REMEDIATION OF HEAVY METAL CONTAMINATED SOIL BY DIFFERENT CHELATING AGENTS

Researcher

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PINSTECH, Islamabad

Department of Environmental Science

Faculty of Basic & Applied Sciences

INTERNATIONAL ISLAMIC UNIVERSITY, ISLAMABAD

(2016)

Lead - pollution

Cadmium

Soil pollution.

Chelating agents.

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بسم الله الرحمن الرحيم

In the name of Allah,

The Supremely Merciful, the Most Kind

Department of Environmental Sciences

International Islamic University Islamabad

Dated: __________

FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. Barira Bilal and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Environmental Science.

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A thesis submitted to Department of Environmental Sciences, International Islamic University, Islamabad as a partial fulfillment of requirement for the award of the

Degree of MS Environmental Sciences

Dedicated to

My Parents

My Mentor Dr. Azhar Mashiatullah

&

Respected Teachers of IIU

Whose encouragement, assistance, guidance and advice made this uphill task possible

I am forever indebted to them

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree

Date __________

____

Barira Bilal
(175-FBAS/MSES/F13)

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Barira Bilal
(175-FBAS/MSES/F13)

LIST OF ABBREVIATIONS AND SYMBOLS

Symbol/Abb.	**Description**
Å	Ångström
As	Arsenic
APCAs	Aminopolycarboxylic acid
Fe (NH4)2(SO4)2.6H2O	Ammonium Iron(II) Sulfate Hexahydrate
NH_4Ac	Ammonium Acetate
Cd	Cadmium
Cr	Chromium
Cu	Copper
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium Chloride
^0C	degree Celsius
DTPA	Diethylene triamine penta acetic acid
dS/m	deciSiemens per meter
EDTA	Ethylene diamine tetra acetic acid
FRTR	Federal Remediation Technologies Roundtable
HCl	Hydrochloric acid
$NH_2OH \cdot HCl$	Hydroxylamine hydrochloride
HF	Hydrofluoric acid
HEDP	1-Hydroxyethylidene-1,1-diphosphonic Acid
H_2O_2	Hydrogen peroxide
HF	Hydrofluoric acid
INECAR	Institute of Environmental Conservation & Research
Fe	Iron
Pb	Lead
$PBCl_2$	Lead chloride
λ	Lambda
HNO_3	Nitric acid
NTA	Nitrilotriacetic acid

NTMP	Nitrile tri methylene phosphonic acid
Hg	Mercury
Mn	Manganese
Mg	Magnesium
µ mol/ L	micro moles per litre
M	molar
Mg/kg	milligram per kilogram
ug/g	Microgram per gram
ug/L	Microgram per Litre
mg/L	Milligram per litre
mM	millimoles
mg/g	milligram per gram
mS/m	milliSiemens per meter
$MgCl_2$	Magnesium Chloride
µm	micro meter
nm	Nano meter
pH	Power of Hydrogen Ion
H3PO4	Phosphoric acid
K^{+2}	potassium ion
$K_2Cr_2O_7$	Potassium Dichromate
ppm	part per million
$HClO_4$	Perchloric acid
rpm	rotation per minute
Na+	Sodium ion
NaOH	Sodium hydroxide
NaF	Sodium fluoride
H_2SO_4	Sulphuric acid
Ti	Titanium
θ	theta
USEPA	United State Environmental Protection Agency
U	Uranium
XRD	X Ray powder Diffraction
ZnS	Zinc Sulphide
$ZnCl_2. H_2O$	Zinc Chloride
Zn	Zinc

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ABSTRACT

The heavy metal contamination of soil is a major environmental concern. Various remediation methods are being used to decontaminate soil from heavy metals. Soil washing technology is extensively used to minimize the concentration of heavy metals in soil. The basic proposition of soil washing method is using chelators for maximum efficiency to extract heavy metals from soil. The aminocarboxylic acids and low molecular weight organic acids are used as chelating agents to remove heavy metals from soil. The purpose of this study was to determine the removal efficiency of selected chelators for Cd, Cr, Pb and Zn.

In present investigation EDTA, DTPA and citric acid were used as a chelating agents. The soil was artificially contaminated with heavy metals chloride salt of Cd, Pb and Zn, while Potassium dichromate was used for Cr spiking. The Soil was equilibrated for sufficient time for heavy metal adsorption. The physical and chemical analysis of contaminated soil classified it as mildly alkaline with silty clay texture. The XRD analysis determined that experimental soil had high proportion of quartz (53%) dominant clay montmorillonite (14 %) and calcite content was 12 % which gave soil its calcareous characteristic. The sequential extraction of heavy metals from different soil fractions showed that Cd Cr and Zn were partitioned five fractions in followed order reducible fraction > oxide bound > carbonate bound > exchangeable > organic bound fraction. Lead followed sequence of oxide bound fraction > residual > carbonate > organic fraction > exchangeable fraction.

The selected chelators efficiency was studied at different contact time (3, 6, 9, 12 and 24 hours). The concentration of citric acid used in this study were 0.1M, 0.5M, 1M and 1.5 M. EDTA concentrations were 0.01 M , 0.05 , 0.1 and 0.5 M and DTPA were 1mM , 3 mM , 5 mM and 7mM. The optimal contact time for heavy metal removal with citric acid was 9 hours with 1 M concentration at which all heavy metals had maximum removal efficiency percentage. The maximum removal of Cd was 23.4 %, Cr was 37 %, Pb 27.4 % and Zn was 38.6 %. The maximum removal efficiency for Cd (37.7%), Cr (16.3 %), Pb (32.8%) and Zn (28.5%) was observed with 7mM DTPA after 24 hours. EDTA maximum removal for Cd was 32.5%, Cr 27.8%, Pb 40.3 % and Zn 36.5% with 0.5M solution after 24 hours of contact time.

The pH and temperature control experiment was performed with 1 M citric acid at 9 hours, 0.5 M EDTA and 7mM DTPA at 24 hours of contact time. The control solution was without any pH adjustment. Citric acid 1M solution was adjusted to pH 2 , 4 and 6 to study the change in removal efficiency at temperature 25 ^{0}C , 30 ^{0}C and 35 ^{0}C. The pH 2 of citric acid showed increase in removal efficiency of Cd and Cr at 25 ^{0}C, while control solution of citric acid showed better removal efficiency for Pb and Zn. The increase in temperature to 35^{0}C showed decrease in Pb and Cd removal efficiency, Cr remained unchanged while Zn showed slight increase in removal efficiency. EDTA removal efficiency improved with increasing temperature while pH 4 performed better than pH 7 and pH 10 solution. DTPA showed good result with pH4 and control solution. Cd removal efficiency was 40.2 % at 25 ^{0}C, and it decreased to 38.6 % at 35 ^{0}C. Cr removal efficiency increased with increasing temperature removing 30.8% with pH4 at 35 ^{0}C. Pb maximum removal efficiency was recorded with DTPA control solution removing 32.8 %. Zn removal was 28 % with control solution and showed decreased efficiency with increasing temperature. DTPA proved to be a good chelator for Cd, citric acid for Cr and Zn, while EDTA was a suitable chelator for Pb.

CHAPTER 01

INTRODUCTION

1.0 INTRODUCTION

Soil contamination with heavy metals is major environmental problem throughout the world because of their persistence and danger to environment (Uraguchi *et al.,* 2006). Heavy metals pollution cause a long term effect on biological, physical and chemical properties of soil and its fertility (Nicholson, 2003).The rapid urbanization and industrialization has negative impact on environment. The main sources of heavy metals contamination of soils are industrial emissions from coal combustion and atmospheric deposition of metals from aerosols, mine tailings, land use of fertilizers, paints and leaded gasoline, wastewater irrigation and discharge of untreated industrial effluent on land (Khan *et al.,* 2008 ; Zhang *et al.,*2010). The transport exhaust and fine heavy metal particles spread from industrial waste heap to surrounding areas causes detrimental heavy metal contamination (Pb, Zn, Cd, Cr, Cu, etc.) of the ambient air and soils (Falahi-Ardakani, 1984; Viard *et al.,* 2004).

Heavy metals contamination is are also associated with fertilizers. The long-term excessive application of fertilizers and pesticides results in the heavy metal contamination of soils. The large amount of pesticides are organic compounds, and a few are inorganic compound or pure mineral, and some pesticides contain Hg, As, Cu, Zn and other heavy metals (Arao *et al.,* 2010). Heavy metals exposure is possible through daily use commodities such as; mercury disinfectants (like mercurochrome), antifungal agents, toiletries and creams. Cadmium exposure is due to the use of nickel/cadmium batteries and artist paints, lead through mirror coatings, batteries, old paints and tiles. (McCluggage, 1991).

Some heavy metals are necessary for living body for nourishment. These heavy metals are required in trace amount and therefore refer to as trace elements, micronutrients and microelements in literature. Trace element plays an important role in chemical, biological, biochemical, metabolic, catabolic, and enzymatic reactions in the living cells of plants, animals, and human beings (Waseem *et al.,* 2014). Heavy metals such as iron, manganese and molybdenum are important micronutrients to living cells. Other, such as zinc, nickel, copper, cobalt, chromium and vanadium are useful for living organism in trace amount, but these elements above their permissible trace limits causes cell toxicity.(Schützendübel and Polle, 2001; Meharg, 2005) .

Toxic metals are non-essential for biological roles and are very harmful even at low concentration such as Cd, As, Hg, TI and U (Alloway *et al.,* 1990). Toxicity from heavy metals depend on the form of metal specie, duration of dose exposure and also from long term accumulation of heavy metals due to consumption of nontoxic sources (Kakkar and Jaffery 2005).

1.1 Environmental and Health Concerns of Heavy Metals Contamination

Soil plays a major role in terrestrial environment by cycling elements (Cottaine and Verlo, 1984). Soil helps to immobilize many contaminates, reduces the risk of transport of toxic substances and their bioavailability by acting as a buffer (Alam *et al.,* 2003). The contamination of soils by metals can have long-term ecological and health implications. Heavy metals to some extent are necessary for plant growth, but if the concentration exceeds the plant threshold tolerance limit then plant will become poisoned (Chao *et al.,* 2014).

Heavy metals buildup in soil can result in loss of many crucial soil functions which raises concern about productivity and fertility of soil, environmental quality and human health. Soils contaminated with heavy metals are poor in nutrients and microbial diversity (White *et al.,* 2006). The presences of higher concentration of heavy metals in soil cause chlorosis in plants, damage root system and enzymatic disorders which are caused growth inhibition in plants (Sanitadi and Gabbrielli, 1999; Agarwal, 2002).

Heavy metals like zinc, copper, nickel is required by human body in trace amounts but there excess cause serious health damages. Heavy metals in soil are transferred to human diet through crops, vegetables grown in contaminated soil. The dairy products from grazing animals are also exposed to risk of heavy metal contamination by ingesting soil and foliage with high concentration of toxic metals. (Nicholson *et al.,* 2003; Kabata-Pendias *et al.,* 2007). Heavy metals exposure to humans may induce tumors and mutations (Groten and Vanbladeren, 1994; Dianne *et al.,* 1999). The consumption of food contaminated with Pb, Hg, As, Cd and other metals reduce body stores of iron, vitamin C and other vital nutrients, leading to growth retardation, decreased immunological defense and disabilities associated with malnutrition (Iyengar and Nair, 2000).

1.2 Heavy Metals Contamination in Pakistan

The environmental problems in Pakistan are rooted in poor urban planning, disposal of industrial and sewage effluents in water bodies and open fields, use of sewage water to irrigate agricultural land, unabated use of pesticides, exhaust from industries and automobiles. All these problems have been aggravated with rapid urbanization and haphazard economic growth in various cities of Pakistan has led to deterioration of natural resources. In Pakistan various studies established the transfer of heavy metals from consuming crops, vegetables grown in contaminated soil or irrigated with waste water (Hassan *et al.,* 2013; Mahmood and Malik, 2014 ; Abbas *et al.,* 2010; Achakzai *et al,* 2011; Amin *et al.,* 2013). Access to clean water has become a main problem in Pakistan, in Azad Jammu Kashmir 80 % of illness are attributed to contaminated water (Javed *et al.,* 2008). Rahbar *et al.,* (2002) in their found that 80% of children of Karachi had high blood lead concentrations due to the presence of elevated levels of lead in the air from automobile exhaust and food contaminated with road side dust.

1.2.1 Cadmium

Cadmium (Cd) is considered very toxic metal commonly percolate from industrial wastes. Cadmium is present in all soils and rocks, as well as coal and mineral fertilizers. It can cause interference in crop photosynthesis and protein synthesis (Acar and Alshawabkeh, 1993; Kale, 1993). Elevated Cadmium level in soils causes alteration in plants mineral and water uptake cycle, carbohydrate metabolism, and decreased crop productivity (Hossain *et al.,* 2010; Toppi and Gabereilli, 1999). Cadmium is considered to be strongly absorbed metals by living cells. It causes softening of bones and kidney failure which eventually lead to death of individuals.

In the mid 1950's the effect of Cd poisoning (Itai-Itai disease) was reported in Japan. The source was the consumption of rice from nearby paddies fields irrigated with river water contaminated by mining operations (Almeida and Stearns, 1998).The effect of cadmium toxicity on cabbage and bean seedling was studied by Qin *et.al.* (1994).They found that concentration of cadmium when exceeds 30 μ mol/ L in soil caused stunted growth in cabbage and bean seedlings. In Pakistan, the large variation of Cd in soil were found which ranged from 0.02 to 184 mg/kg from normal to contaminated soil mainly associated with mining and other anthropogenic activities (Parveen *et al.*,2012) .

1.2.2 Chromium

Chromium (Cr) occurs in many valence states (from -2 to +6) but trivalent and hexavalent state is most stable in the terrestrial environment (Kimbrough *et al.*, 1999). The concentrations of chromium (III) and chromium (V1) is increased by anthropogenic activities are steel, leather, textile manufacturing and chemical leather, textile manufacturing respectively (Martin and Griswold, 2009). The uptake of too much as well as shortage of Cr^{+3} ions can cause adverse health effects may cause heart conditions, disruptions of metabolisms and diabetes. But Chromium (VI) compounds are toxins and known human carcinogens. (Nwaedozie *et al.*, 2013). The hexavalent chromium causes both acute and chronic toxicity by inhalation and ingestion of hexavalent chromium compounds, dermatitis, allergic and eczema like skin diseases, asthma, bronchial carcinomas and ulceration of mucous membrane (Baruthio, 1995).

The accumulation of Cr in soil cause phytotoxicity and increased movement of Cr and its related compounds into the food chain (Mahimairaja *et al.*, 2000). The symptoms of Cr toxicity in plants are decreased seed germination, retarded growth, low yield, inhibition of enzymatic activities, damaged photosynthesis, nutrient and oxidative imbalances, and mutagenesis (Oliveira, 2012). In Pakistan, Parveen *et al.*, (2012) reported a high content of Cr 3.74 and 7.56 mg/kg in leaf and edible portion of vegetables respectively. Lone et al., (2003) reported high Cr 3.93 mg/kg in spinach irrigated with chromium contaminate waste water while same crop irrigated with clean water resulted in 0.004 mg/kg of Cr.

1.2.3 Lead

Soils can be contaminated by lead by natural weathering of rocks, exhaust of fumes from automobiles and industries, smelting of Pb ores, batteries effluent and disposal of sewage sludge in soil (Eick *et al.,* 1999). Lead is considered as protoplasmic poison for plants showed harmful effects on seedling length, germination, reduced chlorophyll production and gaseous exchange in leaves (Latif *et al.,* 2008). Lead poisoning in humans causes suppression of hemoglobin synthesis, deterioration in the kidneys, joints and reproductive systems, cardiovascular system and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS) (Ogwuebgu and Muhanga, 2005). Lead contamination in Pakistan is mostly associated with application of sewage sludge in agricultural fields (Amin *et al.,* 2013) and vehicular emissions (Faiz *et al.,* 2009).

1.2.4 Zinc

Zinc (Zn) is present in the form of oxides of zinc/sphalerite (ZnS). Zinc commonly comes from water in acid mine drainage, natural ores, coal burning and municipal wastewater (Cesur and Balkaya, 2007). Zinc contamination in road side dust is mostly associated with vehicular traffic (Khan *et al.,* 2011). Zinc is comparatively non-toxic, however, it can result in growth impairment and reproductive dysfunction when exceeds limit (INECAR, 2000; Nolan, 2003). Zinc poisoning has same signs of illness as does lead, and can mistakenly be diagnosed as lead poisoning (McCluggage, 1991). Elevated levels of Zn in soil adversely affect microbial facilitated soil processes (Wang *et al.,* 2007). In Pakistan the concentration of Zn in soil and dust varies from > 0.1 to 1193 mg/kg, while in highly contaminated areas it is 29755 mg/kg (Muhammad *et al.,* 2011).

The heavy metals contamination has harmful effects on human health and environment and it is highly desirable to apply remediation approaches to contaminated soil .The demand for new soil treatment methods which are cost effective, efficient and environmental friendly are growing gradually. The excavation and disposal of contaminated soil is being replaced by developing safe and long term remediation technologies (Khulman and Greenfield, 1999; Mann, 1999).

1.3 Remediation of Contaminated Soil

The heavy metal contaminated soil remediation approaches are scrutinized by regulatory authorities and only those strategies are favored which reduces metal contamination and bioavailability in long term (Martin and Ruby, 2004). It is required that physical, chemical characteristics of soil, type and level of contamination must be obtained earlier for the accurate assessment of remediation techniques (Wuana and Okieiman, 2011).

The preliminary study of site is necessary to determine whether soil requires *in-situ* (on site) or *ex-situ* (off site) approaches for cleanup. *In situ* or in place means that the contaminated soil is treated in its original place without moving or excavation. *Ex situ* means that the contaminated soil is moved, excavated, or removed from the site (USEPA, 2007). These methods are divided into biological and non-biological methods. The non-biological methods are subdivided in physical-chemical methods, thermal methods and others methods (e.g., supercritical extraction and electrokinetic) (Castelo-Grande *et al.,* 2003).

1.3.1 Biological Methods

The biological method involves use of plants and microbes to clean contaminated soil which is known as phytoremediation.

- Bioremediation uses microorganisms or microbial processes to degrade and transform environmental contaminants into harmless or less toxic forms.
- Phytoremediation, also called green remediation, can be defined as an in situ remediation strategy that uses vegetation and associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render environmental contaminants harmless (Cunningham and Ow, 1996; Helmisaari *et al.,* 2007).

1.3.2 Non Biological Methods

The non-biological treatment of heavy metal contaminated soil is divided into:

1.3.2.1 Solidification/Stabilization

Solidification involves the addition of binding agents to a contaminated material to impart physical/dimensional stability to contain contaminants in a solid product and reduce access by external agents through a combination of chemical reaction, encapsulation, and reduced permeability/surface area. Stabilization (also referred to as fixation) involves the addition of reagents to the contaminated soil to produce more chemically stable constituents (Evanko and Dzombak, 1997; Anderson and Mitchell, 2003).

1.3.2.2 Vitrification

Vitrification technology uses a powerful source of energy to heat soil at extremely high temperatures (1600–2000^{0}C), immobilizing inorganic and destroying organic pollutants by pyrolysis (Acar and Alshawabkeh, 1993; FRTR, 1999).The three main process of vitrification are electrical process, thermal process and plasma process. The advantage of this method is that it immobilizes contaminant for long term. The main disadvantage is the requirement of a very high temperature, which raises the cost of treating gases released in the process.

1.3.2.3 Electrokinetic remediation

This technology is based on the phenomenon of pollutant migration under electric field influence. Electrodes are inserted into the ground on opposite sides of the contaminated area. Contaminants under the influence of an electromagnetic field migrate through the soil within the cathode or anode area, where they are removed by chemical precipitation, and adhesion to the electrodes surfaces (Lindgren *et al.,* 1994). The applicability of this method is limited by alkalinity of soil and requirement for high moisture (Castelo-Grande *et al.,* 2003).

1.3.2.4 Solvent Extraction

This ex situ method does not destroy wastes but it separates the hazardous contaminants from soils, sludges and sediments. The solvent extraction technology uses organic solvents for reducing the contaminants in soil. Solvent extraction is commonly used in combination with other technologies, such as solidification/stabilization, incineration, or soil washing, depending upon site-specific conditions. The limitation of this technology is that presence of solvent residue in treated soil. The toxicity of the solvent is an important consideration while using this method (Pavel and Gavrilescu, 2008).

The above mentioned methods are useful for many contaminated sites but their main disadvantage is long treatment duration, dependence on weathering pattern, risk of food chain contamination, disposal treatment cost of process by products, inability to extract strongly bound contaminants. Among these developed remediation technologies, soil washing process gives high removal efficiency for heavy metal contaminated soil by using appropriated chelating agent (USEPA, 2001).

1.3.2.5 Soil Washing

Soil washing technology reduce or minimize the contaminants. It can be physical or chemical process or may employ both physical and chemical techniques. Soil washing technology is generally an *ex situ* method (Peters, 1999).

Some advantages of ex situ soil washing are;

- complete removal of heavy metal contaminants
- rapid cleanup of contaminated soil
- reduces or eliminates long-term liability
- cost-effective solution (GOC, 2003).

Soil washing method utilizes exchange solutions of acid, bases, chelating agents and other additives (Griffiths 1995). In soil washing technology, soil particles are cleaned by selectively transferring the contaminants attached on the soil into chemical solution. The effectiveness of washing is based on the ability of extracting solution to dissolve metals from contaminated media. The strong bonding between soil colloidal particles and metal makes the washing difficult. Therefore, extracting solution which can extract large quantities of metals are preferred (Tejowulan and Hendershot, 1998).

Several factors effect soil washing process such as;

- soil clay content which makes removal of contaminant difficult
- high humic content which inhibits contaminant removal
- concentration of soluble metals
- complex waste mixture which affects the selection of appropriate treatment fluid
- soil particle mineralogy and texture (USEPA, 1993).

Several types of chemicals are used for soil washing such as surfactants, chelating agents and organic acids (Zhang *et al.,* 2009). Water is mainly used for pretreatment of subsequent extractions, but not very effective. The mineral acid like nitric acid, sulfuric acid and hydrochloric acid is used to wash contaminated soil but require large solution to solid ratio. Complexing agents or chelating like EDTA, DTPA and organic acids are useful in extracting heavy metals from soil. It requires low concentration of solvent and less cost of equipment. While soil washing does not reduce toxicity but it does provides meaning of reducing the volume of the contaminant (Devgun *et al.,* 1993).

1.4 Chelating Agents

A chelating agent is a multidentate ligand that can bound metal from any matric. This type of bonding forms a heterocyclic ring. The process of formation of chelate ring is called chelation and resulting complex is called chelate. If one ligand molecule is involved in the formation of a chelate, the compound is monodentate chelate or complex. If many ligands form complex with a metal ion then it is called multidentate chelator (Tan, 2011). A shorter word for chelating agent is “chelant” or “chelator” (Haworth, 1998; Nowack and Van Briesen, 2005).The chelating agents extract heavy metal from the soil in two steps adsorptions, in which a rapid desorption within the first hour was followed by a subsequent gradual release that occurred over the following hours (Peters and Shem, 1992). The multidentate ligands have many coordinating sites that can penetrate into soil matrix to bind metals to form stable complexes. The chelating agents prevents precipitation and do not release their metal ions unless soil pH drops significantly. The strong acid solutions cause deterioration in the physico-chemical properties of the soil, using chelating agents is less environmentally disruptive (Hong *et al.,* 1995; Stumm and MorGan; 1996 Xu and Zhao, 2005).

The extensive research on chelating agent to wash contaminated soil helps to understand the important issues in this approach which should be considered before remediation process. The chelating agent should be selected on the following criteria;

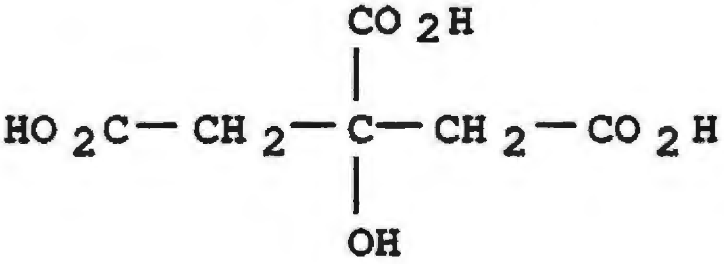
- chelator should form strong and stable complexes over a wide pH range.
- chelator should be cost effective
- have low toxicity
- should be recycled or reused and has low biodegradability in soil (Peters and Shem, 1992; Hong and Jiang, 2005).

1.4.1. Types of Chelating Agents

Chelating agents are broadly classified into natural and synthetic groups. The natural and synthetic chelators are used to increase the soil nutrient uptake for improving plant biomass and metal uptake (Sherameti and Varma, 2015). Proteins, polysaccharides and poly nucleic acids are the example of naturally occurring chelators in soil (Kaushik, 2015). The heavy metals extraction from contaminated soil also uses naturally occurring organic acids or low molecular weight organic acids (LMWOA) like oxalic, citric, formic, acetic, malic, succinic, malonic, lactic, aconitic, and fumaric acids. The organic acids are secreted by plant roots, microbes in soil and decomposition product of plants and animals (Naidu and Harter, 1998).

❖ Natural Chelators

The natural chelators known as low molecular weight organic acids are secreted by plant roots. Their release is enhanced when plants are expose to iron stress and low concentration of calcium and phosphorus (Uren and Reisenauer, 1988).



$$\mathrm{HO_2C-CH_2-\overset{\displaystyle CO_2H}{\underset{\displaystyle OH}{C}}-CH_2-CO_2H}$$

Fig 1.1: chemical structure of citric acid

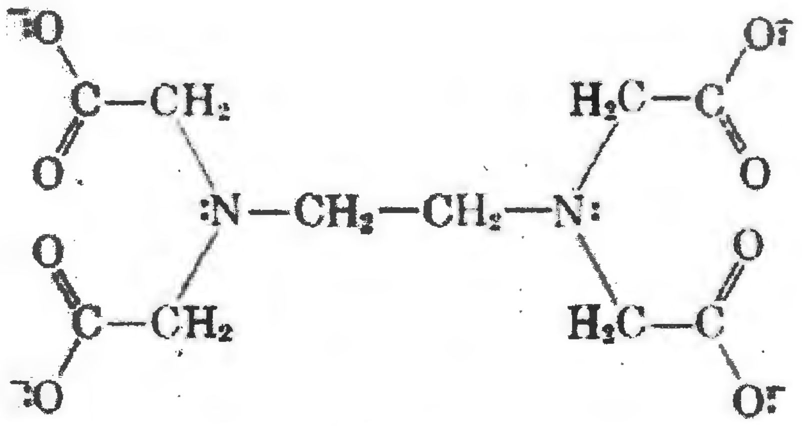
Citrate is most predominate acid in soil and rapidly biodegradable. Citric and tartaric acids were found to reduce Cr (VI) to Cr (III) in the soil (Tian *et al.*, 2010). Citric acid is most effective natural chelator in desorption of heavy metals followed by malic, acetic, tartaric and oxalic acid (Wu *et al.*, 2003; Chiang *et al.*, 2011). The chemical reaction of citrate is related to its functional groups and capacity to form complexes with alkaline earth metals or metallic cations (Rashid, 2012).

The removal of heavy metals from contaminated soil depend on concentration and degradation of the organic acids, pH, and concentration of competing cations (Lombnaes *et al.*, 2008; Wen *et al.*, 2009). These organic chelators are effective in leaching heavy metals from exchangeable, carbonate and reducible fractions of soil (Peters, 1999).

❖ Synthetic Chelators

Synthetic chelating agents belongs predominantly to two different groups i.e. Aminopolycarboxylates (APCAs) and Polyphosphonates (Nowack, 2007; Knepper, 2003). Phosphonates include DTPPH (diethylene triamine penta (methylene phosphonic acid)) and NTMP (nitrile tris (methylene phosphonic acid)), HEDP (1-hydroxyethane-1, 1-diphosphonic acid) are important. HEDP is generally used as main ingredient of detergents (Kołodyńska, 2011).

Among the aminocaboxylates chelators, EDTA (ethylene diamine tetraacetic acid), diethyl triamine penta acetic acid (DTPA) and nitrilotriacetic (NTA) are widely studied chelators for soil washing (Maier *et al.,* 2001). NTA is class II carcinogen (Borgmann and Norwood, 1995). EDTA is most widely studied chelator because of its metal cations mobilization efficiency and minor impact on soil physical and chemical properties (Lee and Marshall, 2003). It can form four or six bonds with a metal ion, and it forms chelates with both transition-metal ions and main group. EDTA is a nonselective chelator and has ability to complex other metals like Fe, Mg, Al and Ca (Kim *et al.*, 2003).



EDTA

Fig 1.2: Chemical Structure of EDTA

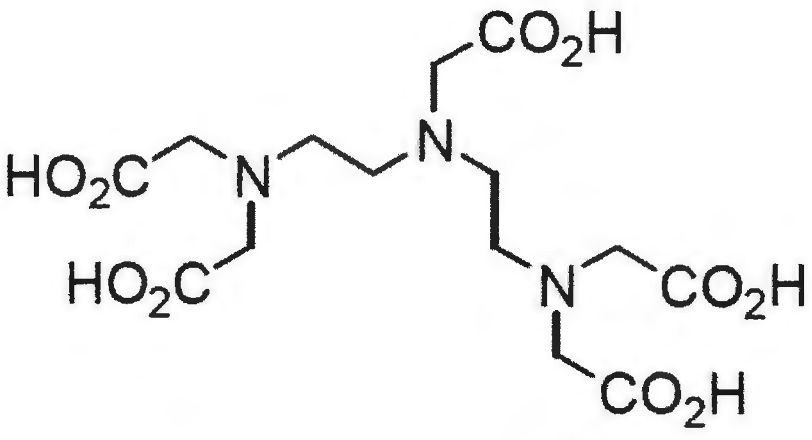


Fig 1.3: Chemical Structure of DTPA

The coordinating groups in EDTA are two amine nitrogen and four carboxylic oxygens. The six electron pairs are capable of coordinating to a metal ion, the hexadentate EDTA ion wraps completely around the metal and is very difficult to dislodge (https://chem.libretexts.org/Textbook_Maps/General_Chemistry_Textbook_Maps/Map%3A_ChemPRIME_(Moore_et_al.)/22Metals/22.09%3A_Chelating_Agents).

The major factor that effects efficiency of EDTA is soil texture and Calcite content. Calcium is a major competing cation that competes soil metals and affect the leaching of targeted metals in contaminated media (Theodoratos *et al.,* 2000; Manouchehri *et al.,* 2006).

Diethylenetriaminepentaacetic acid (DTPA) is an aminopolycarboxylic acid consisting of a diethylenetriamine backbone with five carboxymethyl groups. The molecule can be viewed as an expanded version of EDTA and is used similarly. DTPA has two CH_2CH_2 bridges (fig 1.3) linking three nitrogen atoms and is octadentate (http://www.wikiwand.com/en/Aminopolycarboxylic_acid#/Structure). DTPA estimates the bioavailability of heavy metals in soil and sludge due to its capacity to chelate a wide range of metallic elements. It can also be used to remediate the contaminated soil (Francis, 1999).

1.4.2. Chelating Agents Use in Soil Washing

Chelating agents can improve the soluble metal contents in soil solution and have been extensively used in remediation techniques for contaminated soils (Novak, 2002). Many studies provide the information about the distribution of heavy metals in soil and the efficiency of chelating agents to remediated contaminated soil. EDTA is used by **Papassiopi *et al.*, (1999)** to treat the calcareous soil contaminated with Pb and Zn. The removal efficiency was 50 to 98% for Pb and 50 to 100% for Zn. The study also demonstrated the consumption of available EDTA by dissolution of calcite content of soil.

In another study, **Khodadoust *et al.*, (2005)** had studied the removal of metals (Pb, and Zn) from a silty textured soil with 3 % organic matter by using various concentrations of EDTA and DTPA. The efficiency of both EDTA and DTPA increased with increasing concentration of chelators. The EDTA efficiency was better than DTPA except 0.2 M DTPA which performed better for Pb. The increase in removal efficiency with increasing the molar concentration was also reported by **Moghal *et al.*, (2015).** They studied the removal efficiency of Cu^{+2} and Pb^{+2} ions with deionized water, 0.1 M, 0.2 M and 0.5 M solution of EDTA in batch washing test. They reported threefold increase in removal efficiency with 0.1 M EDTA as compared to deionized water. The increasing molarity of EDTA solution increases the removal efficiency 90 to 95 %. **Di Palma and Mecozzi (2007)** compared efficiency of EDTA and citric acid in removal of heavy metals Cu, Pb, Zn from Italian harbor sediments by soil washing. They observed the increased dissolution of cations such as Ca^{+2} and Fe^{+2} increased with higher concentration of EDTA in extraction solution. Citric acid showed high affinity for Fe^{+2} under strong acidic condition, therefore, was unable to remove Cu and Pb effectively. However, it removed 80% Zn.

The soil textural class plays key role in the removal efficiency of chelators. In one study, **Mohantay and Mahindrakar (2011)** reported the removal efficiency of Cr and Pb by using EDTA. The removal of Pb and Cr increased with increasing EDTA concentration from 0.005 to 0.1M. The removal of Pb varied from 41.99 % to 53.87 %. The Cr removal efficiency was reported to be from 39.21 % to 48.35 % with increasing concentration of EDTA. Cr was less dependent on EDTA concentration due to its strong adsorption to soil clay particles. Another factor that effects the removal of chromium from contaminated soil is its speciation and particle size of soil. For example, **Cheng *et al.*, (2011)** demonstrated that both soil particle size and type of chromium chemical specie in contaminated soil effects the chelator efficiency. The citric acid (0.5 M) effectively removed the Cr bound to organic fraction of soil than using 0.5M HCl. The separation of particle size for washing does not significantly improve the Cr extraction.

The pH of soil is vital factor in the mobilization or removal of heavy metals from contaminated soil. The neutral or near alkaline soil pH has a very low heavy metals mobility. On the other hand, acidic soils have high metal mobility. At pH 7, Pb did not show any relationship to pH except with increasing soil organic matter. The Pb solubility increases at pH 7, with increase dissolution of organic matter promoting formation of organo- Pb complexes **(Sherene, 2010)**.

In another study, **Lim *et al.*, (2004)** employed EDTA, NTA and DTPA to extract Cd, Cr and Pb at pH 7.0 from artificially contaminated soil. The chelating agents were found to be effective as compared to HNO_3, $CaCl_2$ and deionized water. The removal of chromium was poor but cadmium and lead were rapidly extracted after 15 minutes. The extraction efficiency dependent on heavy metal speciation in solid phase and required high concentration of chelators and longer contact time to remove Cr from contaminated soil.

Citric acid was studied by **Bassi *et al.*, (2000)**. They evaluated Cd, Cu, Zn, and Pb removal from contaminated sandy soil with 0.5 M citric acid over 24 hours. The concentration of heavy metals Cd, Cu, Zn, and Pb was reduced from 0.01, 0.04, 0.42, and 41.52 mg/g to 0, 0.02, 0.18 and 5.21 mg/g, respectively. The heavy metal removal was negligible after 24 hours washing time. While, **Del Dacera and Babel (2006)** studied citric acid efficiency with controlled pH of citric acid solution and contact time. They found that citric acid can leach Cr and Zn at 100% efficiency at pH 2.33. Lead removal was high at 95% but it required 11 day leaching time.

Wasay *et al.*, **(2001)** compared the strong chelating agents EDTA and DTPA and different weak organic acids like citrate, tartrate and oxalate to study the remediation efficiency on different textural class soils artificially contaminated in laboratory. The clayey loam soil washed with citrate and tartrate removed Cr, Mn, Hg and Pb at levels of 43 to 45, 37 to 41, 91 to 92 and 75%, respectively. EDTA and DTPA could only leach Pb from clay loam soil. For the loam, citrate leached 98 and 89% of Cd and Pb respectively, while tartrate leached 91 % Cd and 87% of Pb. The sandy clay loam soil was effectively washed by EDTA, DTPA citrate and tartrate.

Wuana *et al.*, **(2010)** compared the efficiency of EDTA, citric acid and tartaric acid for soil washing experiment on sandy loam soil. Citric acid removed 43.50 % Zn, 38.40 % Cd and 31.00 % Pb after 6 hours. The extraction efficiency followed EDTA > CA > TA order for all metal studied. EDTA performed better because of its degree of selectivity of metals and ability to complex free metal ions.

Park *et al.*, **(2013)** reported the extraction efficiencies of Cu, Cu,. Zn and Pb with citric acid. The sequential extraction of heavy metals from contaminated soil showed that citric acid was able to extract metals from soil exchangeable, carbonate and Fe-Mn bound fraction of soil.

Yang *et al.*, **(2012)** evaluated the use of different concentrations of Na_2EDTA and HCl to remove heavy metals from calcareous soil. HCl was not very effective but the removal efficiencies of DTPA-extractable Cd and Pb increased when using combination of acids in sequential washing experiment. The removal efficiency for HCl and Na_2EDTA was increased more than 8.3% and 27.92%, respectively.

Akhtar *et al.*, **(2014)** used three chelators EDTA, DTPA and NTA to study the effect on solubility of heavy metals Cd, Cr, Cu and Pb. The study demonstrated that by increasing the dose concentration of three chelators at 5mM, the solubility of metals also increased. In incubation experiment, more Cu and Cd was extracted by DTPA. EDTA showed better extraction for Pb (22.8 ppm) from contaminated soil.

Gitipour *et al.*, (2016) compared acetic acid, ethylene diamine tetra acetic acid (EDTA) and hydrochloric acid to remove Cd and Cr contaminated sludge samples. Acetic acid extracting efficiency was less than EDTA and HCl. EDTA 0.1 M solution extracted 66.81% of cadmium and 72.52% of chromium from the sludge.

1.5 Sequential Extraction of Heavy Metals

Sequential extraction procedures (SEP) are extensively applied for the assessment of heavy metal mobility in sediments , soils (Steve *et al.,* 2001; Mossop and Davidson , 2003) and waste materials (Alvarez *et al.,* 2002). Sequential extraction methods used a series of selective reagents that solubilize in succession the different mineralogical fractions of heavy metals (Gleyzes *et al.* 2002). The use of sequential extractions process provides the detailed information about the origin, occurrence, biological and physicochemical availability, mobilization and transport of trace metals from bioavailable to inert fraction and vice versa. Sequential extraction experiments shown to determine the metals associated with the principal accumulative phases in sedimentary deposits and information on binding of heavy metals and soil components (Rauret *et al,* 1999; Ure and Davidson, 2001; Kierczak *et al.,* 2008; Rao *et al.,* 2010; Nemanti *et al.,* 2011; Aiju *et al.,* 2012).

The sequential extraction by Tessier *et al.,* (1979) is generally followed method to assess the distribution of heavy metals in soil. The theory behind SEP is that the most mobile metals are removed in the first fraction and continue in order of decreasing of mobility. Heavy metals in soil are distributed among several phases which include water soluble phase, exchangeable phase, and organic associated phase, carbonate associated phase, bound to oxides and in residual phase within the primary mineral lattice phase (Li and Shuman, 1996).

The first fraction is exchangeable which adsorbed weak chemical species and known as water or weakly acid soluble fraction. The carbonate fraction and exchangeable are bioavailable fraction of heavy metals. The Fe/Mn oxide bound fraction is called reducible fraction. The fourth fraction is organic matter bound released in the oxidizable step. It contained high molecular weight humic substances. The residual fraction is considered inert and associated with silicate in soil (Bacon and Davidson, 2008; Jensen *et al.,* 2006).

1.6 Significance of Study

Heavy metals are considered as well-known contaminates in the nature. Some of them are very toxic and potentially carcinogenic. The degradation of soil quality due to heavy metal contamination is a foremost environmental concern because of the risks and hazards it may pose to humans directly or indirectly. The remediation of soil is a difficult task because of the diverse nature of contaminants present in soil and also the remediation methods are very site specific. Soil physical and chemical characteristics and its fractional distribution of heavy metals in soil provides insight into heavy metals bioavailability, sources of contamination, chemistry, and risk of ecological damage due to metal contamination. The remediation method helps to understand the intrinsic relationship between contaminant and its specific environment.

Various remediation methods are being used to decontaminate soil from heavy metals. The recent research is being focused on methods that are inexpensive, rather quick, safe, environmental friendly use less resources and produce nontoxic by product. The soil washing method is used just for that reason and it is constantly being explored with various variations in chemicals, chelators and control on optimum experimental conditions. The basic proposition of soil washing method is using chelators for maximum efficiency to extract heavy metals from soil and the better understanding of soil physical chemical characteristics and its relation to washing treatments.

The purpose of this study to evaluate chelators to remove maximum concentration of heavy metals from contaminated soil. The study tends to explore various factors that might have influence on chelating agents' efficiency to extract heavy metals. The most commonly used chelator EDTA DTPA and citric acid are applied to remove heavy metals from soil. .The low molecular weight organic acid like citric acid is used in this study to understand the extraction efficiency and applicability in soil washing because of its biodegradable properties. This study will also evaluate selected chelators heavy metals removal efficiency and their applicability under variable physical parameters such as temperature, pH and contact time. The sequential extraction procedure will be able to show the distribution and mobility of selected heavy metals in different soil fractions.

1.7 Objectives of the Study

In view of above discussion, the proposed work was carried out to achieve the following objectives:

- evaluating the efficiency of different chelating agents like EDTA, DTPA and citric acid to remove heavy metals (Cd, Cr, Pb, Zn) from laboratory contaminated soil.
- studying the effect of different concentration of chelators and optimal contact time at which chelator maximally remove heavy metals from contaminated soil.
- to determine the effect of pH and temperature variations of chelating agents solution on removal efficiency of heavy metals.
- to study the fractional distribution of heavy metals in spiked and non-spiked soil.

1.8 Scope of Work

The scope of this thesis is the evaluation of EDTA, DTPA and citric acid efficiency for chromium, cadmium, lead and zinc from artificially contaminated soil in the laboratory. All analysis of heavy metals concentration in non-spiked and spiked soil was analyzed by ICP-OES (iCAP 6500 from Thermo Fisher UK) equipment. This work does not include studying the chemical reactions involved during experiment or evaluation of reaction kinetic

CHAPTER 02

MATERIALS & METHODS

2.0 Materials and Methods

2.1 Experimental Scheme

This study was aimed at evaluating the efficiency of chealting agents (EDTA, DTPA, citric acid) to remove heavy metals (Cd,Cr,Pb,Zn) from contaminated soil and changes in removal efficiency over different contact times . The present work studied effect of pH and temperature variations on removal efficiency of chelating agents. The soil was artificially contaminated with salts of Cd, Cr, Pb, and Zn. **Figure (2.1 & 2.2)** below shows the sequence of experiment.

❖ Step 1

The selected soil samples were prepared and preliminary analyzed to study the effect of selected chelators in heavy metal removal.

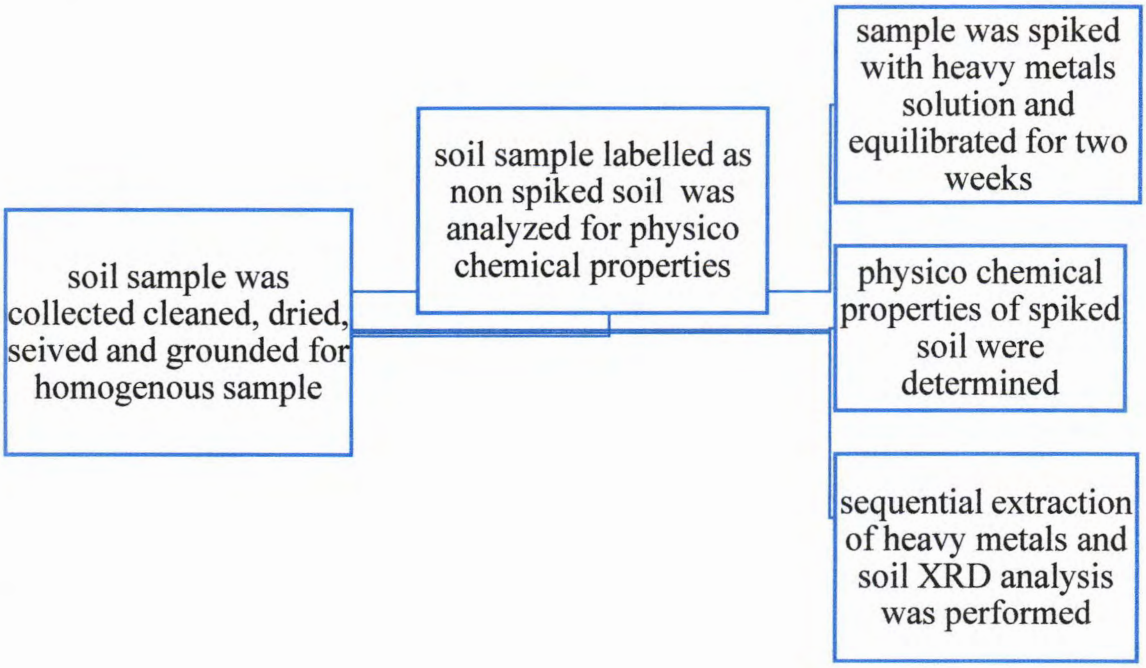


Fig 2.1: Experimental Scheme for soil physico chemical properties

❖ Step 2

In this step the experimental conditions of three selected chelators EDTA, DTPA and Citric acid were studied.

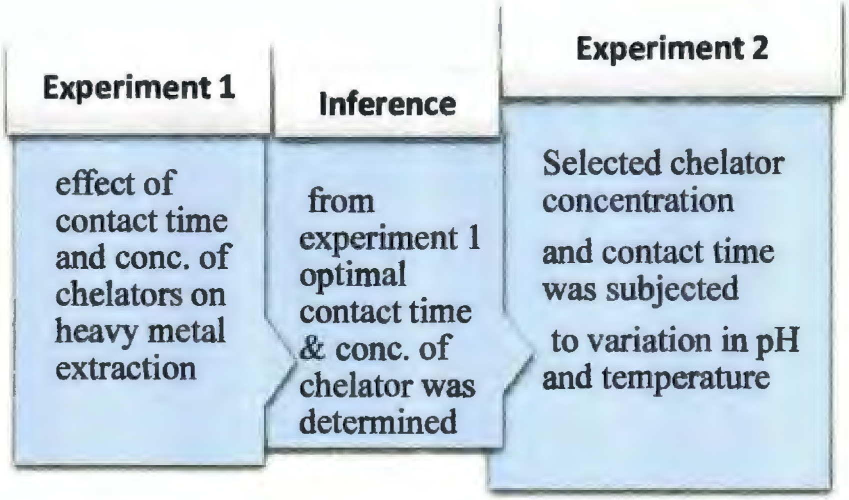


Fig: 2.2: Experimental scheme for chelating agents

Fig: 2.3: Soil Sampling Site

2.2 Soil Sampling Site

The soil sample was collected from Village Chirah located in Islamabad (Federal Capital of Pakistan). This village was adjacent to Pakistan Institute of Nuclear Science & Technology (PINSTECH). The soil sampling site was susceptible to waste disposal from nearby poultry farm. The collected soil was artificially contaminated. The surface soil (0-20 cm) was collected at 30 different points and made into composite sample. The soil sample was air dried for a week. The large stones, leaves and twigs were hand-picked. Soil was then grounded and sieved from < 2mm sieve to get 1 kg of sample.

2.3. Materials

2.3.1 Chemical and Reagents

All chemicals used were of analytical grade. The details are ; Wattmann filter paper # 42, distilled water , Cadmium Chloride $CdCl_2$(MW 183.3 g/mol) , Potassium Dichromate $K_2Cr_2O_7$(MW 294.1), Lead chloride $PbCl_2$ MW 278.106 g/mol, Zinc Chloride Hexahydrate ($ZnCl_2.6H_2O$)(244.3g/mol), EDTA tetra sodium salt ($C_{10}H_{14}N_2Na_4O_8$) (MW:380.1g/mol) , DTPA ($C_{14}H_{23}N_3O_{10}$)(MW 393.1 g/mol) , Citric Acid ($C_6H_8O_7$) MW(192.12 g/mol).

Concentrated Sulfuric Acid (H_2SO_4), NaOH, Na_2CO_3 anhydrous salt, HNO_3, H_2O_2, 1N HCl, H_3PO_4 85%, H_2SO_4 96%, NaF, 1N $K_2Cr_2O_7$ (105^0C oven dried 1L solution), 0.5N Fe++ solution (196.1 g of ($NH_4)_2(SO4)_2.6H_2O$ was dissolved in 800 ml water containing 20 ml concentrated H_2SO_4 and made up to 1 L solution) and Ferrion indicator, Phenolpathelene indicator and Methyl orange indicator.For sequential extraction of heavy metals following extraction solutions were prepared:

1M $MgCl_2$ solution adjusted to a pH of 7.0 with NaOH pallets , 1.0 M NaAc solution adjusted to a pH of 5.0 with HAc , 0.04 M $NH_2OH{\cdot}HCl$ in 25% acetic acid solution , 0.02 M HNO3 H_2O_2 30% solution adjusted to a pH of 2.0; 3.2 M NH4Ac and 20 % HNO_3 solution , aqua regia (HF+$HClO_4$ 1:2).

2.3.2. Glass wares

Volumetric flasks, Erlenmeyer flasks of 500 ml and 150 ml capacity, Conical glass flask 250 ml, Glass burette 25 ml, beakers, digestion tubes, pipettes, funnels, 10 and 20 ml dispensers, 50 ml Polyethylene centrifuge tubes, 50 ml high density polyethylene storage bottles. All glassware was borosilicate and dipped in 14 % HNO_3 solution overnight, washed with tap water and then again with distilled water.

2.3.3. Instruments

Following instruments were used during the study;

- Balance machine (ER-182A, 0SAKA Japan)
- Centrifuge (Gallehamp, UK)
- Electric shaker (KA-VIBRAX-VX8, Germany)
- pH meter (Crison, France)
- EC meter (Model WTW–LF 95)
- Block digester (Velp Scientifica)
- Shaking water bath
- ICP-OES instrument (Model iCAP 6500, Thermo Fisher, UK)

2.4 Experimental Methods

2.4.1. Soil Spiking Procedure

The soil was spiked with solutions of heavy metals. The metal stock solutions of Zinc in the form of $ZnCl_2 .6H_2O$, Lead as $PbCl_2$ and Cd as $CdCl_2$ and Cr as $K_2Cr_2O_7$ was added to soil according to methods of Wauna *et al.,* (2010) with slight modification. The 150 ml solution of 150 ppm Pb and Zn was prepared by dissolving salt in distilled water. While Cd and Cr was prepared as 100 ppm solution. About 500 ml of heavy metal solutions of Cd, Cr, Pb and Zn was added to 1000g soil. This mixture was then supersaturated with deionized water after spiking and then mixed periodically for two weeks. The wetting and air dry cycle procedure was repeated five times adequate mixing of heavy metals in soil to mimic field conditions . The wet aging stage was crucial for even distribution of heavy metals in soil (Shanbleh *et al.,* 1996; Lin *et al.,* 1998). Soil was then completely air dried, once again grounded and sieved to get fine textured homogenous soil sample. This soil was stored in air tight plastic bag at 4^0C until further analysis.

2.4.2. Soil pH and Electroconductivity (EC)

Soil samples were dissolved in water at 1:5 (w/v), soil: distilled water suspension for pH and electroconductivity (EC) measurements. This was done by weighing 10 g air-dry soil into a beaker. Then 50 mL of deionized water were added and mixed with clean glass rod for 20 minutes .This mixture was allowed to stand for 1 hour to allow soluble salts to dissolve and ionic exchange to reach equilibrium prior to measuring pH and EC. Soil EC was recorded with a calibrated EC meter (Model WTW–LF 95) and pH using a Crison pH meter.

2.4.3. Soil Texture Analysis

The soil textural analysis sieving method was adopted. The sieves were four in a set with Sigma Aldrich sieves # 10 is for the largest fraction, # 30 used for medium sand, # 60 and # 230 was the smallest used in this test. The brass sieves were stacked together with largest mesh size on top followed by decreasing mesh size. A 100 g air dried soil was placed in top sieve and shacked for two minutes so that sieve below it collects small particles. Then remaining soil from sieve 1 was put on a pre weighed paper and measured on a balance. The sieve 2 was shacked as above and measured. The percentage of Sand silt and clay were measure as % sand = mass of sand /total soil mass x100, for silt % = mass of silt /total mass of soil x 100, and for clays % = mass of clays/ total soil mass x 100.

2.4.4. Soil Organic Matter Analysis

Soil is composed of minerals (derived from the weathering of the rocks and minerals), organic matter (derived from plants and animals), and also from the living organisms. The sorption distribution coefficient of soil depends upon the content of organic matter in the soil as the heavy metals tend to sorb onto the soil organic matter, which makes it difficult for the metal species to mobilize. The Walkley Black (WB) method used for determining Soil Organic Matter (OM) utilizes a specified volume of acidic dichromate solution reacting with a determined amount of soil in order to oxidize the OM. The oxidation step is then followed by titration of the excess dichromate solution with ferrous sulfate .The OM is calculated using the difference between the total volumes of dichromate added and the volume titrated after reaction.

Procedure

- 1 g of air dried soil was transferred in 500ml Erlenmeyer flask and 10 ml of 1N $K_2Cr_2O_7$ was added to soil
- Then, 20 ml of concentrated H_2SO_4 was added by dispenser and swirled gently. It was allowed to stay for 30 minutes and was diluted with 200 ml distilled water.
- 10 ml of 85 % H_3PO_4 and 0.2 g NaF was added to complex Fe^{+3}
- Then 10 drops of ferrion indicator was added prior to titration. The solution was titrated with 0.5N Fe^{++} solution until wine red end point was reached
- Two reagents blanks were performed without soil according to above procedure.

Calculations

oxidizable organic C % was calculated by using following formula ;

$$C\ \% = \frac{(B-S) \times N \text{ of } Fe^{+2} \times 12 \times 100}{\text{g of soil} \times 4000}$$

Where:

B = ml of Fe^{++} solution used to titrate blank

S = ml of Fe ++ solution used to titrate sample,and

12/4000 = equivalent weight of C in g

The C % was divided by 0.77 for total carbon. Organic matter was then calculated by using following formula;

$$\% \text{ O.M.} = \frac{\% \text{ total C x1.72}}{0.58}$$

2.4.5. Soil $CaCO_3$ Analysis

Soil calcium carbonate was determined by acid neutralization method. In this method soil was treated with an excess of standard hydrochloric acid, destroying carbonates. The amount of excess acid was determined by titration with standard sodium hydroxide, after separation from the soil by filtration. The acid dissolved a certain amount of iron and aluminum from oxides and soil materials and these metals were precipitated as hydroxides when the pH rises towards the end of the filtration with alkali. The amount of acid destroyed in dissolving the metals was equivalent to the amount of alkali used to precipitate their hydroxides.

HCl Normality

10ml of Na_2CO_3 was pippeted in 100ml flask and 2 drops of methyl orange indicator was added. This solution was titrated against 1N HCl .The solution changed color from light to dark orange.

The normality of HCl was calculated as:

$$\text{N HCl} = \frac{\text{10 x Normality of Na2Co3}}{\text{Vol. of HCl}}$$

NaOH Normality

10 ml of 1N HCl was taken in 100ml flask and 2 drops of Phenopathalene indicator was added and titrated against 1 N NaOH.

NaOH normality was calculated as;

$$\text{N NaOH} = \frac{\text{10 x normality of HCl}}{\text{Vol. of NaOH}}$$

Procedure

- 1 g of air dried soil was taken in a 100 ml flask and 10 ml of 1 N HCl was added to to it with pipette.
- The flask was heated at 50 to 60 ^{0}C on a hot plate for 20 minutes. This mixture was allowed to cool. Then 100 ml of deionized water was added with graduated cylinder.
- 2-3 drops of phenopathelen indicator was added to mixture and titrated with 1 N NaOH until faint pink color developed.
- The reading R was noted.

$$\mathbf{CaCO3\ \% = \frac{(10\ X\ N\ HCl) - (R\ X\ NaOH)\quad X\ \ 100\ X\ 0.05}{Wt.\ of\ soil}}$$

2.4.6 Heavy Metal Analysis of Soil

The U.S. EPA method 3050B was followed for heavy metal analysis for non-spiked and spiked soil. For total heavy metal analysis of test soil, one gram each spiked and non-spiked soil was digested. The sample mixture was digested on bloke digester till solid sample was dissolved. The detailed procedure is as following:

- 10 ml of 1:1 HNO3 was added to 1 g soil and refluxed for 10 minutes
- 5 ml of concentrated HNO3 was added and refluxed for 30 minutes. This step was repeated till brown fumes disappeared and evaporated to 5 ml and cooled. Then 2 ml of distilled water and 3 ml of 30% H_2O_2 was added. 1 ml aliquots of H_2O_2 were continuously added until bubbling subsided
- The digestate was reduced to 5 ml and 10 ml concentrated HCl was added to the digestate and refluxed for 15 minutes.
- Then this digestate was centrifuged at 1000 rpm for 5 minutes and filtered using Whatman filter paper # 42 and made up to 100 ml in a volumetric flask for ICP-OES analysis.

2.4.7 Mineralogical Phase Analysis of Soil (X-Ray Difractometry)

Qualitative X-ray diffraction (XRD) analysis of soil was performed to characterize mineral phases. The unique properties of the X-rays make it one of the most important non-destructive tool for qualitative and quantitative characterization of clays, minerals and rock samples.

An XRD diffractometer works when X-rays interact with a crystalline substance or powder, the angle and intensity of the diffracted beam recorded by a detector forms a diffraction pattern, called a diffractogram which provides information about a sample. Results are commonly presented as peak positions at 2θ (theta) angle and X-ray counts (intensity) in the form of a table or an x-y plot. Peak position (angle 2θ), the interplanar atomic spacing or d-spacing provides information about atoms arrangement within the crystalline compounds. Peak width gives the crystallite size Peak intensity or counts (I) relates to the nature of the atoms and their concentration. A measurement procedure that ensures high peak/background ratios, low sensitivity to sample density variations, and a good counting statistics, while the strongest peaks of 0.334 nm quartz stay within the linearity range of the counter, and the beam stays within the preparation for the lowest 2θ angle, meets the optimal conditions for XRD analysis.

Procedure:

In the present study, soil mineralogical XRD analysis was conducted by an X–Ray Difractometer available in the Materials Division, PINSTECH. Soil sample about 10 g previously air dried and sieved at 2mm was crushed and pulverized in an inert atmosphere to get less than 75 micron fine powdered soil sample. The samples were submitted to XRD after the following pre-treatments: air-dried, ethylene glycol solvated, and heated at 550 °C for one hour. Then 30 % soil suspension was prepared and 6 ml was added to a glass slide. The glass slide was scanned 0 to 75 degrees using Cu k alpha radiation ($\lambda = 1.5418\ A^0$).

X-ray diffraction patterns were recorded using the computer controlled powder diffractometer (model DMAX IIIC of M/S Rigaku Corporation, Japan). The Cu k α ($\lambda = 1.5418\ A^0$) radiation monochromatized by a graphite crystal placed in the diffracted beam, was used at room temperature. Intensities were recorded at 0.020 degrees/ minute using NaI (sodium Iodide) detector. Indexing and refinement of unit cell parameters were accomplished using the computer program ITO (1967) and PDP 11 (1990). The semi quantitative analysis was calculated by using the counts for each peak over the total counts obtained for the scan according to the data from JCPDS (Joint Committee on Powder Diffraction Standards) files.

2.4.8 Sequential Extraction of Heavy Metals

Sequential extraction experiment was undertaken to determine the distribution and chemical fractions of Cd, Cr, Pb, and Zn and to assess the mobility of heavy metals in spiked and non-spiked soil. This method was helpful in determining the degree of contamination with in soil profiles. For this purpose 1 gram of soil was used in the sequential extraction process. The Tessier (1979) scheme was followed with slight modification.

Procedure

- **Fraction 1: Soluble and exchangeable fraction**

The soluble and exchangeable metals from soil were extracted by adding 20 mL of a 1 M $MgCl_2$ at pH 7.0 to soil in a 50 ml centrifuge tube. This solution was shaked on a mechanical shaking for 1 hour. The solution was centrifuged and filtered for residue to be used in next step.

- **Fraction II: Bound to carbonates**

The carbonates in the residue from the fraction 1 were extracted with 20 mL of a 1.0 M NaAc solution adjusted to a pH of 5.0 with acetic acid by continuously shaking for 5 hours at room temperature.

- **Fraction III: Bound to iron and manganese oxides**

Residue from the fraction II was subjected to determine metals bound to iron and manganese oxides by shaking with 50 mL of a 0.04 M $NH_2OH{\cdot}HCl$ in 25 % acetic acid solution. The extraction was performed at 96±2°C for 6 hours in a shaking water bath.

- **Fraction IV: Bound to organic matter**

Residue from above was washed with distilled water. then 7.5 ml of 0.02M HNO_3 and 12.5 ml of 30% H_2O_2 (pH 2.0) was added to the residue, and the mixture heated in a water bath at 85°C for 2 hours with occasional agitation. An additional 7.5 ml of 30% H_2O_2 (pH 2.0) was added and the mixture heated for 3 hours. This solution was then cooled to room temperature. An aliquot of 12.5 mL of a 3.2 M NH4Ac/ 20 % HNO_3solution was added and shaken for 30 minutes.

- **Fraction 5: Bound to residual fraction**

The residue from the fraction IV was washed with distilled water, and the supernatant discarded. The residue was quantitatively transferred to digestion tubes and concentrated HNO_3 and HF (3:1) were added to the residue and the sample was digested until reflux conditions were achieved. The digestate were filtered and analyzed for residual bound metal ions.

This experiment was performed in duplicates with blank solution subjected to same experimental conditions in each fraction. The liquid was separated from solid phase by centrifuging at 3000 rpm for 15 minutes. The clear supernatant was removed and filtered in clean polyethylene plastic bottles for heavy metal analysis by ICP-OES. The residue from each fraction was washed with 8 ml deionized water. After centrifugation for 15 minutes the supernatant was discarded.

2.5 Experimental Conditions

2.5.1 Contact Time and Concentration of Chelators

In this experiment different operating variables like contact time, concentration of chelator, pH and temperature were studied. The kinetic experiment was performed in 50 ml polyethylene centrifuge tubes. Soil sample of 2 g and 20 ml of chelating solution (L/S 10:1) was added. Soil solution was shaken at 180 rpm on a mechanical shaker to study the effectiveness of EDTA, DTPA and Citric acid over varying concentrations and contact time. Batch extractions of heavy metal contaminated soil was conducted by using different concentration of selected chelators. The concentration were 0.1 M, 0.5 M, 1 M, and 1.5 M for citric acid and for EDTA 0.01, 0.05, 0.1 M and 0.5 were selected. The concentration for DTPA in this study was 1mM, 3 mM, 5mM and 7mM were used. Molar solutions were prepared by dissolving required dry reagent in distilled water. The contact time or shaking time was 3, 6, 9, 12, 24 hours for all selected chelators. The solution after each shaking time was centrifuged at 3000 rpm for 15 minutes and filtered with Whatmann filter paper #42. The filtrate was collected in 50 ml volumetric flask made up to volume by distilled water and transferred to 50 ml clean polyethylene bottle for storage at 4^0C. All experiments were performed in duplicates results are presented as average of duplicate experiment.

2.5.2 Effect of pH and Temperature Variations

The pH experiment was conducted to evaluate the extraction efficiency of chelators with variations in pH of chelator solution. The pH selected for chelators were 4, 7 and 10 for EDTA and DTPA. Citric acid pH was adjusted to 2, 4 and 6 point. The pH was set by NaOH pellets and dilute HNO_3. The temperature selected for this study was 25^0C, 30^0 C and 35^0C. The soil samples were shacked with chelator solution set at different pH solutions. The control sample was run to evaluate the efficiency of chelator with or without pH adjustment of parent chelator solution. The control in this experiment was chelator solution without pH adjustments. The samples were shacked in 50 ml polyethylene tubes, centrifuged and filtered in a 50 ml volumetric flask and stored in polyethylene bottles at 4 ^{0}C until analysis. These experiments were performed in triplicates and results were averaged.

2.6 Heavy Metal Analysis

ICP-OES Analysis

Metal analyses for Cd, Cr, Pb, and Zn were carried out by ICP-OES instrument (Model iCAP 6500, Thermo Fisher, UK) available at the Central Analytical Facility Division, PINSTECH (Fig.). This instrument is equipped with high performance solid state CID camera system having RACID 86 detector containing a 512 x 512 array of charge injection devices (CID). A high speed precision peristaltic pump of the instrument having 12 rollers, 4 channels with adjustable speed (0 – 125 rpm) was used for sample introduction into the plasma.

Principle

The ICP-EOS works on the principle that the atoms of elements take up energy from Inductively Coupled Plasma (ICP) and thereby, excited and fall back into their ground state again emitting a characteristics radiation. The identification of spectrum of this emitted radiation by the spectrometer gives the qualitative analysis of the sample. A quantitative determination is then performed on the basis of proportionality of radiation intensity and elemental concentration in the calibration and analysis samples.

The clear supernatant solutions obtained from all the experiments were analyzed for Cd,Cr, Pb and Zn using the ICP-OES (iCAP 6500 from Thermo Fisher UK) equipment. The concentration of metals (µg/g) in each sample was calculated on dry basis. The ICP-OES instrument was also calibrated using standard aqueous mixtures. These standard mixtures were prepared from Specpure Multielement Standard Solution 4 as well as Specpure 1CAL2 standard solution B, both obtained from Alfa Aesar USA. Analysis was performed using emission line selected for different elements. **(Table 2.01).**

2.7 Calculation of Removal Efficiency

The heavy metal removal efficiency for (Cd, Cr, Pb, and Zn) was calculated using the following formula.

$$\textbf{Removal efficiency \%} = [(C_{\text{initial}} - C_{\text{final}}) / C_{\text{final}}] \times 100$$

Where

C_{initial} is metal concentration (mg/kg) before soil washing

C_{final} heavy metals load after soil washing

Table 2.01: List of selected Emission Lines for ICP-OES

Element	**ICP-Optical Emission Lines (nm)**
Cd	228.802
Cr	357.869
Pb	261.418
Zn	213.856

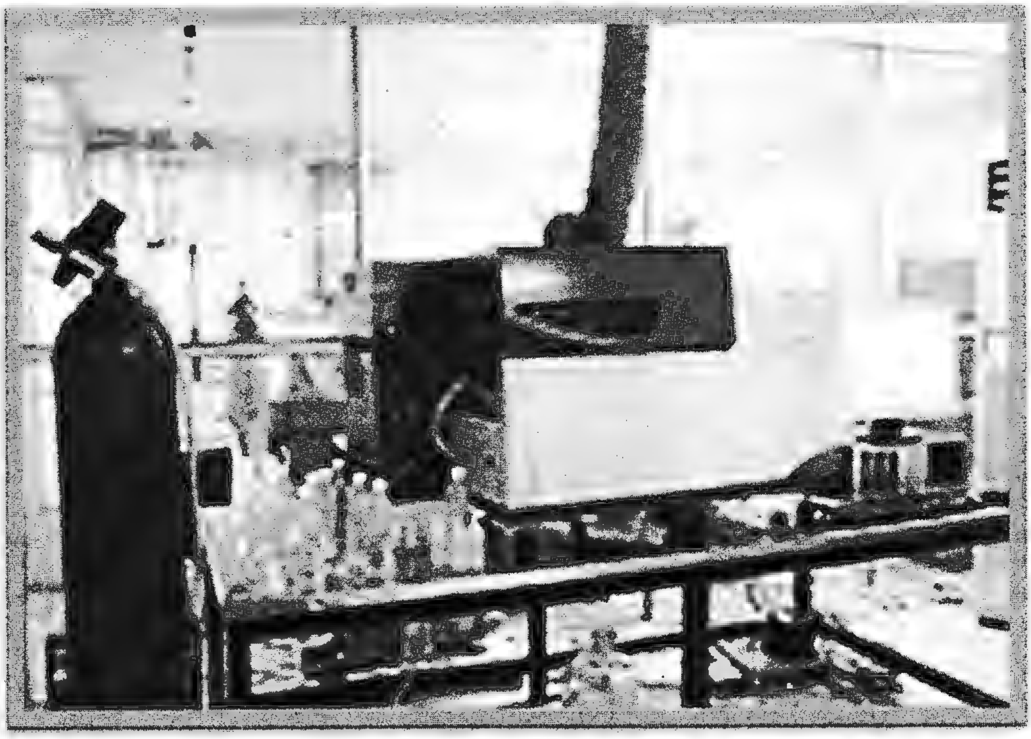


Fig. 2.4: ICP-OES Instrument

CHAPTER 03

RESULTS & DISCUSSION

3.0 RESULTS AND DISCUSSION

Removal efficiency of cadmium, chromium, lead and zinc at various experimental conditions viz contact time, concentration of chelators, pH and temperature had been evaluated using three chelators EDTA, DTPA and Citric acid.

3.1 Physical and Chemical Properties of Experimental Soil

Table 3.01 showed the physical and chemical properties of spiked and non-spiked soil. Soil pH plays an important role in the sorption of heavy metals because it directly controls the solubility and hydrolysis of metal hydroxides, soil carbonates and phosphates (Tokalioglu *et al.,* 2006). The pH of non-spiked soil was increased from 7.9 to 8.2 with spiking procedure. The pH increase indicated that soil contained considerable soluble salts that had undergone alkaline hydrolysis such as sodium carbonate. The inability of the displaced Na^+ ions to inactivate or combine with OH^- ions results in increased soil pH, which is usually greater than 8.2 (Abrol *et al.,* 1988; Lukman *et al.,* 2014).

The exchangeable cations hydrolyses depends on capacity to compete with H^+ ions for exchange sites. The Ca^{2+} and Mg^{2+} are more tightly held than Na+, that is why exchangeable Na^{+2} and K^{+2} are hydrolyzed to a much greater extent and produce a higher pH than do exchangeable Ca^{2+} or Mg^{2+}. The hydrolysis of $CaCO_3$ and of $MgCO_3$, is limited due to their low solubility and therefore they produced a pH in soils no higher than about 8.0 to 8.2. (http://www.fao.org/docrep/x5871e/x5871e05.html).

Electrical conductivity (EC) is the ability of a material to conduct (transmit) an electrical current. It is commonly expressed in units of milliSiemens per meter (mS/m). Alternatively, electrical conductivity measurements can be expressed in deciSiemens per meter (dS/m). EC depends upon the water content of soil, organic matter, soil texture, concentrations of dissolved electrolytes, composition and types of collides present in soil (Rhodes *et al.,* 1976). Sands have a low conductivity, silts have a medium conductivity and clays have a high conductivity. The non-spiked soil has EC of 0.35 dS/m which increased to 0.47 dS/m after spiking it with heavy metals solution.

The soil had a low organic matter content 1.12 %, and it belonged to silty clay textural class, silt being the major fraction of mineral particles. The fine textured soil had clay particles coagulated together that caused less soil organic matter absorbance per unit of weight, thus had enhanced ability to trap heavy metals. In coarse grained soils the clays are individual particles and hold more organic matter (Hassink *et al.*, 1997). The experimental soil also had considerable amount of $CaCO_3$ 12.3%.

An alkaline soil is associated with the presence of CaCO3 (Wali *et al.*, 2013). Alkaline soils have increased capacity to adsorb manganese and iron hydroxides, which are strong natural adsorbents (Bozkurt *et al.*, 2000). The free calcium carbonate in soil also decreases the mobility of heavy metals by increasing the adsorptive capability of soil (Gasparatos *et al.*, 2001). On the other hand, the lower pH of acidic soils have increased mobility of heavy metals. (Papafilippaki *et al.*, 2008).

3.1.1 Heavy Metals Concentration

The concentration of heavy metals in spiked and non-spiked soil was determined by EPA 3050 B method analyzed by ICP-OES. The concentration of Pb and Zn were 116 mg/kg and 121 mg/kg respectively, in spiked soil (**Table 3.02**). The non-spiked soil contained Cd around 0.5 mg/kg. The total concentration of Cr was also low at 1.5 mg/kg. Similarly, the total concentration of Pb and Zn was at 6.7 mg/kg and 14 mg/kg which made this soil perfect for spiking procedure. Cd concentration in spiked soil was 57 mg/kg and Cr was at 62 mg/kg. The recovery of these heavy metals are low against the addition of 100 ppm solution of these metals. Cd can be lost from sample through adsorption on container walls or lost through volatilization during sample treatment and concentrated acid digestion (Forstner, 1980). Chromium VI is sorbed by iron, aluminum oxides, kaolinite and to lesser degree by soil montmorillonite (Jesus et al, 1999). It is also readily reduced in the presence of Fe^{+2} (Zachara *et al.*, 1989).

Table 3.01: Physicochemical properties of non-spiked and spiked soil

Parameter	Non spiked soil	Spiked soil
pH	7.9	8.2
Electrical Conductivity at 25^0C (dS/m)	0.35	0.47
Organic matter (%)	1.12 %	-
$CaCO_3$ (%)	12.3 %	-
Soil Textural Class	Silty clay Silt : 58.60 % Clay: 39.12 % Sand: 2.30%	

Table 3.02: Total Concentration of heavy metals in non-spiked and spiked soil with acid digestion method (US EPA 3050 b)

Heavy metal	Non spiked soil conc. mg/kg	Spiked soil conc. mg/kg
Cd	0.5	57
Cr	1.5	60
Pb	6.7	116
Zn	14	121

3.1.2: Major Mineral Composition of Soil

The XRD analysis of spike and non-spiked soil was performed to further establish the mineralogical profile of soil clay fraction. Based on this analysis, the major mineralogical phases present in the soil are shown in **Table 3.02.** The dominant clay minerals were identified by peaks producing d spacing (Fig **3.1; Fig 3.2**). It is evident that the clayey fraction in soil mainly consists of Montmorillonite / Chamosite (14%) produced peaks at 14.071Å, 7.07 Å, Calcite (12 %) produced peak at 3.23Å, Muscovite (9%) and Albite (4%). The higher % of Montmorillonite than the other clays in the test soil gives a swelling character to the test soil. Further, the test soil also hosts significant proportions of calcite (12%) which acts as a buffer during metal adsorption process. The presence of Albite (6%) indicates the abundance of Na+ ions in soil and this is likely to occur under increasing pH conditions. Apart from this, Calcite presence is the indicator of poorly drained soil (Sparks, 1995). Significantly high contents of Quartz (53%), and moderate contents of clay minerals shows that the test soil represents silty clay texture.

Table 3.03: Major Mineral phases percentage in Heavy Metal Spiked Soil

Mineral Phases	Percentage (%)
Quartz	53
Muscovite	9
Albite	6
Calcite	12
Kaolinite	4
Montmorillonite / Chamosite	14

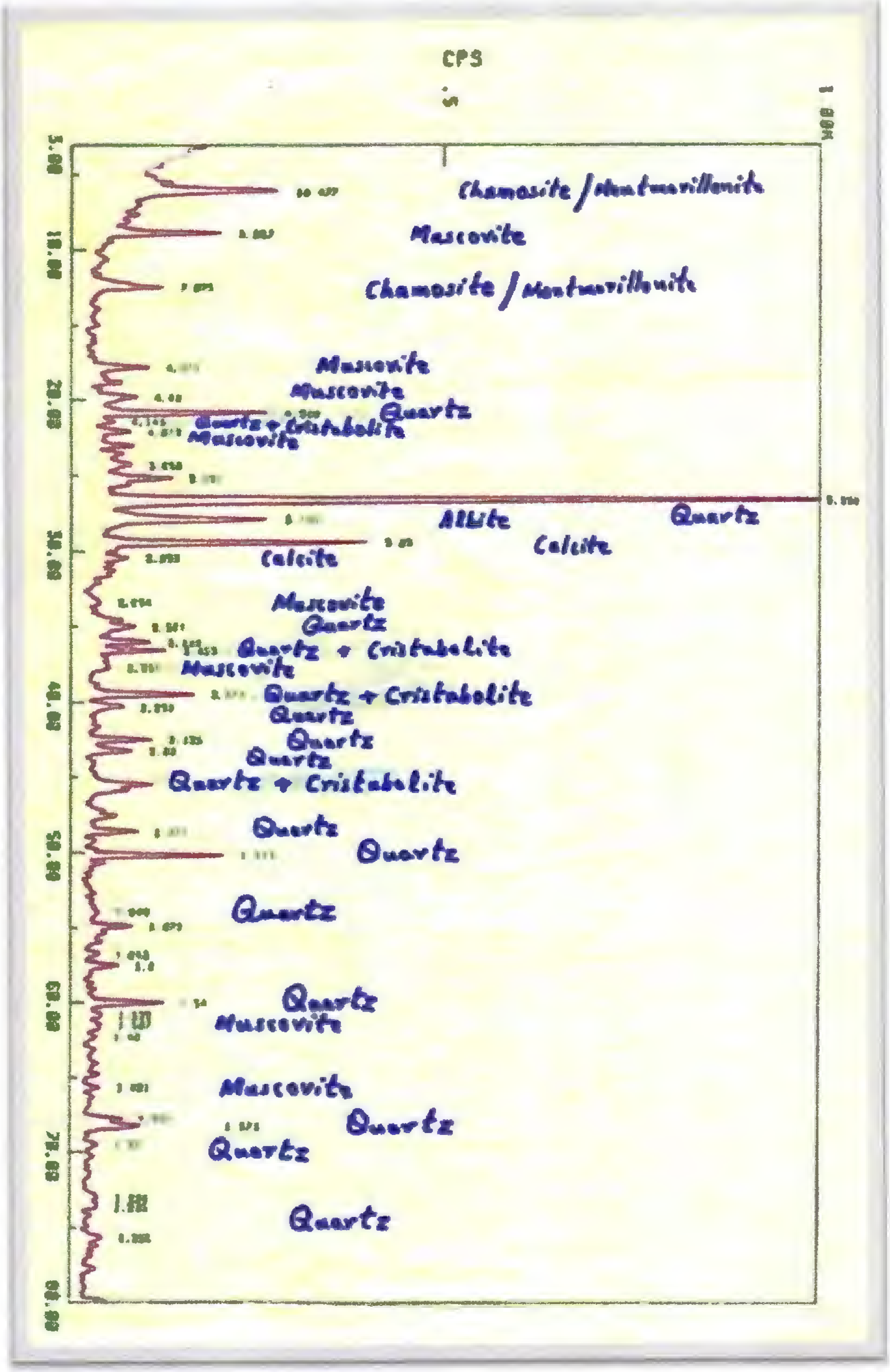


Fig 3.1: XRD mineralogical phase spectrum of non-spiked soil

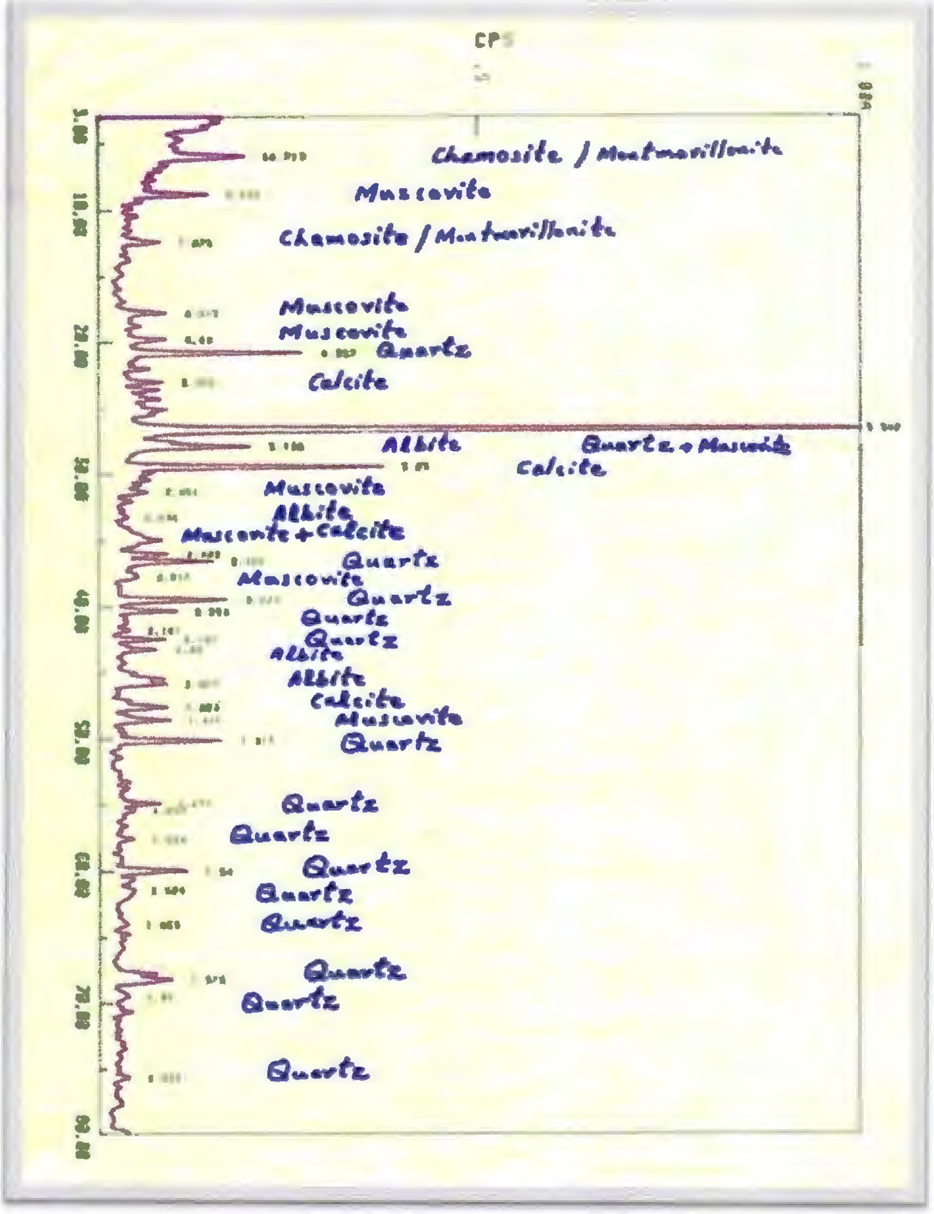


Fig 3.2: XRD mineralogical phase spectrum of spiked soil

3.2 Sequential Extraction of Heavy Metals

Table 3.04 showed the result of fractional distribution of heavy metals from non-spiked and spiked soil. The sequential extraction analysis gave the detailed assessment of heavy metals distribution in different soil fractions before and after spiking the soil. When heavy metals enter the soil, reactions with soil components cause progressive conversion to insoluble forms. Heavy metals are incorporated into soil minerals or bound to various soil phases through different mechanisms, particularly absorption, ion exchange, co-precipitation, and complexation (Navas and Lindhorfer, 2003). This study follows the Tessier *et al.,* (1979) sequential extraction of heavy metals scheme in five fractions i.e. Exchangeable, carbonate bound, bound to Fe/Mn oxide, bound to organic matter and residual fraction.

Figure 3.3 shows the percentage of heavy metals extracted from different fractions in non-spiked soil. The sequential extraction of non-spiked soil revealed that high concentration of heavy metals were found in oxide bound and residual fraction of soil. The heavy metals in exchangeable and carbonate fraction of soil are bioavailable, as they are more mobile in environment. The exchangeable and carbonate fraction of non-spiked soil was not able to extract Cd and Cr. This indicated that soil did not have labile fraction of these heavy metals. The acid digestion of non-spiked soil also gives very low concentration of Cd and Cr which confirms that these two metals were not mobile in non-spiked soil.

Lead and Zn were major heavy metals in non-spiked soil. The results showed that these metals were partitioned in different phases of soil. The exchangeable fraction extracted 6 % Pb and Zn 8 %. In carbonate fraction of non-spiked soil Pb and Zn were 13 and 14 % respectively. The third fraction is known as reducible fraction contains hydrous oxides of manganese and iron. These Fe/Mn oxides are extracted together, and known as 'sinks' in the surface environment for heavy metals (Stone and Droppo, 1996; Baruah *et al.,* 1996). These oxides are abundant in soil and sediments. The sequential extraction data shows that Pb was dominant in this fraction with 35 % followed by Cd at 30 %, Cr extracted in oxide bound fraction were 25 % of total Cr and Zn was low at 19 %. The fourth fraction is organic bound fraction. The non-spiked soil fractional data indicated that Pb and Zn were low with 10 % and 8 % of total concentration, respectively. However, Cd and Cr were 20 % of total concentration found in organic bound faction. Heavy metals in the residual fractions remained stable and inert and they are not easily released (Tesseir *et al.,* 1979). The residual fraction of non-spiked soil contained higher concentration of heavy metals. The residual Zn is 51 % and Pb is 36 % of total proportion. The amount of Cd extracted were 50 % and Cr was 55 % in residual fraction.

The high percentage of Cr in residual fraction shows that native minerals of Cr are practically inert (Pais and Benton, 1997). The distribution of Cd and Cr in non-spiked soil follows order ; residual > oxide bound > organic. Lead in non-spiked soil followed Oxide > Residual > carbonate > organic > Exchangeable fraction. Zn fractioned in following order residual > oxide > carbonate > organic > exchangeable.

The fractional distribution of Cd, Cr, Pb and Zn in spiked soil is shown in **Fig. 3.4.** When soluble metal salts are added to soil, it enters soil pore water and the concentration of heavy metals increased initially. The mobility of heavy metals decreases very slowly and equilibrium is usually attained after decades (Koen and Janssen, 2003).The heavy metals redistributed from weakly binding sites to strongly binding sites relative fo contact time (McLaughlin, 2001). This process plays a key role in heavy metals availability, extraction and soil toxicity status (Zapusek & Lestan, 2009).

In spiked soil both Cd and Cr were extracted in exchangeable and carbonate bound fraction which is associated with bioavailable fraction of heavy metals in soil. The concentration of heavy metals in exchangeable fraction were Cd 8 %, Cr 6 %, Pb 3 % and Zn at 9 %.The carbonate bound fraction showed slightly higher concentration of heavy metals with Cd extracted 20 % and Cr was 18 % . The bioavailable fraction of Cd and Cr has increased after spiking the soil with Cd and Cr salt solution. This showed that Cd and Cr were chemically reactive and posed an ecological risk (Zhang and Shan, 2008). Carbonate bound Pb accounted for 19 % and Zn was at 16 %. The oxide bound Pb was 41 %, Cd extracted from this fraction was 23 %. Cr was 26 % while Zn bound to this fraction was 22 %.

Table 3.04: Sequential distribution of heavy metals Cd, Cr, Pb and Zn in spiked and non-spiked soil in mg/kg

Soil	Fraction	Cd	Cr	Pb	Zn
Concentration in mg/kg					
Non spiked soil	Exchangeable	0	0	0.5	1.2
	Carbonates	0	0	0.7	1.8
	Fe/Mn oxides	0.3	0.5	2.9	2.2
	Organic	0.2	0.4	0.6	1
	Residual	0.5	1.1	3.0	7
	Total	**1**	**2.0**	**7.7**	**13.2**
Spiked soil	Exchangeable	4.9	4.2	4.4	11.8
	Carbonates	13.4	12.5	23	21
	Fe/Mn oxides	15.38	17.7	49.4	29
	Organic	2.1	7	4.5	6.7
	Residual	30	28	40	62
	Total	**65.7**	**69.4**	**121.3**	**130.5**

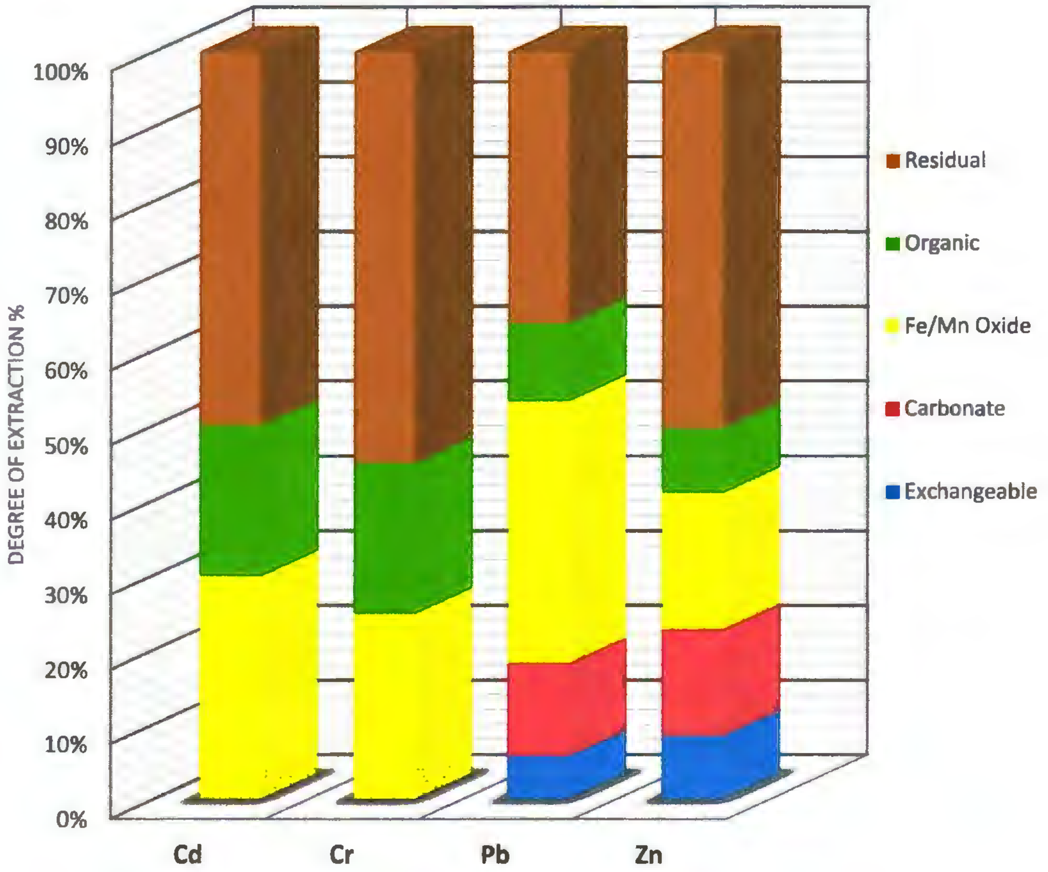


Fig 3.3: Heavy metals percentage distribution in different soil fractions with sequential extraction of non-spiked soil

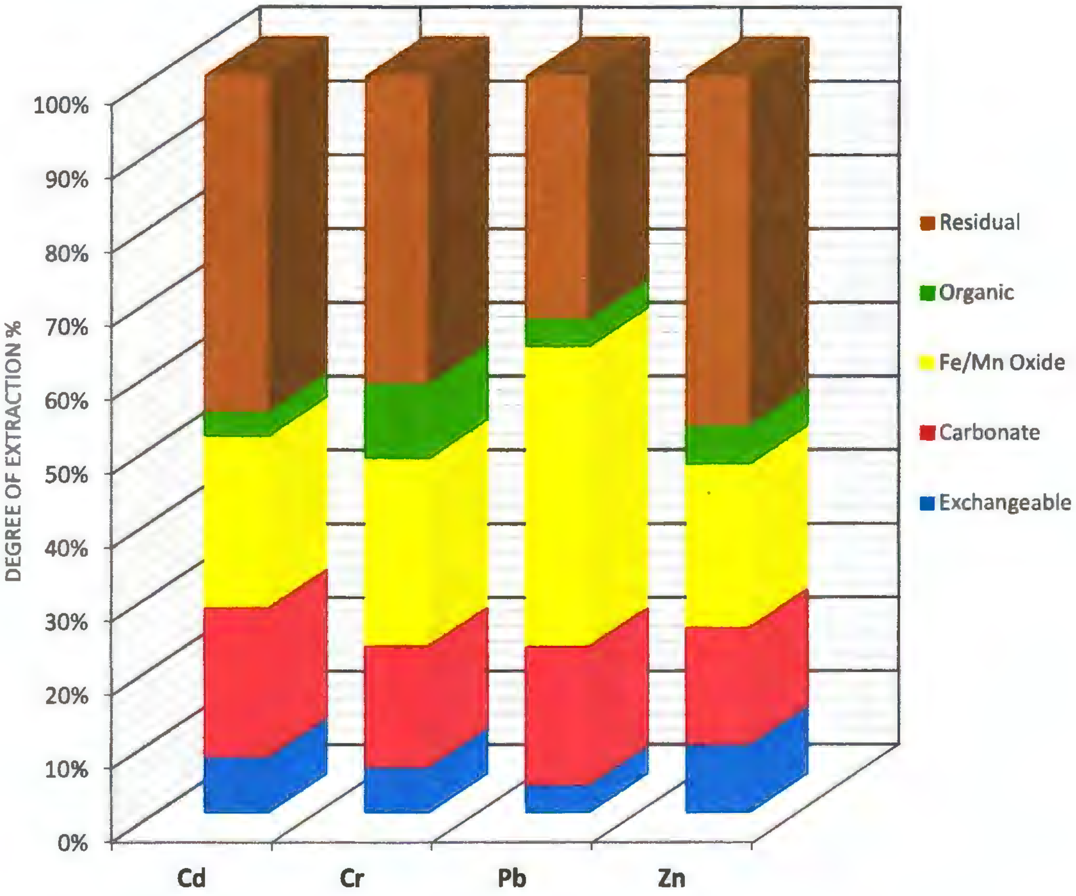


Fig 3.4: Heavy metals percentage distribution in different soil fraction with sequential extraction of spiked soil

Lead is mostly associated with silt fraction of soil. The highest fraction of Pb in Iron / manganese oxide bound fraction has been reported by various authors (Anju *et al.,* 2013; Farkas *et al.,* 2007 and Stone and Marsalek 1996). This is because of presence of reactive sesquioxides in silt fraction which bind Pb in this fraction (Sutherland, 2003). The drying of soil accelerates the crystallization of iron and manganese oxides and promoted the oxidation of Fe, Mn and S. This process caused increased in heavy metals affinity to Fe/Mn oxyhydroxides. (Bordas, 1998).

The organic bound fraction of spiked soil contained very low amount of heavy metals except Cr which is about 10 %.The organic fraction is negligible in surface soils or those which have low organic matter percentage (Kabala and Singh, 2001). The experimental soil had a low percentage of organic matter, and heavy metals bound to his fraction were also low. Lead was 4 % in organic fraction Cd was 3 % and Zn was found to be 5% in organically bound fraction. Both Cd and Zn were significantly partitioned in residual fraction of soil.

The residual fraction is considered inert and about 46 % of Cd and 48 % Zinc was retained in residual fraction. Cr was extracted at 40 % and 33 % of Pb was extracted from residual fraction. Zn follows the same pattern of distribution in extractable fractions as non-spiked soil. Jaradat *et al.,* (2006) has reported presence of high concentration of Zn in residual fraction. Kabala and Singh (2001), and Rivero *et al* (2000) reported high concentration of Zn in residual fraction in calcareous agricultural soil.

The order of heavy metals in residual fraction were; Zn > Cd > Cr > Pb. The order of heavy metals (Cd, Cr, Zn) in five fractions follows Residual > Fe/Mn oxide bound > carbonate bound > exchangeable > organic matter bound except for Pb which follows oxide bound > residual > carbonate > organic fraction > exchangeable fraction. The experimental soil had silty clay texture with alkaline pH. Kim *et al.,* (2003) reported that soil pH greater than 5.6 resulted in very low percentage of exchangeable heavy metals in soil.

Reddy *et al.,* (2010) reported sequential extraction observations of silty sand and silty clay soil contaminated with heavy metals. The silty clay soil had more concentration of heavy metals in residual and Fe /Mn oxide fraction than organic bound or other fractions because silty clay soil strongly adsorbed heavy metals to its clay particles than silty sand soil. The organic bound fraction of heavy metals in silty clay soil was lower than silty sand soil. Similar observations of heavy metals distribution were made in this study.

3.3 Heavy Metals Removal Efficiency with Citric Acid

3.3.1 Effect of Concentration and Contact Time

The removal efficiency percentage of citric acid for Cd, Cr, Pb and Zn was studied Vis molar concentration of citric acid and contact time in batch extraction method. The relationship between citric acid concentration and contact time **(Fig.3.5).** The concentration of citric acid were 0.1 M , 0.5 M , 1 M and 1.5 M and contact time was 3 , 6 , 9 , 12 and 24 hours at room temperature (26 ^{0}C – 28 ^{0}C). Heavy metals were extracted with distilled water in comparison to chelators solution. The 0 M indicates the extraction performed with distilled water without any chelator.

The results showed that distilled water was a poor extractant because of low water soluble fraction of heavy metals in contaminated soil. The highest removal of heavy metals by distilled water was observed with Zn at 4.3 %. The distilled water could remove 5.2 mg/kg of Zn at 12 hours of shaking time. The removal of Pb by distilled water was 1.8 mg/kg at 12 hours. The Cd and Cr removed by distilled water were 1.9 mg/kg at 12 hours and 0.5 mg/kg at 24 hours respectively. The citric acid concentration 0.1 M, 0.5 M, 1 M and 1.5 M successfully mobilized heavy metals in contaminated soil. The higher removal efficiency of heavy metals were recorded with 1 M citric acid.

The variations in contact time showed that removal efficiency increased from 6 to 12 hours, while 24 hours contact time could not bring much change in removal efficiency of heavy metals. The results showed that citric acid was a good chelating agent for Cr and Zn, while low removal efficiency for Cd and Pb was observed. The clay particles and oxides are central adsorption media for heavy metals in soils. That is why clay soil retains high metals as compared to sandy soils (Sherene, 2010). Citric acid function as organic ligand by increasing heavy metals mobility through ligand exchange reactions, it dissolves the metal oxides from

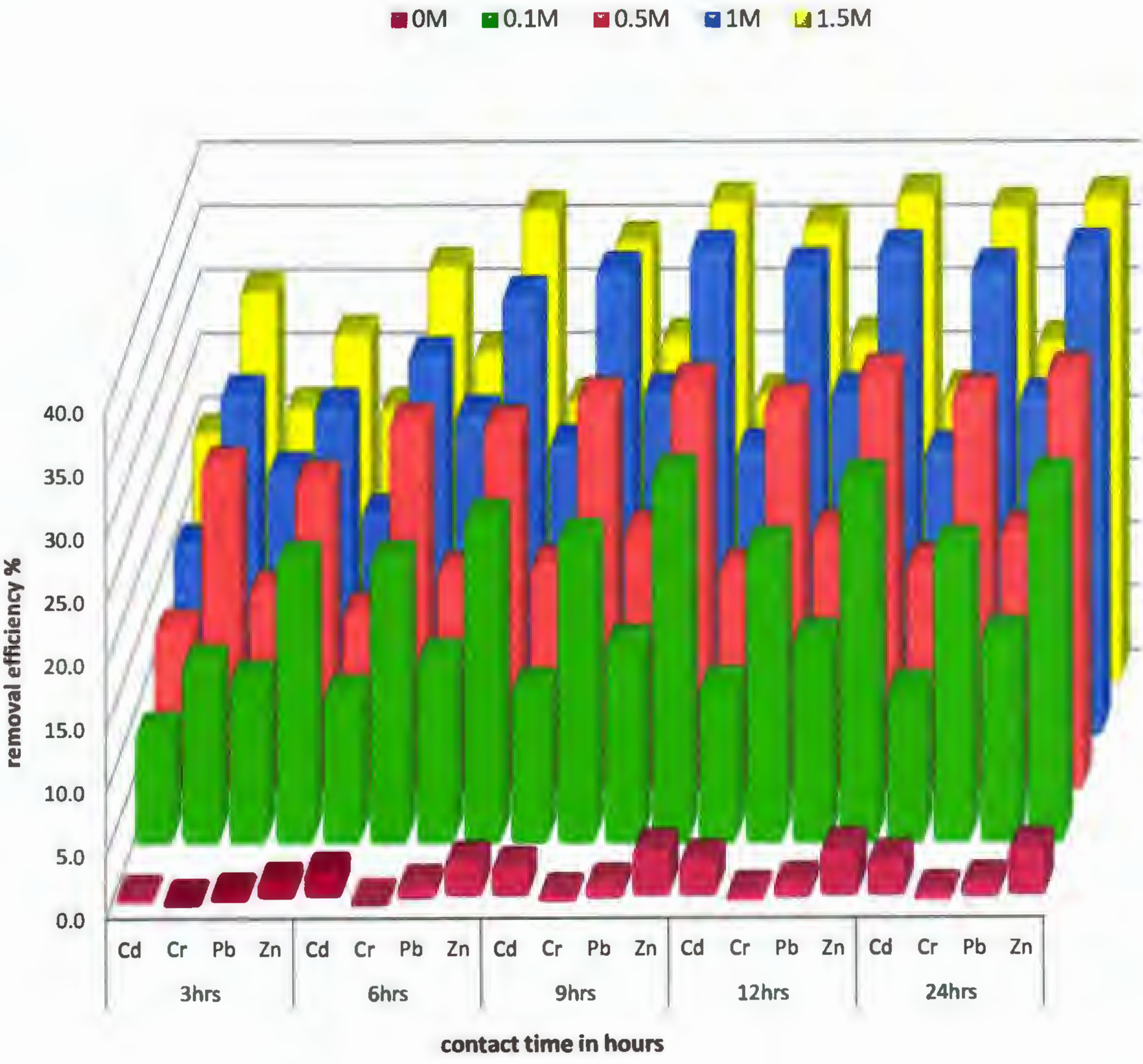


Figure 3.5: Citric acid removal efficiency with different concentration and contact time for Cd, Cr, Pb and Zn

surfaces that adsorbed metals and by forming the metal-organic complexes in solution (Stone, 1988; Pohlman and McColl, 1986; Banks *et al.,* 1994; Burckhard *et al.,* 1999).

The lowest removal efficiency of Cd by citric acid was 9.3 % after 3 hours with 0.1 M solution.it increased to 12.8 % after 12 hours. The shacking time up to 24 hours showed slight reduction in removal efficiency removing 12.5 % Cd. The highest removal efficiency of citric acid for Cd was achieved with 1 M and 1.5 M, removing 23.4 % at 9 and 12 hours of contact time respectively as shown in **Fig. 3.6**. The removal of Cd was initially high with 1 M concentration, it increased to 23.4 % after 9 hours. The 1 M citric acid removal efficiency slightly reduced after 24 hours of contact time removing 22.8 % of Cd. The 1.5 M citric acid showed initial increase in removal efficiency after 6 hours removing 22.1 % Cd, it showed almost same removal efficiency after 9 hours.

The removal efficiency of Cr by different concentrations of citric acid is shown in **Fig. 3.7**. It was observed that Cr could not be extracted with distilled water even after 24 hours of contact time. The concentration of Cr with distilled water was recorded at 0.5 mg/kg and removal efficiency was less than 1%. The minimum removal efficiency of Cr was 14.6 % observed with 0.1 M solution of citric acid at 3 hours. There was a slight decrease in removal efficiency from 12 hours removing 23.9 % of Cr, the 0.1 M solution reached its equilibrium and 24 hours of shacking did not remove any more Cr. The 0.5 M of citric acid removed 31.3 % from 9 to 24 hours of contact time. The highest removal efficiency of Cr removal by citric acid was achieved by 1 M solution removing 37 % to 37.2 % at 9 hours and reached equilibrium after that. The 1.5 M citric acid performed better removing 31.1 % Cr which gradually increased with 24 hours of contact time removing 37.5 % of Cr at 24 hours. The 1.5 M solution slowly released Cr while other applied concentration reached equilibrium after 9 hours of contact time. Citric acid performed better for Cr removal because it can remove soluble Cr which is mainly Cr VI and reduce it to Cr III by lowering pH of solution.

Cr III is removed by complexing reactions of citric acid (Wang Xing Run *et al.,* 2012). The higher molar concentration of citric acid performed better as compared to 0.1 M, the higher concentration lowered soil pH and partially solubilizing metal precipitates. Similar results were reported by Reddy *et al.,* (2000) studied removal efficiency of citric acid on glacial till soil. The citric acid removed 24 to 47 % of Cr. The citric acid in this study removed 14.6 % to 37.5 % of Cr.

The removal efficiency percentage of citric acid for Pb is shown in **Fig 3.8.** The lowest removal efficiency was observed with 0.1 M concentration of citric acid removing 13.3 % at 3 hours and highest removal efficiency for Pb was 16.7 % after 24 hours. The lowest removal from 0.5 M citric acid was 16.2 % at 3 hours. The subsequent time removed 20.8 % of Pb and remained unchanged after 24 hours. The 1 M citric acid removal efficiency at 3 hours was 21.3 %, while highest removal was noted at 9 hours with 27.4 %. The 1.5 M solution of citric acid behaved like 1 M solution removing 27.4 % of Pb at 9 hours. The 1.5 M citric acid showed no significant change in the removal efficiency of Pb.

Nascimento (2006), studied low molecular weight organic acids like citric acid, oxalic acid, vanillic acid, and gallic acid in desorbing Cd, Pb, Zn, Cu, and Ni from soil. All of these lower molecular organic acids were least effective for lead desorption from contaminated soil. The main reason seemed was low water solubility of these acids as compared to DTPA and EDTA, which leads to decrease in reaction with metals bound to soil collides or precipitated. This assumption supports the low extraction of Cd and Pb by citric acid in this study.

Figure 3.9 shows the result of citric acid removal efficiency for Zn. Some of the Zn was present in water soluble form and it could be removed by distilled water. In this case, distilled water removed 4.3 % after 12 hours of shaking time. Citric acid had been a better chelator for Zn as compared to Cd, Cr and Pb. The highest efficiency of Zn was achieved with 1 M citric acid solution removing 38.6 % after 9 hours. The citric acid lowered the pH considerably to mobilize Zn from contaminated soil. The fractional data results showed that nearly 50 % of Zn is bound to residual fraction, while 50 % is distributed among bioavailable and oxide fraction. The initial extraction of all applied concentration showed that citric acid is extracting from same bioavailable fraction of Zn and then from other fractions with increase in time and concentration of chelator. The 1.5 M citric acid showed similar behavior with 1 M solution in removal efficiency.

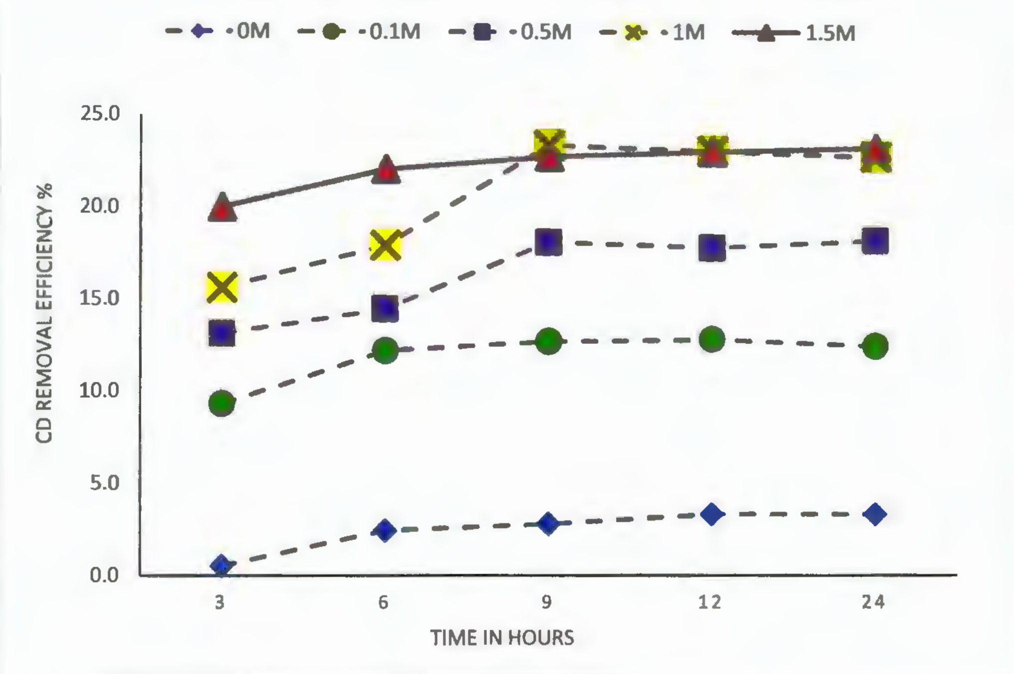


Fig 3.6: Removal efficiency of citric acid for Cd

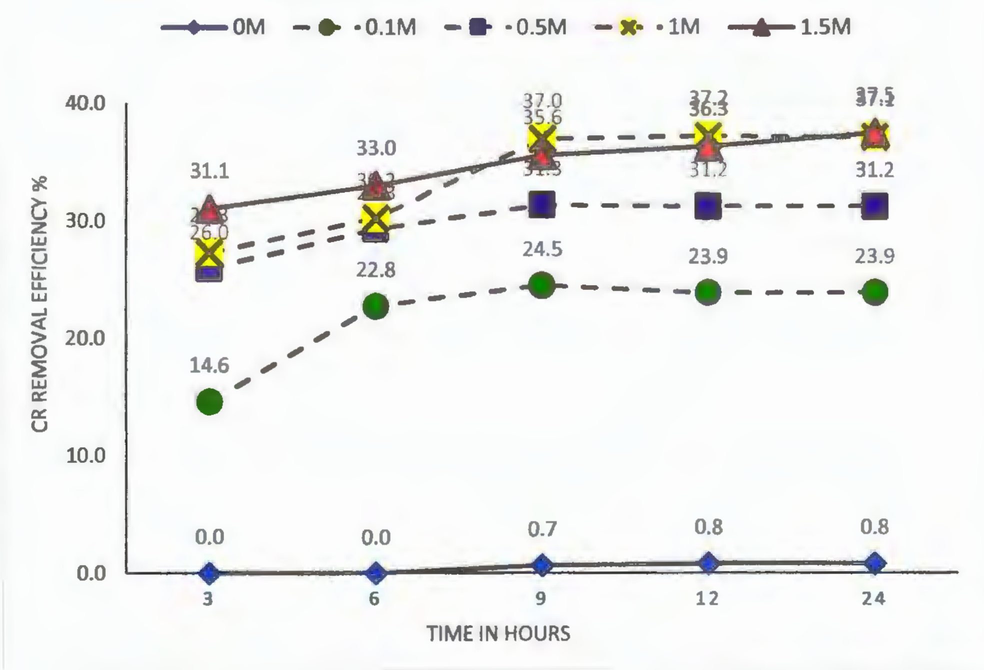


Fig 3.7: Removal efficiency of citric acid for Cr

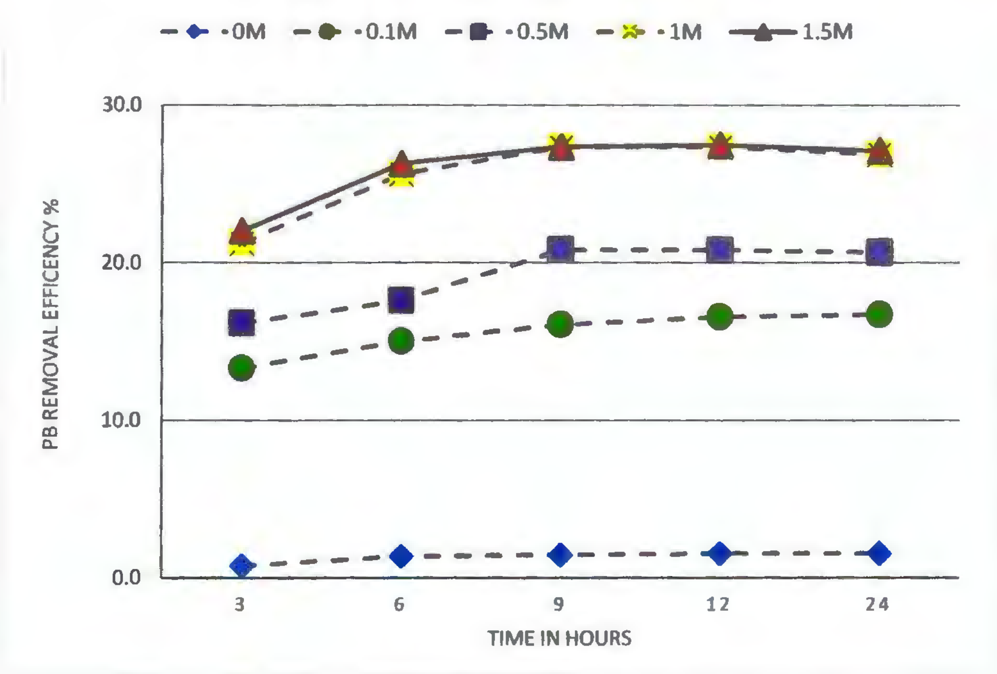


Fig 3.8: Removal efficiency of citric acid for Pb

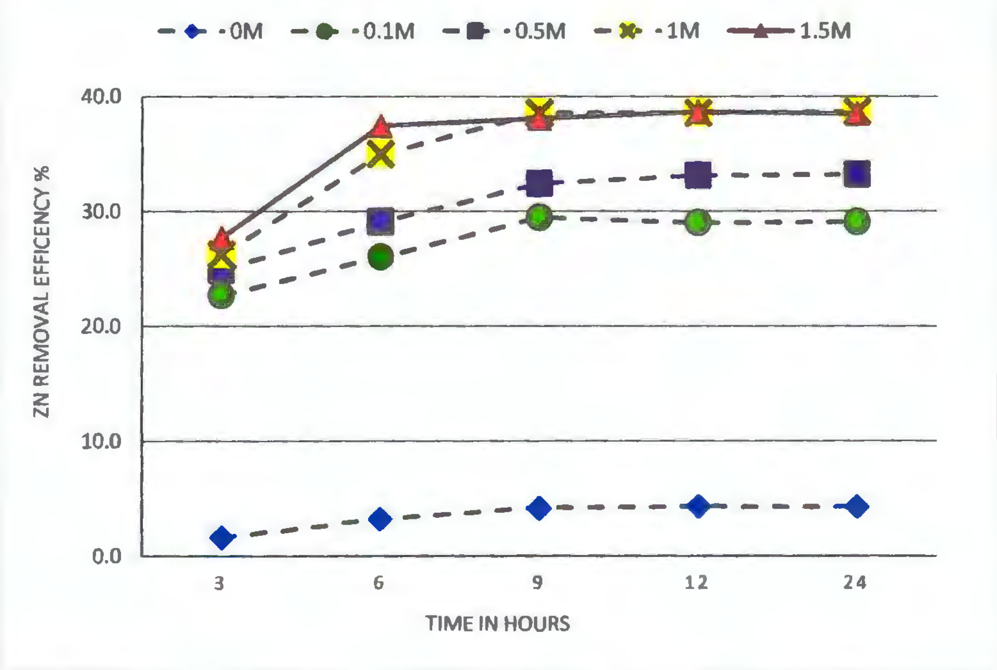


Fig 3.9: Removal efficiency of citric acid for Zn

The above experiment showed that increasing the citric acid concentration to 1.5 M did not improved the removal efficiency of heavy metals. The high concentration of citric acid was required to lower the pH from alkaline to acidic to release heavy metals from tightly bound clay particles. The lower pH of system generally leads to the protonation on the ionized chelant species resulting in competition for binding sites between the hydrogen and metal ions, which led to decrease in metal solubilization (Neale *et al.,* 1997).

3.3.2. Effect of pH and Temperature on Citric acid removal efficiency

The experiment with pH and temperature variations was performed to observe change in removal efficiency of citric acid for cadmium, chromium, lead, and zinc. The contact time and concentration experiment showed that heavy metals are best extracted with 1 M citric acid after 9 hours contact time. The same parameters was selected for pH and temperature variation experiment. The 1 M solution of citric acid was adjusted to pH 2, 4 and 6 , with temperature adjusted to 25 ^{0}C , 30 ^{0}C and 35 ^{0}C.

The control solution was citric acid 1 M solution mixed with contaminated soil without any pH adjustment shacked along pH treated solutions at same experimental condition. The pH of 1 M citric acid is highly acidic $\leq$ 1. The removal efficiency of 1 M citric acid at 25 ^{0}C with pH variations for cadmium, chromium, lead, and zinc was shown in **Fig. 3.10.**

The highest removal efficiency for heavy metals was observed for pH 2 of citric acid. The solution without any pH changes resulted in slightly lower percentage of heavy metals except Pb and Zn. The control solution removed 35 % of Cr while pH 2 removed 40.9 %. Cd removal with control was 21.6 % which improved with pH 2 removing 24.9 %. Zinc removal with control was 33.6 % while pH 2 removed 31.6 % at 25 ^{0}C. The removal of Pb with citric acid control solution was 26.3 % while pH 2 removed 20.5 %. This showed that Pb extraction was favored at very low citric acid pH because subsequent changes in pH resulted in lower extraction of Pb.

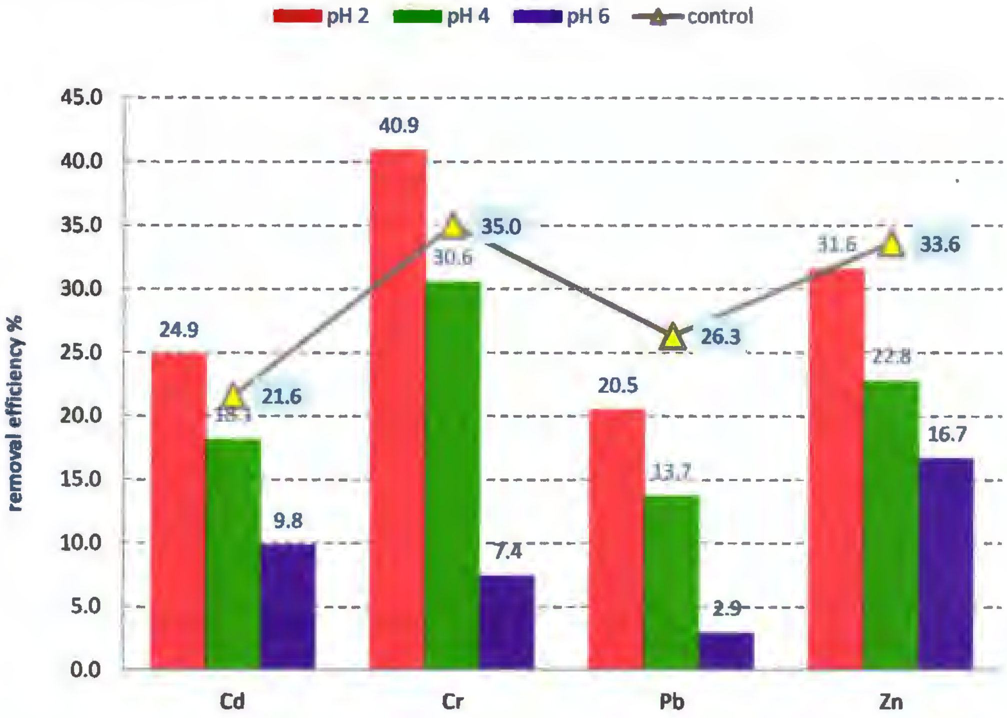


Fig 3.10: Removal efficiency of 1 M citric acid for heavy metals with pH variations at 25 ^{0}C

It was observed that lowering pH resulted in less removal efficiency of all studied heavy metals. For instance, pH 4 removed 18.1 % Cd, 30.6 % Cr and 22.8 % Zn, while pH 6 resulted in poor extraction of heavy metals as compared to all other solutions. The lowest extraction of heavy metals was observed with pH 6 removing 9.8 % Cd, 7.4 % Cr and 16.7 % Zn.

Figure 3.11 shows the increase in temperature of citric acid solution resulted in increased removal of Cd, Cr and Zn with control solution, while Pb removal efficiency lowered at 30 ^{0}C as compared to removal at 25 ^{0}C. The highest removal efficiency of Cd was observed with control solution at 30 ^{0}C.It removed 28.3 % of Cd ,while pH 2 removed 19.5 % , pH 4 removed 14.3 % and lowest removal efficiency was observed with pH 6 removing 9.4 %. The removal of Cr increased with increasing temperature. The control solution removed 37.3 % of Cr, pH 2 removed highest Cr at 30 ^{0}C with 41.9 %, pH 4 and pH 6 removed 32.1 % and 10.2 % of Cr which is slightly higher than removal at 25^{0}C.

Pb showed lower removal efficiency with increase in temperature. The control solution removed highest percentage of Pb 18.2 % at 35 ^{0}C, pH 2 removed 15.7 %, pH 4 removed 11 % while lowest removal was observed with pH 6 removing 2.8 % Pb. Here, it was observed that pH 6 at temperature 25 ^{0}C and 30 ^{0}C showed a little change in removal of Pb. Zinc showed increase in removal efficiency with increase in temperature. It removed 30.8 % Zn with citric acid control solution. The highest removal efficiency 33.1 % observed with pH 2 and pH 4 solution removed 23.8 % and pH 6 removed lowest percentage of Zn at 15.8 %.

The effect of temperature increase from 30 ^{0}C to 35 ^{0}C, resulted in similar observation except some changes in removal efficiency of control solution. **Figure 3.12** showed that the control solution of citric acid at 35 ^{0}C did not bring much change it was recorded at 28.6%. But the pH 2 showed no significant change in removal efficiency and remained at 19.1 %. While, pH 4 and pH 6 on the other hand, showed decreased removal efficiency with increasing temperature removing 10.9 % and 8.3 % respectively. Cr showed no significant change at 35 ^{0}C. The highest removal was observed with using pH 2 with 41.7 %, the control solution removed 38.6 % Cr. The pH 4 removed 31.8 % of Cr, however pH 6 showed decrease in extraction efficiency removing 5.5 % Cr with increasing temperature.

Lead removal efficiency with increasing temperature further decreased. The highest removal efficacy was observed at pH 2 solution removing 9.8 %. The lowest removal efficiency was observed with pH 6 solution. It removed 2.1 % Pb. The Zn removal efficiency was improved with increasing temperature. The highest removal efficiency was observed and 5.6 % of Zn. The control solution of citric acid at 35 ^{0}C extracted 14.8 % Pb and 21.2 % Zn. pH 4 also showed decrease in removal efficiency at 35 ^{0}C removing 5.3% Pb and 11.3 % of Zn.

It was observed that increase in temperature caused decrease in removal efficiency of Cd and Pb. Citric acid is a ligand with the ability to complex heavy metals over wide pH range (Cheng *et al.*, 2012). Yan *et al.*, (2014) observed the leaching abilities of organic and inorganic acids at pH 2-6 for Cu, Cd, Pb and Zn. Their result indicated that increase in pH caused decrease in leaching concentration of metals and organic acids are better chelating agents with pH 2 as compared to inorganic acids. In present study Zn extraction was increased with increasing temperature with pH 2 and pH4 solution of citric acid. The pH 6 solution showed decrease in extraction of Zn with increasing temperature.

Veeken and Hamelers (1999) showed that the rate of extraction for Zn increases with increasing temperature and citric acid concentration. It was reported that citric acid was the most effective ligand for Cr (VI) mobilization, due to its ability to render the mineral matrix soluble and the competition for the surface sites with Cr (VI) (Jean *et al.*, 2007). Lead showed overall decrease in extraction efficiency with controlled pH and temperature as compared to experiment done without pH variations. Mahvi *et al.*, (2005) observed in a study that recovery and desorption of lead from silt clay loam soil was increased under strong acidic conditions which become decreased as the pH increased.

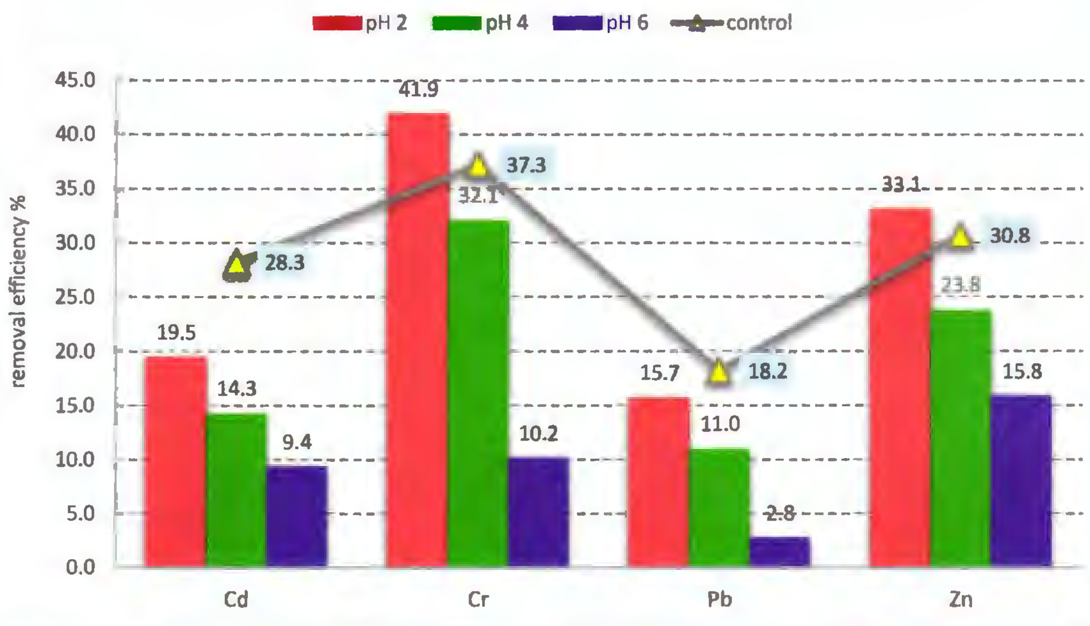


Fig 3.11: Removal Efficiency of Citric acid 1M for heavy metals with pH variations at 30 ^{0}C

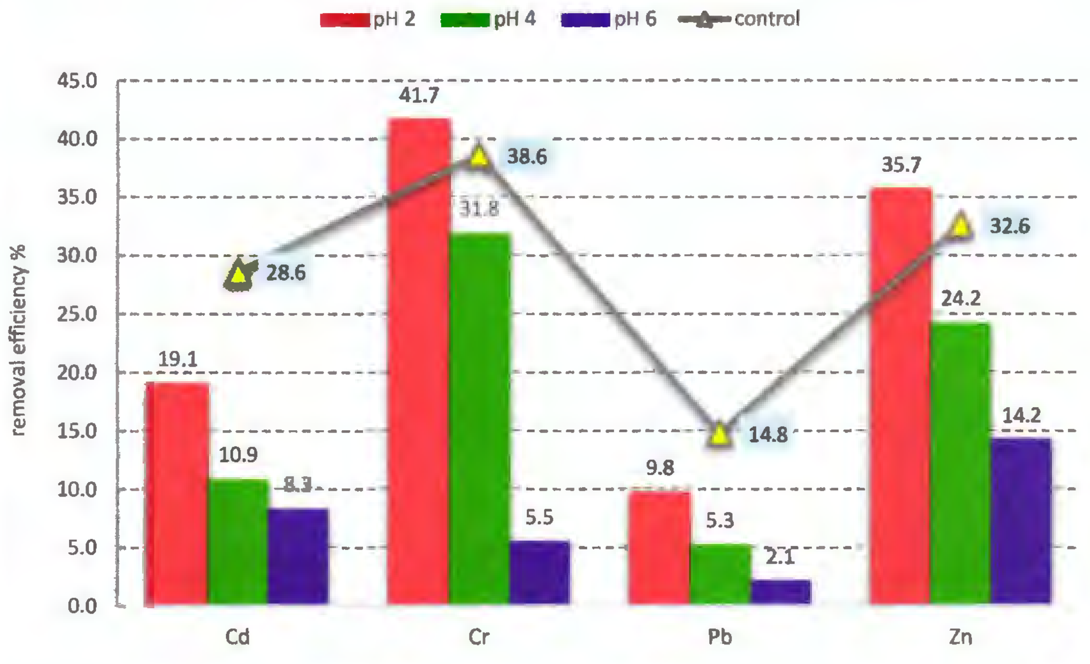


Fig 3.12: Removal Efficiency of Citric acid 1 M for heavy metals with pH variations at 35 ^{0}C

3.4 Heavy Metals Removal Efficiency with DTPA

3.4.1 Effect of Contact time and Concentration

The concentration of DTPA in this experiment was selected at 1mM, 3mM, 5mM and 7mM. The soil and chelator solution was shacked for 3, 6, 9, 12 and 24 hours at room temperature. Effect of variation in contact time and concentration of DTPA on the removal efficiency of Cd, Cr, Pb, and Zn **(Fig. 3.13).**

The figure showed that DTPA was successful in mobilizing heavy metals (Cd, Cr, Pb, Zn) from soil. The control in the experiment was contaminated soil in distilled water which was shaked along soil and chelator solution at same experimental conditions. The optimum concentration of DTPA in shacking experiment was 7 mM at which maximum removal efficiency of Cd, Cr, Pb and Zn was achieved. The concentration 7mM was found to have a similar behavior as 5 mM, and it did not significantly showed variation in removal efficiency of heavy metals from soil. It was observed that heavy metals were maximally removed after 24 hours of contact time.

From **Fig 3.14** it was observed that Cd removal efficiency increased with increasing concentration from 1mM to 5mM. The 7mM DTPA showed slightly less removal concentration than 5mM. The concentration of 1 mM extracted lowest percentage of Cd 12.7 % and showed rapid change in removal efficiency from 3 hours to 6 hours. The further increase in extraction time did not bring any significant change in removal efficiency. The highest removal efficiency by 1mM solution was 24.7 % at 24 hours. The lowest removal efficiency of Cd by 3mM was 27.7 % and highest efficiency was recorded at 24 hours removing 32.2 % of Cd. The 5 mM removal efficiency after 3 hours of contact time was 30.4 % of Cd, the subsequent shacking removed highest percentage of Cd with 5 mM at 38.2 % after 12 hours. The shacking up to 24 hours did not change the removal efficiency. The 7 mM concentration showed rapid removal efficiency from 3 to 6 hours removing 34.1 % to 35.5 %. The further increase in time up to 24 hours removed 37.7 % of Cd.

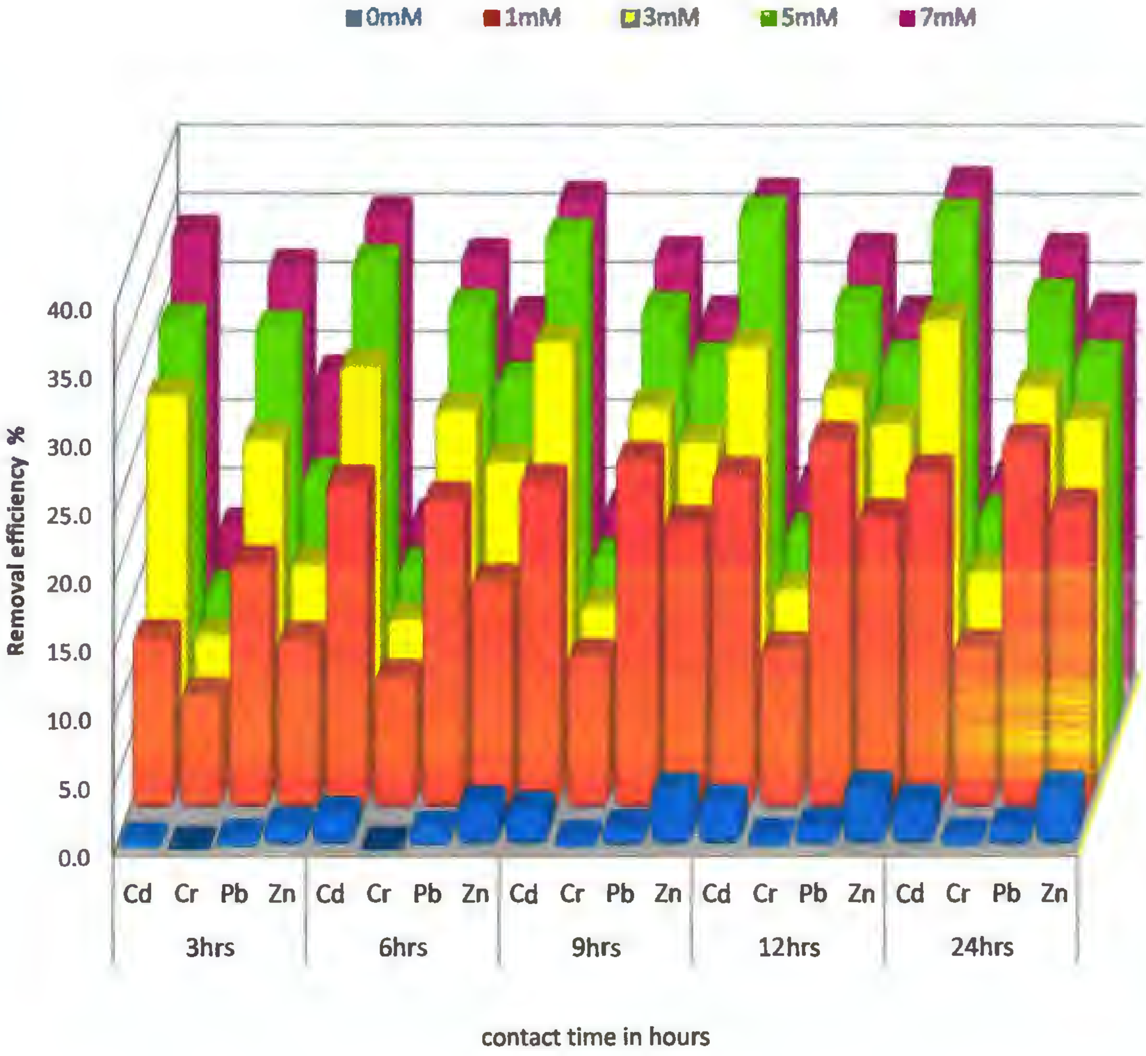


Figure 3.13: DTPA removal efficiency with different concentration and contact time for Cd, Cr, Pb and Zn

Cadmium is highly labile metal even more than lead. Its bioavailability increased from non-organic sources for the soil environment (Galloway, 1990). In the study conducted by Khanmarzei *et al.,* (2013) Cd was predominately attached to the carbonate fraction (about 40-44%) in highly calcareous soils. The soil used in this study had moderate amount of $CaCO_3$ (12 %).The sequential extraction of contaminated soil used in this study revealed that 20 % of Cd is present in labile fraction of soil. DTPA was successful in extraction of Cd from bioavailable fractions.

Removal efficiency of Cr is quite low with DTPA as shown in **Fig 3.15.** DTPA extraction efficiency for Cr was low. The 1mM concentration of DTPA mobilized 8.5 % Cr after 3 hours of shaking that slowly reached to 12 % after 24 hours. The maximum removal efficiency of Cr was recorded between 16.2 % and 16.5 % with 5 and 7 mM of DTPA after 24 hours. It was observed that increase in concentration and contact time gradually extracted Cr. DTPA low removal efficiency for Cr had been reported in sewage sludge amended soil by Fuentes *et al.* (2006) and Walter *et al.* (2006). This was due to Cr solubility, which decreased above pH 4, with complete precipitation above pH 5.5 (Alloway, 1995). Another reason for low extraction is the slow kinetics for the formation of Cr-DTPA complexes (Byegård *et al.,* 1999) which supports the results as efficiency was increasing with time .

DTPA removal efficiency for Pb is shown **Fig 3.16.** The minimum removal efficacy for Pb was recorded 18.1 % with 1 mM of DTPA. The maximum removal efficiency with 1 mM was 27 .2 % after 12 hours. The further shaking did not removed Pb from contaminated soil. The removal efficiency of 1 mM and 3 mM after 24 hours did not bring any significant change in removal efficiency. The applied concentration initially removed maximum percentage of Pb and reached equilibrium after 6 hours of contact time. The Maximum removal of Pb from 5 mM solution was 32.2 % while 7 mM removed 32.8 %.

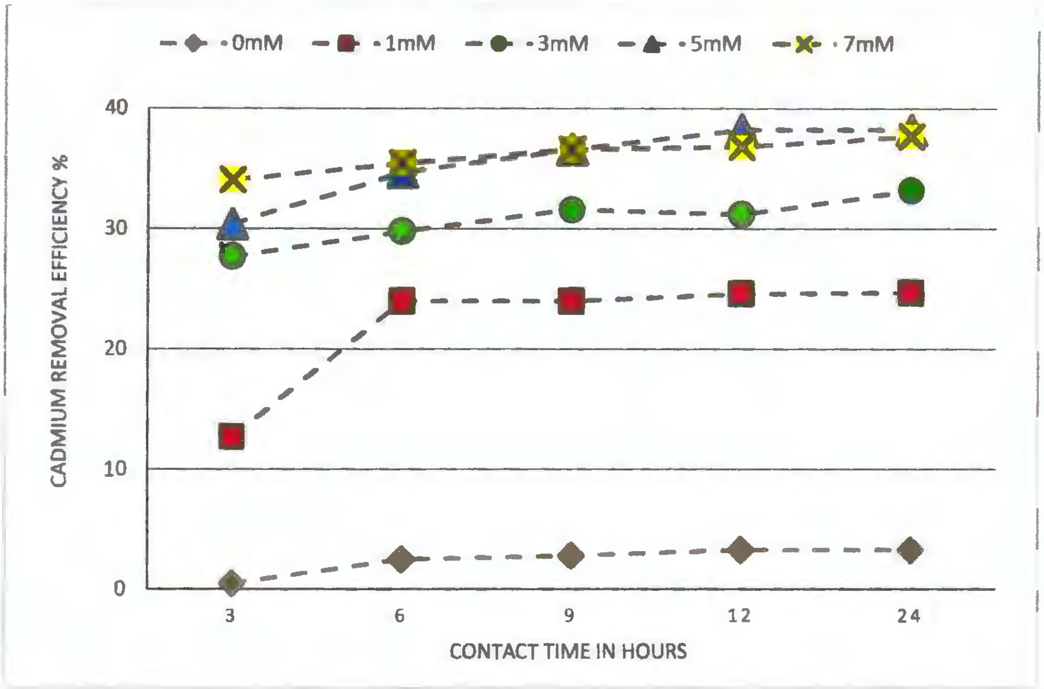


Fig: 3.14: Effect of DTPA concentrations and contact time on Cd removal efficiency

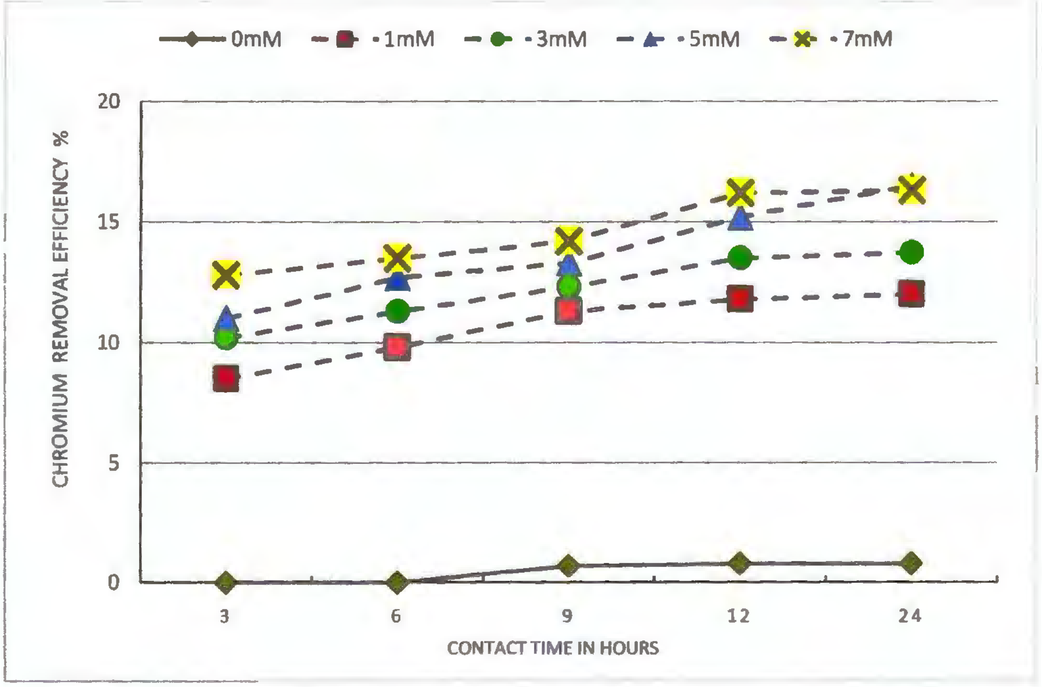


Fig 3.15: Effect of DTPA concentrations and contact time on Cr removal efficiency

The concentration of DTPA solution did improve removal efficiency of Pb but extending time up to 24 hours was not able to bring significant increase in removal efficiency, except 1mM solution of DTPA. Similar observation were made by Akhtar *et al.*, (2014) extracting Pb, Cd and Cr with DTPA. Pb was increased with increasing concentration but shacking time had little impact on removal efficiency. While increasing the contact time also increased Cr solubility

The **Fig 3.17** showed the removal efficiency of Zn by DTPA. The minimum removal efficiency of Zn was observed with 1mM DTPA at 12.6 % with 1 mM after 3 hours. The removal percentage gradually increased to 22.2 % after 24 hours. The maximum concentration of Zn was removed by 3mM was 26 %. All applied concentration of DTPA showed marked increase in removal efficiency from 3 to 6 hours and further increase in contact time did not bring significant change in removal efficiency. The maximum removal by 5 mM was 27.8 % after 24 hours. While 7mM has slightly more removal efficiency and removed 28.5 % of Zn after 24 hours of shaking.

The low extractability of Zn by DTPA was reported by Khodadoust *et al.*, (2005) even after using 0.2 M of DTPA which removed approximately 12 to 14 % of Zn. This may be attributed to the non-selective nature of DTPA and pH sensitivity of chelation process which sometimes results in lower extraction even after using high concentration of chelator. DTPA removed heavy metals in increasing order of removal efficiency Cd > Pb > Zn > Cr.

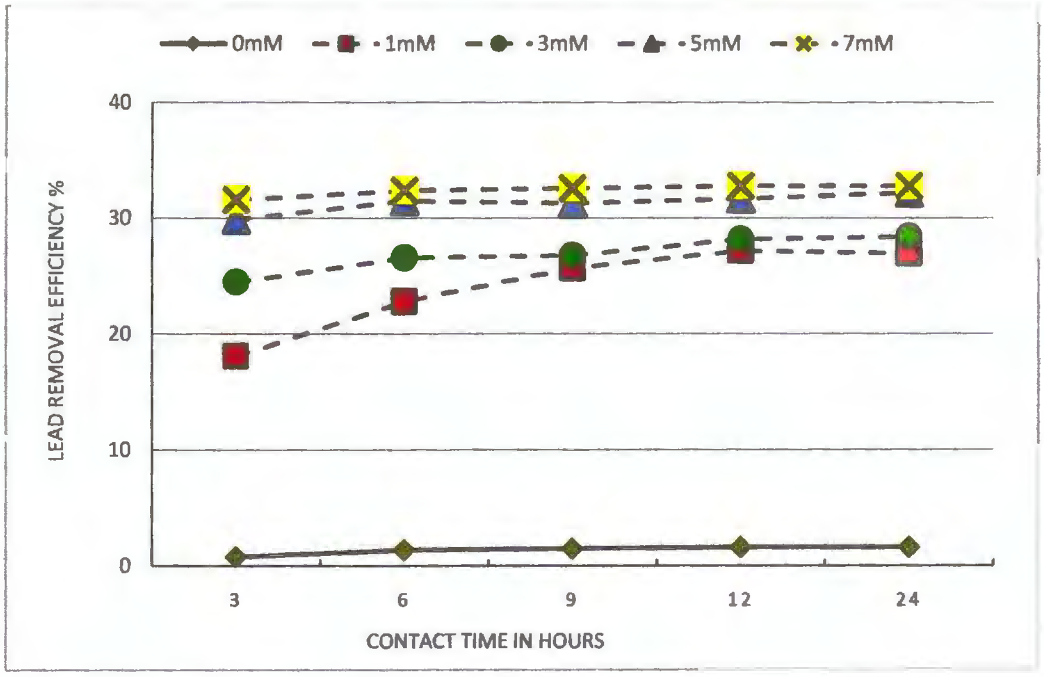


Fig 3.16: Effect of DTPA concentrations and contact time on Pb removal efficiency

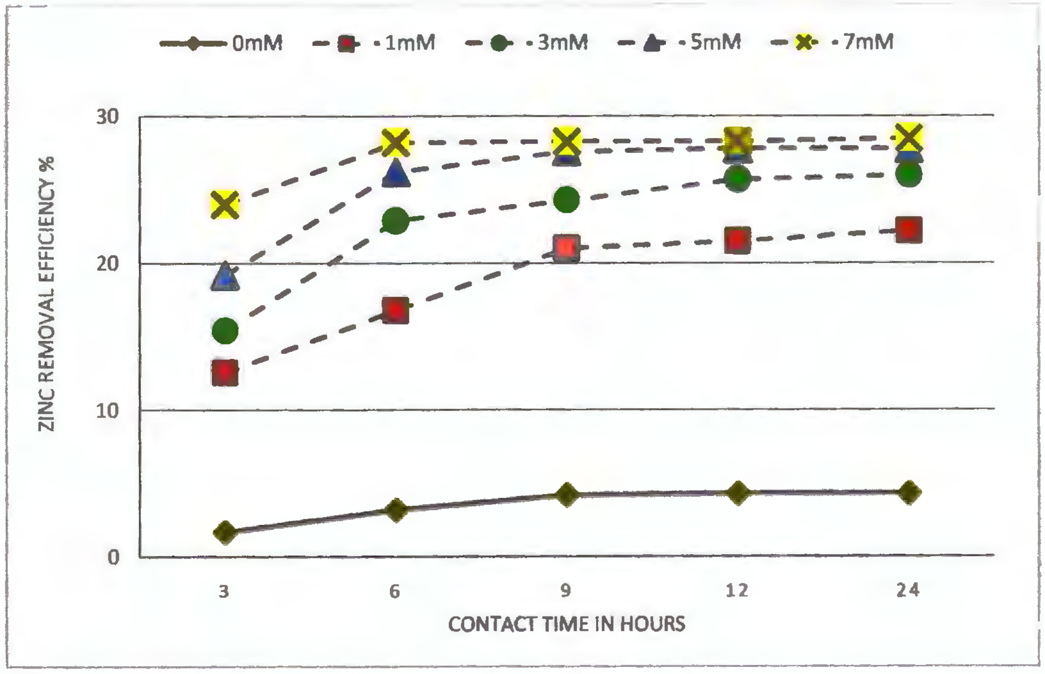


Fig 3.17: Effect of DTPA concentrations and contact time on Zn removal efficiency

3.4.2 Effect of pH and Temperature variations on DTPA removal efficiency

This experiment was designed to see the changes in removal efficiency by selecting optimum concentration and contact time from above experiment. The above kinetic experiment suggested that DTPA has optimum removal efficiency with 7mM solution after 24 hours of contact time. The pH and temperature experiment was performed with 7 mM of DTPA in 1: 10 ratio shacked for 24 hours. The DTPA 7 mM solution was adjusted to pH 4, 7 and 10. Each selected pH was subjected to temperature variations ranged from 25 ^{0}C, 30 ^{0}C and 35 ^{0}C in a shaking water bath. The control in the experiments were soil: chelator solution but without any pH adjustment. All experimental procedures were same as kinetic experiment. The 7 mM of DTPA had pH ≈.11.6

The variations in pH of DTPA 7 mM at 25^{0}C is shown in **Fig 3.18.** The percentage removal efficiency of heavy metals decreases with change in pH as compared to control in the experiment. DTPA pH 7 solution seemed to be good chelator for Cd and Pb. The highest removal efficiency of Cd was recorded at 40.2 % with control solution, followed by pH 7 solution at 34.0 %. pH 4 solution removed 30.7 % while pH 10 solution had lowest removal efficiency at 23.7 %. In fact, pH 10 solution of DTPA performed low for all the metals in this study. The maximum removal efficiency of Pb 32.2 % was also achieved with control solution and pH 7 solution had removal efficiency 30.2 %. The removal efficiency of Pb with pH 4 solution was 28.4 % and the lowest removal efficiency of Pb was 22.8 % at pH 10.

In this experiment Cr showed highest removal efficiency 16.7 % with pH 7 solution, while minimum removal efficiency was 15.5 % with pH 10. However, the control solution removed 16.5 % of Cr and pH 4 removal efficiency was 15.8 %. The pH variations did not significant effect on the removal efficiency of Cr. The removal efficiency of Zn with pH variations at 25 ^{0}C showed that pH 4 had better removal efficiency with 17. 4 % but it showed marked decrease in removal efficiency when compared to the control. The highest removal efficiency was 28.0 % with control and the lowest removal efficiency was recorded 13.2 % at pH 10.The removal efficiency of Zn with pH 7 was 15.7 %.

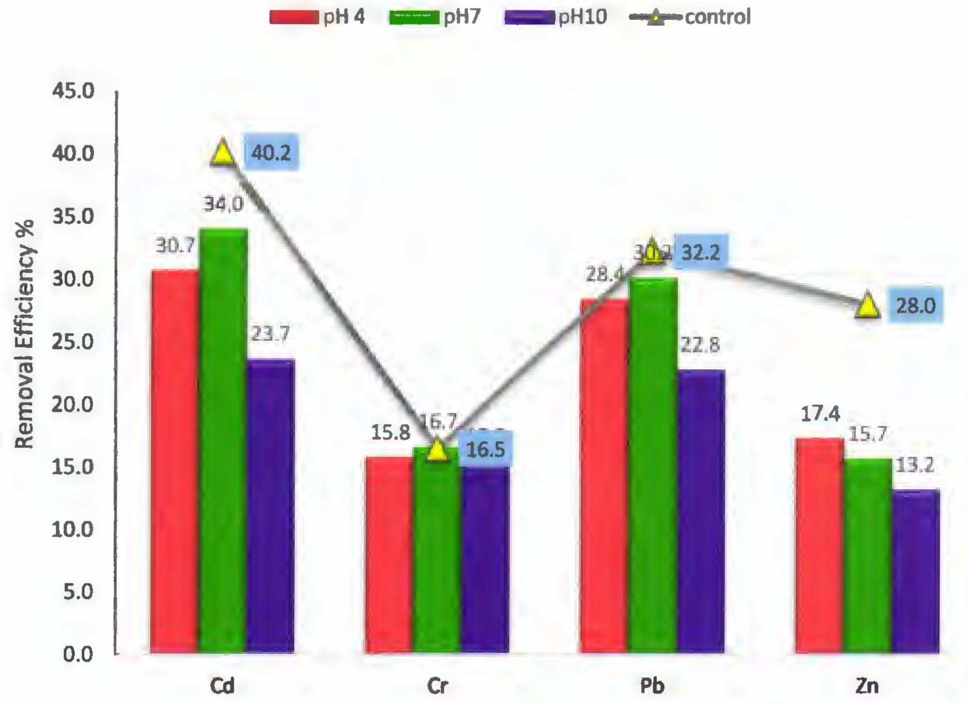


Fig 3.18: Removal efficiency of 7 mM DTPA at 25 ^{0}C with pH variations

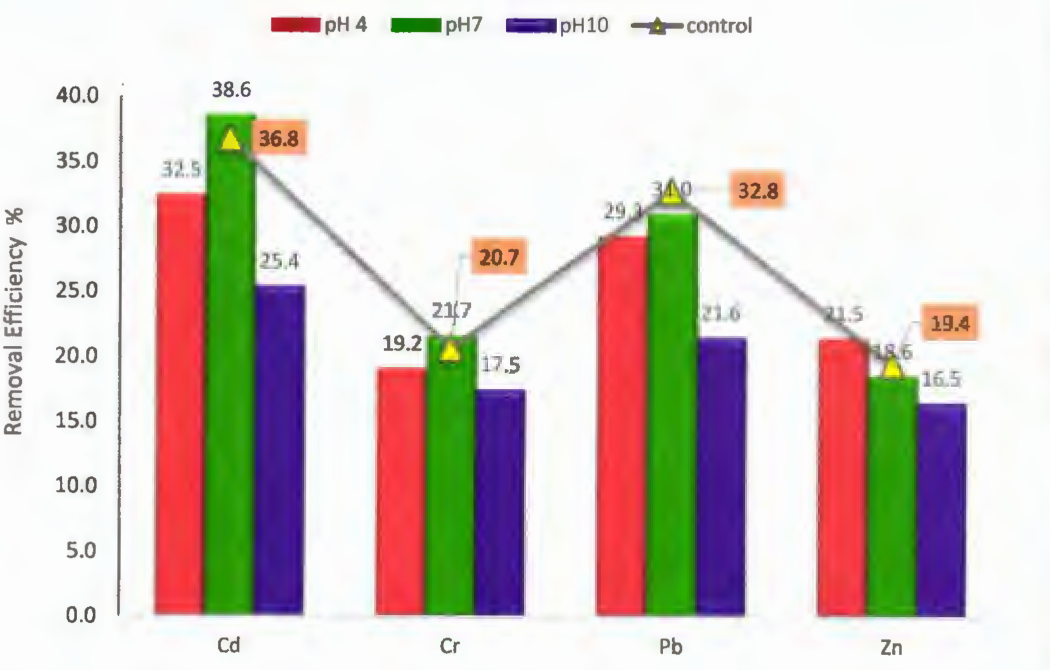


Fig 3.19: Removal efficiency of 7 mM DTPA at 30⁰C with pH variations

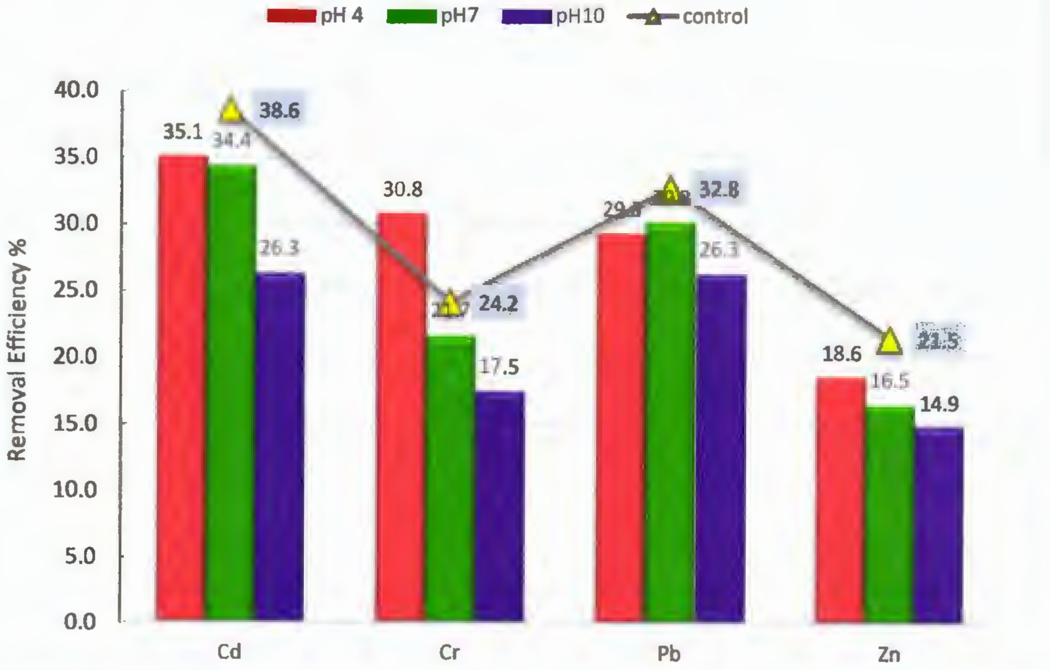


Fig 3.20: Removal efficiency of 7mM DTPA at 35 ⁰C with pH variations

Effect of variation in pH at 30 ^{0}C on the removal efficiency of cadmium, chromium, lead and zinc is shown in **Fig 3.19.** The maximum removal efficiency of Cd was recorded at pH 7 with 38 %. While the control solution had removal efficacy slightly lower at 36.8%. The removal efficiency of pH 4 solution was 32.5 % and lowest efficiency was recorded for pH 10 at 25.4 %.the highest removal efficiency for Cr was 21.7 % at pH 7. The removal efficiency of control solution was little lower at 20.7%.The highest removal efficiency of Pb was recorded at 32.8 % with control solution followed by 31 % with pH 7 solution .The pH4 solution removed 29.3 % of Pb at 30 ^{0}C. The lowest removal efficiency for Pb and Zn was also recorded at pH 10 removing 21.6 % and 16.5 %, respectively. Zinc showed better removal efficiency with pH 4 solution removing 21.5 %.The control solution removed 19.4 % of Zn followed by 18.6 % at pH 7.

The removal efficiency of DTPA at 35 ^{0}C with pH variations is shown in **Fig 3.20.** The control solution of DTPA at 35 ^{0}C removed 38.6 % of Cd which was lower than removal efficiency at 25 ^{0}C. Removal of Cr and Pb by control solution was highest at 24.2 % and 33.3 % respectively as compared to 30^{0}C. However, Zn showed no change from 30 ^{0}C to 35 ^{0}C in removal efficiency with control solution it remained at 19.4 %. The removal efficiency of Cd at pH 4 was recorded at 30.5 % and pH 7 had 35.1 % which was higher than both pH 4 and pH 10 solution. The highest removal efficiency for Cr was recorded 27.8 % at pH4. The lowest efficiency for Cr was recorded at 35^{0}C was 16.7 % at pH10. Removal efficiency of Pb was slightly increased with control solution. It did not showed any change from 30 ^{0}C to 35 ^{0}C and remained at 29.3% at pH 4. The pH 7 solution of DTPA removed 31.9 % of Pb and lowest efficiency was recorded at pH 10 with 24.0%. Zn decreases from 21.5 % at 30^{0}C to 20.5 % at 35 ^{0}C with pH 4 solution. Similarly, pH 7 also showed a slight decrease in efficiency removing 18.2 % and lowest removal efficiency of Zn was recorded at 14.9% with pH 10.

Overall, pH 7 solution of DTPA showed no change in removal efficiency for Cd and Pb with increasing temperature while pH 4 and pH 10 removal efficiency changed with increasing temperature for Cd and Pb. The change in removal efficiency of Cr with pH and temperature variation was noticeable at 35 ^{0}C with pH 4 solution of DTPA. The removal efficiency of Cr with pH 7 increased between 25^{0}C to 30^{0}C from 16.7 % to 21.7 %. It showed no change at 35 ^{0}C .The pH 4 and pH10 of DTPA solution showed dependence on temperature variation for Cr removal. Zinc was optimally extracted with pH 4 and pH 7 at 30 ^{0}C. It showed decrease in removal efficiency at 25 ^{0}C and 35^{0}C.

3.5 Heavy metals Removal Efficiency with EDTA

3.5.1 Effect of Contact Time and Concentration

The removal efficiency percentage for cadmium, chromium, lead and zinc with EDTA molar concentration of 0.01 M, 0.05 M, 0.1 M and 0.5 M was studied in a batch experiment for 3 ,6 , 9 ,12 and 24 hours. **Fig.3.21** showed the removal efficiency of heavy metals viz time and molar concentration. The figure showed that there was an increase in heavy metals mobility with increase in molar concentration of EDTA. The 0.5 M concentration had higher removal efficiency for all heavy metals. In this study distilled water (0M) was a poor extracting agent for heavy metals.

Fig.3.22 showed the removal efficiency of EDTA for cadmium. The 0.5 M concentration of EDTA removes the highest percentage of Cd from contaminated soil. The highest removal efficiency 32.5 % was observed after 12 hour. The lowest removal efficiency was observed for 0.01 M removing 12.1 % of Cd after 3 hours of contact time. The removal efficiency percentage by 0.01 M reached equilibrium after 9 hours removing 18.1 % Cd. The 0.05 M of EDTA showed sharp increase in removal efficiency from 3 to 6 hours removing 24.4 % of Cd, it slowly increased to 25 % after 24 hours of contact time. The 0.1 M of EDTA showed the similar extraction behavior, removing 21.6 % after 3 hours which increased rapidly to 27.3 % after 6 hours. The further increase in time removed 28.2 % Cd after 24 hours. The 0.5 M showed good removal efficiency with 32.5 % of Cd removal after 24 hours.

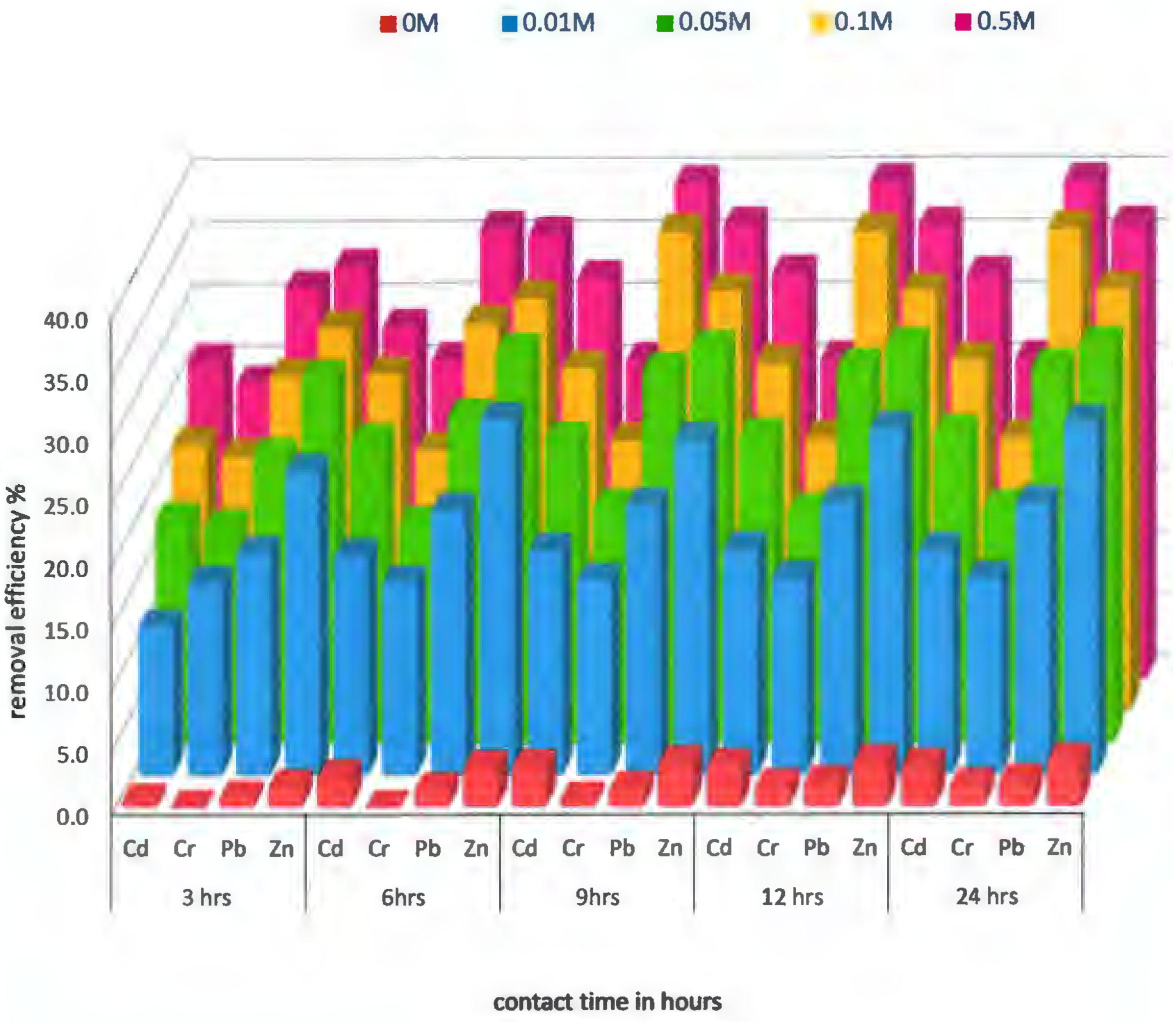


Fig 3.21: EDTA removal efficiency with different concentration and contact time for Cd, Cr, Pb and Zn

Figure 3.23 showed removal of Cr by different concentration of EDTA over 24 hours contact time. The low removal of Cr was observed with 0.01 M EDTA removing 15.5 % after 3 hours, it increased to 15.6 % after 6 hours and further contact time up to 24 hours removed 15.8 % of Cr. The 0.05 M EDTA removed 17.5 % of Cr after 3 hours and slight increase at 19.1% was observed after 9 hours. The 0.1M of EDTA removed 20.6 % after 3 hours. The 6 hours of contact time removed 21.1 % Cr, further contact time showed minor effect removing 22.0 %. The 0.5 M removed 23.9 % Cr after 3 hours it increased to 25.8 % after 6 hours. The further contact time did not bring any change in removal efficiency and it remained at 25.8 % after 24 hours.

Figure 3.24 shows the removal of Pb with different concentration of EDTA at various contact time. The extraction efficiency of Pb increased with increasing the concentration of chelator. The 0.01 M EDTA removed 17.9 % of Pb after 3 hours. The contact time increased the removal efficiency removing 21.8 % after 12 hours which remained unchanged after 24 hours.. The 0.1 M of EDTA maximum removal efficiency was recorded 38.6 % of Pb after 9 hours of contact time and it remained unchanged up to 24 hours. The 0.5 M EDTA showed highest removal of Pb removing 40.3 % after 24 hours of contact time. The 0.1M and 0.5 M concentration showed initial difference in removal efficiency from 3 to 6 hours while increase in contact time resulted in a little change in removal efficiency of Pb .

Figure 3.25 showed the removal efficiency of EDTA for Zn. The lowest removal of Zn was observed with 0.01 M EDTA, while highest removal efficiency was observed with 0.5 M EDTA. The 0.01 M EDTA removed 24.5 % Zn after 3 hours. It showed increase in removal efficiency after 6 hours removing 27.7 % of Zn. The increase in contact time up to 24 hours removed 28.4 % of Zn. The 0.05 M EDTA removed 29.5 % of Zn after 3 hours and highest removal of Zn was observed after 12 hours of contact time removing 32 % Zn and remained unchanged after 24 hours. The 0.1 M EDTA removed 31 % after 3 hours which increased 33.3 % after 6 hours , further contact time after 24 hours removed only 33.9 % Zn .

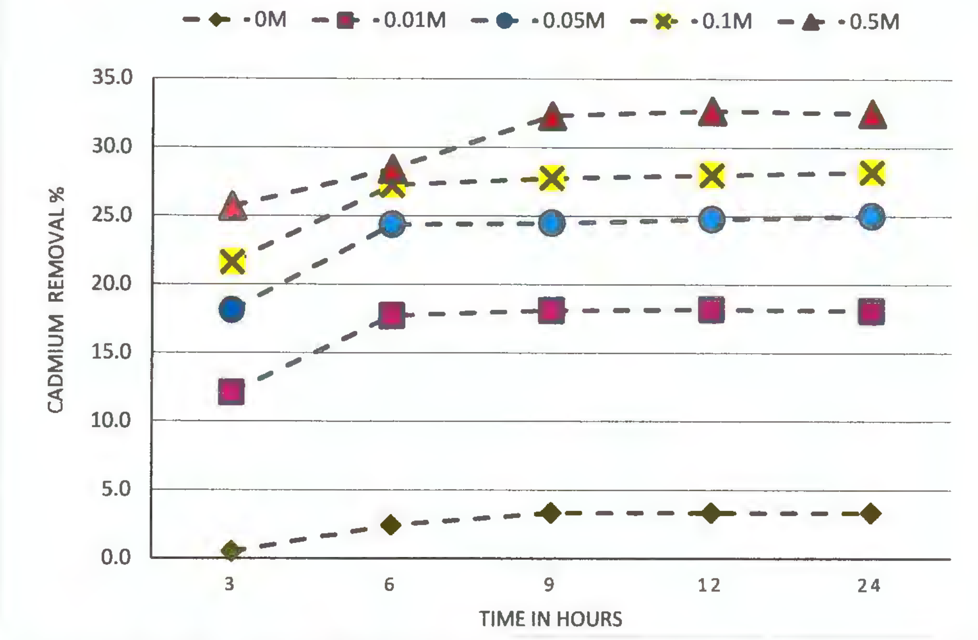


Fig 3.22: EDTA removal efficiency for Cd at different time and concentration

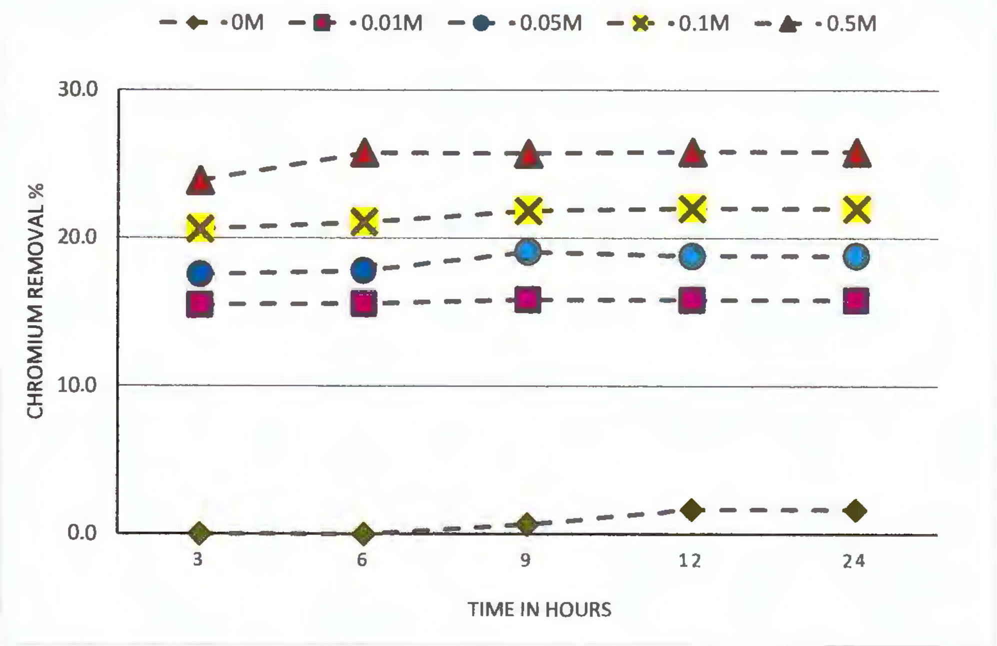


Fig 3.23: EDTA removal efficiency for Cr at different time and concentration

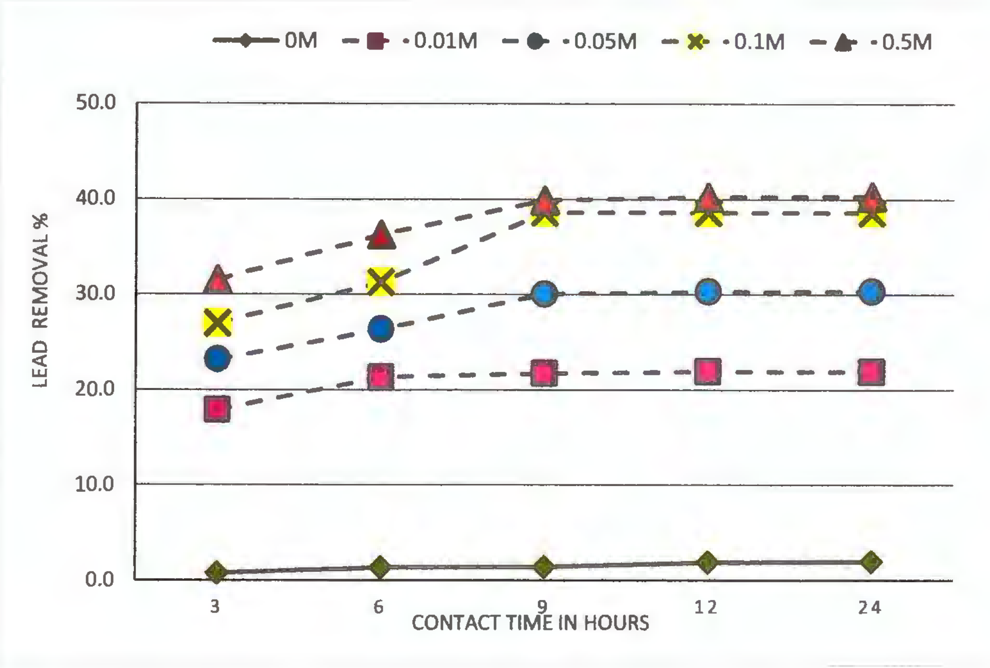


Fig: 3.24: EDTA removal efficiency for Pb at different time and concentration

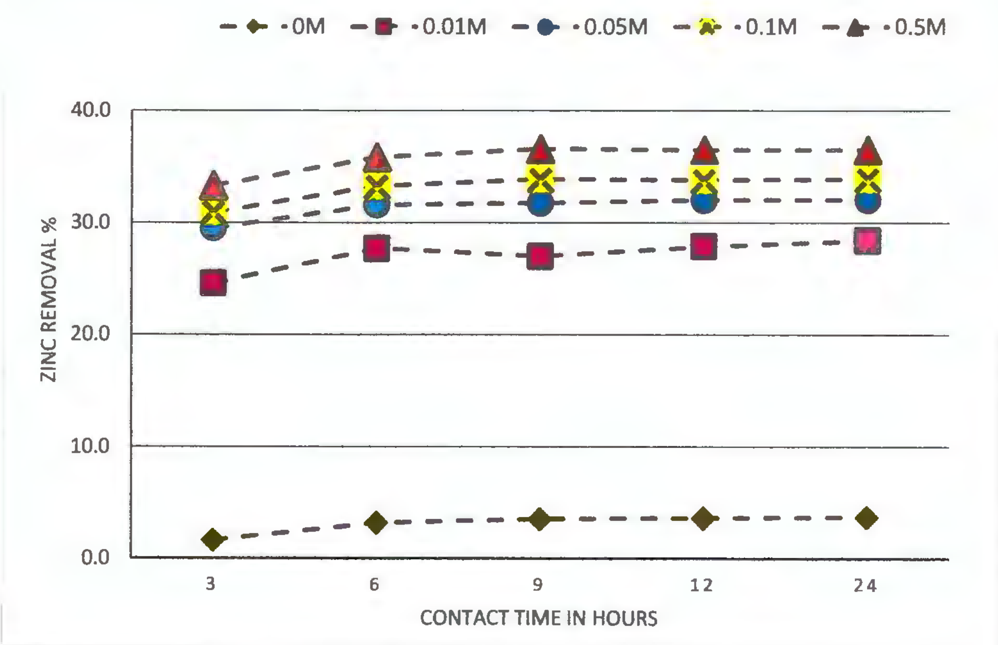


Fig: 3.25: EDTA removal efficiency for Zn at different time and concentration

The 0.5 M EDTA behaved like 0.1 M after 3 hours of washing time removing 33.3 % of Zn. The highest removal efficiency for EDTA was observed with 0.1 M removing 36.5 % Zn after 9 hours of contact time, further contact time had no effect on removal efficiency.

The heavy metals removed by different concentrations of EDTA were in increasing order Pb > Zn > Cd > Cr. Abumaizar and Smith (1999) found in a study that EDTA favored Pb extraction over Zn and Cd and had little impact on Cr removal. EDTA removed heavy metals by two mechanisms; mobilizing the complexation reactions between cationic heavy metals which targets the weak bonds between soil and heavy metals and by mobilizing the heavy metals that are bound to oxides and organic matter. Barona and Romero (1996) found that EDTA is most effective for Pb extraction from soils in which Pb is primarily partitioned in Oxide bound and organic matter bound fraction. The sequential extraction result from this study showed that Pb was mainly associated with oxide bound fraction of soil.

Kirpictchikova (2006) also observed that rapid desorption of heavy metals by EDTA is due to labile species , the second or long term removal is due to refill of labile pool from more recalcitrant species. This cause disruption in soil structure, therefore EDTA is useful in overall removal of heavy metals (Yip *et al.,* 2009). The heavy metals were rapidly mobilized in first 6 hours, the further increase in time from 9 to 24 hours caused a subtle increase in heavy metals removal efficiency. Chromium was not much effected by increase in contact time.

Tejuwulan and Hendershot (1998) observed that EDTA removal efficiency is depended on chelator concentration. The different concentration of EDTA used in the study showed that with increase of molar concentration of EDTA removal efficiency increased as well. The Cr removal efficiency varies from 15.5 % to 28.1 % with increasing molar concentration of EDTA. Zn did not showed much change in efficiency with increase in concentration of EDTA solution. The 0.1 M EDTA (31 to 33.9 %) and 0.5 M (33.3to 36.5 %) showed little increase in removal efficiency with increasing the contact time.

Khalkhalaini *et al.,* (2006) reported 54.5 % to 100 removal efficiency for Cd, Pb and Zn by using 0.1 M EDTA at low pH. The 0.1 M EDTA preferentially extracted Pb over Cd and Zn. This study however, removed 12 % to 40.3 % of Cd, Pb and Zn but removal of Pb was higher than all heavy metals.

The most common soil cation Ca and Fe compete with other heavy metals to form complexes with EDTA (Kim *et al.,* 2003). This could have resulted in low extraction efficiency of EDTA as contaminated soil was moderately calcareous. Finzgar and Lestan (2007) also reported the interference major soil cation Fe^{+2} and Ca^{+2} on the removal efficiency of Pb and Zn. The Fe - EDTA had high stability constant log K_8 = 25.1. while, Pb and Zn 18 and 16.5, respectively at 25 ^{0}C (Martell and Smith, 2003).

Papassiopi *et al.,* (1999) reported the reduced efficiency of EDTA for the removal of Zn, Pb and Cd from calcareous soils. The simultaneous dissolution of calcite consumed 90 % of available EDTA. The alkaline pH of soil and calcium carbonate reduce the removal efficiency of heavy metals (Bermond *et al.,* 2004; Palma and Ferrantelli, 2005). This could have resulted in low extraction efficiency of EDTA as contaminated soil was moderately alkaline and calcareous.

3.5.2: Effect of pH and Temperature Variations on EDTA removal efficiency

The result from above kinetic experiment gave the optimum concentration and time of EDTA efficiency for the maximum removal of heavy metals. In this experiment, the EDTA concentration of 0.5 M was subjected to pH 4, 7 and 10. The solution was shacked for 24 hours in a water bath at temperature 25 ^{0}C, 30 ^{0}C and 35 ^{0}C. The solution was then filtered and analyzed for heavy metal removal efficiency.

Figure **3.26** showed the heavy metals removal efficiency with pH variations at 25 ^{0}C. The removal efficiency of heavy metals were decreased as pH increased. The control was 0.5 M solution of EDTA without pH adjustment. The 0.5 M EDTA had pH value 5.97. It was observed that control solution had slightly lower extraction efficiency than pH 4 solution for Cr and Pb , but Zn and Cd were better extracted with control solution. The more alkaline pH, in this case pH 10, had a very low extraction efficiency because at higher pH hydrolysis is favored over complexation by EDTA. The low pH solution favors heavy metals mobilization because low pH promote oxide dissolution (Peters, 1999).

Cadmium showed highest removal efficiency with control solution removing 31.9 %, while pH 4 solution removing 27.1 %. pH 7 removed 221.2 % of Cd, while pH 10 showed lowest Cd removal with 17.8 % . Cr showed highest removal efficiency with pH 4 removing 25.3 % which was slightly greater than control solution removing 24 %. The pH 7 solution of EDTA removed 19.6 of Cr and pH 10 was poor extractant removing 4.8 %. Pb showed good removal efficiency with pH 4 removing 36.3 %. The control solution removed 33.4 % of Pb . The pH 7 removed 29.7 %. While, pH 10 removed 10.4% of Pb. Zinc removal efficiency was better with both control solution (34.8 %) and pH 4 solution of EDTA (36.3 %). The removal efficiency was dropped with using pH 7 which removed 24.1 % Zn and pH 10 removed 15.9 % Zn.

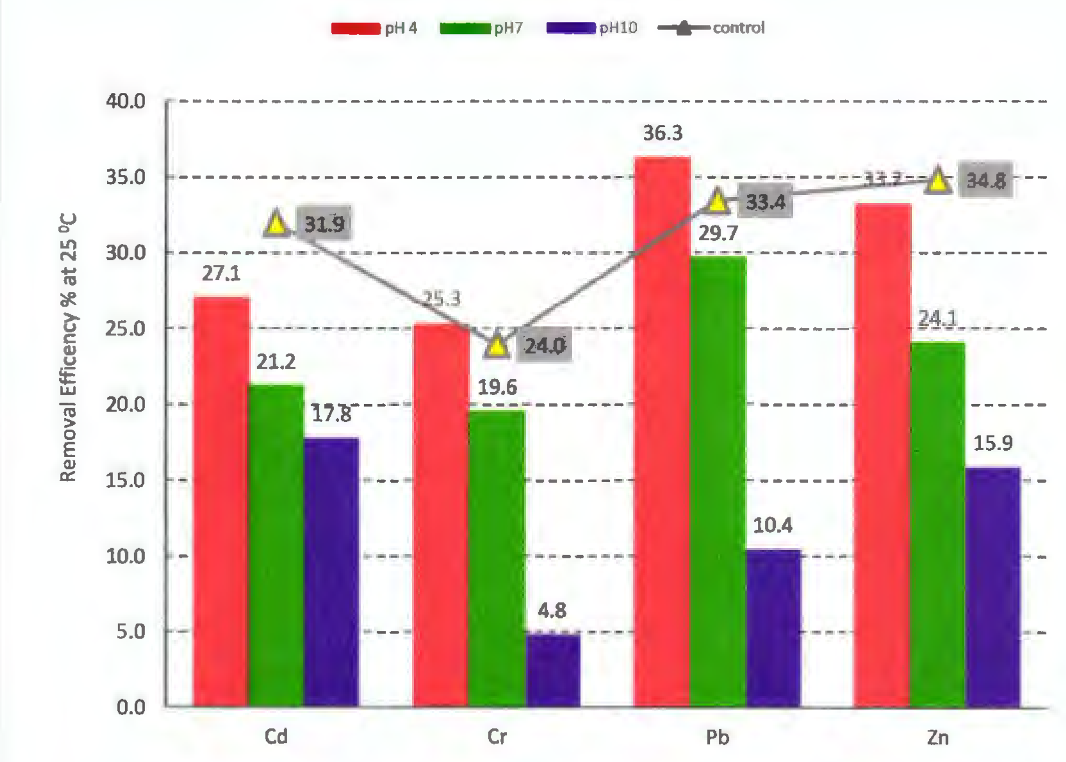


Fig 3.26: Effect of EDTA pH solution on heavy metals removal efficiency at 25 ⁰C

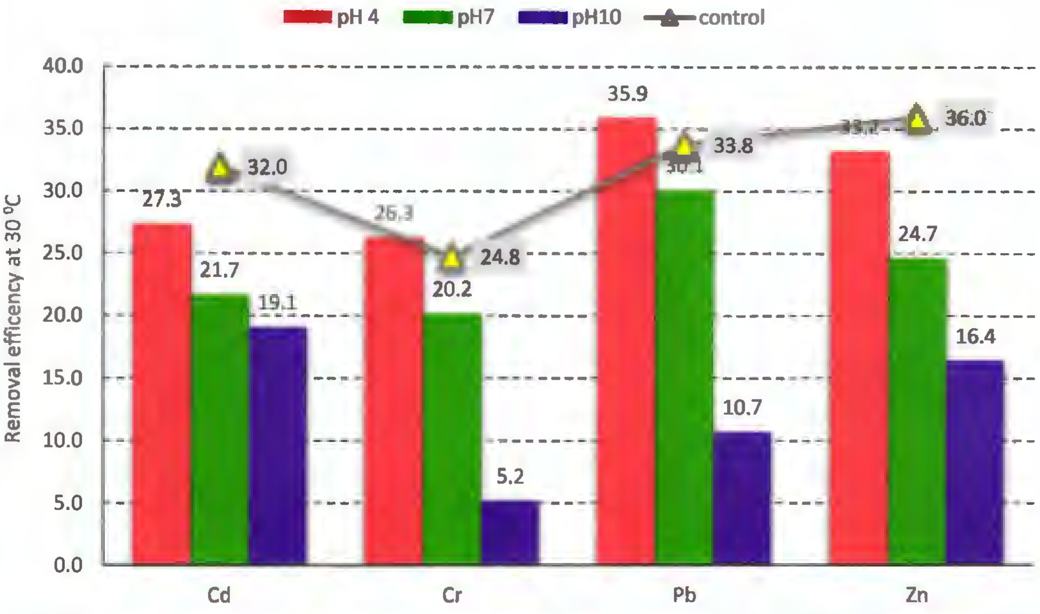


Fig 3.27: Effect of EDTA pH solution on heavy metals removal efficiency at 30 °C

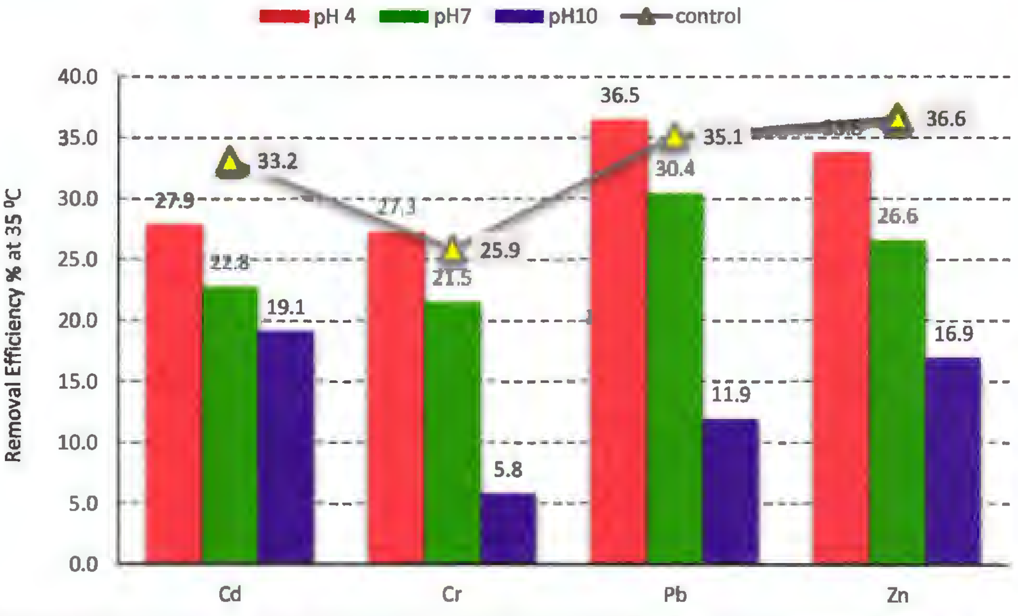


Fig 3.28: Effect of EDTA pH solution on heavy metals removal efficiency at 35 °C

The **Fig 3.27** shows the removal efficiency of EDTA at 30 ^{0}C with pH variations. It was observed that increasing temperature slightly enhanced the removal efficiency of heavy metals. The Cd removal efficiency for control solution was not changed and remained at 32 %. While, pH 4, pH 7 and pH 10 solution showed increase in removal efficiency. The pH 4 solution removed 27.3 % of Cd, pH 7 removed 21.7 % and pH 10 showed significant increase from 17.8 to 19.1 % removal with increase in temperature. The control solution removed 24.8 % Cr, pH 4 showed increase from 25.3 % to 26.3 % in removal efficiency. The removal efficiency for Pb was slightly lowered from 36.3 % to 35.9 % at 35 ^{0}C. Zinc with increasing temperature showed little improvement of removal efficiency. The control solution was by temperature change and removed 36 % Zn. pH 4 did not show change in removal efficiency and removed 33.2 % and pH 7 removed 24.7 % of Zn. However, pH 10 showed increase from 15.9 % to 16.4 % removal efficiency of Zn.

Fig 3.28 shows the removal efficiency of EDTA pH solution at 35 ^{0}C. The increase in temperature increase the removal efficiency of EDTA. The control solution also showed increase in removal efficiency removing 33.2 % Cd, 27 % Cr, 35.1 % Pb and 36 .6 % Zn. The highest removal efficiency for Cd with pH variations was observed with pH 4 removing 27.9%, Cr showed much improvement with 27.3 %, Pb at 36.5 % and Zn at 33.8 %. The pH 7 solution of EDTA at 35 ^{0}C showed increase for Cd (22.8 %), Cr (21.5 %) and Zn from 24.7 % to 26.6 %. Pb showed minor change from 30.1 % to 30.4 %. pH 10 solution did not showed increase in efficiency for Cd at increased temperature and remained at 19.1% and Pb 11.9 % and Zn removal was 16.9 %. Cr removal was 5.8 % at pH 10.

Ya and Klarup (1994) reported their observation on EDTA pH and concentration effect on heavy metals solubilisation. The lower the pH and higher the concentration of EDTA, greater would be extraction efficiency of heavy metals. Lim *et al.,* (2004) observed that EDTA extraction efficiency for Cd and Pb increased at pH 4.7 as compared to high pH extractions. Palma *et al.,* (2012) studied the Cr removal from polluted soil by EDTA 0.1 M adjusted to pH 5, 7 and 9. Cr removal was 25 % at pH 5 and reduced with increasing pH.

Similar observation were observed in this study with 0.5 M EDTA. It showed increase in removal efficiency for Cr with increasing temperature with pH4. Sheaffer *et al.,* (1980) reported that organic acids from soil increased the heavy metals mobility at high temperatures. The increased temperature promotes degradation of organic matter which increase organic acids in soil. The contaminated soil in this study had very low organic matter, but Cr percentage was more in organic bound fraction of soil than other heavy metals. This factor caused more Cr to be released with increasing temperature but Cd, Pb and Zn showed minor increase with temperature variations.

The study evaluated that the removal efficiency of Cd, Cr, Pb and Zn varied with the chelator used and its different concentration. DTPA showed highest removal of Cd (40.2 %) as compared to EDTA and Citric acid. The Cr (37.1 %) and Zn (38.7 %) showed better removal efficiency with citric acid. EDTA showed good extractability with Pb (40.3 %) **Fig 3.29).** Citric acid is comparatively a weaker chelating agent than EDTA and it has strong affinity towards Ca as compared to Pb, this factor could result in low extraction of Pb (Kirpichtchikova *et al.,* 2006).

Heavy metal removal efficiency depend on soil characteristics and heavy metal fractionation (Dermont *et al.,* 2008). EDTA is most widely studied chelator and considered to be most effective chelator for heavy metals bound to exchangeable, carbonate and organically bound heavy metals while it is in effective for oxide bound and heavy metals in reducible fraction (Abumaizer and Smith 1999; Wasay *et al.,* 2001; Elless and Blaylock 2000; Tandy *et al.,* 2004). Wasay *et al.,* (1998) observed that soils that contain low concentrations of heavy metals are hardest to remediate because the heavy metals preferentially adsorbed by the highest energy binding sites. Such soils would require very high concentration of chelators to remove heavy metals. This inference could be applied to present study, because heavy metals showed affinity for solid phase of soil. The sequential extraction data clearly demonstrate that cadmium, chromium and zinc were mainly partitioned in reducible fraction, while lead was dominant in oxide bound fraction **(Fig.3.4)**.

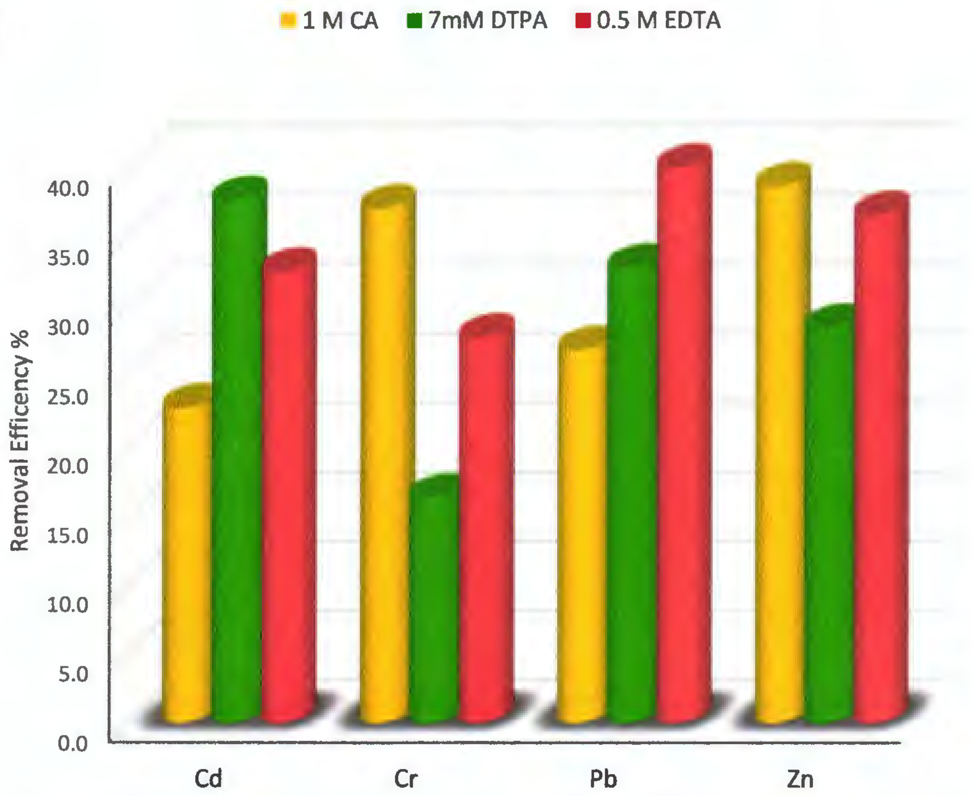


Fig. 3.29: Maximum removal efficiency percentage of EDTA, DTPA and Citric acid for Cd, Cr, Pb and Z

Citric acid had low extraction efficiency for Cd at higher concentration due to calcium citrate formation (Heideri *et al.*, 2015). The present study used high concentration of citric acid 0.1, 0.5, 1, 1.5 M because the soil was moderately alkaline. The heavy metal removal from alkaline soil required the lowering of pH of soil considerably. The 1.5 M citric acid did not showed good removal efficiency. DTPA is extensively used to check the bioavailability of heavy metals in soil for plant uptake (Guan *et al.*, 2011). It extracts both the carbonate bound and organic fraction for plant uptake (Walter *et al.,* 2006). DTPA was successful in mobilizing heavy metals in this study.

According to a study by Kovacevic *et al.,* (2002) EDTA had good affinity for acidic soils but poor affinity for neutral and alkaline soils. On the other hand, DTPA had good affinity for neutral and alkaline soils but poor affinity for acidic soils (Feng *et al.,* 2005). DTPA was successful in removing highest percentage of Cd from soil than EDTA and citric acid. This study showed that the chelator's concentration, contact time, pH, temperature and most importantly soil physical chemical characteristics determine the extent of heavy metals mobility from contaminated soil. The removal efficiency for cadmium was DTPA > EDTA > Citric acid, for Cr and Zn it was Citric acid > EDTA > DTPA. While for Pb it was EDTA > DTPA > Citric acid.

3.6 Conclusions

This study was focused on evaluating the removal efficiency percentage of different chelators EDTA, DTPA and citric acid for cadmium, chromium, lead and zinc. The removal efficiency of chelators were optimized at different parameters such as chelator concentration, contact time, temperature and pH. The present work gives us the following results:

- The contaminated soil had silty clay texture with alkaline pH and moderate carbonate content. The sequential extraction data revealed that all added heavy metals were bound to residual fraction mostly. The bioavailable portion of heavy metals were quite low. The order of heavy metals (Cd, Cr, Zn) in five fractions follows Residual > Fe/Mn oxide bound > carbonate bound > exchangeable > organic matter bound except for Pb which follows oxide bound > residual > carbonate > organic fraction > exchangeable fraction.

- The removal efficiency of EDTA and DTPA increased with increasing concentration while for Citric acid the higher concentration did not improved the removal efficiency of heavy metals. Distilled water was ineffective for heavy metals removal from contaminated soil. The maximum removal efficiency with EDTA was observed with 0.5 M, for DTPA it was 7mM and citric acid performed better with 1 M solution.

- The maximum removal efficiency for Cd was 37.7 % with DTPA 7mM solution. The maximum removal efficiency for Cr and Zn was observed with Citric acid. Cr removal was 37% with 1 M solution while Zn removal was 38.7 % after 9 hours. The maximum removal of Pb 40.3 % was observed with EDTA 0.5 M solution after 24 hours.

- The heavy metals and different chelators concentration used in this study behaved differently with increasing contact time. The higher concentration of DTPA 5mM and 7mM removed heavy metals over 24 hours while 1 mM and 3 mM reached equilibrium at 6 to 9 hours.

- EDTA showed rapid change in removal efficiency for Cd in first 6 hours, the further time slowly improved efficiency till 12 hours, while Cr was not much effected by shaking time. For Pb and Zn , EDTA required 24 hours for maximum removal of heavy metals .Citric acid's all applied concentration showed rapid extraction of heavy metals within 9 hours.

- The removal efficiency of heavy metals varied with increase in pH and temperature for EDTA, DTPA and citric acid. The result showed that control solution of DTPA was a better chelator than DTPA 7mM pH 4, 7 and 10. The increase in temperature improved the removal efficiency for Cr from 25 ^{0}C to 35 ^{0}C, but for other heavy metals temperature increase from 30^{0}C to 35^{0}C did not bring any change in removal efficiency.

- EDTA removal efficiency decreased with increasing the pH from 4 to pH 10, but increased with increasing temperature. The pH 4 and control solution had better removal efficiency as compared to pH 7 and pH 10. The 35 ^{0}C slightly increased the removal efficiency of heavy metals, maximum Cd and Zn removal efficiency was 33.2 % and 36.6 % respectively, with control solution. Cr and Pb were had maximum removal efficiency with pH 4.

- Citric acid showed decreased removal efficiency with increasing pH and temperature. The highest Pb (26.3%) was achieved with control solution of Citric acid without any pH alterations. The maximum removal efficiency for Cd was 24.9 % with pH 2 at 25 ^{0}C. It decreased with increasing temperature to 19.1 %. Cr was maximally removed with pH 2 (41.9 %) at 30 ^{0}C. Similar behavior for Zn (35.7%) was observed which showed increased removal efficiency with pH 2 at 35 ^{0}C.

Future Considerations

- For future study, the washed soil should be analyzed for sequential distribution of heavy metals. This would provide an insight into chelator removal efficiency from various soil fractions.

- The chelators efficiency should be evaluated with naturally polluted soil to determine the effectiveness and limitations of soil washing technique.

- The evaluation of soil major cations and their influence on extraction efficiency of cheating agents is important.

- The different types of soil physical characteristics should be explored for heavy metal remediation with chelating agents. The coarse textured soil have high desorption capacity to release heavy metals as compared to fine textured soils. The silty clay soil should be explored for landfill lining material. Most of the heavy metals for this soil showed high affinity for soil inert fraction which is resistant to heavy metals leaching even at high chelators concentration.

- Soil washing with chelating agents should employ methods to reuse and degrade the used chelator solution for environmental safety and economic purposes.

CHAPTER 04

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