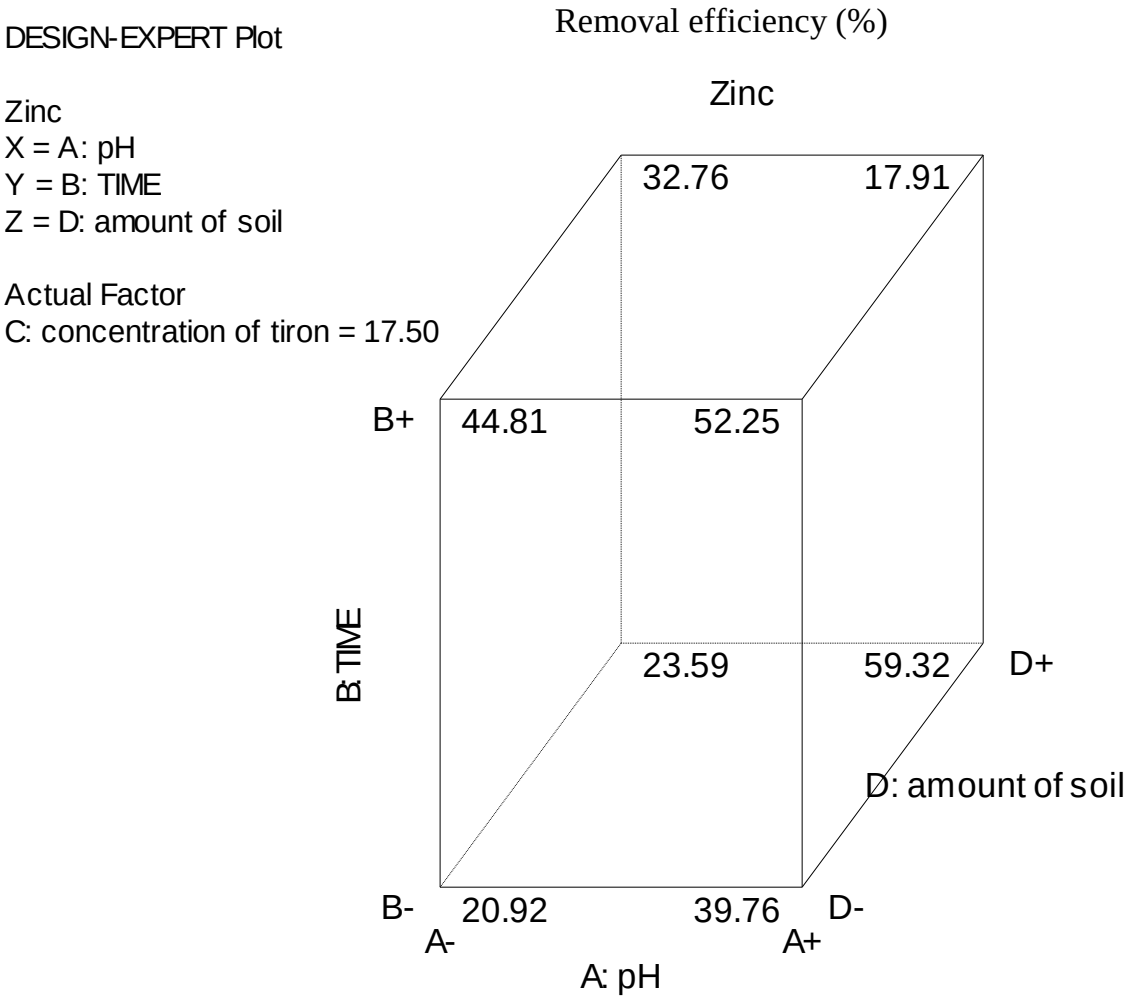


Figure 4.7: Cube plot for the absorption of zinc using tiron and interaction between pH, time and concetration.

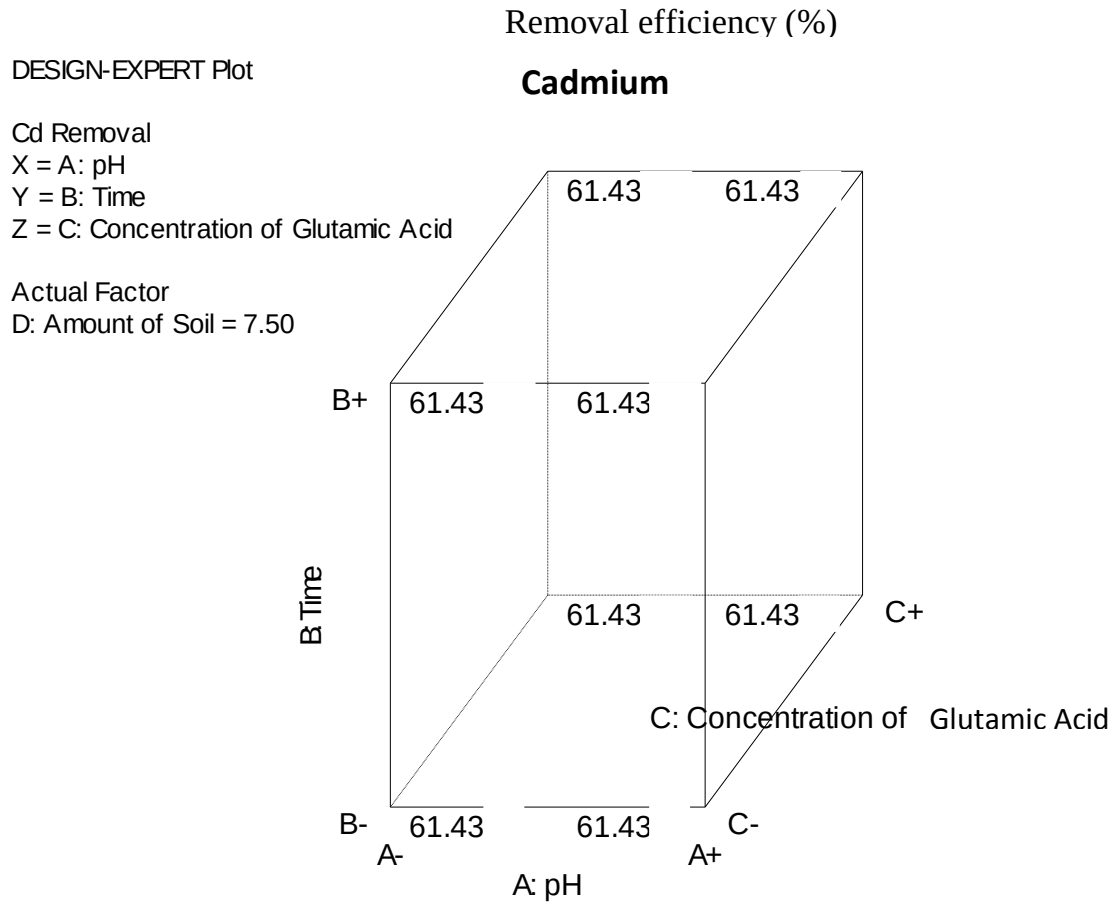


Figure 4.8: Cube plot for the absorption of cadmium using glutamic acid and interaction between pH, time and concetration.

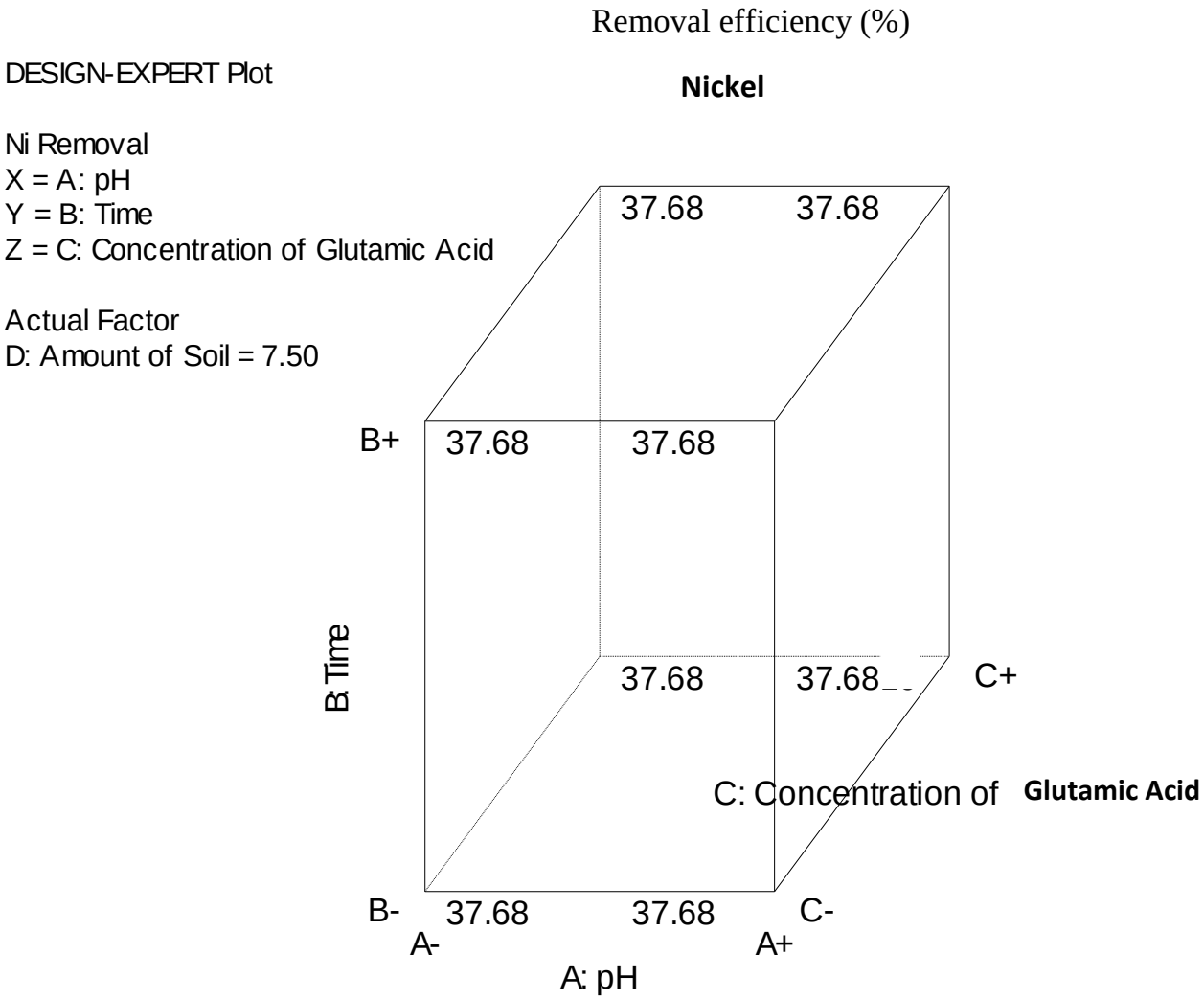


Figure 4.9: Cube plot for the absorption of nickel using glutamic acid and interaction between pH, time and concetration.

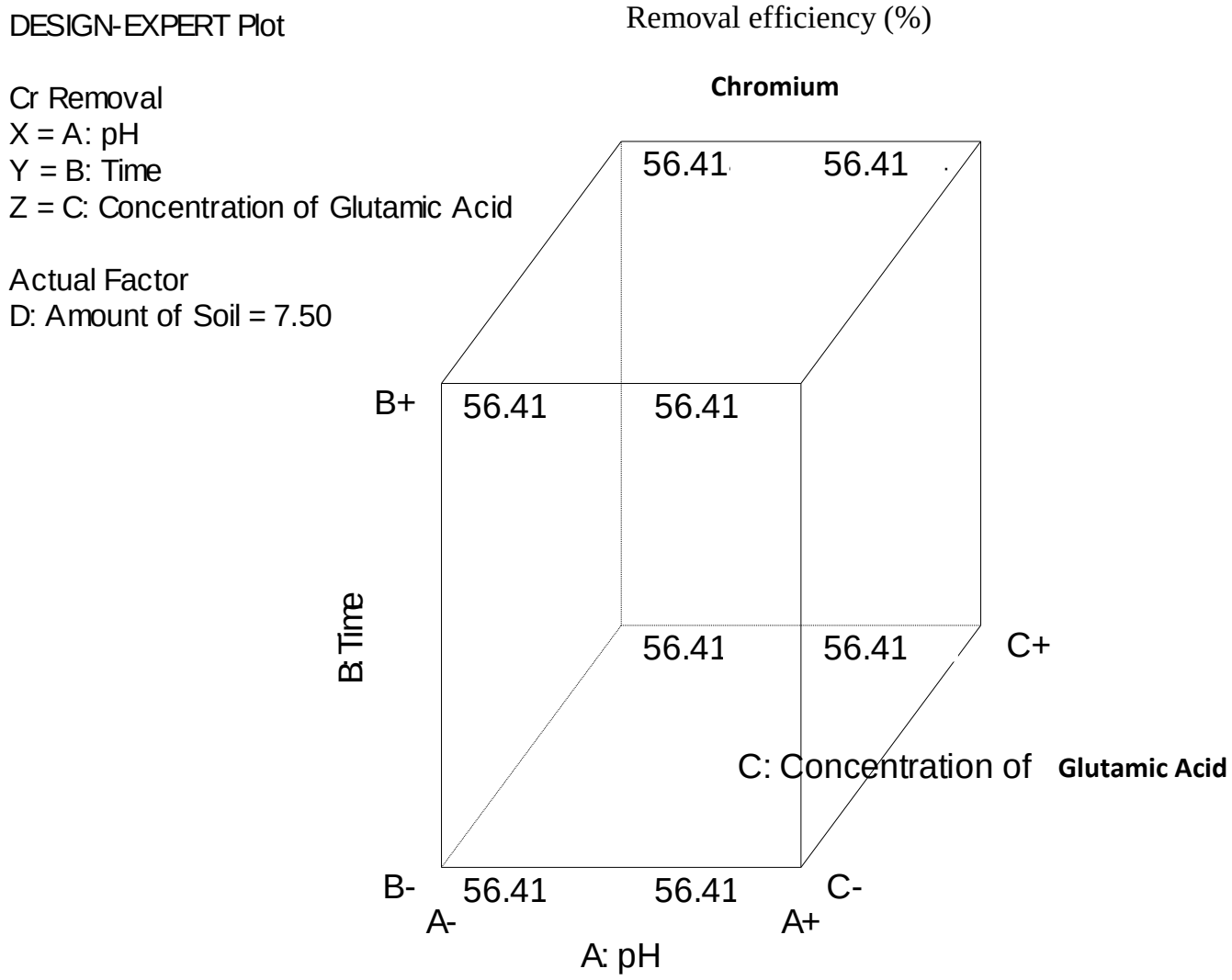


Figure 4.10: Cube plot for the absorption of chromium using glutamic acid and interaction between pH, time and concetration.

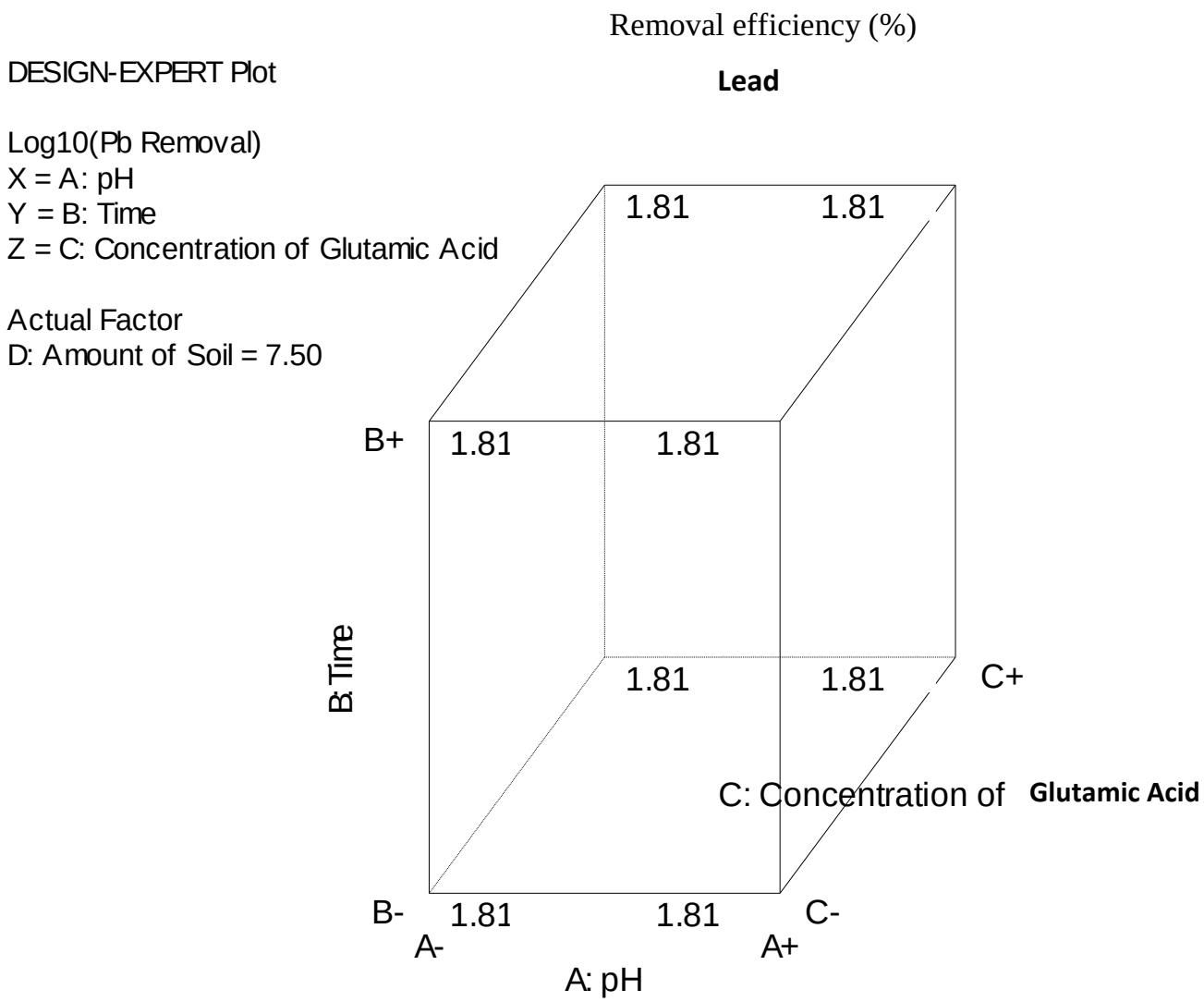


Figure 4.11: Cube plot for the absorption of lead using glutamic acid and interaction between pH, time and concetration.

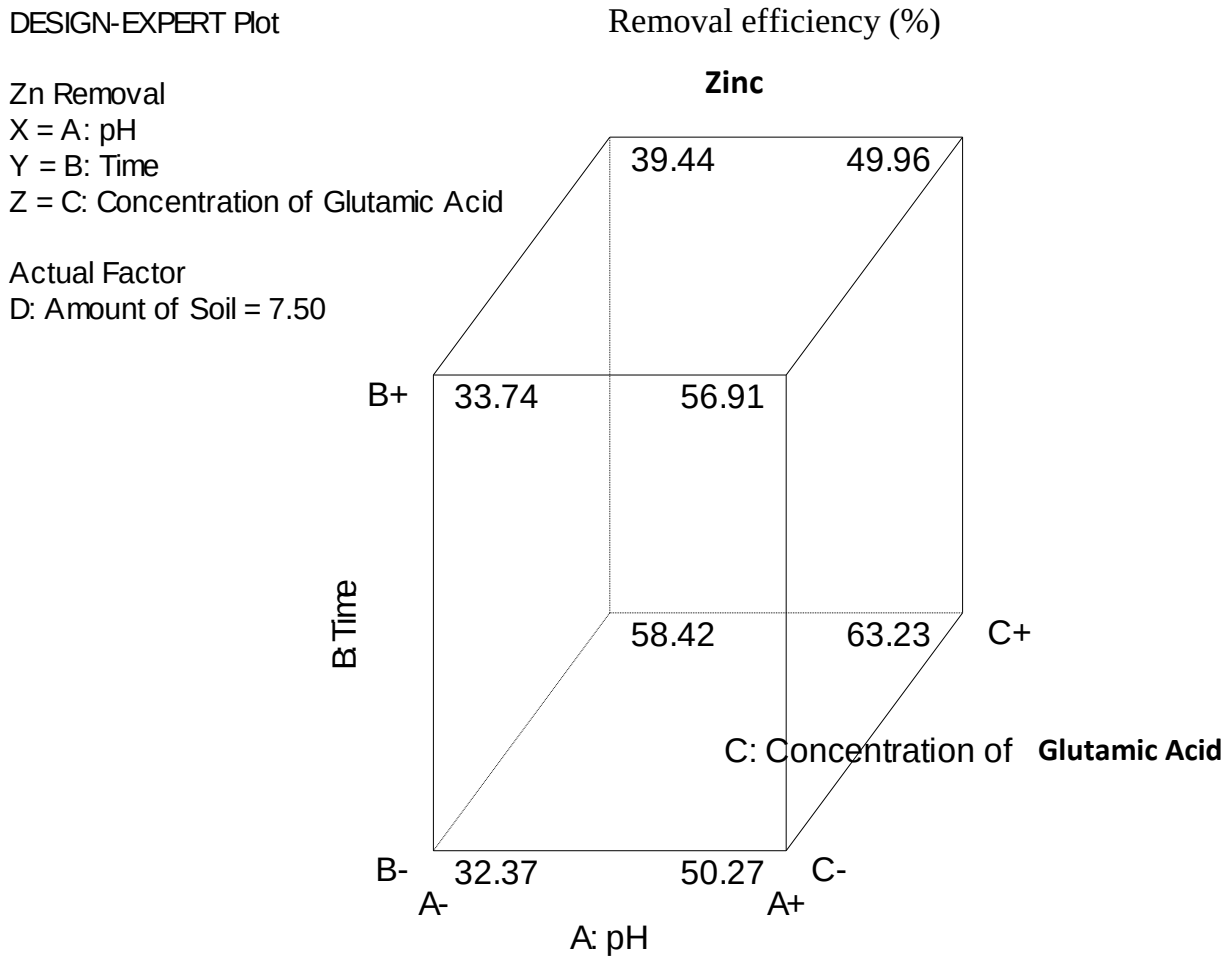


Figure 4.12: Cube plot for the absorption of zinc using glutamic acid and interaction between pH, time and concetration.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Properties of Soil

Particle size analysis across all sampling locations showed the preponderance of sand fraction ranged from (67.33 - 80.00%), followed by silt and clay ranging from (12.67 - 19.67%), thus classifying the parent soils as loamy-sand (soil survey staff,1998). The CEC parameter particularly measures the ability of soils to allow for easy exchange of cations between its surface and solution. The relatively low levels of silt, clay and high CEC indicate the high permeability (Eshan *et al.*, 2006; Atafar, 2010). Preliminary characterization also revealed very high metal concentration. Consequently, there was no need for the soil to be spiked with a mixture of the metal nitrates to achieve higher levels of Cd, Cr, Ni, Pb and Zn. The pH values recorded for the soils in Samaru and Tudun-wada is within the range of agricultural soils, while that recorded slightly higher than the range of agricultural soils. Soil pH play a major function in heavy metals as it directly influences ion-pair formation (Tokalioglu *et al*., 2006). Each soil had low carbonate and bicarbonate content and high CEC ranging between (11-20). The relatively low levels of carbonate and bicarbonate, and high CEC indicate high permeability hence leachability of heavy metals in the soil. (Eshan *et al*., 2006; *Atafar et al*., 2010). Particle size distributed across the sampling locations indicate very high percentage of sand, and low levels of clay and silt respectively, classifying the soils as sandy-loam.

5.2 Comparism to Other Used Complexing Agents

Many studies have compared EDTA to other chelating agents, acids and surfactants and found it better suited than or equal to its competitors for the extraction of toxic metals from soils. Between 45 and 100% Pb, 54 – 100% Zn and 47 – 98% Cu were extracted from various contaminated soils by EDTA. The maximum extraction efficiencies of other chelating agents for Pb were found to be the following: 63% for ADA (N – 2 (acetamido) iminodiacetic acid), 60% for NTA, 58% for PDA (pyridine -2, 6, dicarboxylic acid. For Zn maximum extraction efficiencies were reported as 41% with DPTA, 12% with citric acid, and for Cu they were 32% with DPTA and 11% with acid. Citric and tartaric acids and their salts have been identified as the best chelating organic acids (80 – 100%) whose binding efficiencies are close to that of EDTA and DTPA (75 – 99.9%) (Wasay *et al*., 1998).

The results of the batch washing of heavy metals from the contaminated soils with different concentrations of tiron gave a binding efficiency Pb (16.7 – 76.16%), Zn (7.33 – 62.63%), Cd (29.00 – 83.95%), Ni (8.49 – 86.00%) and Cr (21.43 – 98.81%) while glutamic acid gave Pb (31.79 – 99.98%), Zn (17.45 – 99.98%), Cd (29.00 – 99.98%), Ni (17.74 – 53.26%) and Cr (35.00 – 99.98%).

The concentrations of heavy metals and extraction yields varied considerably with nature of the compexing agent. At the end of washing experiment, glutamic acid gave best percentage sorbed of Cd, Cr, Pb and Zn than tiron for all metals under consideration. Suggesting it is suited for the extraction of heavy metals from soils than tiron. Tiron gave a better optimum percentage sorbed Ni than glutamic acid, indicating it is better suited for the removal of Ni from

contaminated soils (Tables 4.2 and 4.3). Results from this study has shown that tiron and glutamic acid have close extraction efficiencies compared to EDTA and other complexing agents like EDDS, IDSA, NTA and MGDA. The result also showed glutamic acid as the most promising compared to tiron

5.3 Heavy Metal concentration in Metal-Works Site

The level of metal concentrations across Samaru, Sabon-gari and Tudun-wada is shown in Figure 4.1. The height of each bar gives the level of the metal ion concentration.

The mean level of Cd in the soils of the study areas ranged from 1.35 – 119.68 mg kg^{-1} The mean Cd value obtained for the metal-work site soils studied were higher than those reported by Omoniyi *et al.,* (2013) for soils of Kaduna metropolis dumpsite, Nigeria, and studies of Yahaya *et al.,* (2009) for dumpsite soil of abattoir dumping site in Yauri, Nigeria, likewise that by Ukpong *et al.,* (2013) for dumpsite soil in Uyo metropolis.From the results, the level of Cd in the soils may lead to environmental hazard. The high concentration of cadmium in the study areas could be attributed to human activities around the site. Cadmium concentrations in the soils were above the standard limits (0.01 mg kg^{-1}) set by Federal Environmental Protection Agency (FEPA) 1991 and (3.0 mg kg^{-1}) set by European Commission (1986).

The mean level of Cr across the metal-work site soils ranged from 40.83 mg kg^{-1} – 119.97 mg kg^{-1}. Chromium concentration in the polluted soils were generally above standard limits (0.03 mg kg^{-1}) set by Federal Environmental Protection Agency (FEPA) 1991 and (50 mg kg^{-1}) set by European commission (1986) with exception of the soil from Tudun-wada which exceeds the standard limits. The concentration range reported in this study for the Cr was higher

than the range of values reported by Adelekan and Alawode (2011) being (13.15 – 75.55 mgkg^{-1}) for municipal dumpsite in Ibadan. Nigeria. The levels of Cr recorded in this study were lower than those recorded by Awokunmi *et al*., (2010) (212.00 – 2020.00 mg kg^{-1}) for soils within Ikere and Ado Ekiti Metropolis dumpsites soils, Ekiti State, Nigeria and Oluyemi *et al*., (2008).

Zinc concentration in the metal-work site was higher for the soil in Tudun-wada, almost equal with the soil from Sabon-gari and lower than the soil from Samaru according to standard limits (138 mg kg^{-1}) set by European Commission (1986).The levels of Zn recorded in this study were higher than the levels reported by Opaluwa *et al.,* (2012) with range (0.82 – 0.91 mg kg^{-1}) for soils around polluted site in Lafia metropolis, Nigeria.

The mean Ni concentration value recorded at across the sampling locations were generally above the standard limits (0.1 mg kg^{-1}) set by Federal Environmental Protection Agency (FEPA) 1991 and the soils from Sabon-gari and Tudun-wada with values of 130 mg kg^{-1} and 168 mg kg^{-1} had higher values than the (75 mg kg^{-1}) limit set by European Commission (1986), thus implying that the soils are contaminated with Ni. The concentration range reported in this study was higher than the concentration range reported by Adelekan and Alawode (2011) being 4.35 – 49.80 mg kg^{-1}, and the range (1.50 – 54.80 mg kg^{-1}) reported by Ukpong *et al.*, (2013), Opaluwa *et al.,* (2012) (0.21 – 0.31) and range (7.10 – 14.90 mg kg^{-1}) reported by Akpofure (2012) in soils of municipal dumpsites in Ibadan, Nigeria, in soils of waste dumpsites in Uyo metropolis, Akwa Ibom State, in soils around dumpsites in Lafia metropolis, Nasarawa State, Nigeria and in soils of abandoned dumpsites in Effurun, Nigeria.

Lead had values ranging from 20.42 mg kg^{-1} to 199.97 mg kg^{-1}.The levels of Pb recorded in this study were lower than the range of 35.7 – 635.31 mg kg^{-1} reported by Yahaya *et al.*,(2009) in

the study of seasonal variations of heavy metal concentration in abattoir dumping site soil in Yauri, Nigeria, Ukpong *et al.,* (2013) reported a range of (3.00 – 117.50 mg kg^{-1}) for dumpsite soil in Uyo metropolis, Nigeria and Adelekan and Alawode (2011) reported a range 45.00 – 624.50 mg kg^{-1} for soil from municipal dumpsite in Ibadan. Nigeria. On the other hand, the concentrations of Pb reported in this study were higher than those reported by Opaluwa *et al.,* (2012) with range 0.90 – 4.20 mg kg^{-1} for soil around dumpsites in Lafia metropolis, Nigeria. Higher values were recorded for lead in this study, these values were generally found to be above the 0.05 mg kg^{-1} limit of Pb in soil as recommended by Federal Environmental Protection Agency (FEPA) 1991, but were below the standard limit set by European Commission (1986) being 100 mg kg^{-1}, though they are within the tolerance limit set by EU. In general, the ranking of the average metal contents in the study area was Pb> Ni> Zn> Cr> Cd.

5.4 The Effect of Interaction of Three Variables on The Removal Efficiency of Cadmium Using Tiron and Glutamic Acid

The cube plot to show the effects of the interaction of three variables on the removal of Cadmium by tiron is presented in figure 4.3. The results show that for 7.5 g amount of soil, the optimum removal efficiency of 9.16% and minimum removal of 6.39%. This result implies that, for the interaction of these three variables when amount of soil is 7.5 g, minimum removal efficiency of 6.39% obtained at 7.5 g amount of soil, pH 3, 10 mg dm^{-3} concentration of tiron and 30 minutes contact time (Table 3.2). Maximum removal efficiency of 9.16% was achieved at pH 3, 10 mg dm^{-3} concentration of tiron and 60 minutes contact time. This shows that the factor influencing absorption of cadmium by tiron is the contact time.

The effect of interaction between contact time, pH and, concentration of glutamic acid on the percentage removal of cadmium showed that for the interaction of these three variables when amount of the soil is 7.5 g, optimum removal efficiency of 61.43% was obtained at pH 3, concentration of 10 mg dm^{-3} of glutamic acid and 30 minutes contact time while a minimum removal efficiency of 61.43% was also obtained under the same conditions (Figure 4.8). This shows equal effect of all variables on the removal of cadmium by glutamic acid.

5.5 The Effect of Interaction of Three Variables on The Removal Efficiency of Chromium Using Tiron and Glutamic Acid

The cube plot for the effect of interaction between pH, contact time and concentration of tiron for the removal of chromium is presented in figure 4.4. The results shows minimum removal efficiency of 45.24% (this corresponds to the least value on the plot). The minimum value was achieved at 10 mg dm^{-3} concentration of tiron, 5 g amount of soil, and 30 minutes contact time at pH 5. The optimum value of 83.22% (which corresponds to the highest value on the cube plot) was achieved at 30 minutes contact time, 25 mg dm^{-3} concentration of tiron 10 g amount of soil and pH 4 (Table 3.2). This revealed that the factor influencing absorption of chromium by tiron are amount of soil, concentration of tiron and pH.

The cube plot for the effect of interaction between contact time, concentration of glutamic acid and amount of soil on the removal of chromium is presented in figure 4.10. The optimum removal efficiency achieved was 56.41% obtained at pH 5, 25 mg dm^{-3} concentration of ligands, 60 minutes contact time and 7.5 g amount of soil (Table 3.2). This implies all variables have equal impact on the absorption of chromium.

5.6 The Effect of Interaction of Three Variables on The Removal Efficiency of Lead Using Tiron and Glutamic Acid

In figure 4.6, the effect of interaction of pH, contact time and concentration of tiron on the removal efficiency of lead indicates good removal efficiency of 69.86% were obtained at (highest value on the cube plot). This optimum value was obtained at 7.5 g amount of soil, 25 mg dm^{-3} concentration of ligands, 30 minutes contact time and pH 5. A minimum percentage removal of 23.40% (least value on the cube plot) was obtained as 7.5 g amount of soil, 25 mg dm^{-3} concentration of tiron, 30 minutes contact time and pH 3 (Table 3.2). From the values of optimum and minimum removal obtained, the variable influencing the removal of lead by tiron is pH. This means when pH is high, more removal of lead could be obtained.

The cube plot for the effect of interaction between pH, contact time and concentration of glutamic acid is presented in figure 4.11. Optimum removal efficiency of 1.81% was achieved at 7.5 g amount of soil, 60 minutes contact time and pH 3 (Table 3.2). This implies that all the variables have equal effect on the absorption of lead by glutamic acid.

5.7 The Effect of Interaction of Three Variables on The Removal Efficiency of Nickel Using Tiron and Glutamic Acid

The cube plot to show the effect of interaction between pH, concentration in $mgdm^{-3}$, and contact time of tiron on the removal of nickel is presented in figure 4.5. The result showed optimum removal efficiency of 61.03% (highest value on the plot). Optimum value of 61.03% removal efficiency for the interaction was obtained at 7.5 g amount of soil, $25mgdm^{-3}$ concentration of tiron, 30 minutes contact time and pH (Table 3.2). A minimum percentage

removal of 12.81% (least value on the plot) was achieved at 7.5 g amount of soil, 10 mg dm^{-3} concentration, 30 minutes contact time and pH 3(Table 3.2). In this case concentration is affecting the removal of nickel by tiron.

Figure 4.9 is the cube plot for the effect of interaction of pH, contact time, concentration of glutamic acid on the removal of nickel. This plot has revealed that optimum removal efficiency of 37.68% was achieved at 25 mg dm^{-3} concentration of glutamic acid, 60 minutes contact time, 7.50 g amount of soil and pH 3 (Table 3.2). Minimum removal efficiency of 37.68% was also obtained under the same conditions. From the optimum and minimum removal values, it can be inferred that all the factors have the same effect on the absorption of nickel.

5.8 The Effect of Interaction of Three Variables on Removal Efficiency of Zinc Using Tiron and Glutamic Acid

In figure 4.7 is the cube plot to show the effect of interaction of pH, contact time, concentration of tiron. This plot has shown the optimum value of 59.32% (highest value on the cube plot). The optimum value of 59.32% was achieved at 10 g amount of soil, pH 5, 30 minutes contact time and 17.5 mg dm^{-3} concentration of tiron (Table 3.2). A minimum removal efficiency of 20.92% (least value on the plot) was achieved at 5 g amount of soil, pH 3, 30 minutes contact time and 17.5 mg dm^{-3} concentration of tiron. This revealed amount of soil and pH are the dominant factors influencing the absorption of zinc by tiron.

Shown in figure 4.12 is the cube plot for the effect of interaction of concentration of glutamic acid, pH, and contact time. The results show that at 7.5 g amount of soil, optimum removal efficiency of 63.23% (highest value on the plot). Therefore, for the interaction of these

three variables when amount of soil is 7.5 g, removal efficiency was achieved at pH 5, concentration of 25 mg dm^{-3} and 30 minutes contact time (Table 3.2). The minimum percentage removal was 32.37% (least value on the plot) obtained at 7.5 g amount of soil, pH 3, concentration of 10 mg dm^{-3} and 30 minutes contact time. This shows that the factors influencing the removal of zinc by glutamic acid are pH and concentration of glutamic acid.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This study has allowed the determination of the level of some important heavy metals at locations where blacksmith activities are carried out. The blacksmith (metal-work) site is getting polluted particularly with Pb, Zn, Cr and Ni. This is a reflection of anthropogenic contribution which might partly result from continuous smelting of iron and aluminum containers. Based on the concentration of heavy metals in the soils, the soils can generally be classified as moderately polluted. The entire blacksmith site soils are considered to be of pollution concern. There was an indication of uneven distribution of the metals in soils from various sampling locations.

The polluted soil samples was treated separately with aqueous solution of tiron and glutamic acid using full factorial design of experiment. The effect of pH, concentration of complexing agent, amount of soil and contact time on the absorption of: Cd, Cr, Ni, Pb and Zn on the absorption process was also investigated. Eighteen (18) experimental runs including two (2) central points were used for each complexing agent as suggested by the 2^4 full factorial design generated from design expert 6.0 software.

The interactions between variables were evaluated by response surface methodology and cube plots. The study clearly shows that factorial design of experiment is one of the suitable methods for optimizing absorption process. Statistical analysis of results proved the significance of the

models from optimization data. The removal efficiency of tiron was Cd 9.16%, Cr 83.22%, Ni 61.03%, Pb 69.86%, and Zn 59.32%.

Removal efficiency of glutamic acid was 61.44% for Cd, 56.41% for Cr, 37.68% for Ni, 64.79% for Pb and 63.23% for Zn, with large surface presented by high low amount of soil, low pH and high initial concentration interacting to enhance removal efficiency.

6.2 Conclusion

From the results of the various studies carried out in this investigation the following conclusions are hereby made:

i. Total metal concentration across all sampling locations in Samaru and Sabon-gari is moderate but high in Tudun-wada, indicating that metal work activities in this area impact the environment negatively due to metal ion.

ii. The relatively low levels of silt, clay, organic matter and cation exchange capacity indicates high permeability and hence leachability of heavy metals.

iii. There was variation in metal removal efficiency. The removal efficiency showed that complete solubilization of metals did not occur, as not all the chelating agent added to the soil, binded the target metal. This might be due to the presence of other ions such as Ca and Fe which have relatively high stable complexes.

iv. Glutamic acid gave more optimum yield of Cd, Cr, Pb and Zn than tiron for all the metals under consideration. This could be due to entropy resulting from an increase in the number

of free molecules liberated as the chelate is formed. This can be readily explained in terms of thermodynamic factors. Large values of stability constant K are favoured by large negative enthalpy and positive entropy changes. For example, in the reaction:

$$[M(H_2O)_6]^{2+} + Gl \rightarrow [M(H_2O)_{4\,Gl}]^{2+} + 2H_2O$$

Two water molecules are replaced by bi-dentate Gl ligand. This process increases the number of particles in the system and hence its disorder and entropy. Thus, $[M(H_2O)_{4\,Gl}]^{2+}$ is more stable than $[M(H_2O)_6]^{2+}$. The size and number of rings, substituents on the rings and the nature of the metal and donor atoms are among the factors that can affect the thermodynamic stability of these chelate systems (Chao *et al*., 1998).

v. Ni was extracted more by tiron for all metals under consideration, this suggest that Ni is more labile in solution than Cd, Cr, Pb and Zn. Cd, Cr, Pb are more bonded to the soil and less labile.

6.3 Recommendations

i. Further work on the removal of heavy metals by complexing agents may include the use of other complexing agents like heme, porphine, malate, histidine.

ii. Additionally the essential metals Ca, Fe, Mg and Mn may be studied with respect to negative side effects of extraction.

iii. The application of tiron and glutamic acid on other polluted sites like dumpsites and heavily fertilized soils, are suggested.

iv. It is also recommended that government should provide appropriate places that will serve as blacksmith village where metal works to be being carried out.

v. Education and legislation on management of wastes in Blacksmith sites should be intensified to forestall the effect of waste-related problems on the environment.

vi. Also, full factorial design of experiment at other levels and factors above four to include temperature may be investigated.

REFERENCES

Ademoroti, C. M. O. (1996) *Standard methods for water and effluents analysis.* Foludex Press Ltd, Ibadan, Nigeria, pp. 29-118.

Adriano, D. C. (1986). Trace elements in the terrestrial environment. Springer Verlag: New York. pp. 553.

Adriano, D.C., Bolan, N.S., Vangronsveld, J., and Wenzel, W.W. (2005): Heavy Metals. *Encyclopedia of Soils in the Environment*, pp. 175-182.

Adriano, DC. (2001): Trace Elements in Terrestrial Environments: Biochemistry, Bioavailability and the Risks of Metals. 2nd ed. Springer. New York. pp. 29-59

Agarwal, S., Zaman, T., and Tuzcu, E.M. (2011). Heavy metals and cardiovascular disease: Results from the National Health and Nutrition Examination Survey 1999-2006. *Angiology*, 62(5): 422-429

Allen SE (1974). Chemical analysis of ecological materials, Backwell scientific Publications, Oxford. Pp. 13-106.

Alloway, B, (2013). Heavy metals in soils- trace Metals and Metalloids in soils and their Bioavailability. *Springer Dordrecht Heidelberg New York London*, Pp: 613.

Alloway, B.J. and Agres, D.C., (1997).Chemical Principles of Environmental Pollution Blackie Academy and Professional, London.

Alloway, B.J., (1990). Heavy metals in soil. First edition, Blackie Academy and Professional Publishers. John Wiley and Sons New York. Pp.5, 6 34 and 100.

Alvarez, J.M., Lopez-Valdivia, L.M., Novillo, J., Obrador, A., and Rico, M.I. (2006).Comparison of EDTA and Sequential extraction tests for phytoavailability prediction of Manganese and Zinc in Agricultural Alkaline Soils. *Geoderma,* 132: 450-463.

American Society for Testing and Materials. (1985) Standard test method for particle-size analysis of soils D 422-63.

Andriano, D.C., (1986). Trace elements in the terrestrial environment, Springer Verlay, New York. Pp. 553.

Ann, M., and Clain, J. (2005). Basic Soil Properties. *Soil and Water Management*Module1.pp. 1-3

Annadurai, G; Juang, R.S; and Lee, D.J (2000) Factorial design analysis of adsorption of activated carbon on activated carbon incorporated with calcium alginate. *Advanced Inorganic Environmental Resources*, 6: 191- 198

Antoniadis, V., Tsadilas, C.D., and Ashworth, D.J. (2007). Monometal and competitive adsorption of heavy metals sewage sludge-amended soil. *Chemosphere,* 68: 489-494.

Arinola, O.G., Nwozo, S.O., Ajiboye, J.A., and Oniye, A.H. (2008). Evaluation of trace elements and total antioxidant status in Nigerian cassava processors. *Pakistan Journal of Nutrition*, 7(6): 770-772.

Asagba, S.O. (2007). Alterations in activities of tissue enzymes in oral cadmium toxicity. *Nigeria Journal of Science and Environment*, 6: 91-102.

Asagba, S.O. (2009). Role of diet in absorption and toxicity of oral cadmium. *African Journal of Biotechnology*, 8(25): 7428-7436.

Asagba, S.O., and Eriyamremu, G.E. (2007). Oral cadmium exposure alters haematological and liver function parameters of rats fed with Nigerian-like diet. *Journal of Nutrition and Environmental Medicine*, 16(1-3): 267-274.

Asagba, S.O., and Obi, F.O. (2000). Effect of cadmium on kidney and liver cell membrane integrity and antioxidant enzyme status: implications for Warri River cadmium level. *Tropical Journal of Environmental Science and Health*, 3(1): 33-39.

Asagba, S.O., and Obi, F.O. (2005). A comparative evaluation of the biological effects of environmental cadmium contaminated control diet and laboratory cadmium supplemented test diet. *Biometals* 18: 155-161.

Asagba, S.O., Eriyamremu, G.E., Adaikpoh, M.A., and Ezeoma, A. (2004). Levels of lipid peroxidation, superoxide dismutase and Na+/K+- AT Pase in some tissues of rats exposed to a Nigerian diet and cadmium. *Biological Trace Elements Research*, 100(1): 75-86.

Asagba, S.O., Isamah, G.K., Ossai, E.K. and Ekakitie, A.O. (2002). Effect of oral exposure to cadmium on the levels of vitamin A and lipid peroxidation in the eye. *Bulletin of Environmental Contamination and Toxicology*, 68: 18-21.

Atafar, Z., Mesdaghinia, A.R., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, and Mahvi, A.H. (2010). Effect of fertilizer application on soil heavy metal concentration. *Environmental Monitoring Assessment,* 160(1-4) 83-89.

Atanassova, I., and Okazak, M. (1997). Adsorption – desorption characteristics of high Levels of copper in soil clay fractions. *Water, Air and Soil Pollution*, 98: 213-228

Auta, M. Hameed, B.H (2011). Optimized waste tea activated carbon for adsorption of methylene blue and acid blue29 dyes using response surface methodology. *Chemical Engineering journal*, 175: 233- 243.

Adelekan B.A. and Alawode, A.O. (2011). Concentrations of municipal refuse dumps to heavy metal concentrations in soil profile and ground water Ibadan, Nigeria. *Journal of Applied Biosciences*, 40: 2727-2737.

Awokunmi, A., Asaolu, S. and Ipinmoroti, K. (2010). Effect of leaching on heavy metals concentration in some dumpsites. *African Journal of Environmental Science and Technology*, **4**(8): 495-499.

Bagchi, D., Bagchi, M., Hassoun, E.A. and Stohs, S.J. (1996). Cadmium-induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague-Dawley rats. *Biological Trace Elements Research*, 52(2): 143-154.

Barekzai, A. and Mengel, K. (1993). Effect of microbial decomposition of mature leaves on soil-pH. *Journal of Plant Nutrition and Soil Science*, 156: 93–94.

Barona, A., Aranguiz, I., and Elias, A. (2001). Metal Associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. *Environmental Pollution,* 113: 79–85.

Basu, P.K. (2011). Nano materials and chemical sensors. *Sens Transducers,* 134(11): 1–31.

Batra, J. and Seth, P.K. (2002). Effects of iron deficiency on developing rat brain. *Indian Journal of Clinical Biochemistry*, 17(2): 108-114.

Bennett LE, Burkhead JL, Terry N, Pilon M, PIioln–smits EAH (2003). Analysis of transgenic Indian mustard plants for phytoremediation of metals-contaminated mine tailings. J. Environ. Qual. 32: 432- 440.

Bermond, A., Varrault, G., Sappin-Didier, V., and Mench, M. (2005). A Kinetic Approach To Predict Soil Trace Metal Bioavailability: Preliminary Results. *Plant and Soil,* 275: 21-29.

Bermond, A., Yousfi, I., and Ghestem, J.P. (1998). Kinetic Approach to the Chemical Speciation of Trace Metals in Soils. *Analyst,* 123: 785-789.

Black, C.A. (1965). Methods of Soil Analysis. *American Society Agronomy Journal.* Madison, Wisconsin USA. 2: 1-19.

Bordas, F. and Bourg, C.M. (1998). Effect of Complexing Agents (EDTA and ATMP) on the Remobilization of Heavy Metals from a Polluted River Sediments. *Geochemistry, 4: 201-214.*

Bouyoucos, G.J. (1962). Hydrometer method improved for making particle size analysis of soil. *Agronomy Journal,* 54: 464-465.

Box, G.E.P; and Wilson, K.N. (1951). On the experimental attainment of optimum conditions. J R Stat Soc Ser B13: 1- 45.

Bradl, H.B. (2004). Adsorption of heavy metal ions on soils and soil constituents. *Journal of Colloid Interface Science,* 277: 1-18.

Brady, N. and Weil, R. (2002). The Nature and Properties of Soils, 13th Edition. Prentice Hall. Upper Saddle River, New Jersey. Pp 960.

Bricka, R.M. (1993). Technology Assessment of Currently Available Development Techniques for Heavy Metal-Contaminated Soil Treatment. Technical report IRRP-93-1, U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS. *Environmental Progress and Sustainable Energy,* 16 (4): 274–280.

Buchanan, B., Grusen, W., Jones, R. (2000). Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists Maryland pp 1367.

Bucheli Witscheli, M., and Egli, T. (2001). Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiology Reviews,* 25: 69–106.

Buokhalfa H, Lewis JG and Crmbliss AL, (2004). Beryllium, (II) Binding to ATP and ADP: potentiometric determination of thermodynamic constants and implications for in vivo toxicity, Biometals, 17, 105

Campbell, J.R. and Estey, M.P. (2013). Metal release from hiprostheses: cobalt and chromium toxicity and the role of clinical laboratory. *Clinical Chemistry and Laboratory Medicine*, **52**(1): 213-220.

Casagrande, C. and Alleoni, R.F. (2001). Ionic Strength and pH Influence on Zn adsorption in a Variable Change Soil. In proceedings of 6th International Conference on the Biogas Chemistry of Trace Elements. Guelph. pp 7-11

Chaignon, V., Sanchez-Neira, I., Herrmann, P., Jaillard, B., and Hinsinger, P. (2003).Copper Bioavailability and Extractability as Related to Chemical Properties of Contaminated Soils from a Vine-Growing Area. *Environmental Pollution,* 123: 229-238.

Chao, J.C., Hong, A., Okay, R.W., and Peters, R.W. (1998). Selection of chelating agents for remediation of radionuclide-contaminated soil. *Proceedings of the 1998 Conference on Hazardous Waste Research,* 142-155.

Chapman, H.D. (1965). Cation Exchange Capacity. In: C.A. Black (ed). Methods of Soil Analysis Chemical and Microbiological properties. *Agronomy, 9: 891–901*.

Chater, S., Douki, T., Favier, A., Sakly, M. and Abdelmelek, H. (2009). Changes in antioxidant status and biochemical parameters after oral cadmium administration in female rats. *Acta Biologica Hungarica*, 60(1): 79-88.

Chen, H., and Cutright, T. (2001). EDTA and HeDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus. Chemosphere,* 45: 21–28.

Chen, S.B., Zhu, Y.G. and Ma, Y.B. (2006). The Effect of Grain Size of Rock PhosphateAmendment on Metal Immobilization in Contaminated Soil. *Hazardous Material*, 134(1-3): 74-79. doi:10.1016/j.jhazmat.2005.10.027

Chen, S.H, Atken, M, D., 1999. Salicylate stimulates the degradation of high molecular weight polycyclic aromatic hydrocarbons by pseudomonas saccharophila p15. *Eviron. Sci Technol.* **33,** 435-439.

Cline, S. R., and Reed, B. R. (1995). Lead Removal from Soils via bench-scale soil washing Techniques. *Journal of Environmental Engineering*, 121(10): 700–705

Cross, D.P., Gurumurthy, R., and Wattenberg, E.V. (2001). Mixtures of Nickel and Cobalt Chlorides Induce Synergistic Cytotoxic Effects: Implications for Inhalation Exposure Modeling. Annas of Occupational Hygiene, 45(5): 409-418

Crossgrove, J., and Zheng, W. (2004). Manganese toxicity upon overexposure.*NMR in Biomedicine*, 17: 544-553.

Davis, A. P., and Singh, I. (1995). Washing of zinc (II) from contaminated soil column. *Journal of Environmental Engineering*, 121(2): 174-185

Degryse, F., Smolders, E., and Merckx, R. (2006). Labile Cd Complexes Increase availability to Plants. *Environmental Science & Technology*, 40: 830-836.

Dembitsky. V (2003). Natural occurrence of arseno compounds in plants, lichens, fungi, algal species, and microorganisms. Plant Sci., 165: 1177-1192.

Detzner, H.D., Schramm, W., Doring, U., and Bode, W. (1998). New technology of mechanical treatment of dredged material from hamburg harbour. *Water Science Technology*, 37: 337–343

Di Palma, L. and Mecozzi, R. (2007). Heavy Metals Mobilization from Harbor Sediments using EDTA and Citric Acid as Chelating Agents. *Journal of Hazardous Material*, 147: 768–775.

Di Palma, L. and Medici, F. (2002). Recovery of copper from contaminated soil by flushing. Waste *Management*, 22: 883-886.

Dodge, C.J., Francis, A.J. (1994). Photodegradation of uranium-citrate complex with uranium recovery. *Environmental Science and Technology*, 28: 1300–1306.

Donald, S. R., and Quirine, K. (2011). Recommended methods for determining soil cation exchange capacity. *Cooperative Bulletin*, 493: 75–85

Doumett, S.; Lamperi, L.,; Checchini, L., Azzarello, E.; Mugnai,S.; Mancuso, S.; Petruzzelli, G.,; Del Bubba, M., (2008). Heavy metal distribution between contaminated soil and Pualownia tomentosa, in a pilot-scale assisted phytoremediation study: Influence of different complexing agents. *Chemosphere*, 72 (10), 1481- 1490.

Dowdy, R.H., and Volk, V.V. (1983).Movement of heavy metals in soils. In chemical mobility and reactivity in soil system. Nelson, D.W. *et al*., (ed) SSSA spec. publ. LLSSSA madison, WIPP. 227-240.

Ducic, T. and Polle, A. (2005). Transport and Detoxification of Manganese and Copper in Plants. *Brazilian Journal of Plant Physiology*, 17, 103-112.

Ducic, T. and Polle, A. (2007). Manganese toxicity in two varieties of Douglas fir (*Pseudotsugamenziesii* var. viridis and glauca) seedlings as affected by phosphorus supply. *Functional Plant Biology*, 34, 31-40.

Dudka S, Adriano DC (1997). Enviromental impacts of metal ore mining and processing: A review. *Journal of Environmental Quality*, 26: 590-602.

Ehsan , S., Prashner, S.O. and Marshall, W. D. (2006). A washing procedure to mobilize mixed contaminants from soil: Heavy metals. *Journal of Environmental Quality*, 35 (6): 2084 - 2091.

Elibol, M (2002). Response surface methodological approach for inclusion of perfluorocarbon in actinorhodin fermentation medium. Process Biochemistry, 38: 667- 773.

Elless, M.P. and Blaylock, M.J. (2000). Amendment of optimization to enhance lead extractability from contaminated soils for phytoremediation. *International Journal of Phytoremediation*, 2(1): 75-89.

Epstein, A.L., Gussman, C. D., Blaylock, M. J., Yermiyahu, U., Huang, J. W., Kapulnik, Y., and Orser, C. S. (1999). EDTA and Pb-EDTA accumulation in Brassica Juncea grown in Pb-amended soil. *Plant soil*, 208: 87–94.

European Commission (EC) (1986). European Commission office for official publications of the European Communities, Luxembourg, council directive 66/27/EEC on the protection of environment and in particular of soil, when sewage sludge is used in agriculture.

Eriyamremu, G.E., Asagba, S.O., Onyeneke, E.C. and Adaikpoh, M.A. (2005). Changes in carboxypeptidase A, dipeptidase and Na^{+}/K^{+}-AT Pase activities in the intestine of rats orally exposed to different doses of cadmium. *Biometals* 18: 1-6.

Evangelou, M.W.H., Ebel, M. and Schaeffer, A. (2006). Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco Nicotiana tabacum. *Chemosphere*, 63: 996–1004.

Fangueiro, D., Bermond, A., Santos, E., Carapuc¸a, H., and Duarte, A. (2002). Heavy Metal Mobility Assessment in Sediments based on a Kinetic Approach of the EDTA Extraction: Search for Optimal Experimental Conditions. *Analytica Chimica Acta*, 459: 245-256.

Fangueiro, D., Bermond, A., Santos, E., Carapuc¸A, H., and Duarte, A. (2005). Kinetic Approach to Heavy Metal Mobilization Assessment in Sediments: Choice of Kinetic Equations and Models to Achieve Maximum Information. *Talanta,* 66: 844-857.

Feng, M.-H., Shan, X.-Q., Zhang, S.-Z., and Wen, B. (2005). A Comparison of the Rhizosphere-Based Method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ Extraction Methods for Prediction of Bioavailability of Metals in Soil to Barley. *Environmental Pollution,* 137: 231-240.

Figueiredo-Pereira, M.E., Yakushin, S. and Cohen, G. (1998). Disruption of the intracellular sulhydryl homeostasis by cadmium-induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. *Journal of Biological Chemistry*, 273: 12703-12709.

Finkelstein, Y., Markowitz, M.E., and Rosen, J.F. (1998). Low-level lead-induced neurotoxicity in children: an update on central nervous system effects. Brain Research Reviews, 27:168-76.

Fox, T.R., and Comerford, N.B. (1990). Low Molecular-Weight Organic Acids in selected Forest Soils of the South-Eastern USA. *Soil Science Society of America Journal,* 54: 1139-1144.

Freeman, H.M., and Harris, E.F. (1995). Harzadous waste materials: innovative treatment. *Technomic publishing company inc., Lancaster Basel*. 2nded, pp 5-11.

Galazyn-Sidorczuk, M.,Brzoska, M.M., Jurczuk, M. and Moniuszk-Jaconiuk, J. (2009).Oxidative damage to proteins and DNA in rats exposed to cadmium and or ethanol. *Chemico-Biological Interactions*, 180(1): 31-38.

Gao, K., Zhang, W. (1992) Biochemical Eng., 2001, 451-459

Garcia, R., and Baez, A, P. (2012).Atomic Absorption Spectrometry, Akhyar Farrukh (Ed), pp 1-5

Gardiner, D.T. and Miller, R.W. (2004). Soil in our Environment. 10th ed Pearson Education, inc. upper Saddle River, New Jersey. pp 641.

Gee, G.W., and Boudre, J.W. (1986). Particle-size analysis. In klote, A. (Ed) method of soil analysis, part 1 (2nd edition Agronomy No.9) Madison WI.PP.404-409.

Ghestem, J.P. and Bermond, A. (1998). EDTA extractability of trace metals in polluted soils: a chemical-physical study. *Environmental Technology,* 19: 409-416.

Gibson, M.J., and farmers, J.G. (1986).Multi-stop sequential chemical extraction of heavy metal from urban soils. Environ. pollut.11:117-135.

Gismera, M.J., Lacal, J., da Silva, P., Garcia, R., Sevilla, M.T., and Procopio, J.R. (2004). Study of Metal Fractionation in River Sediments. A Comparison between Kinetic and Sequential Extraction Procedures. *Environmental Pollution*, 127: 175-182.

Gleyzes, C., Tellier, S., and Astruc, A. (2002). Fractionation Studies of Trace Elements in Contaminated Soils and Sediments: A Review of Sequential Extraction Procedures. *Trends in Analytical Chemistry*, 21: 451-467.

Goldstein, G.W. (1992). Neurological concepts of lead poisoning in Children. *Pediatric Annals*, 21(6): 384–388.

Gramss, G., Voigt, K.D. and Bergmann, H. (2004). Plant availability and leaching of heavy metals from ammonium-, calcium-, carbohydrate- and citric acid-treated uranium-mine dump soil. *Journal of Plant Nutrition and Soil Science,* 167: 427–471.

Grcman, H., Velikonja-Bolta, S., Vodnik, D., Kos, B., and Lestan, D. (2001). EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant Soil*, 235: 105 – 114.

Grcman, H., Vodnik, D., Velikonja-Bolta, S., and Lestan, D. (2003).Ethylene diamine dissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *Journal Environmental Quality,* 32: 500–506.

Griffths, R.A. (1995). Soil washing technology and practice. *Journal of Hazardous. Material,* 40: 175–189.

Gurevitch, J., Scheiner, S.M. and Fox, G.A. (2002). The Ecology of Plants. Sinauer Associates incorporated, Sunderland Massachuselts. pp 523.

Gutzman, D.W., and Langford, C.H. (1993). Kinetic Study of the Speciation of Copper (II) Bound to a hydrous ferric oxide. *Environmental Science & Technology*, 27: 1388-1393.

Gzar, H.A., Abdul-Hameed, A.S. and Yahya, A.Y. (2014). Extraction of Lead, Cadmium and Nickel from Contaminated Soil Using Acetic Acid. *Open Journal of Soil Science*, 4: 207-214.

Han, F.X., and Banin, A. (1999). Long-term transformation and redistribution of potentially toxic heavy metals in arid-zone soils: II. Incubation at the field capacity moisture content. *Water, Air, and Soil Pollution* 114, 221–250.

Harris RR, Santos MCF (2000). Heavy metal contamination and physiological variability in the Brazilian mangrove crabs *Ucides cordatus* and *Callinectes danae* (Crustacea: Decapoda). Marine Biology, 137: 691-703.

Health Organization International Program on Chemical Safety (IPCS). Geneva, Switzerland.

Hendershot, W.H., Lalande, H. and Duquette, M. (1993). Soil reaction and Exchangeable acidity. In soil sampling and methods of analysis, carter, M.R. (Ed), can soc. Sci. Lewsis, Boca Raton, FL, PP 141-145.

Hong, A.P.K., Chelsea, L., Banerji, S.K., and Wang, Y. (2002). Feasibility of metal recovery from soil using DTPA and its biostability. *Journal of Hazardous Materials,* B94: 253–272

Hrudey, S.E., Chem, W and rousseaux, C.G. (1995). Bioavailability in environmental risk assessment, lewis publ. Boca Raton FL.

Huang, F.C., Brady, P.V., Lindgren, E.R. and Guerra, P. (1998b). Biodegradation of uranium-citrate complexes: implications for extraction of uranium from soils. *Environmental Science and Technology*. 32, 379–382.

Huang, J. W., Chen, J., Berti, W. B., and Cunningham, S. D. (1997). Phyto-remediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science Technology*, 31: 800–805.

Huang, J.W., Blaylock, M.J., Kapulnik, Y. and Ensley, B.D. (1998a). Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyper accumulation in plants. *Environmental Science and Technology* 32, 2004–2008.

Huang, P.M. and Germida, J.J. (2002). Chemical and biochemical processes in the rhizosheremetal pollutants. In P.M. Huang interactions between soil particles and microorganisms impact on terrestrial ecosystem. John Wiley and sons, New York. 381-438.

Ikhuoria EU, Okieimen, FE (2000). Scavenging cadmium, copper, lead, nickel, and zinc ions from aqueous solution by modified cellulosic sorbent. Int. J. Environ Studies

Institute, T., Morose, G., Harriman, E., Civie, P. and Tenney, H. (2013). Industrial uses of lead. Retrieved from http://www.eoearth.org/view/article/153827

Ishii, R., Otsuka, S.I. and Shiro, I. (1967), Microbial production of amino acids from hydrocarbons. (1) Isolation of good hydrocarbon utilizers and amino acids production by autoxotrophs, *J.Gen. Appl. Microbiol* 13, 217-225.

Jackson, M.L. (1962). *Soil Chemical Analysis*. Prentice Hall, New, York.

Jean, L., Bordas, F. and Bollinger, J. (2007). Chromium and Nickel Mobilization from a Contaminated Soil using Chelant. *Environmental Pollution, 147: 729-736.*

JECFA (Joint FAO/WHO) Expert Committee and Food Additives, (1987), L-glutamic acid and its ammonium, extraction, monosodium and potassium salts, in: *Toxicological Evaluation of certain food additives*, WHO food additive services, Cambridge University Press 22, 97-162.

Jerome, L., Fabrice, M., Alain, B., Philippe, C., Christelle, F., Isabelle, L. and Folkert,V.,(2007). Kinetic Extraction to assess mobilization of Zn, Pb, Cu and Cd in a metal-contaminated soil: EDTA *vs* citrate. *Environmental Pollution, 152: 693–701*.

Jung, C., Maeder, V., Funk, F., Frey, B., Sticher, H., Frossard, E, 2003. Release of phenols from *Lupinus albus L*. roots exposed to Cu and their possible role in Cu detoxification. Plant Soil 252, 301-312.

Kabata-Pendas, A. (1995) Agricultural Problems Related to Excessive Trace Metal Contents of Soil. In: Salomons, W., Forstner, U. and Mader, P., Eds., Heavy Metals (Problems and Solutions), Springer Verlag, Berlin, Heidelberg, New York, London, Tokyo, 3-18.

Kabata-pendias, A and pendias, H, (1992). Trace elements in soil and plants. 2nd ed. Lewsis, Boca Raton FL, pp. 365-367.

Kabata-pendias, A, and pendias, H. (2001). Trace Elements in soil and plants, 3rd Ed. CRC press LLC, Boca Raton, FL.

Ke, X.; Li, P.; Zhou, Q,; Zhang, Y., Sun, T., (2006). Removal of heavy metals from contaminated soil using Tartaric acid. *J. Environ. Sci*. (China), 18 (4), 727- 733

Kharasch, N. (1983). Biological action and medical application of dimethyl sulfoxide. *Annals of the New York academy of sciences*, 391-402.

Kim, C. and Ong, S.K. (1998). The selective reaction of lead and amorphous iron with EDTA in lead sulfate-contaminated soil system. *Environmental Engineering Research*, 3(3): 167-174.

Kim, C., Lee, Y., and Ong, S.K. (2003). Factors affecting EDTA extraction of lead from lead-contaminated soil. *Chemosphere*, 51(9): 845-853.

Kinoshita. S., Udaka and Shimono. M. (1957). Amino acid fermentation: production of L-glutamic acid by various microorganisms, *J. Gen. Appl. Microbiol.* 3, 193-205.

Kirpichtchikova, T.A., Manceall, A., Spandini, L., Panfili, F., Marcus, M.A. and Jacquet, T. (2006). Speciation and solubility of heavy metals in contaminated soil using fluorescence, EXAFS spectroscopy chemical extraction and thermodynamic modeling. *Geochimica et Cosmochimica Acta*, 70: 2163-2190.

Kochian, L., Hoekenga, O. and Piñeros, M. (2004). How do crops plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. *Annual Review Plant Biology*, 55, 459-93.

Koedrith, P. and Seo, Y.R. (2011). Advances in carcinogenic metal toxicity and potential molecular markers. *International journal of molecular science*, 12(12): 9576-9595.

Krishina, R.R. and Supraja, C. (2000). Comparison of Extractants for removing heavy metals from contaminated clayey soil. *Soil and Sediment Contamination*, 9(5):449-462.

Krishnamurti, G.S., and Naidu, R. (2003). Solid-solution equilibria of cadmium in soils. *Geoderma*, 113: 17-30

Krishnamurti, G.S., Smith, L.H., and Naidu, R. (2002). Method for Assessing Plant Available Cadmium in Soils. *Australian Journal of Soil Research*, 38: 823-836.

Kumar, P.B.A.N., Dushenkov, V., Motto, H., and Raskin, I. (1995). Phytoextraction –the use of plants to remove heavy metals from soils. *Environmental Science Technology*, 29: 1232–1238.

Lall, S.B., Das, N., Rama, R., Peswin, S.S., Khattar, S., Gulatti, K. and Seth, S.P. (1997). Cadmium-induced nephrotoxicity in rats. *Indian Journal of Experimental Biology*, 35(2): 151-154.

Lee, C.C. and Marshall, W.D. (2002). Recycling of complexometric extractants to remediate a soil contaminated with heavy metals. *Journal of Environmental Monitoring*, 4:325-329.

Letan, D., Hanc, A.and Finzgar, N. (2005). Influence of ozonation on extractability of Pb and Zn from contaminated soils. *Chemosphere,* 61:1012-1019.

Li, J., Xu, R., Tiwari, D., and Ji, G. (2006). Mechanism of Aluminium Release from Variable Charge Soils Induced by Low Molecular-Weight Organic Acids: Kinetic Study. *Geochimica et Cosmochimica Acta,* 70: 2755-2764.

Li, Z. and L.M. Shoman. (1991). Heavy metal movement in metal contaminated soil profiles. Soil Science. 16(10): 656-666.

Lim, T.T., Tay, J.H. and Wang, J.Y. (2004). Chelating-agent-enhanced heavy metal extraction from a contaminated acidic soil. *Journal of Environmental Engineering,* 130: 59-66.

Lo, I.M.C., and Yang, X.Y. (1999). EDTA Extraction of Heavy Metals from Different Soil Fractions and Synthetic Soils. *Water, Air, and Soil Pollution,* 109: 219-236.

Magaye, R., Zhao, J., Bowman, L., and Ding, M. (2012). Genotoxicity and carcinogenicity of cobalt, nickel and copper based nano particles. *Experimental and Therapeutic Medicine,* 4(4): 551-561.

Manohar S, Jadia CD, Fulekar MH (2006). Impact of ganesh idol immersion on water quality. Indian J. Environ. Prot, 27(3): 216-220.

Manouchehri, N., Besancon, S., and Bermond, A. (2006). Major and Trace Metal Extraction from Soil by EDTA. Equilibrium and Kinetic Study. *Analytica Chimica Acta,* 559(1): 105-112.

Marschner, H. (1995). Mineral Nutrition of Higher Plants. Academic Press, San Diego, 889

Masakazu, N., Koudai, N., and Kenji, A. (2008). Removal of lead from contaminated soils with chelating agents. *Materials Transactions*, 49(10): 2377-2382.

Mature, K., and Reddy, K.R. (2008). Extraction of mixed contaminants from different soil types. *Soil Sediment Contamination,* 17: 586-608.

McCready, S.; Birch, G.F.; and Taylor, S.E. (2003). Extraction of Heavy Metals from Harbor Sediments using 1M HCl and 0.05M EDTA and Implications for Sediments. *Australian Journal of Earth Sciences,* 50: 249–255

McGrath, D. (1996). Application of Single and Sequential Extraction Procedures to Polluted and Unpolluted soils. *Science of the Total Environment,* 178: 37-44.

Meeker, J.D., Rossano, M.G., Protas, B., Diamond, M.P., Puscheck, E., Daly, D., Paneth, N. and Wirth, J.J. (2008). Cadmium, lead and other metals in relation to semen quality: Human evidence for Molybdenum as a male reproductive toxicant. *Environmental Health Perspective*, 116: 1473-1479.

Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., and Tack, F.M.G. (2005).Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosphere,* 58: 1011-1022.

Millaleo, R., Reyes-Díaz, M., Ivanov, A.G., Mora, M.L. and Alberdi, M. (2010).Manganese as Essential and Toxic Element for Plants: Transport, Accumulation and Resistance Mechanisms. *Journal of Soil Science Plant Nutrition,* 10 (4): 476-494.

Moutsatsou, A., Gregou, M., Matsas, D., and Protonotaris, V. (2006). Washing as a remediation technology applicable in soils heavily polluted by mining metallurgical activities. *Chemosphere,* 63: 1632–1640.

Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (2001). An Evaluation of Technologies for Heavy Metal Remediation of Dredged Sediments. *Hazardous Materials,* 85(1-2): 145-163. Doi: 10.1016/S0304-3894(01)00226-6

Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. (2000). Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA.

Muthukumar, M.D, Sargunmani, N., Selvakumar, J., and Venkkata, R., (2004). Optimization of ozone treatment for color and COD removal of acid dye effluent using central composite design experiment. Dyes Pigments, 63: 127- 134.

Myers, R. H; Mentgomery, D.C; and Anderson-cook, C.M. (2009). *Response surface methodology, process and product optimization using designed experiments*, 3rd edition. Wiley, Hoboken, USA.

Naidu, R., and Harter, R.D. (1998). Effects of Different Organic Ligands on Cadmium Sorption and Extractability from Soils. *Soil Science Society of America Journal*, 62: 644 - 650.

Nakayama, K., Sato, Z., Kinoshita, S. (1964). Growth of a glutamic acid producing bacterium and related bacteria (1) effect of iron salts, ferrichrome, Amino acids and some other Compounds, *J. Gen. Appl. Microbiol.***10**, 143-153.

Nakayama, K., Sato. (Kinoshiya, S. (1964). Growth of a glumatic acid producing bacterium and related bacteria (i) effect of iron salts, ferrichrome, amino acids and some other compounds, *J. Gen. Appl. Microbiol.,* **10:** 143-155.

Needleman, H.L., Schell, A., Bellinger, D., LEvinto, E.N. (1990). The long term effect of exposure to low doses of lead in childhood. An 11 years follow up report. New England. Med 322 (2): 93- 98.

Nicholas, K.A., Wright, S.F. Liebig, M.A. and Pikul, J.L. (2004). Functional Significance of Glomalin to Soil Fertility. Proceedings from the Great Plains Soil Fertility Conference Denver Co. pp 23–30.

Niragu, J.O. (1992). Toxic metal pollution in Africa. *Science of the Total Environment*, 121: -37.

Nirala S., Bhadauria M., Mathur R., Mathur A. (2007): Amelioration of beryllium induced alterations in hepatorenal biochemistry and ultramorphology by co-administration of tiferron and adjuvants. *Journal of biomedical Science*, **14:** 331-345.

No, H.K. and Meyers, S.P. (2000). Application of chitosan for treatment of wastewaters. *Review of Environmental Contamination and Toxicology*. 163:1-28

Nolan, A. L., Zhang, H.; McLaughlin, M. J., (2005). Predition of zinc, cadmium, lead and copper availability to wheat in contaminated soils using chemical speciation, diffusive gradients in thin films, extraction and isotopic dilution techniques. J Environ. Qual., 34 (2), 496-507.

Nouri. J., Mahvi, A. H.; Babeal, A.; Ahmadpour (2006). Regional pattern distribution of groundwater fluoride in shush aquifer of Khuzestan County Iran Fluoride, 39 (4), 321-325.

Nowack, B. (2002). Environmental chemistry of aminopolycarboxylate chelating agents. *Environmental Science and Technology,* 36: 4009 – 4016.

Nowack, B., Schulin, R., and Robinson, B.H. (2006). A critical assessment of chelant enhanced metal phytoextraction. *Environmental Science and Technology,* 40: 5225–5232.

Nriagu J.O. (1978). Lead in the atmosphere, in the Biogeo-chemistry of lead in the environment,

Nriagu J.O. (1996). Toxic metal Pollution in Africa. Science, 223: 272

Omoniyi, K.I., Ekwumemgbo, P.A, and Sanni, H.A. (2013). An evaluation of the As, Cd, Mn and Hg contamination in soils and plants (*Amaranthus spinusis*) of Kaduna Metropolis dumpsites. *Physical Review and Research International*, **3**(4): 541-555.

Opaluwa O.D., Aremu, M.O., Ogbo, L.O., Abiola, K.A., Odiba, I.E., Abubakar, M.M. and Nweze, N.O. (2012). Heavy metal concentrations in soils, plant leaves and crops grown around dump sites in Lafia Metropolis, Nasarawa State, Nigeria. *Advances in Applied Science Research*, **3**(2): 780-784.

Oluyemi, E.A., Feuyit, G., Oyekunle, J.A.O. and Ogunfowokan, A.O. (2008). Seasonal variations in heavy metal concentrations in soil and some selected crops at a landfill in Nigeria. *African Journal of Environmental Science and Technology*, **2**(5): 89-96.

Ottaway, J.H. (1978). The Biochemistry of pollution, condot press Ltd. London, pp. 70-72.

Paff, S. (1995). Heavy metal removal via bench–scale soil washing techniques. Private Communication, *Hazardous Materials*, 8: 87–94.

Pal, R., Nath, R. and Gill, D.K. (1993). Influence of Ethanol on cadmium accumulation and its impact on lipid peroxidation and membrane- bound functional enzymes (Na^+/K^+ ATPase, acetylcholinesterase) in various regions of adult rat brain. *Neurochemistry International*, 235: 451-458.

Panday, K.K.; Prasad, G.; Singh, V. N.; Copper (II) removal from aqueous solutions by fly ash, Wat. *Res*. Vol. 19, 1985, pp. 869.

Papassiopi, N., Tambouris, S., and Kontopoulos, A. (1999). Removal of heavy metal from calcareous contaminated soils by EDTA leaching. *Water, Air and Soil Pollution,* 109:1-15.

Paya`-Pe´rez, A., Sala, J., and Mousty, F. (1993). Comparison of ICP-AES and ICPMS for the Analysis of Trace Elements in Soil Extracts. *International Journal of Environmental Analytical Chemistry*, 51: 223 - 230.

Pendias, K., Pendias, H. (1992). Trace elements in soils and plants. USA, CRR Press, pp365.

Perez, J. and Jeffries, T.W. (1992). Roles of manganese and organic-acid chelators in regulating lignin degragation and biosynthesis of peroxidases by phanerochaete-chrysosporium. *Applied and Environmental Microbiology*, 58: 2402–2409.

Peters, R.W. (1999). Chelant Extraction of Heavy Metals from Contaminated Soils. *Hazardous Materials, 66(1-2): 151-210.*

Peters, R.W., and Shem L. (1992). Adsorption/desorption Characteristic of lead on various types of soil. *Environmental Progress, 11(3): 234-240.*

Petruzelli G, and Lubruna L. (1994). Soil sorption of heavy metals as influenced by sludge addition. Enviorn Sci. health. A29: 31-50.

Pichtel, J., and Pichtel, T.M. (1997). Comparison of solvent for ex-situ removal of Chromium and lead from contaminated soil. *Environmental Engineering Science*, 14(2): 97-104

Pichtel, J., Vine, B., Kuula-Väisänen, P., and Niskanen, P. (2001) Lead extraction from soils as affected by lead chemical and mineral forms. *Environmental Engineering Science*, 18(2): 91-98.

Polettini, A., Pomi, R., Rolle, E., Ceremigna, D., De Propris, L., Gabellini, M., and Polycyclic aromatic hydrocarbons by pseudomonas saccharophila p15. *Environ. Sci. Technol*. **33**, 435- 439.

Qi, D.**,** Jing, L.**,** Qing, L.**,** Kangcheng, C.**,** Zhenfang, L.**,** Yuefei, S.**,Xiao Bo**, Y. (2013).Interaction of occupational manganese exposure and alcohol drinking aggravates

the increase of liver enzyme concentrations from a cross-sectional study in China. *Environmental Health*, 12: 30-36

Qin, F., Wen, B., Shan, X.-Q., Xie, Y.-N., Liu, T., Zhang, S.Z., and Khan, S.U. (2006).Mechanisms of competitive adsorption of Pb, Cu, and Cd on peat. *Environmental Pollution*, 144: 669-680.

Quirine, K., Shaw, R., and Renuka, R. (2007). Cation Exchange Capacity. Cornel Agronomy Fact sheet 22. Accessed 30th April 2014 at http: // www. nmsp. cals. cornell. edu/ guidelines/ factsheet.html.

Rampley, C.G., and Ogden, K.L. (1998). Preliminary studies for removal of lead fromsurrogate and real soils using a water soluble chelator. Adsorption and batch extraction. *Environmental Science Technology*, 32(7): 987 – 993.

Reddy, K. R. and Chinthamreddy, S. (2000). Comparison of Extractants for RemovingHeavy Metals from Contaminated Clayey Soil. *Soil and Sediment Contamination*, 9(5): 449-462.

Reddy, K.R.; Chinthamreddy, S., (2000). Comparison of extractants for removing heavy metals from contaminated soils. Soil Sediment Contaminant, 9 (5), 449- 462.

Reddy, M.S., Basha, S., Kumar, V.G.S., Joshi, H.V. and Ramachandraiah, G. (2004) Distribution, Enrichment and Accumulation of Heavy Metals in Coastal Sediments of Alang-Sosiya Ship Scrapping Yard, India. Marine Pollution Bulletin, 48, 1055-1059. http://dx.doi.org/10.1016/j.marpolbul.2003.12.011.

Reddy S.S., Kotaiah B. (2006). Comparative evaluation of commercial and sewage based activated carbons for the removal of textile dyes from acqueous solutions. *Iran journal of Environmental Health Science Engineering*, 3(4) : 239- 246.

Reed, B.E., Carriere, P.C., and Moore, R. (1996). Flushing of a Pb (II) contaminated soil using HCl, EDTA and $CaCl_2$. *Journal of Environmental Engineering*, 122(1): 48 – 50.

Rhodes J.D (1982). Cation exchange capacity in page A.L., Miller, R.H., Keeny, D.R., (eds). Methods of soil analysis part II. 2nd (eds): Agron. Monogr.No.9. ASA and SSSA Madison WI. Pp. 149 – 158.

Rivera-Mancia, S., Rios, C., and Montes, S. (2011). Manganese accumulation in the CN Sand associated pathologies. *Biometals,* 24: 811-825.

Sanborn, M.D., Alan, A., Campbell, M., and Weir, E. (2002). Identifying and managing adverse environmental health effects: Lead exposure. *Canadian Medical Association Journal,* 166(10):1287-1292.

Sarker, S., Yadar, P., Trivedi, R., Bansal, A.K. and Bhatnagar, D. (1995). Cadmium induced lipid peroxidation and the status of the antioxidant system in rat tissues. *Journal of Trace Elements in Medicine and Biology,* 9(3): 144-149.

Sarret, G., Vangronsveld, J., Manceau. A., Musso, M., D'Haen, J., Menthonnex, J.J., and Hazemann, J.L. (2001). Accumulation forms of Zn and Pb in *Phaseousvulgaris* in the presence and absence of EDTA. *Environmental Science Technology* 35: 2854-2859.

Sastre, S., Sahuquilo, A., Vidal, M. and Rauret, G. (2002). Determination of Cd, Cu, Pb and Zn in Environmental Samples: Microwave-Assisted Total Digestion versus Aqua Regia and Nitric Acid Extraction. *Analytical Chimca Acta,* 462: 59-72.

Satarug, S., and Moore, M.R. (2004). Adverse health effects of chronic exposure to low level cadmium in foodstuffs and cigarette smoke. Environ Health Perspective112:1099-1103.

Schmidt, U., 2003. Enhancing phytoextraction: the effect of chemical soil manipulation on Mobility, plant accumulation, and leaching of heavy metals. J. Environ. Qual. 32, 1939-1954.

Schoeters, G., Elly, D.H., Moniek, Z., RIMA, N., Peter, V.D., Nikolaos, S., and Roberto,R. (2006). Cadmium and children: Exposure and health effects. *Acta Pædiatrica;* 95(453): 50-54.

Schramel, O., Michalke, B. and Keltrup, A. (2000). Study of Copper Distribution in Contaminated Soil of Hop Field by Single and Sequential Extraction Procedure. *The Science of the Total Environment,* 263(1-3): 11-22.

Semer, R. and Reddy, K.R. (1996). Evaluation of Soil Washing process to remove mixed contaminants from a sandy loam. *Journal of Hazardous Material,* 45(1): 45 – 57.

Sharma, H.D. and Reddy, K.R. (2004) Geo-Environmental Engineering: Site Remediation, Waste Containment and Emerging Waste Management Technologies, John Wiley, New Jersey.

Sharma, H.D. and Reddy, K.R. (2004) Geo-Environmental Engineering: Site Remediation, Waste.

Sharma P., John S., Shukla S. (2000). Berylium-induced toxicity and its prevention treatment by chelating agents. *Journal of Applied Toxicity* 2000, **20:** 313-318.

Sharma P., Shah Z. and Shukla S., Protective effect of tiron (4, 5-dihydroxylbenzene-1, 3-disulphonic and disodium salt) against beryllium induced maternal and fatal toxicity in rats, *Arch toxicol,* 76(2002) 442.

Shen, Z.G., Li, X.D., Wang, C.C., Chen, H.M., and Chua, H. (2002). Lead Phytoextraction from contaminated soils with high biomass plant species. *Journal of Environmental Quality,* 31: 1893 – 1900.

Sherene, T. (2010). Mobility and Transport of Heavy Metals in Polluted Soil Environment. *Biological Forum,* 2(2): 112-121.

Shi, J. G., Yuan, Z. X., Zhang, S. F., Huang, G. H., Dai, F., Li, J. B., Chakma, A., Qin, X. S. and Liu, H. L. (2004): Removal of cadmium and lead from sediment by rhamnolipid. *Transactions of Nonferrous Metals Society of China*, 14, 66-70.

Shiio, I. and Ujigawa, K. (1980), presence and regulation of α-ketoglurate dehydrogenase complex in a glutamate producing bacterium, B. flavum, Agric. *Biol. Biol. Chem., 42,* 1987-1904.

Shiio, L., Otsuka, S. and Katsuya, N. (1963), cellular permeability and extracellular formation of Glutamic acid in *Brevibacterium favum, J. Biochem*. **53,** 333-40.

Shio, I. (1982). Metabolic regulation and overproduction amino acids, in: proceedings of FEMS symposium on overproduction of microbial metabolites, V. Krumphansi, B. Sikyta and Z. Vaneks (eds), Academic Press, London, 463-472.

Shiskowski, D.M., and Viraraghavan, T (1993). Removal of chromium from waste-water by peat filters. *Journal of Environmental sciences*. 2(10): 80-86.

Shilu, T., Yasmin, E. and Tippawan P. (2000). Environmental lead Exposure: A Public Health Problem of Global Dimensions. *Bulletin of the World Health Organization*, 78(9): 1068-1077.

Shina, G. and Alok, G. (2010). Effect of Chelating Agents on Mobilization of Metal from Waste Catalyst. *Hydrometallurgy*, 101(3-4): 120 – 125.

Shio, I., (1982), Metabolic regulation and over production of amino acids, in*: Proceedings of FEMS symposium on overproduction of microbial metabolites,* V. Krumphansl, B. Sikyta and Z. Vanek (EDS), Academic Press, London, 463-472.

Shiio, I., Otsuka, S. and Katsuya, N. (1963), Cellular and extra cellular formation of Glutamic acid and *Brevibacterium favum, J. Biochem.* 53, 330-340.

Shiio I., O and Ujigawa, K. (1980), Presence and regulation α-ketoglutamate dehydrogenase Complex in a glutamate producing bacterium, *B. flavum, Agric. Biol. Chem.***42,** 1897-1904.

Shiio, I., Ozaki, H. (1986), concentrated inhibition of isocitrate dehydrogenase by glyoxylate plus oxaloacetate, *J. Biochem* 64, 45-53.

Shiio, I. Ujigawa (1978), Enzymes of the glutamate and aspartate synthetic pathways in glutamate producing bacteria, *B. flacbum, J. Biochem.*, 84, 647-657.

Shiskowski, D.M., and Viraraghavan, T. (1993). Removal of chromium from waste water by Peat filters. *Journal of Environmental Science*, 28 (5), 967- 981.

Shrestha, R., Fischer, R. and Rahner, D. (2003): Behavior of cadmium, lead and zinc at the sediment-water interface by electrochemically initiated processes. *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 222, 261-271

Shumilin, E., Meyer-Willerer, A., Marmoleji-Rodriguez, A.J.,Morton-Bermae, O.,Galicia Parez, M.A., Hernandez, E. and Gonzalez-Hernandez, G. (2005). Iron, cadmium, chromium, Copper, lead and zinc distribution in the suspended particulate matter of the tropical Marabasco River and its estuary, colina, Mexico. *Bulletin of Environmental Contamination and Toxicology*, 74, 518-525

Siegel, F.R. (2002). Environmental Geochemistry of Potentially Toxic Heavy Metal;Springer:

Berlin, Germany.pp 59-62

Simonsen, L., Harbak, H., and Bennekou, P. (2012). Cobalt metabolism and toxicity- a brief update. *Science of total environment*, 15(432): 210-215.

Sindhu, P.S. (2005). "Environmental Chemistry" New Age International Ltd. Publishers, New Delhi; pp.191, 224 and 226.

Singh, B.R and Fernandez-Deschamps, D. (2008): Cadmium accumulation in oat crop after long term use of P fertilizer at different pH levels. *Journal of Trauma and Acute Care Surgery*, 11(1): 56 – 68.

Smith, J.L. and Doran, J.W. (1996). Measurement and use of pH and Electrical Conductivity for Soil Quality Analysis. In Methods for Assessing Soil Quality. Soil Science Society of America Special Publication. 49: 169-182.

Soetan, K.O., Olaiya, C.O. and Oyewale, O.E. (2010). The importance of mineral elements for human, domestic animals and plants: A Review. *African Journal of Food Science*, 4(5): 200-222.

Somerson, N.L. and Philips. (1962), *Belg patent* No. 593807.

Spark, D.L. (2003). Environmental Soil Chemistry. 2nd Edition Academic Press, San Diego. pp. 299-307.

Spark, D.M., Johnson, B.B., and Wells, J.D. (1995). Characterizing Heavy Metal Adsorption on oxides and hydroxides. *European Journal of Soil Science, 46(4): 621-631*.

Sposito, G., (1989). The chemistry of soil. Oxford university press. New York, pp.5-6.

Srivastava, R.K., Tiwari, R.P., and Bala Ramuda, P. (2007). Electrokinetic Remediation Study for Cd Contaminated Soil. *Iranian Journal of Environmental Health, Science and Engineering*, 4(4): 207 – 214.

Steele, M.C., and Pichtel, J. (1998). Ex-situ remediation of metal contaminated superfund soil using selective extractants. *Journal Environmental Engineering* 124(7): 637-645.

Sun, B., Zhao, F. J., Lombi, E. and McGrath, S. P. (2001). Leaching of Heavy Metals from Contaminated Soil Using EDTA. *Environmental Pollution*, 113(2): 111-120.

Sun, B.; Zhao, F. J.; Lombi, E.; McGrath, S.P. (2001). Leaching of Heavy metals from contaminated soils using EDTA. Environ. Pollut., 113 (2). 111-120

Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., and Nowack, B. (2004). Extraction of heavy metals from soils using biodegradable chelating agents. *Environmental Science and Technology*, 38(3): 937-944.

Tejowulan, R. S., Hendershot, W. H., (1998). Removal of trace metals from contaminated soils using EDTA incorporating resin trapping techniques. *Environ. Pollut*. 103 (1), 135-142.

Tessier, A., Campbell P.G.C and Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem.51: 844-850.

Tessier, A., Campbell P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem*. **51**, 844-851.

Ukpong, E.C., Antigha, R.E. and Moses, E.O. (2013). Assessment of heavy metals content in soils and plants around waste dumpsites in Uyo Metropolis, Akwa Ibom State. *International Journal of Engineering and Science*, **7**(2): 2319 – 1805.

Tokalioglu, S., Kartal, S. and Gultckin, A. (2006) Investigation of heavy metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method. *International Journal of Environmental Analytical Chemistry,* 86 (6): 417-430.

Tornato, A. (2006). A Kinetic Study of Chelant–Assisted Remediation of Contaminated Dredged Sediment. *Journal of Hazardous Material*, 137: 1458 –1465.

Tyler, L.D and McBride, M.B. (1982). Mobility and extractability of Cd, Cu, Ni and Zn in organic, in mineral soil columns. Soil sci. 134: 198-205.

U.S. Environmental Protection Agency (1993). Selecting Remediation Techniques for Contaminated Sediments, EPA-823-B93-001, Washington, DC, pp 1-25

U.S. Environmental Protection Agency (2001). Treatment of Arsenic Residuals from Drinking Water Removal. EPA/600/R-01/033, Cincinnati, OH, pp 1-85

Udosen, E.D, Benson, N.U, Essein J.P and Ebong, G.A. (2006).Relation between Aqua-regia Extractable heavy metals in soil and Manthot Utilissima within a municipal Dumpsite. Intl. soil sci., 1:27-32.

US Environmental Protection Agenct (US EPA) 2001. 40 CFR Part 745. Lead; identification of Dangerous levels of lead; final rule. Code of Fed. Reg. 66, pp. 1206-1240.

US Environmental Protection Agency (US EPA), 2000. Eliminating childhood lead poisoning: a Federal strategy targeting lead paint hazards. US Government Printing Office, Washington, DC. Available from: http://www.epa.gov/lead/fedstrategy 2000.pdf>.

USEPA (1995). Contaminants and remedial options at selected metal contaminated sites, EPA/542/2-90/017, Office of Research and Development, Washington, D.C.

Usman, A.R.A. (2008). The relative adsorption selectivities of Pb, Cu, Zn, Cd and Niby soil developed on shale in new valley, Egypt. *Geoderma,* 144: 334-343.

Valine, S.B., Chilcote, D.C., and Zambrano, A.R. (1989). Development of a soil washing system, Pro. 44th purdeuc ind. *Waste conference,* 44: 83 – 89

Van Hees, P.A.W., and Lundstrom, A.S. (2000). Equilibrium Model of Aluminum and Iron Complexation with Different Organic Acids in Soil Solution.*Geoderma,* 94: 201-221.

Van Hees, P.A.W., Jones, D.L., and Godbold, D.L. (2002). Biodegradation of Low Molecular Weight Organic Acids in Coniferous Forest Podzolic Soils. *Soil Biology and Biochemistry,* 34: 1261-1272.

Van Oort, F., Jongmans, A.G., Citeau, L., Lamy, I., and Chevallier, P. (2006). Micro Scale Zn and Pb Distribution Patterns in Subsurface Soil Horizons: An Indication for Metal Transport Dynamics. *European Journal of Soil Science,* 57: 154-166.

Van-Benschoten, J.E., Matsumoto, M.R., and Young, W.H. (1997). Evaluation and Analysis of Soil Washing for seven lead contaminated soils. *Journal of Environmental Engineering,* 123: 217-224.

Varrault, G. and Bermond, A. (2004). Application of a Kinetic Fractionation of Trace Elements (Cd, Cu and Pb) in Polluted Soil Samples. *Environmental Technology, 25(3): 293-300.*

Vassil, A.D., Kapulnik, Y., Raskin, I., and Salt, D.E. (1998). The role of EDTA in lead transport and accumulation in Indian mustard. *Plant physiology*, 117: 447-453.

Walkley, A., and Black, I.A. (1934). Method of Determining Soil Organic Matter and Proposed Modification of the Chromic Acid Titration Method. Soil Science, 37: 29-38.

Waller. P.A., and Pickering, W.P. (1992).Effect of time and PH on liability of Copper and Zinc Sorbed on humic acid particles.Chemistry, speciation bioavailability. 4: 29-41.

Wasay, S.A., Barrington, S.F., and Tokunaga, S. (1998). Remediation of soils polluted by heavy metals using salts of organic acids and chelating agents. *Environmental Science Technology,* 19:369-379.

Wasay, S.A., Barrington, S. and Tokunga, S., (1998). Retention form of heavy metals in three polluted soils. *Journal of Soil Contamination* 7(1): 103-119.

Wei, J., Tao, T., and Zhiming, L. (2011). Removal of heavy metals from contaminated soil with chelating agents. *Open Journal of Soil Science,* 1: 70-76.

Weihua, Z.; Hao, H.; Fenfang, F.; Hong, W. and Rongliang, O. (2010) Influence of EDTA Washing on the Species and Mobility of Heavy Metals Residual in Soils. *Analytical Chimica Acta*, 173(1-3): 369-376

Wenzel, W.W., Unterbrunner, R., Sommer, P., and Pasqualina, S. (2003). Chelate assisted Phytoextraction using canola (*Brassica napus L.*) in outdoors pot and lysimeter experiments. *Plant Soil*, 249: 83-96.

Wepener, V., Van Vuren, J.H.J., and DuPreez, H.H. (1992). Effect of Manganese and Iron at a Neutral and Acidic pH on the Hematology of the Banded Tilapia (*Tilapia sparrmanii*). *Bulletin of Environmental Contamination and Toxicology,* 49(4): 613-619.

Wong SC, Li XD, Zhang G, Qi SH, Min YS (2002). Heavy metals in agricultural soils of the pearl,

World Health Organization (1992). Cadmium. *Environmental Health Criteria,* 134. World

World Health Organization (1995). Inorganic lead. *Environmental Health Criteria*, 165 Geneva,

Wu, L.H., Luo, Y.M., Christie, P., Wong, M.H., 2003. Effects of EDTA and low molecular Weight organic acids on soil solution properties of a heavy meal polluted soil. Chemosphere **50**, 819- 822

Wu, Y.T. & Grant, (2002). Effect of chelation chemistry of sodium polyaspartate on the Dissolution of calcite, *langmiur*, Vol. 18, pp. 6813-6820.

Wuana, R.A., Okeieimen, F.E., and Imbervungu, J.A. (2010). Removal of heavy metals from a contaminated soil using organic chelating acids. *International Journal of Environmental Science and Technology*, 7(3): 485-496.

Xian, X., (1989).The effect of chemical forms of cadmium and lead in polluted soil on their uptake by cabbage plants. Plant soil 113:257-264. In Maand Rao (1997).Envi. Qual. 26: 257-264.

Xie, T., and Marshall, W.D. (2001). Approaches to soil remediation by complexometric extraction of metal contaminants with regeneration of reagents. *Journal of Environmental Monitoring*, 3(4): 411-416.

Yamashita T, Ando Y, Nakamura M, Obayaski K, Terzaki H, Haraoka K, Gou SX, Ueda M and Uchino M, Inhibitory effect of α-tocopherol on methyl mercury-induced oxidative stress, *Environ Health Prev Med,* 9 (2004) 111

Yan, F., Schubert, S. and Mengel, K. (1996). Soil pH increase due to biological decarboxylation of organic anions. *Soil Biology and Biochemistry*, 28: 617-624.

Zachara, J.M., Cowan, C.E. and Resch, C.T. (1991). Sorption of Divalent Metals on Calcite. *Cosmochim Acta 55: 1549.*

Zhang,F.,V. Romheld, and H, Marschner, 1989. Effects of zinc efficiency in wheat on the release of zinc and iron mobilizing root exudates. Zeitschrift fur pflanzen-ernahrung und Bodenkunde 152: 205-210

Zhou, Q.X. and Song, Y.F. (2004). Remediation of Contaminated Soil: Principles and Methods. 8th ed. Science Press, Beijing. pp. 24–46.

APPENDIX A

ANOVA for the factorial model for the removal of lead using Tiron [Parial sum of squares]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	4917.57	15	327.84	33452.88	0.0043	Significant
A	1629.54	1	1629.54	1.663E+005	0.0016	
B	49.18	1	49.18	5017.87	0.0090	
C	19.56	1	19.56	1995.77	0.0142	
D	153.45	1	153.45	15658.18	0.0051	
AB	766.60	1	766.60	78224.25	0.0023	
AC	109.36	1	109.36	11159.11	0.0060	
AD	172.07	1	172.07	17558.04	0.0048	
BC	253.53	1	253.53	25870.00	0.0040	
BD	1.91	1	1.91	195.03	0.0455	
CD	116.59	1	116.59	11896.53	0.0058	
ABC	1246.97	1	1246.97	1.272E+005	0.0118	
ABD	28.70	1	28.70	2928.86	0.0037	
ACD	285.02	1	285.02	29083.55	0.0525	
BCD	1.43	1	1.43	146.33	0.0069	
ABCD	83.68	1	83.68	8538.44	0.0070	Significant
Curvature	81.62	1	81.62	8328.18		
Pure Error	9.800E-003					
Cor Total	4999.20	17				

Std. Dev.	0.099	R^2	1.0000
Mean	44.05	R^2_{adj}	1.0000
C.V.	0.22	CL	95%

APPENDIX B

ANOVA for the factorial model for the removal of zinc using Tiron [Partial sum of squares]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	4099.74	15	273.32	45176.16	0.0037	Significant
A	555.90	1	555.90	91884.05	0.0021	
B	4.30	1	4.30	709.96	0.0239	
C	14.54	1	14.54	2402.51	0.0130	
D	146.11	1	146.11	24150.03	0.0041	
AB	959.61	1	959.61	1.586E+005	0.0016	
AC	36.45	1	36.45	6025.03	0.0082	
AD	7.28	1	7.28	1202.73	0.0184	
BC	404.92	1	404.92	66928.10	0.0025	
BD	1177.35	1	1177.35	1.946E+005	0.0014	
CD	145.87	1	145.87	24110.08	0.0041	
ABC	59.41	1	59.41	9819.10	0.0025	
ABD	383.47	1	383.47	63384.18	0.0054	
ACD	82.67	1	82.67	13665.05	0.0122	
BCD	105.42	1	105.42	17425.05	0.0014	Significant
ABCD	16.46	1	16.46	2721.21		
Curvature	1216.44	1	1216.44	2.011E+005		
Pure Error	6.050E-003	1	6.050E-003	0.0048		
Cor Total	5316.18	17				

Std. Dev.	0.078	R^2	1.0000
Mean	39.32	R^2_{adj}	1.0000
C.V	0.20	CL	95%

APPENDIX C

ANOVA for the factorial model for the removal of cadmium using Tiron [Partial sum of squaers]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	17.12	15	1.14	6.366E+007	< 0.0001	Significant
A	1.22	1	1.22	6.366E+007	< 0.0001	
B	0.64	1	0.64	6.366E+007	< 0.0001	
C	3.48	1	3.48	6.366E+007	< 0.0001	
D	2.90	1	2.90	6.366E+007	< 0.0001	
AB	0.58	1	0.58	6.366E+007	< 0.0001	
AC	1.33	1	1.33	6.366E+007	< 0.0001	
AD	0.10	1	0.10	6.366E+007	< 0.0001	
BC	3.27	1	3.27	6.366E+007	< 0.0001	
BD	0.48	1	0.48	6.366E+007	< 0.0001	
CD	0.33	1	0.33	6.366E+007	< 0.0001	
ABC	0.27	1	0.27	6.366E+007	< 0.0001	
ABD	1.77	1	1.77	6.366E+007	< 0.0001	
ACD	0.19	1	0.10	6.366E+007	< 0.0001	
BCD	0.47	1	0.19	6.366E+007	< 0.0001	
ABCD	1.16	1	0.47	6.366E+007	< 0.0001	Significant
Curvature	0.000	1	1.16			
Pure Error		1	0.000			
Cor Total	18.29	17				

Std. Dev.	0.000	R^2	10000
Mean	7.82	R^2_{adj}	1.0000
C.V	0.000	CL	95%

APPENDIX D

ANOVA for the factorial model for the removal of Nickel using Tiron [Partial sum of squares]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	5129.25	15	341.95	6.366E+007	< 0.0001	Significant
A	157.25	1	157.25	6.366E+007	< 0.0001	
B	36.72	1	36.72	6.366E+007	< 0.0001	
C	143.40	1	143.40	6.366E+007	< 0.0001	
D	736.31	1	736.31	6.366E+007	< 0.0001	
AB	0.89	1	0.89	6.366E+007	< 0.0001	
AC	156.75	1	156.75	6.366E+007	< 0.0001	
AD	28.73	1	28.73	6.366E+007	< 0.0001	
BC	1317.69	1	1317.69	6.366E+007	< 0.0001	
BD	1.66	1	1.66	6.366E+007	< 0.0001	
CD	41.15	1	41.15	6.366E+007	< 0.0001	
ABC	1269.85	1	1269.85	6.366E+007	< 0.0001	
ABD	615.29	1	615.29	6.366E+007	< 0.0001	
ACD	546.16	1	546.16	6.366E+007	< 0.0001	
BCD	11.70	1	11.70	6.366E+007	< 0.0001	
ABCD	65.69	1	65.69	6.366E+007	< 0.0001	
Curvature	3066.02	1	3066.02	6.366E+007	< 0.0001	Significant
Pure Error	0.000	1	0.000			
Cor Total	8195.27	17				

Std. Dev.	0.000	R^2	1.0000
Mean	39.68	R^2_{adj}	1.0000
C.V	0.00	CL	95%

APPENDIX E

ANOVA for the factorial model for the removal of Chromium using Tiron [Partial sum of squares]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	6157.35	15	410.49	6.366E+007	< 0.0001	Significant
A	87.33	1	87.33	6.366E+007	< 0.0001	
B	240.25	1	240.25	6.366E+007	< 0.0001	
C	746.38	1	746.38	6.366E+007	< 0.0001	
D	431.39	1	431.39	6.366E+007	< 0.0001	
AB	3.71	1	3.71	6.366E+007	< 0.0001	
AC	88.08	1	88.08	6.366E+007	< 0.0001	
AD	71.83	1	71.83	6.366E+007	< 0.0001	
BC	444.79	1	444.79	6.366E+007	< 0.0001	
BD	472.19	1	472.19	6.366E+007	< 0.0001	
CD	130.19	1	130.19	6.366E+007	< 0.0001	
ABC	3040.97	1	3040.97	6.366E+007	< 0.0001	
ABD	278.39	1	278.39	6.366E+007	< 0.0001	
ACD	0.73	1	0.73	6.366E+007	< 0.0001	
BCD	117.72	1	117.72	6.366E+007	< 0.0001	
ABCD	3.40	1	3.40	6.366E+007	< 0.0001	
Curvature	52.76	1	52.76	6.366E+007	< 0.0001	Significant
Pure Error	0.000	1	0.000			
Cor Total	6210.11	17				

Std. Dev.	0.000	R^2	1.0000
Mean	62.20	R^2_{adj}	1.0000
C.V	0.000	CL	95%

APPENDIX F

ANOVA for the factorial model for the removal of lead using glutamic acid

[Partial sum of squares]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	0.000	0				
Curvature	4.004E-003	1	4.004E-003	0.33	0.5753	not significant
Residual	0.20	14	0.012	0.26	0.9304	not significant
Lack of Fit	0.16	15	0.010			
Pure Error	0.040	1	0.040			
Cor Total	0.20	17				

Std. Dev.	0.11	R^2	0.0000
Mean	1.82	R^2_{adj}	0.0000
C.V.	6.09	CL	95%

APPENDIX G

ANOVA for the factorial model for the removal of nickel using glutamic acid
[Partial sum of squares]

Source	Sum of squares	DF	Mean square	F Value	Prob >F	Remark
Model	1572.35	15	104.82	311.79	0.0444	significant
A	11.04	1	11.04	32.83	0.1100	
B	153.95	1	153.95	457.90	0.0297	
C	171.94	1	171.94	511.41	0.0281	
D	80.78	1	80.78	240.26	0.0410	
AB	52.74	1	52.74	156.88	0.0507	
AC	324.99	1	324.99	966.66	0.0205	
AD	335.16	1	335.16	996.92	0.0202	
BC	55.84	1	55.84	166.09	0.0493	
BD	73.40	1	73.40	218.33	0.0430	
CD	0.81	1	0.81	2.40	0.3652	
ABC	28.65	1	28.65	85.21	0.0687	
ABD	0.98	1	0.98	2.90	0.3380	
ACD	183.53	1	183.53	545.91	0.0272	
BCD	17.33	1	17.33	51.54	0.0881	
ABCD	81.23	1	81.23	241.60	0.0409	
Curvature	0.021	1	0.021	0.062	0.8449	not significant
Pure Error	0.34	1	0.34			
Cor Total	1572.71	17				

Std. Dev.	0.58	R^2	0.9998
Mean	39.69	R^2_{adj}	0.9984
C.V.	1.54	CL	95%

APPENDIX H

ANOVA for the factorial model for the removal of Zinc using Glutamic acid

[Partial sum of squares]

Source	Sum of Squares	DF	MeanSquare	Value	Prob>F	Remark
Mode	6570.55	15	438.04	1.13	0.6392	not significant
A	795.38	1	795.38	2.04	0.3886	
B	146.96	1	146.96	0.38	0.6492	
C	356.55	1	356.55	0.92	0.5139	
D	1386.26	1	1386.26	3.56	0.3102	
AB	30.11	1	30.11	0.077	0.8273	
AC	165.57	1	165.57	0.43	0.6321	
AD	297.65	1	297.65	0.76	0.5426	
BC	405.32	1	405.32	1.04	0.4935	
BD	699.73	1	699.73	1.80	0.4080	
CD	721.06	1	721.06	1.85	0.4034	
ABC	0.052	1	0.052 1.	330E-004	0.9927	
ABD	200.01	1	200.01	0.51	0.6041	
ACD	103.99	1	103.99	0.27	0.6963	
BCD	0.066	1	0.066 1	704E-004	0.9917	
ABCD	1261.85	1	1261.85	3.24	0.3227	
Curvature	41.29	1	41.29	0.11	0.7995	not significant
Pure Error	389.21	1	389.21			
Cor Total	7001.05	17				

Std. Dev.	19.73	R^2	0.441
Mean	48.58	R^2_{adj}	0.1052
C.V40.61CL	95%		

APPENDIX I

ANOVA for the factorial model for the removal of Cadmium using Glutamic acid

[Partial sum of squares]

Source	**Sum of Squares**	**DF**	**MeanSquare**	**Value**	**Prob>F**	**Remark**
Mode	0.000	0				
Curvature	410.94	1	410.94	0.87	0.3657	not significant
Residual	7586.12	16	474.13	18.91	0.1788	not significant
Lack of Fit	7559.48	15	503.97			
Pure Error	26.65	1	26.65			
Cor Total	7997.06	17				
Std. Dev.	21.77				R^2	0.0000
Mean	63.13				R^2_{adj}	0.0000
C.V.	34.49				CL	95%

APPENDIX J

ANOVA for the factorial model for the removal of chromium using Glutamic acid

[Partial sum of squares]

Source	Sum of squares	DF	Mean square	F Value	Prob> F	Remark
Model	4673.45	15	311.56	676.14	0.0302	Significant
A	50.52	1	50.52	109.63	0.0606	
B	170.76	1	170.76	370.57	0.0330	
C	715.16	1	715.16	1552.00	0.0162	
D	1.18	1	1.18	2.57	0.3553	
AB	57.34	1	57.34	124.44	0.0569	
AC	24.43	1	24.43	53.01	0.0869	
AD	152.09	1	152.09	330.06	0.0350	
BC	330.24	1	330.24	716.67	0.0238	
BD	407.13	1	407.13	883.53	0.0214	
CD	28.22	1	28.22	61.25	0.0809	
ABC	1.59	1	1.59	3.46	0.3141	
ABD	911.59	1	911.59	1978.27	0.0143	
ACD	1013.63	1	1013.63	2199.71	0.0136	
BCD	43.53	1	43.53	94.46	0.0653	
ABCD	766.04	1	766.04	1662.42	0.0156	Significant
Pure Error	0.461					
Cor Total	4859.7617					

Std. Dev.	0.68	R^2	0.9999
Mean	55.28	R^2_{adj}	0.9984
C.V.	1.23	CL	95%

APPENDIX K



