REMEDIATION OF METAL CONTAMINATED SOILS IN BANGLADESH BY CHEMICAL TECHNOLOGY

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(Registration No. 23/2012-2013)

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DEPARTMENT OF SOIL, WATER AND ENVIRONMENT
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Dedicated
to
My Respected Parents,
Beloved Wife
and
Beautiful Daughter Manha Sharmeen

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A DISSERTATION

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Declaration

I do hereby declare that the submitted thesis titled "**Remediation of Metal Contaminated Soils in Bangladesh by Chemical Technology**" has been composed by me and all the works presented herein are of my own experimental findings. I further declare that this work has not been submitted anywhere for any academic degree, prize or scholarship and not published anywhere.

Date: **(Mohammad Moniruzzaaman)**

(Registration No. 23/2012-2013)

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Abstract

To remediate metal contaminated soils by chemical technologies soil samples were collected from six metal contaminated industrial areas. The Pb concentrations were high (245 and 164 mg kg^{-1}) in soils of battery industries of Madhupur tract (acidic soil) and Ishwardi (calcareous soil), respectively. Soil of tannery industrial area contained high amount of chromium (256 mg kg^{-1}). The saline soil of ship breaking yard, Shitakunda had high amounts of Pb, Cr, Ni, Cu and Zn of 93, 71, 69, 192, 845 mg kg^{-1}, respectively. Other two soil samples were collected from cable industry of Kushtia (calcareous soil) and Chittagong (slightly saline), which contained 196 and 151 mg kg^{-1} of Cu, respectively. The mobility indexes of metals were found high (above 40%) in calcareous soils.

Different synthetic extractants (NH_4OAC, $CaCl_2$, HCl, EDTA, DTPA and distilled water) were applied to spiked soils for removal of metal. Among the metals the highest amount of Cd and lowest amount of Cr were removed from soils by these extractants. The metal removal efficiencies of extractants were found higher in light textured soils than that of heavy textured soils. Among the extractants distilled water removed the lowest amount of metals from all soils. Between the salt solutions, buffered NH_4OAc extracted comparatively higher amounts of metals than that of $CaCl_2$. The extractants 0.1 M HCl extracted significantly high amount of metals, but the extraction markedly reduced soil pH and also leached a high amount of plant essential elements like Ca, Mg, Fe and Mn. Moreover HCl could not extract much metal from calcareous soils. The chelating agents EDTA and DTPA extracted considerable amounts of metals from all types of soils. These extractants effectively lowered the mobility indexes of metals below 10% in acidic and saline soils and 8 to 29% in calcareous soil. Among the chelating agents, EDTA removed the highest amount of metals from soils, but its use is also problematic one if persists in the environment.

Soluble humic substances (HS) might be used instead of synthetic chemicals. Humic substances act as chelating agents for metal ion extraction which are also less expensive, environment friendly, less destructive to soil structure and improve soil properties. Different concentrations of humic and fulvic acids (0.1 to 0.5%) were used for removal of metal from soils. Fulvic acid extracted comparatively higher amount of metals from heavy textured soils of Madhupur, Ishwardi and Kushtia due to its lower molecular structure than that of humic acid. In contrast humic acid leached somewhat higher amount of metals from light textured soil of Hazaribagh and Shitakunda due to having more contact time with soil particles.

Humic substances (HS) reduced metal mobility in soils but the values were comparatively higher than that of EDTA, because HS extracted comparatively lower amounts of metals from soils (especially from calcareous soil) compared to EDTA. The extraction capacities of humic substances were significantly increased when combined with small amount of EDTA. Combined HS + EDTA extractants reduced the mobility indexes which were also lower than that of individual HS and EDTA treatments. In heavy textured soils of Madhupur tract and in calcareous soils 0.4 % fulvic acid in combination with 0.04 M EDTA extracted similar amounts of metals as was done by only 0.1 M EDTA solution, whereas humic acid required 0.06 M EDTA. Both humic and fulvic acids required lower amounts of EDTA (0.04 M) to remove metals from light textured soils of Hazaribagh and Shitakunda as was done by EDTA alone.

Pot experiments were conducted with red amaranth and rice plants in six different contaminated soils to study the mobility and bioavailability of metals from soil to plant after reclamation with different extractants. Each contaminated soil was treated with only HA, FA, EDTA and also with combined HA + EDTA and FA + EDTA extractants. The contaminated soils treated with these extractants showed reduced metals toxicity and created positive impacts on the growth and yields of plants. In Pb contaminated Madhupur tract soil only 0.4 % FA extractants created almost similar effects on the minimum content of Pb in plants as were obtained with combined FA + EDTA treatment. Soils treated with 0.4 % FA + 0.04 M EDTA extractant yielded the lowest Pb and Cu concentrations in plant materials grown in contaminated calcareous soils of Ishwardi and Kushtia, respectively. The contaminated soil of Hazaribagh treated with only 0.4 % HA and 0.4 % FA was enough to reduce the Cr concentration in both plant materials. In contaminated soil of ship breaking yard the combined application of 0.4 % HA + 0.04 M EDTA and 0.4 % FA + 0.04 M EDTA extractants were equally effective in reducing high concentrations of metals in soil and plant materials. The Cu contaminated soil of Chittagong treated with only 0.4 % HA and 0.4 % FA was enough to reduce Cu concentration in plants. In all pot experiments, soils treated with only 0.1 M EDTA extractants increased metals concentration in labile fraction due to further dissolution of metals from non-labile fraction with time, which enhanced metal uptake by plants. But residual humic substances in soil reduced the mobility of metals by forming immobile HS-metal complex which inhibited metal accumulation in plant.

LIST OF ABBREVIATIONS

AAS	:	Atomic Absorption Spectroscopy
AEZ	:	Agro-ecological Zone
AOAC	:	Association of Analytical Communities
APHA	:	American Public Health Association
ANOVA	:	Analysis of Variance
BAB	:	Bangladesh Accreditation Board
BADC	:	Bangladesh Agricultural Development Corporation
BARC	:	Bangladesh Agricultural Research Council
BCSIR	:	Bangladesh Council of Scientific and Industrial Research
BRRI	:	Bangladesh Rice Research Institute
CC	:	Cable Industry, Chittagong
$CaCl_2$	:	Calcium Chloride
CEC	:	Cation Exchange Capacity
CSTPA	:	Council on Soil Testing and Plant Analysis
DMRT	:	Duncan’s Multiple Range Test
DTPA	:	Diethylenetriamine pentaacetic acid
DoE	:	Department of Environment
EC	:	Electrical Conductivity
EDTA	:	Ethylene diamine tetra acetic acid
EEA	:	European Environment Agency
EPZ	:	Export Processing Zone
FAO	:	Food and Agricultural Organization
FA	:	Fulvic Acid
FTIR	:	Fourier Transform Infrared Spectroscopy
GWRTAC	:	Groundwater Remediation Technologies Analysis Center
GIS	:	Geographical Information System
GPS	:	Global Positioning System
HS	:	Humic Substances
HCl	:	Hydrochloric Acid
HA	:	Humic Acid
HMs		Heavy metals
HT	:	Tannery Industrial area, Hazaribagh, Dhaka

LIST OF ABBREVIATIONS

IHSS : International Humic Substances Society

IUSS : Integrated Underwater Surveillance System

IB : Battery Industry, Ishurdi

KC : Cable and Metal Industries, Kushtia

MB : Battery Industry, Madhupur Tract, Gazipur

MEFQ : Quebec Ministry of Environment and Wild Life

MI : Mobility Index

MOP : Muriate of Potash

NSC : National Safety Council

NMR : Nuclear Magnetic Resonance

NH_4OAC : Ammonium Acetate

QC/QA : Quality Control and Quality Assurance

TSP : Triple Super Phosphate

SOC : Soluble Organic Carbon

SOM : Soluble Organic Matter

SRDI : Soil Resource Development Institute

USDA : United States Department of Agriculture

USEPA : United States Environmental Protection Agency

WHO : World Health Organization

SSy : Ship breaking yard, Sonaichhari, Shitakunda

SSSA : Soil Science Society of America

m : Minute

s : Second

h : Hour

1N : 1 Normal

MW : Molecular weight

g : Gram

mg : Milligram

kg : Kilogram

ng : Nanogram

µ : Micron

µm : Micrometer

LIST OF ABBREVIATIONS

µM : Micro molar

µl : Micro liter

µg : Microgram

µS cm^{-1} : Microsiemens Per Centimeter

% : Percentage

µM : Micro molar

µl : Micro liter

°C : Degree centigrade

i.e. : id est (that is)

et al. : et alii (and others)

viz. : Videlicet, Namely

No. : Number

Fig(s). : Figure(s)

Vol. : Volume

W/V : Weight/Volume

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1. INTRODUCTION

Soil is characterized as a complex and dynamic system. It is an integral part of the eco-system and essential to all life on Earth. Besides contributing to the maintenance of all forms of life that occur in the terrestrial surface, soil plays an importapnt role in protecting the groundwater acting as a collector filter of organic and inorganic residues, helping in sequestering possible toxic compounds (Sousa *et al.*, 2008).

Heavy metals are natural constituents of the earth crust. These metals at trace levels present in natural water, dusts, soils and sediments, play an important role in human life (Isaac *et al.*, 2004). But high metal concentration can reduce soil fertility, which leads to accumulate toxic metals in food stuffs, and ultimately can endanger human health. Heavy metals and trace elements are also a matter of concern due to their non-biodegradable nature and long biological half-lives (Denti *et al.*, 1998; Sandaa *et al.*, 1999; Arantzazu *et al.*, 2000; Krzyztof *et al.*, 2004).

Generally, heavy metals are persistent, and undegradable unlike other organic pollutants (Islam *et al.*, 2012). Normally, soil works as a natural adsorbent which immobilizes heavy metals and decreases their bioavailability through different mechanisms (precipitation, adsorption process and redox reactions), but when the concentrations of heavy metals exceed the soil capacity, the heavy metals become mobilized, resulting in serious contamination of agricultural products or ground water. Contamination of the soil by heavy metals has a great impact on the soil parameters, and it may negatively affect the soil characteristics and limit their productive and environmental functions (Alghanmia, 2015). Furthermore, the presence of heavy metals in the soil may lead to a great change in the physiological and biochemical processes in plants, which causes growth reduction, and accordingly reduces crop yield (Chibuike and Obiora,2014).

Heavy metal pollution of soils has become one of the most important environmental problems in developed as well as developing countries. Soils also receive large quantities of heavy metals from a variety of industrial wastes and municipal sewage sludge. In most cases, soil polluted by heavy metals such as Cd, Zn, Cr, Pb, Cu, As and Hg is disposed in a secured landfill. But those systems are always accompanied by a risk of secondary pollution (Asami, 1984).

Now-a-days, contamination of soils with heavy metals is a widespread and serious problem all over the world for human health and environmental quality. Soils might be contaminated with heavy metals from various anthropogenic activities, including agricultural practices, transport, industrial activities, and waste disposal (Nilgun *et al.*, 2004).

At present soils are gradually degrading due to many reasons and industrial pollution is one of them. Many large and small industrial units are discharging their wastes and effluents into the natural ecosystems in most cases without any treatment thus causing environmental pollution especially with heavy metals and organic toxics (Tadesse *et al.*, 1994), Bangladesh is not an exception. Rapid and unorganized industrialization and urbanization have contributed to an elevated level of heavy metals in the urban environment of the developing countries such as China (Wong *et al.*, 2003), India (Tripathi *et al.*, 1997; Sharma *et al.*, 2008a,b) and Bangladesh (Alam *et al.* 2003; Islam *et al.*, 2005; Naser *et al.*, 2009). Developing countries lag behind in the area of heavy metals research.

Metal pollution results from either discharge of industrial wastes, run off from contaminated sites, or metal deposition through emission from metal smelter, resulting from metal mines and metal processing industries (Tecsult, 1993). One of the negative environmental impacts arising from the heavy metal pollution of soil is surface and groundwater contamination due to rainwater runoff (Matschullat *et al.,* 1992).

Conventional remediation techniques are costly when the soil is polluted by heavy metals because the metals are tightly bound to various soil fractions. It has been estimated that the clean-up cost for one hectare over a depth of one meter ranges between $0.6 to 3 million depending of the pollution load on the soil (Moffat, 1995).

Heavy metals are associated with the various components of the soil in different ways, and these associations indicate both their mobility in the soils as well as their bioavailability (Ahumada *et al.*, 1999). The degree at which metals are associated with different chemical forms depends on various physical, chemical and biological processes acting on soil media and such soil properties as pH, amount of organic matter, redox conditions and soil texture and also cation exchange capacity, clay minerals, calcium carbonate, and Fe/Mn oxides in soil (Rieuwerts *et al.*, 2006).

In cases of soil contamination by heavy metals, it is important to identify the available and unavailable forms of the heavy metals to ensure that the soil is managed in such a way as to prevent the unavailable forms from becoming available (Cottenie *et al.,* 1980). Bioavailability and the mobility of metals are also related to each other. Higher concentration of mobile toxic metals (Pb, Cd, Cr, Ni etc.) in the soil column increases the potential for plant uptake, and animal/human consumption (Tack and Verloo, 1995; Ratuzny *et al.*, 2009).

Soils are complex porous aggregates usually possessing a high metal-binding capacity, primarily due to their small, high surface active components such as clay particles, organic materials and hydrous metal oxides. Heavy metals may be retained in the soil as exchangeable metals, carbonates, hydroxides, Fe/Mn-oxides, or may be bound to organic matter. In most cases, heavy metals are retained in the upper horizon of soils (<0.5 m), depending on local environmental conditions (Wasay, 1998). The scientific and technological challenges are to:

1. Understand the interactions between soil and heavy metals,
2. Establish a better scientific basis for deciding:
    a. what remediation approach to take at a given site
    b. which heavy metal forms in the soil are removable
3. Develop new cleanup technologies or enhance existing ones
4. Develop a remediation process for heavy metals which is cost effective and environmentally friendly.

-(Wasay, 1998).

At a particular site it is, therefore, important to know how the contaminants are held in the soil before designing a remediation process. Sequential extraction is thought to be the most suitable method to study how heavy metals are retained in different components of the soil and to evaluate the relative binding strength and hence the solubility, mobility and bioavailability of heavy metals in contaminated soils (Liu and Dezfulian, 1995).

Remediation of polluted soils can be carried out either by *ex situ* or *in situ* leaching and *in-situ* immobilization (fixation) of heavy metals. The soil is first excavated (dug out) from the site and then submitted to soil washing; one of the most suitable processes for removing heavy metals from the soil. Heavy metals are mainly retained within few cms of surface soil depending on the soil condition, which can he excavated and treated by soil washing process through proper drainage system. The washing solution may contain acids, bases, or chelating agents (Benschoten *et al.,* 1994).

In situ immobilization or fixation of heavy metals in soil is another remediation process. Different immobilization agents (such as chelate ion exchange resins, Portland cement/silicate, natural materials-clays, molecular sieves, greensand and other additives like hydrated lime, silylated silica gel, insoluble starch xanthate, ferrous sulfate) can he added to the soil to reduce the mobility of heavy metals (Liu and Dezfulian, 1995; Czupyrna *et al.,* 1989).

Soil washing can also be carried out *in-situ* (without digging out the soil). The flushing (washing) solution for contaminated sites may be the same as for *ex-situ* remediation. This process is the most appealing because it generally minimizes surface damage, requires a minimal amount of facilities reduces the potential for human exposure to contaminants, is less expensive and, when effective, reduces or removes contaminants. However, this remediation process is limited to permeable soils and cannot be applied easily to clay soils (Wasay, 1998).

Soils cleaned by means of a specific remediation technology, should meet a standard level. Washed soils are considered to be sufficiently clean if the heavy metal concentrations have been reduced to meet a specification (MEFQ, 1994).

Some organic acids such as acetic, citric and oxalic acids have been tested to treat soils artificially contaminated with heavy metals (Farrah and Pickering, 1978). Humic substances (HS) play an important role in the behavior of heavy metals in the natural environment due to the formation of complexes that can modify the mobility of these ions (Christman *et al.*, 1983; Stevenson and Fitch, 1986; Yin *et al.*, 1997). Humic substances comprises a highly complex mixture of organic compounds ranging from small water-soluble molecules to large insoluble compounds formed during mainly microbial degradation of dead organic materials (Stevenson and Cole, 1999). Due to the content of carboxylic acid and phenolic groups, HS possesses ability to form complexes with di- and trivalent metal cations such as many heavy metals (Christensen and Christensen, 1999; Weng *et al*., 2002; Hruˇska *et al*., 2003). Accordingly, a substantial capacity of soluble HS to dissolve (extract) heavy metals through complex formation has been demonstrated in many studies (Raulund-Rasmussen *et al*., 1998; Jensen *et al*., 2000; Strobel *et al*., 2001a, 2005; Zhao *et al*., 2007). However, despite a likely potential as cleaning (washing) agent for heavy metal polluted soils, HS does not seem to have been tested for that purpose. Humic substances can extract the most bioavailable and leachable heavy metal fractions, it seems to be a suitable substitute for the environmentally problematic synthetic soil cleaning agent (Borggaard *et al.*, 2009). The advantages associated with the use of such organic acids are: low cost, biodegradable, therefore environment friendly, less destructive to soil structure than mineral acid, more specific to heavy metals and less likely to leach soil macronutrients and improve soil properties (Wasay, 1998).

1.1. Objectives

The objectives of this study are:

- To survey the metal contaminated soils in Bangladesh and ascertaining the specific site characteristics.
- To categorize the metal contaminated soils according to the level of metal concentration.
- To do speciation of heavy metals and measure the relative proportion of heavy metals retained by each soil fraction (water soluble, exchangeable, carbonate bound, oxides bound, organic matter bound and residual form).
- To remediate metals-contaminated soils by using chemical extraction.
- To introduce some natural extracting agents in remediation technologies.
- To observe the combined effects of natural and synthetic extractants on the remediation of metal contaminated soils.
- To determine the mobility and bioavailability of these metals from soil to plant before and after remediation.
- To evaluate the efficiency of the suitable chemical extractant for remediation of particular metal contaminated soils in Bangladesh.

2. LITERATURE REVIEW

Heavy metals, one of the contaminant groups considered to be most noxious, enter the environment through natural as well as anthropogenic activities. These metals are produced naturally due to the weathering of rock minerals. These are then accumulated in soils. On the other hand, the direct input of metal ions to the environment due to anthropogenic activities causes serious environmental threats. The anthropogenic sources consist of all contributions made by vehicle emissions, metal plating or finishing operations, automobile battery production, mining and metallurgy, urban and industrial wastes, and uncontrolled sewage and agricultural inputs to all segments to the environment.

Soils are the major sink for heavy metals released into the environment by aforementioned anthropogenic activities and most metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.*, 2006), and their total concentration in soils persists for a long time after their introduction (Adriano, 2003).

Some of the heavy metals are required in minute quantities for normal growth and development of plants and animals, but turn out to be toxic once their available concentration exceeds a particular level. They are known to accumulate in living organisms and to have deleterious biological effects. Since the metals are not degraded in biological systems, their concentrations generally increase at each trophic level through the process of biomagnification. Almost all groups of living organisms have been reported to be affected by different heavy metals. The death tolls experienced in Japan due to the Minamata disease, caused by the consumption of mercury contaminated fish from Minamata Bay, and Itai-Itai, a condition of cadmium poisoning, are enough to show the magnitude of harm that metals can cause.

Soils may become contaminated by the accumulation of heavy metals through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan *et al.*, 2008).

Heavy metals are included in soil minerals as well as bound to different phases of soil particles by a variety of mechanisms, mainly absorption, ion exchange, coprecipitation, and complexation. Moreover, soil properties such as contents of organic matter, carbonates, oxides as well as soil structure and profile development influence the heavy metal mobility (Kabata-Pendias and Pendias, 2001). The knowledge of the binding of metals with the different soil phases and components is of major interest to assess the connections with other biotic and abiotic elements of the environment (Hirner, 1992).

2.1 Heavy Metals

Heavy metals are conventionally considered to include metallic elements with atomic number greater than 23 (vanadium), except rubidium and strontium. They have also been defined as metals having density more than five times that of water or a density more than 6,000 kg m^{-3} (Wild, 1988). Heavy metals are used and/or discharged by industries e.g. cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), arsenic (As), tin (Sn) and zinc (Zn). Toxic metals have adverse effects on plants, animals and humans. They are capable of decreasing crop production due to the risk of bioaccumulation and biomagnification in the food chain. Heavy metals have peculiar characteristics including that –

- They do not decay with time;
- They can be necessary or beneficial to plants at certain levels but can be toxic when exceeding specific thresholds;
- They are always present at a background level of non-anthropogenic origin,
- They strongly interact with the soil matrix, consequently, heavy input in soils being related to weathering of parent rocks and pedogenesis and
- They often occur as cations which metals in soils can become mobile as a result of changing environmental conditions (Facchinelli *et al.*, 2001).

Pollution of biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution. (Maier *et al.*, 2000).

Lead (Pb): Typical Pb concentration for surface soils worldwide averages 32 mg kg^{-1} and ranges from 10 to 67 mg kg^{-1} (Kabata-pendias and Pendias, 2001). Lead ranks fifth behind Fe, Cu, Al, and Zn in industrial production of metals. About half of the Pb used for the manufacture of Pb storage batteries. Other uses include solders, bearings, cable covers, ammunition, plumbing, pigments, and caulking. (Manahan, 2003). Ionic lead, Pb(II), lead oxides and hydroxides, and lead metal oxyanion complexes are the general forms of Pb that are released into the soil, groundwater, and surface waters (GWRTAC, 1997). The predominant insoluble Pb compounds are lead phosphates, lead carbonates (form when the pH is above 6), and lead hydroxides (Raskin and Ensley, 2000). Lead is not an essential element. It is well known to be toxic and its effects have been more extensively reviewed than the effects of other trace metals. Lead can cause serious injury to the brain, nervous system, red blood cells, and kidneys (Baldwin and Marshall, 1999). In general, plants do not absorb or accumulate lead. However, in soils testing high in lead, it is possible for some lead to be taken up by plant. Higher concentrations are more likely to be found in leafy vegetables and on the surface of root crops (Rosen, 2002).

Cadmium (Cd): Cadmium is not known for any essential biological function. In its compounds, Cd occurs as the divalent Cd (II) ion. The most significant use of Cd is in Ni/Cd batteries, as rechargeable or secondary power sources. Other uses of cadmium are as pigments, stabilizers for polyvinyl chloride (PVC), in alloys and electronic compounds. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products (Campbell, 2006). The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes increases the total concentration of Cd in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree (Weggler *et al.*, 2004). Cadmium in the body is known to affect several enzymes. It is believed that the renal damage that results in proteinuria is the result of Cd adversely affecting enzymes responsible for reabsorption of proteins in kidney tubules (Manahan, 2003).

Chromium (Cr): Chromium is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Major sources of Cr contamination include releases from electroplating processes and the disposal of Cr containing wastes (Smith *et al.* 1995). Chromium (VI) is the form of Cr commonly found at contaminated sites. Chromium can also occur in the (+III) oxidation state, depending on pH and redox conditions. Chromium (III) is the dominant form of Cr at low pH (<4). Chromium (VI) is the more toxic form of chromium and is also more mobile. Chromium (III) mobility is decreased by adsorption to clays and oxide minerals below pH 5 (Chrostowski *et al.*, 1991). Most of Cr released into natural waters is particle associated, however, and is ultimately deposited into the sediment (Smith *et al.,* 1995).

Nickel (Ni): In neutral to slightly alkaline solutions, Ni precipitates as nickelous hydroxide, $Ni(OH)_2$, which is a stable compound. This precipitate readily dissolves in acid solutions forming Ni(III) and in very alkaline conditions; it forms nickelite ion, that is soluble in water. In acidic regions, however, these solids dissolve producing Ni^{2+}(Pourbaix, 1974). Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries. The most common application of Ni is an ingredient of steel and other metal products. The major sources of nickel contamination in the soil are metal plating industries, combustion of fossil fuels, and nickel mining and electroplating (Khodadoust *et al.*, 2004). The larger part of all Ni compounds that are released to the environment will adsorb to sediment or soil particles. In acidic soils, however, Ni becomes more mobile and often leaches down to the adjacent groundwater (Raymond and Okieimen, 2011).

Copper (Cu): Copper is a transition metal. It is the third most used metal in the world (VCI, 2011). Copper is an essential micronutrient required in the growth of both plants and animals. In plants, Cu is especially important in seed production, disease resistance, and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. In the soil, Cu strongly complexes to the organics implying that only a small fraction of copper will be found in solution as ionic copper, Cu(II). The solubility of Cu is drastically increased at pH 5.5 (Martinez and Motto, 2000).

Zinc (Zn): Zinc is also a transition metal. Zinc occurs naturally in soil (about 70 mg kg^{-1} in crustal rocks) (Davies and Jones, 1988), but Zn concentrations are rising unnaturally, due to anthropogenic additions. Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. Industrial sources or toxic waste sites may cause the concentrations of Zn to reach levels that can cause health problems. A consequence is that Zn polluted sludge is continually being deposited by rivers on their banks. Plants often have a Zn uptake that their systems cannot handle, due to the accumulation of high Zn in soils. Finally, Zn can interrupt the activity in soils, as it negatively influences the activity of microorganisms and plants (Greany, 2005).

Soils contaminated with trace metals may pose both direct and indirect threats: direct, through negative effects of metals on crop growth and yield, and indirect, by entering the human food chain with a potentially negative impact on human health. Even a reduction of crop yield by a few percent could lead to a significant long-term loss in production and income. Some food importers are now specifying acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their contaminated crops (Bjuhr, 2007).

2.2 Sources of Heavy Metals in Soils

Heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace and rarely toxic (Pierzynski *et al.*, 2000; Kabata-Pendias and Pendias, 2001). Due to some natural and anthropogenic activities, soils of rural and urban environments may accumulate one or more of the heavy metals above background values high enough to cause risks to human health, plants, animals, ecosystems, or other media (D'Amore *et al.,* 2005). Heavy metals in the soil from anthropogenic sources tend to be more mobile, hence bioavailable than pedogenic, or lithogenic ones (Kaasalainen and Yli-Halla, 2003).

2.1.1. Natural or background sources of heavy metals in soil

Metals are some of the most abundant elements in the earth's crust. Minerals such as those listed in Table 2.1 represent the original source of metals found in soils and aquatic environments.

Table 2.1. Natural sources of metals and elements on the priority pollutant list

Element	Natural Source of Metals or Metallic Minerals
Antimony	Stibnite (Sb_2S_3), geothermal springs, mine drainage
Arsenic	Metal arsenides and arsenates, sulfide ores (arsenopyrite), arsenite ($HAsO_2$), volcanic gases, geothermal springs
Cadmium	Zinc carbonate and sulfide ores, Copper carbonate and sulfide ores
Chromium	Chromite ($FeCr_2O$), chromic oxide(Cr_2O_3)
Copper	Free metal (Cu^0), copper sulfide (CuS_2), Chalcopyrite ($CuFeS_2$), mine drainage
Lead	Galena (PbS)
Mercury	Free metal (Hg^0), Cinnabar (HgS)
Nickel	Ferromagnesium minerals, ferrous sulfide ores, Pentladite ($(Ni, Fe)_9S_8$), nickel oxide (NiO_2), nickel hydroxide ($Ni(OH)_3$)
Zinc	Zinc blende(ZnS), Willemite ($ZnSiO_4$), Calamine ($ZnCO_3$) mine drainage

Source: Novotny (1995)

2.1.2. Anthropogenic sources of heavy metals in soil

There are many anthropogenic sources of heavy metals that accumulate in soils (Table 2.2). The major anthropogenic source of metals to soils and the environment are: chemical and other manufacturing industries, waste disposal, metalliferous mining and smelting, sewage sludges, fossil fuel combustion, metallurgical industries- manufacture, use and disposal of metal commodities, electronics-manufacture, use and disposal of electronic commodities, agricultural and horticultural materials etc.

Table 2.2. Anthropogenic sources of some selected heavy metals in soil.

Element	Sources of Heavy Metals in Soil
As	Pesticides, plant desiccants, animal feed additives, coal and petroleum, mine tailings and detergents
Cd	Electroplating pigments for plastics and paints, plastic stabilizers and batteries
Cr	Tannery industry, Stainless steel, chrome-plated metals, pigments, and refractory brick manufacture
Cu	Cable and metal industry, Mine tailing, fly ash, fertilizers, windblown copper-containing dust
Pb	Battery industry, combustion of coal, gasoline and coal, iron and steel production
Hg	Pesticides, catalysts for synthetic polymers, metallurgy, thermometers
Ni	Combustion of coal, gasoline and oil, alloy manufacture, electroplating, batteries
Zn	Galvanized iron and steel, alloys batteries, brass, rubber manufacture

Source: Moore and Ramamoorthy (1984)

2.3. Forms and fraction of heavy metals in soil

There are various pools or fractions of heavy metals in the soil. In liquid phase they exist as hydrated ions, soluble organic and inorganic complexes and as a component of fine disperse floating colloids. In the solid phase they occur as insoluble precipitates and minerals, on the surface of organic and inorganic colloids in exchangeable and non-exchangeable (specific adsorbed) forms. As soils consist of heterogeneous mixtures of different organic and organo-mineral substances, clay minerals, oxides of Fe, Al, and Mn, and other solid components as well as a variety of soluble substances, the binding mechanisms for trace metals in soils are manifold and vary with the composition of soils and their physical properties.

Trace metals can exist in a variety of forms in soil (Petruzzelli, 1989; Iwegbue *et al.*, 2006a, b). These forms include-

- Water soluble,
- Exchangeable,
- Linked to organic substances,
- Occluded or co-precipitated with oxides, carbonates and phosphates, or other secondary minerals and
- Ions in the crystalline lattices of the primary minerals.

i) Water- soluble Fraction: This phase contains the water soluble species made up of free ions and ions complexed with soluble organic matter and other constituents. It constitutes the most mobile and potentially the most available metal and metalloid species (Petruzzelli, 1989; Iwegbue *et al.*, 2006a, b).

ii) Exchangeable / Non-specifically sorbed fraction: This fraction includes weakly adsorbed metals retained on the solid surface by relatively weak electrostatic interaction, metals that can be released by ion-exchange processes etc. Changes in the ionic composition, influencing adsorption-desorption reactions, or lowering of pH could cause remobilization of metals from this fraction (Ahnstrom and Parker, 2001).

iii) Linked to organic substances/ Organically complexed metal fraction: The trace metal may be associated through complexation or bioaccumulation process with various forms of organic material such as living organisms, detritus or coatings on mineral particles. Metallic pollutants associated with oxidizable phases are assumed to remain in the soil for longer periods but may be mobilized by decomposition processes (Kennedy *et al.*, 1997).

iv) Carbonate phase: Carbonate can be an important adsorbent for many metals when organic matter and Fe- Mn oxides are less abundant in the system (Stone and Droppo, 1996). The carbonate form is loosely bound phase and liable to change with environmental condition.

v) Oxide bound: Hydrous oxides of manganese and iron are extracted together and are the well known “sinks” in the surface environment for heavy metals. The amorphous oxyhydroxides of iron and manganese strongly sorb trace elements, initially in exchangeable forms, but increasingly with time are transformed to less mobile, specifically adsorbed forms (Petruzzelli, 1989; Iwegbue *et al.*, 2006 a, b).

vi) Residual fraction: The residual fraction consists of metals incorporated into the crystal structures of primary and secondary minerals. This fraction is the hardest to remove and requires the use of strong acids to breakdown silicate structures (Petruzzelli, 1989; Iwegbue *et al.*, 2006a, b).

Speciation: For a given element, the term speciation refers to its distribution amongst its chemical forms or species. Total trace metal content of soil is of little importance in determining its uptake by plants. The different forms of metals have different mobilities, bioavailabilities and potential environmental contamination potential. It is more important to know the distribution of each trace metals in the different forms, than just the total content (Petruzzelli, 1989).

The speciation of heavy metals can be done by sequential extraction method. Sequential extraction involves treatment of a sample of soil with a series of reagents in order to partition the trace element content. The various forms of the heavy metals thus sequentially extracted can be classified as dissolved, exchangeable, organically-bound, bound to oxides and residual forms. Although there are several sequential extraction procedures for metal speciation in the literature, the Tessier scheme (Tessier *et al.* 1979) is usually the most adopted methods by various workers. Hence the scheme is given in Table 2.3.

Table 2.3. Operating conditions required in the Tessier sequential extraction procedure

Stage	**Fraction**	**Reagent**	**Experimental conditions**
1	Exchangeable	8 ml of 1 mol l^{-1} $MgCl_2$ (pH 7)	1 h at 25^0C
2	Asoociated with carbonates	8 ml of 1 mol l^{-1} NH_4OAc (pH 5 with acetic acid)	5 h at 25^0C
3	Asoociated with Fe-Mn oxides	20 ml of $NH_2OH.HCl$, 0.04 mol L^{-1} in 25% w/v HOAc (pH 2)	6 h at 96^0C
4	Asoociated with organic matter	3 ml of 0.02 mol L^{-1} HNO_3 / 5 ml of 30% m/v H_2O_2 + 5 ml of 30% m/v H_2O_2 + 5 ml of 3.2 mol l^{-1} NH_4OAc	2 h at 85^0C 3 h at 85^0C 30 min at 25^0C

Source: Tessier et al. (1979)

According to Tessier *et al.* (1979) the order of mobility of the metals considering their abundance in the fractions is: exchangeable > bound to carbonate > bound to oxides > bound to organics > residual.

Depending upon the variability in physical and chemical characteristics of metals, their affinity to soil components governs their speciation. Rule (1999) broadly reviewed recent literature on phase distribution of trace metals in soils, and concluded that the maximum proportions of most metals are found either in residual or in Fe-Mn oxide fractions in contaminated soils. Lead usually show high affinity to the oxide and residual fractions (Silva and Vitti, 2008), where it is strongly bound (Nogueira *et al.*, 2010), which in fact decreases its mobility (Sheppard and Thibault, 1992). A high association with exchangeable fraction was observed only for Cd in soils. Cadmium and zinc were also abundant as carbonate fraction in calcareous soils (Kabata Pendias, 1995). It is now widely recognized that the toxicity and mobility of heavy metals mainly depend on their specific chemical forms and binding states (Ma and Rao, 1997; Kabala and Singh, 2001). Knowledge of the basic chemistry of these heavy metals is necessary in understanding their speciation, bioavailability, and remedial options. In addition, speciation will determine metal mobility and hence potential contamination from soil to plant.

2.4. Transformation of metals in soil

The fate and transport of a heavy metal in soil depends on the chemical form and speciation of the metal. Once in the soil, heavy metals are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity (Shiowatana *et al.*, 2001). This distribution is believed to be controlled by reactions of heavy metals in soils such as (i) mineral precipitation and dissolution, (ii) ion exchange, adsorption, and desorption, (iii) aqueous complexation, (iv) biological immobilization and mobilization, and (v) plant uptake (Levy *et al.,* 1992). Many studies show that, when added to soils, various forms of heavy metals slowly redistribute among their solid-phase components (Ramos *et al.*, 1994). Different physical, chemical and biological processes acting on soil media and various soil properties, including pH, cation exchange capacity, redox potentials (Eh) and contents of organic matter, clay minerals, calcium carbonate, Fe and Mn oxides control the fate of heavy metals in soil.

2.4.1 Adsorption reactions

Adsorption, defined as the accumulation of a substance or material at an interface between the solid surface and the bathing solution, seems to be the most important chemical process controlling the behavior and bioavailability of metals in soils (Sparks, 1995). As a consequence of adsorption, metals are removed from the soil solution and retained on the colloidal soil surface. Many mechanisms are involved in metal adsorption, including non-specific adsorption specific adsorption and Co-precipitation (Sparks, 1995).

Non-specific adsorption: In non-specific adsorption, also known as cation exchange, metals are bound by electrostatic forces resulting in the formation of outer-sphere complexes. The ions in soil solution, such as heavy metals, are in equilibrium with counter-ions that balance the surface charge of the colloids (Harmsen and Vlek, 1985). Non-specific adsorption is a reversible, diffusion-controlled, stoichiometric process, and there is some selectivity or preference of ions by the adsorbent, depending of their valence and degree of hydration. The affinity of soil colloidal surfaces for cations increases as the valence increases. For cations of different valences, the affinity should generally follow the order $M^+ < M^{2+} < M^{3+}$. For cations of the same valence, the adsorption strength should be determined mainly by the hydrated radius of the ions (Ji and Li, 1997).

Specific adsorption: The adsorption of metals by soils - specific adsorption - can involve specific forces. Specific adsorption of ions on colloid surfaces results in the formation of stable molecules, with high bound energy, also called inner-sphere complexes (Sparks, 1995). This mechanism of metal binding is often not reversible, slower than outer-sphere complexation, and is weakly affected by the ionic strength of soil solution (Sparks, 1995). The hydrous oxides of Al, Fe, and Mn and the organic matter are the main soil constituents involved in specific adsorption. Some layer silicates can have the ability of specific adsorption of heavy metals, being in this way similar in properties to the hydroxyl groups on oxide surfaces.

Co-precipitation: Co-precipitation is defined as the simultaneous precipitation of a chemical agent in conjunction with other elements by any mechanism and at any rate (Sposito, 1983). The types of mixed solid commonly formed include clay minerals, hydrous Fe and Mn oxides and calcite in which isomorphous substitution has occurred (Table 2.4). In addition to co-precipitation, replacement of Ca^{2+} cations by Cd^{2+} can also occur in the surface layer of calcite when it comes into contact with solutions containing Cd (Papodopoulos and Rowell, 1988).

Table 2.4. Trace metals normally found co-precipitated with secondary minerals in soils

Minerals	**Co-precipitated Trace Metals**
Fe oxides	V, Mn, Ni, Cu, Zn, Mo
Mn oxides	Fe, Co, Ni, Zn, Pb
Ca carbonates	V, Mn, Fe, Co, Cd
Clay minerals	V, Ni, Co, Cr, Zn, Cu, Pb, Ti, Mn, Fe

Source: Sposito (1983)

2.4.2. Organic complexation

In addition to being involved in cation exchange reactions, solid-phase humic substances such as humic acids also adsorb metals by forming chelate complexes. The stability constants of chelates with metals tend to be in the following decreasing order: Cu > Fe = Al > Mn = Co > Zn. Low molecular weight organic ligands, can form soluble complexes with metals and prevent them from being adsorbed or precipitated. Humic compounds with suitable reactive groups, such as hydroxyl, phenoxyl and carboxyl form coordination complexes with metallic ions. Carboxyl groups play a predominant role in metal binding in both humic and fulvic acids. The maximum amount of any given metal that can be bound is found to be approximately equal to the number of carboxyl groups (Alloway, 1995).

2.4.3 Adsorption as surface complexation

Surface functional groups, including hydroxyl groups on the edges of clays and on hydrous oxides, ditrigonal cavities in the basal plans of clays and carboxyl, amino and phenoxyl groups on the surfaces of organic matter, react with metal species to form surface complexes (Sposito and Page, 1984). These surface complexes can be of two types: (a) inner-sphere complexes, in which no molecule of the bathing solvent (water) is interposed between the surface groups and the ion or molecule it binds, and (b) outer-sphere complexes, in which at least one molecule of the solvent comes between the functional group and the ion.

2.4.4. Precipitation

Heavy metals are typically precipitated as: hydroxides, sulfides or sometime sulfates and carbonates. Many heavy metals are amphoteric. Therefore, their solubility reaches a minimum at a specific pH (different for each metal) (Mellbye and Hart, 2009). Metal sulfides are typically very insoluble. Sulfide precipitation is always conducted under alkaline conditions. Some metals (lead, cadmium, nickel) form insoluble carbonates. Carbonate precipitation takes place only if carbonate ions (CO_3^{2-}) are present. High pH also promotes the precipitation of the metals as hydroxides. Precipitation by hydroxide formation is the most common heavy metal precipitation method.

2.4.5. Ion exchange

Ion exchange refers to the exchange between the counter ions balancing the surface charge on the colloids and the ions in the soil solution. It has the following characteristics: it is reversible, diffusion controlled, stochiometric and, in most cases, there is some selectivity or preference for one ion over another by the adsorbent. Heavy metals entering soil can be transformed by this process.

2.5. Factors affecting heavy metal transformation in soil

Various soil properties, including pH, cation exchange capacity, redox potential (Eh) and contents of organic matter, clay minerals, calcium carbonate, Fe and Mn oxides control the fate of heavy metals in soil. The chemical and physical properties of the soil influence the form of the metal contaminant, its mobility, and the technology selected for remediation.

2.5.1 Particle size distribution

Soil containing a large amount of clay minerals, have the ability to accumulate metallic elements (Nessner and Esposito, 2010). However, sandy soils distinguished by a low sorption capacity and weakly adsorb heavy metals (Sheoran *et al.*, 2009). Heavy soils, compared to light soils, due to large amounts of suspended fraction, have a greater ability to retain metallic elements (Bodar, 2006). The Pb distribution in mineral soils shows a positive correlation with fine soil fraction. Soil texture is an important factor governing Cd contents of soils. This relationship is well illustrated by the Cd geometric mean contents increasing with the content of the soil clay fraction (Kabata-Pendias and Pendias, 2001). Binding of metal cations increases with increase of their valence, atomic weight and ionic potential. The affinity of metal cations relative of clay minerals is arranged in a series of $Cu^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}$ (Gang, 2010).

2.5.2. Soil moisture regime and redox potential

Soil moisture regime can affect pH, Eh, organic matter and $CaCO_3$ contents of soil (Van den Berg and Loch, 2000), and accordingly, may indirectly influence the transformation and repartition of heavy metals in soil, further altering their availabilities to plants. Generally, under saturated conditions, the pH tends to converge to neutral, whether the initial one was acidic or alkaline (McBride, 1994). Soil redox potential can influence the solubility of heavy metals. The Eh decreases by microbial respiration and, as a result, Fe and Mn oxides are reduced under anaerobic conditions, making heavy metals released and redistributed among the solid-phase components (Iu *et al.*, 1981).

2.5.3. Time

Residence time directly relates to the bioavailability of heavy metals in soils (Joner and Leyval, 2001). Generally bioavailability of heavy metals decreases with increasing residence time (McLaughlin, 2001). Such time effect is ascribed to the reactions between metal ions and soils, which mainly include complexation, adsorption, and precipitation of metal ions in the soil particle surface or diffusion into the mesopores and micropores of soil. It is often assumed that residence time effect reduced metal mobility and bioavailability. Lu *et al.* (2005) studied the time effect on the fractionation of heavy metals in soils and found that soluble metals added were transformed from easily extractable fractions to more stable fractions. Mann and Richie (1994) reported that the Cd added was transformed from soluble to less soluble fractions with time. Han and Banin (1997 and 1999) also suggested that added Cd, Cr, Ni, Cu, Pb and Zn were transformed into more stable forms in two arid zone soils after one year incubation under field capacity and flooding moisture regimes.

2.5.4. Soil reaction (pH)

The soil pH is the pre-eminent factor controlling the chemical behaviour of metals and many other important processes in the soil. In general, heavy metal cations are most mobile under acid conditions (Dzombak and Morel, 1987). In acid soils (pH 4.2 - 6.6) the elements Cd, Ni, and Zn are highly mobile (Aydinalp and Marinova, 2003). The mobility of trace elements is reduced with increasing soil pH because of the precipitation as insoluble hydroxides, carbonates, and organic complexes. As soil pH increases, the solubility and availability of these trace nutrients decreases (Mellbye and Hart, 2009).

2.5.5. The presence of hydrous metal oxides of Fe, Al and Mn

The presence of hydrous metal oxides of Fe, Al and Mn can strongly influence metal concentrations because these minerals can remove metals from solution by ion exchange, specific adsorption and surface precipitation. Sorption of metal cations onto hydrous oxides generally increases with pH and is most significant at pH values above the neutral range (Dzombak and Morel, 1987).

2.5.6. The Presence of Competitive Cations

The presence of competitive cations can affect metal adsorption in soils. For instance, Ca^{2+} competes effectively with cationic heavy metals for adsorption sites, and this competition seemed to be greater for Zn and Cd than for Cu and Pb (Kiekens, 1983).

2.5.7. The presence of inorganic and organic ligands

The presence of inorganic and organic ligands in the soil solution can affect the adsorption of trace metals. Puls *et al.* (1991) have demonstrated decrease sorption of Pb in the presence of complexing ligands and competing cations. Lead has a strong affinity for organic ligands and the formation of such complexes may greatly increase the mobility of Pb in soil. Cadmium forms soluble complexes with inorganic and organic ligands.

2.5.8. Cation exchange capacity (CEC)

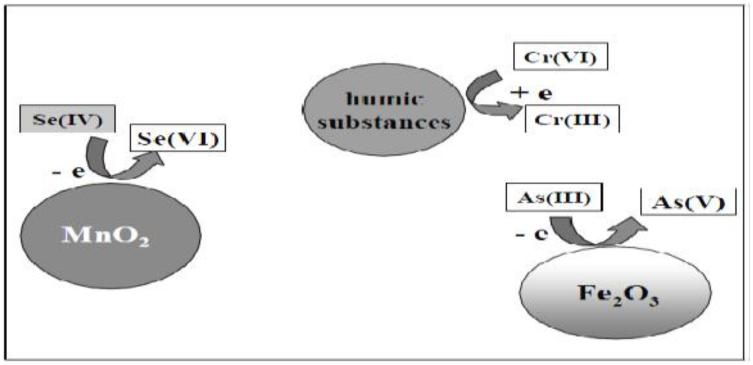
Cation exchange capacity (CEC) refers to the concentration of readily exchangeable cations on a colloidal surface and is often used to indicate the affinity of soils for uptake of cations.

Most heavy metals exist mainly as cations in the soil solution, and their adsorption therefore depends on the density of negative charges on the surfaces of the soil colloids. The CEC is directly related to the soils capacity of adsorbing heavy metals. The greater the CEC values, the more exchange sites on soil minerals will be available for metal retention (Barry *et al.*, 1995).

2.6. Interaction heavy metals with organic matter

The high amount of organic matter (OM) in soil helps to minimize the absorption of heavy metals by plants. When organic matter is added to soil this OM can redistribute heavy metals from soluble and exchangeable forms to fractions associated with OM and the residual fraction (Shuman, 1999). Organic matter can immobilize heavy metals, or work as a factor which inhibits their release. For example, Cu is bound and rendered unavailable chiefly through the formation of complexes with OM (Kirkham, 1977).

Decomposition of organic matter releases humic acids which have a significant impact on the availability of metals. High molecular weight humic acids are known to strongly bind metals which render metals unavailable in the soil solution. On the other hand, low molecular weight humic acids have the ability to chelate metals and prevent their adsorption from solid surfaces, thus enhancing the availability of metals. Thus, low molecular weight humic acids are highly active in the soil environment and have a positive impact on the binding abilities for metals (Amoakwah *et al.,* 2013).



Source: Violante et al. (2010)

Fig. 2.1. Possible abiotic redox processes occurring on the surfaces of humic substances and Fe- and Mn-oxides

The retention of cations by humic acid due to dissociation of functional group is controlled by pH. At pH 6 - 8, humic acids develop the greater surface area and heavy metal retention reaches its peak (Amoakwah *et al.*, 2013). McBride (1989) suggested the following sequence of affinity of divalent metal ions for organic matter: Cu > Ni > Pb > Co > Ca > Zn > Mn > Mg.

In general, the more electronegative the metal ion, the stronger the bond formed with organic matter. Copper is commonly found strongly complexed by organic matter. Evidences from electron paramagnetic resonance (ESR) studies suggested that Cu^{2+} is bonded rigidly as a inner-sphere complex, and that at high metal concentrations, the complex may be mobile (McBride, 1989). This metal may be bound by humic acid, coordinating either with O atoms or with N atoms (Martin-Neto *et al.,* 1991).

Most of the first-row transition metals (Mn^{2+}, Fe^{2+}, Co^{2+}) and alkaline earth metals (Ca^{2+}, Mg^{2+}) form outer-sphere complexes with organic matter (McBride, 1989). Some authors have pointed out that the Pb fixation by soluble organic matter (SOM) is more important than the fixation by hydrous oxides (Li and Shuman, 1996). Basta *et al.* (2005) described that the electronic properties of Pb result in a strong affinity for SOM and formation of inner-sphere metal surface complexes. Logan *et al.* (1997) reported that HA has a high maximum binding capacity for Pb, which is 0.564 mmol Pb^{2+}/g, at pH 5. Pb-SOM sorption increases with increasing pH. Low-molecular-weight OM fractions bound more Cd than do higher-weight fractions, whose affects increased the Cd mobility (Mahara *et al.*, 2007).

Organic matter reacts strongly with metals in soil. Martinez *et al.* (2003) found a paralleled behaviour between dissolved organic carbon and total soluble metal concentrations in OM-rich soil, these increasing with incubation time.

2.7 Maximum allowable limits of heavy metals in soils

The maximum allowable limits of heavy metals in soils and vegetables have been established by standard regulatory bodies such as World Health Organization (WHO), Food and Agricultural Organization (FAO) and Ewers U, Standard Guidelines in Europe as shown in Table 2.5.

Table 2.5. Maximum allowable limits of heavy metal in irrigation water, soils and vegetables.

Chemical Elements	Maximum permissible level in irrigation water (μg ml^{-1})	Maximum permissible level in soils (μg g^{-1})	Maximum permissible level in vegetables (μg g^{-1})
As	0.10	20	-
Cd	0.01	3	0.1
Co	0.05	50	50.0
Cr	0.55	100	-
Cu	0.017	100	73.0
Fe	0.50	50000	425.0
Mn	0.20	2000	500.0
Ni	1.40	50	67.0
Pb	0.065	100	0.3
Se	0.02	10	-
Zn	0.20	300	100

(Chiroma *et al.*, 2014)

European Union Council directive (1986) limits values for concentrations of HMs in arable soils to 3 mg kg^{-1} for Cd, 140 mg kg^{-1} for Cu, 75 mg kg^{-1} for Ni, 300 mg kg^{-1} for Pb, 300 mg kg^{-1} for Zn and 1.5 mg kg^{-1} for Hg.

Table 2.6. Maximum permissible concentrations of potentially toxic elements in soils after application of sewage sludge and maximum annual rates of addition (DoE, 1996).

Metals	Maximum permissible concentration in soil (mg kg^{-1} dry soil)			
	Soil pH value			
	5.0-5.5	5.5-6.0	6.0-7.0	>7.0
Zn	200	200	200	200
Cu	80	100	135	200
Ni	50	60	75	110
	For pH 5.0 and above			
Cd	3			
Pb	300			
Hg	1			
Cr	400			
Mo	4			
Se	3			
As	50			

The limit value for Pb will be reduced to 200 mg kg^{-1} dry soil in a future revision of The Code of Practice for Agricultural Use of Sewage Sludge (DoE, 1996) and the Sludge (Use in Agriculture) Regulations UK (SI, 1989).

2.8. Remediation technologies for metal contaminated soils

Once released into the soil matrix, metals are difficult to remove. Heavy metals must be removed or immobilized to reduce their toxicity in contaminated soil. Various remediation techniques have been developed and employed to decontaminate the metal contaminated sites, and will be discussed in the following sections.

Remediation techniques include-(GOC, 2003; Fawzy, 2008; Nouri *et al.*, 2009; Kord *et al.*, 2010).

(i) *ex situ* (excavation) or *in situ* (on-site) soil washing/leaching/ flushing with chemical agents,
(ii) chemical immobilization/stabilization method to reduce the solubility of heavy metals by adding some non-toxic materials into the soils,
(iii) electrokinetics (electromigration),
(iv) covering the original polluted soil surface with clean soils,
(v) dilution method (mixing polluted soils with surface and subsurface clean soils to reduce the concentration of heavy metals),
(vi) phytoremediation

2.8.1. Excavation: Excavation is a classical method which simply involves physically removing the contaminated soil and disposing of it elsewhere (e.g., a landfill). In addition to the high costs associated with excavation and disposal of the soil, there are also potential runoff and groundwater contamination problems. Considering this technique as among temporary solutions, increasingly stringent measures in most industrialized countries have led to the development of *in-situ* soil treatment technologies, such as flushing or washing, addition of chelating agents, and the application of electrochemical or immobilization techniques.

2.8.2 Immobilization: Immobilization reduces the availability of metal ions by limiting their movement within and out of the soil, either due to capping, vertical and horizontal containment techniques or through solidification/ stabilization technologies (Smith *et al.*, 1995). In the solidification process, metal ions are physically trapped within a solidified matrix in the form of a crumbly soil-like mixture. Stabilization converts the mobile fraction of metal ions to a more immobile form by changing the contaminant chemistry on addition and incorporation of various soil amendments. The above ground treatment is accomplished by mixing the excavated soil with inorganic or organic binders. The commonly used binders are phosphate fertilizers, agricultural limestone, gypsum, Portland-type cements and siliceous pozzolans, which can be added alone or in various combinations. In a study of the effect of soil amendments like phosphate fertilizer and iron oxyhydroxides on soil, Berti and Cunningham (1997) found that the teachable soil Pb was reduced from 30 ppm to below 5 ppm after adding these materials.

In order to eliminate the labor and energy costs that are associated with excavation, transport and disposal of the treated soil, efforts have been made to clean the soil through *in-situ* solidification/ stabilization or simply immobilization of metal ions.

In-situ immobilization of heavy metals in the contaminated soil can be accomplished by adding natural or synthetic chemicals to the soil. Several immobilization materials including chelate ion exchange resins (Amberlite, Duolite, Zeolite), molecular sieves, natural materials such as clays, peat, fly ash, greensand, and additives such as lime, gypsum, silica gel, starch xanthene, etc., have been shown to be suitable metal-fixing agents in heavy-metal contaminated soils (Czupyrna *et al.*, 1989; Berti and Cunningham, 1997; Lin *et al.,* 1998). After screening the metal-binding capacity of 21 additives, Czupyrna *et al.* (1989) concluded that lime and Valfor 200 (sodium alumino silicate) would be a viable treatment for immobilizing Cu, Ni, Cd, and Zn ions in the contaminated soil. Immobilization of Ni, Cu, and Cd ions by addition of polynuclear aluminum and montmorillonite compounds has also been demonstrated (Lothenbach *et al.*, 1997; Badora *et al.*, 1998). However, metal ions immobilized in this fashion could result in future problems such as leaching of heavy metals into the ground water because of biological activities or acidification of the soil.

2.8.3. Electrodialytic remediation: In electrokinetic remediation, pairs of electrodes are placed in the contaminated soil and a direct current potential is applied across them. The contaminants migrate to the electrode wells from where they can be brought to the surface (Acar and Alshawabkeh, 1993; Probstein and Hick, 1993; Hick and Tondrof, 1994; Hansen *et al.,* 1997; Li *et al.*, 1997; Riberio and Mexia, 1997; Viadero *et al.*, 1998). The ground water in the boreholes or externally supplied fluid is used as the conductive medium. This method has the limitation of being used in removing metal ions from saturated soils with nearly static groundwater flow and moderate to low permeability. Secondly, the electrolysis reactions at the electrodes can produce alterations in pH of the pore fluid by generating H^+ and OH^- ions. The pH drops at the anode to below 2 and increases at the cathode to above 12, depending on the total current applied (Acar and Alshawabkeh, 1993). As metal enters the region of high pH at the cathode, it may get re-adsorbed by soil particles, precipitate or form hydroxo complexes, thus, limiting the extent of metal ion migration and the effectiveness of the reclamation process.

Electrodialytic soil remediation is a newly developed method for removal of heavy metals from polluted soils. The method is based on a combination of the electrokinetic movement of ions in soil with the principle of electrodialysis. However, electrodialysis remediation differs from the electrokinetic remediation in having ion exchange membranes instead of passive barriers that are used in the electrokinetic method. The main purpose of using ion-exchange membranes is that no ions can enter the soil from the electrode compartments, which means that the current is carried by the ions that were present originally in the soil (Ottosen *et al.,* 1997). The electrodialytic remediation of Cu-contaminated soil conducted by Ottosen *et al.* (1997) showed the possibility of decontaminating the soil from 1360 mg L^{-1} to below 40 mg L^{-1} of Cu ions. The rate of copper removal increased with an increase in the current. There are few studies on metal ions other than copper.

No doubt, these techniques have recently made significant strides, but methods are still in early stages of development and restricted mainly to the laboratory. Also, the metals must be present in the soil solution, which is not always the case. Finally, as a large amount of electricity is necessary, these techniques are costly.

2.8.4. Bioremediation: Remediation of available metal ions in soil systems can be reduced biologically by immobilization or transformation of metal ions via bioaccumulation, biosorption, oxidation, reduction, methylation, demethylation, and phytoremediation, etc. A brief explanation of these processes is presented below.

Bioaccumulation and Biosorption: Bioaccumulation and biosorption of a wide range of cationic metals by bacteria, algae, and fungi have been demonstrated (Kuyucak and Volesky, 1990; Rao *et al.*, 1993; Churchill *et al.*, 1995; Kapoor and Viraraghavan, 1998; Magna *et al.*, 1998). The mechanism of metal biosorption and bioaccumulation by microorganisms is usually dictated by the preference of metals for different binding sites. For example, Cd^{2+} can be complexed to polyphosphates and to sulfur containing proteins in the cytoplasm (Higham *et al.*, 1984, 1985). Microorganisms produce certain exopolymers, which are acidic in nature, and have acidic functional groups for metal complexation and coordinate binding (Geesey and Yang, 1989).

Oxidation- Reduction: The solubility and toxicity of certain metal ions can vary depending upon their valence. For example, Cr(III) is less toxic and nonmobile in the environment compared to its oxidized forms such as Cr(VI) (Cifuentes *et al.*, 1996). Microorganisms can reduce inorganic contaminants and thereby influence the solubility and mobility. Mercury can be removed from contaminated environments by aerobic bacteria that reduce soluble Hg(II) to volatile Hg(0) (Silver and Walderhaug, 1992). The bacterial gene encodes an enzyme known as mercuric reductase that detoxifies Hg by reducing it to a relatively inert form (Hensen *et al.*, 1984). Metal reducing bacteria are able to reduce U(VI) to U(IV), Cr(VI) to Cr(III), Se(VI) to Se(0), and As(V) to As (III). These metals are soluble in their oxidized forms but are insoluble when reduced (Bolton and Gorby, 1995). When reduced, these metals precipitate and may be removed from aqueous wastestreams or contaminated surface or ground water.

Methylation-Demethylation: A number of microorganisms can methylate or demethylate metal ions. Methylation usually results in the volatile forms of metals, example being methylation of Se ions to volatile dimethyl selenide and dimethyl diselenide (McCarty *et al.*, 1995). The methylation of metals may be a detoxification process to protect the organisms from toxic metal ions.

Phytoremediation: In recent years, many attempts have been made to find heavy metal accumulating plants that are able to extract metals from the soil. Phytoremediation of inorganic contaminants can be further divided into phytostabilization and phytoextraction

(Cunningham *et al.*, 1995). Phytostabilization is the ability of plants to reduce the availability and mobility of toxic substances and prevent their entry into the food chain. In phytoextraction, the plant species extract, transport, and concentrate the toxic substances from soil into roots and aboveground biomass. The advantages of phytoremediation are the low input costs, soil stabilization, and reduced leaching of water and transport of inorganic contaminants in the soil. Some of the tree species also used for this purpose includes Alder, Hybrid Poplar, Lobolly pine, and Juniper etc. The plants can accumulate only those metal ions that can be reached through root growth or that are soluble in soil water. The majority of the metal ions occurring in the soil are not readily available to plant roots. Hence, metal ions below rooting depth will not be extracted. Phytoextraction, which is an economically and environmentally attractive *in situ* method, has been proposed for cleaning soils polluted by heavy metals (Alkorta *et al*., 2004; Leˇstan *et al*., 2008; Johnson *et al*., 2009; Bianchi *et al*., 2010).

This process is economical but passive, and can take years for contaminant concentrations to reach regulatory levels. (Alkorta *et al*., 2004; Jensen *et al*., 2009). To speed up extraction, various chemical compounds have been used to increase the availability (solubility) of the heavy metals, and hence accelerated or chelant-assisted extraction (Alkorta *et al*., 2004; Leˇstan *et al*., 2008; Meers *et al*., 2005).

2.8.5. Soil Flushing

Soil flushing is the enhanced mobilization of contaminants in a contaminated soil for the purpose of recovery and treatment. In simple words, soil flushing uses extraction and injection of aqueous solutions to remove contaminants from the subsurface application without excavation of the contaminated soil. The leaching solution employed should remove the contaminants without harming the *in situ* environment. Flushing solutions are usually contaminant specific. For instance, water will extract water-soluble constituents, whereas acidic solutions, chelating, complexing, and reducing agents may be needed for recovery of metal pollutants. In general, soil flushing is most effective in homogenous permeable soils.

2.8.6. Soil Washing

Soil washing is a water-based process employing physical separation and chemical extraction to remove contaminants from soil. Soil washing using chemical reagents is considered as one of the permanent treatments to remove heavy metals from polluted soils (Dermont *et al.*, 2008). Chemical reagents such as soluble salts, acids/bases, and chelating agents are usually employed to remove the heavy metals from the soils into an aqueous solution. Hydrochloric acid, EDTA, and subcritical water are the most used reagents used for soil washing and removal of metals from contaminated soil due to their high metal removal efficiency (Wasay *et al.*, 2001; Isoyama and Wada, 2007; Rao *et al.*, 2008; Udovic and Lestan, 2007; Voglar and Lestan, 2013). After facilitating remedial treatment, the washed soil is redeposited at the site.

Soil washing is a water based process that employs chemical and physical extraction processes to remove contaminants from the soil. In this process, the excavated soil is screened to remove oversized material, and then washed with water or other cleaning agents to separate the contaminants. Soil washing performance is highly sensitive to the conditions. Tunnell *et al.*, (1996) suggested that the particle size distribution and clay content of the soil are the two most important soil parameters to be evaluated during prescreening activities of soil washing. Soils with relatively large percentages of sand and gravel responded better to soil washing than those with larger percentages of clay and silt. In general, soil washing is most appropriate for soils that contain at least 50% sand/gravel (Tunnell *et al.*, 1996).

There are a number of reports on the remediation of metal-contaminated soils through *in situ* and *ex situ* soil washing and flushing. Soil washing or flushing with tap water, acidic solutions (Mulligan *et al.*, 1999), chelating agent (EDTA, DTPA etc) (Cline and Reed, 1995; Van Benschoten *et al.*, 1994 and 1997; Abumaizar and Khan, 1996; Legiec *et al.*, 1997; Doong *et al.*, 1998; Peters, 1999), reducing agents ($NH_20H.HCl$), surfactants (SDS, TX100, and CTAB etc.), and biosurfactants (surfactin from *Bacillus subtilis*) (Thimon *et al.*, 1992) have been evaluated for efficiency in removing metal ions (Cd^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, and Zn^{2+} ions) from soils.

Soil washing is particularly frequently used in soil remediation because it: (i) completely removes the contaminants, hence ensures the rapid cleanup of a contaminated site (Wood, 1997), (ii) meets specific criteria, (iii) reduces or eliminates long-term liability, (iv) may be the most cost-effective solution and (v) may produce recyclable material or energy (GOC, 2003).

The effectiveness of washing is closely related to the ability of the extracting solution to dissolve the metal contaminants in soils. However, the strong bonds between the soil and metals make the cleaning process difficult. Therefore, only extractants capable of optimally dissolving the metals must be carefully sought during soil washing. All these soil washing extractants have been developed on a case-by-case basis depending on the contaminant type at a particular site. A few studies have indicated that the solubilization/exchange/ extraction of heavy metals by washing solutions differs considerably for different soil types. Strong acids attack and degrade the soil crystalline structure at extended contact times. For less damaging washes, organic acids and chelating agents are often suggested as alternatives to straight mineral acid use (Yu and Klarup, 1994). The chelating organic acids are able to dislodge the exchangeable, carbonate and reducible fractions of heavy metals by washing procedures (Peters, 1999). The identification and quantification of co-existing solid metal species in the soil before and after treatment are essential to design and assess the efficiency of soil washing technology (Kirpichtchikova *et al.*, 2006).

The purpose of this study is to use innovative soil washing and remediation techniques to extract metal ions from the contaminated soil. The objective of the present study was, therefore, to assess the potential of chelating agent, natural organic acids, mild acid and salt solution to decontaminate a soil via soil washing experiments and study the changes in the redistribution of metal forms and bioavailability in the soil before and after washing using chemical speciation analysis and uptake by plant.

2.9 Use of extractants for remediation of metal contaminated soils

The following survey presents the extractants most commonly used and lists the groups of compounds it is presumed that they extract. Reaction times and the ratio of extractant to sample are also important. Reaction times are usually quite short (0.5 - 2.5 h) or adjusted to fit to the working day (e.g., 6 - 7 h or overnight, 16 h). It is not always clear that the composition of an extractant was not changed by its interaction with a sample.

2.9.1. Water

Several workers have measured a water-soluble fraction of the trace metals by shaking soil with water (unspecified) or with distilled or deionized water (Viets, 1962; Gupta and Chen, 1975; Sims and Patrick, 1978; Iyengar *et al*., 1981; Miller and McFee, 1983; Miller *et al*., 1986a,b). Sims and Patrick (1978) divided the water-soluble fraction into free ions and ions complexed by soluble organic matter. Sposito *et al*. (1982) used very pure distilled and deionized water (three repeats of a 2-h extraction) as an extractant for nonspecifically "sorbed" or "adsorbed" ions, after removing exchangeable ions with 0.5 M $KN0_3$. Bradford *et al.* (1975) analyzed "saturation extracts" of rewetted samples of sludge-treated soils as surrogates for their equilibrium solutions.

2.9.2. Simple Salts

Many workers have estimated "exchangeable" (Stover *et al*., 1976; Silviera and Sommers, 1977; Shuman, 1979; Jarvis, 1986, Neilsen *et al*., 1986), "neutral salt exchangeable" (Miller *et al*., 1986a), "water-soluble and exchangeable;' or "nonspecifically adsorbed" (McLaren and Crawford, 1973; Iyengar *et al*., 1981) ions of trace metals from soil, sludge, etc. by extraction with relatively strong solutions of the chlorides or nitrates of potassium, magnesium, and calcium. Such as

KCl	*2 M* (Himes and Barber, 1957)
$KN0_3$	*0.5 M* (Sposito *et al.*, 1982;
	1 *M* (Schalscha *et al.*, 1982; Miller and McFee, 1983)
$CaCl_2$	*0.05 M* (McLaren and Crawford, 1973; Shuman, 1979; Iyengar *et al.*, 1981);
	0.5 *M* (McLaren *et al.*, 1986)
$Ca(NO_3)_2$	*0.25 M* (Miller *et al.*, 1986b);

	0.5 M (Tiller *et al.*, 1972); Miller *et al.*, 1986b)
$MgCl_2$	*0.5 M* (Gibbs, 1977)
	1 *M* (Tessier *et al.*, 1979; Harrison, 1981)
$LaCl_3$	*0.3 M* (Jarvis, 1986).

It is presumed that these reagents act as sources of cations to displace trace metals held on inorganic and/or organic sites predominantly by electrovalent forces. Neutral salts like these have the advantage that they will barely affect the operative pH at the exchange sites.

In general, the cations that are the harder Lewis acids are likely to be better displacents of the metal cations that are held by more covalent forces; for example, magnesium is a stronger displacent than calcium in displacing cobalt and zinc from minerals (Tiller *et al*., 1972). Magnesium salts have been used to estimate "specifically adsorbed" (Tessier *et al*., 1979; Iyengar *et al*., 1981) or "adsorbed" metals (Gibbs, 1973) and more strongly held forms in general (Tiller *et al.*, 1972). Jarvis (1986) found that 0.3 M $LaCl_3$ extracted substantially more exchangeable Al from soils than 1 M KCl, some of it probably from clay inter layers and organic complexes. As between the nitrate, chloride, and sulfate of magnesium, the last two extracted more zinc from soils than the nitrate, which Tiller *et al*. (1972) attributed to the greater stability of their anion complexes with zinc.

2.9.3. Ammonium Acetates

Solutions of ammonium acetate and ammonium chloride (Gibbs, 1973 ; Shuman, 1979) have been widely used to measure soil cation-exchange capacity (CEq, or exchangeable ions, because (i) they are well buffered; (ii) the ammonium ion blocks the slow release of inter lamellar cations from the weathered periphery of clay lamellae (Scott and Welch, 1961) and gives a sharp CEC; and (iii) in the early days, because excess ammonium acetate could be disposed of relatively easily by oxidation or ignition.

They have been preferred to ammonium salts, and particularly to ammonium acetate, on the grounds that ammonium and acetate ions may have specific complexing effects as well (Neilsen *et al*., 1986). Thus, ammonium chloride and acetate have been supposed to dissolve transition metals from coatings on sediment grains, whereas 0.5 M $MgCl_2$ does not

(Gibbs, 1973, 1977). Also, ammonium acetate mobilizes more organic matter than does 0.05 M $CaCl_2$ (McLaren and Crawford, 1973).

Thus, their use has spread to estimates of "exchangeable" trace metal ions in other materials; 1 M ammonium acetate at pH 5 has been used to measure "ion-exchangeable" trace metals (Forstner *et al*., 1981), and, at pH 6.7 or pH 7, to measure "exchangeable," "exchangeable non-specific" or "ion-exchangeable" trace metals (Forstner *et al*., 1981; Iyengar *et al*., 1981; Soon and Bates, 1982); 5 M ammonium chloride at pH 8 has been used to measure "exchangeable" cations (Shuman, 1979). It has been supposed that ammonium acetate removes chromium and zinc from sites on organic matter and iron oxides (Luoma and Jenne, 1976), that it can dissolve oxide coatings in the hydrous oxide fraction (Shuman, 1979), and that ammonium acetate can dissolve or complex transition metals from coatings on sediment grains (Gibbs, 1973, 1977). Ammonium acetate has also been added to other, stronger extractants to block the readsorption of the trace metals they have caused to be released (Forstner *et al*., 1981).

2.9.4. EDTA (Ethylenediaminetetraacetic acid)

EDTA has been used in various forms, not always very precisely recorded, but mainly at pH 6.5-7. The following roles have been claimed for EDTA-

Dissolve carbonates: Solution of 0.1 M EDTA (pH 6.5) as an extractant for carbonates, was shown to dissolve cadmium, copper, lead, and zinc carbonates, and partially to dissolve lead sulfides but not other sulfides (Stover *et al*., 1976); 0.05 M Na_2-EDTA (after NaOH extractants for organically bound elements) to dissolve carbonates but possibly not sulfides (Emmerich *et al*., 1982; Sposito *et al*., 1982); 0.1 M EDTA (pH 7.0) to dissolve carbonates by complexation and thereby mobilize the copper occluded in them (Miller *et al*., 1986b); 0.5 M Na_2-EDTA substantially dissolved pure samples of cadmium, copper, nickel, lead, and zinc carbonates, and some nickel and lead sulfides, but not other sulfides (Lund *et al*., 1985).

Extract trace metals from the oxides or hydroxides of Fe, Mn and Al: EDTA dissolves oxides or hydroxides of Fe, Mn and Al thereby to release trace metals adsorbed on or occluded in them. 0.1 M EDTA + 1 M NaOH to remove cadmium, cobalt, and zinc freshly adsorbed onto iron oxides (Luoma and Jenne, 1976); 0.1 M EDTA to extract trace metals occluded in noncrystalline or poorly crystalline iron oxides (Miller and McFee, 1983); 0.1 M EDTA to extract some oxides (but less rigorously than pyrophosphates) (McLaren and Crawford, 1973); 0.4 M Na_2EDTA extracted cobalt from soil, and specifically from oxide surfaces (McLaren *et al*., 1986); EDTA (pH 10) was used to remove amorphous iron oxides (Jeanroy *et al*.,1986a); 0.1 M Na_2EDTA (pH 4.4-6.0, buffered by ammonium acetate/acetic acid) was believed to extract x-ray amorphous iron oxides (Borggard, 1976);

EDTA release organically bound trace metals: 0.4 M EDTA is usually thought to be an organic extractant (McLaren *et al*., 1986); NaOH/EDTA extracts organically bound iron (Cottenie *et al*., 1979); 0.4 M Na_2EDTA reportedly dissolves cobalt chelated on organic matter (McLaren *et al*., 1986); 0.1 M EDTA (pH 7.0) extracts copper from organic ligands (Miller *et al*., 1986b); 0.1 M EDTA + 1 M NaOH removed cadmium, cobalt, and zinc freshly adsorbed onto organic matter (Luoma and Jenne, 1976).

In addition, ammonium EDTA (pH 7.0) has been used to extract exchangeable and other inorganic forms of aluminium (Jarvis, 1986), although not from alumino silicate minerals, and to remove a general category of adsorbed, chelated, or complexed trace metals (Viets, 1962). Jarvis (1984) used 1% HNaCa-EDTA, in ammonium acetate at pH 8.0, to extract manganese.

2.9.5. DTPA (Diethylenetriamine pentaacetic acid)

DTPA has been used to extract exchangeable and organically bonded trace metals, and to dissolve "precipitates" (Schalscha *et al.*, 1982). More generally, *0.005 MDTPA* + 0.01 M$CaCl_2$ + 0.1 Mtriethanolamine (pH 7.3) has been used (Silviera and Sommers, 1977) to measure "available" forms. Hickey and Kittrick (1984) showed that DTPA-Zn is correlated with pyrophosphate-Zn and assumed that DTPA extracts organically bound zinc. Brown *et al.* (1971) showed that the quantities of zinc extracted from a range of soils by DTPA, EDTA, or dithizone (diphenyl thiocarbazone) were mutually correlated.

2.9.6 Hydrochloric acid

Solutions of 0.1 M hydrochloric acid have been used to extract "exchangeable" (Shuman, 1979) or "H-exchangeable" (Luoma and Jenne, 1976) cations, and have been shown to displace cadmium, cobalt, and zinc freshly adsorbed onto iron and manganese oxides, carbonates, or decomposing organic matter (Luoma and Jenne, 1976); 0.5 M HCI has been used to extract "acid-soluble" copper, and has been shown to dissolve copper oxide and basic copper carbonate, but only a little copper sulfide (Luoma and Jenne, 1976). Mitchell and Mackenzie (1954) reported that 0.05 M HCI dissolves freshly precipitated iron sulfide. Chao and Zhou (1983) used HCI at various concentrations, and found that it dissolved only a little of crystalline iron oxides at room temperature: dissolution was in the order amorphous > magnetite > goethite > hematite.

In conclusion, it must be emphasized:

1. There is a real and urgent need for selective extractants to distinguish and estimate the different forms of combination of trace metals that occur when sludge and soil interact.

2. The development of such extractants requires more effort than it has so far received.

2.10 Remediation of metal contaminated soils by chelating agents

Since heavy metals accumulate in the soils through adsorption, precipitation, and other physicochemical process, attention has been focused on the use of chelating agents which can dislodge and dissolve heavy metals present in soils. The suitable ligand of chelator for extracting metal ions must be strong enough to overcome the competition of metals due to soil surface adsorption and/or surface precipitation. Secondly, the rational of selecting complexing agents for soil reclamation processes is that the chelators should be non-toxic or ecologically safe, strongly complexing, and easily biodegradable. Although chelators have long been used to assess the bioavailable fraction of soil metals, their use for soil clean up is a more recent application. For example, extractability of metal ions such as Zn, Cd, and Ni ions was observed to increase in soils amended with Na_2 -EDTA (Li and Shuman, 1996b). EDTA has also been used to remove lead from artificially contaminated or surrogate wastes with efficiencies ranging from 40% to 80% (Reed *et al.*, 1996).

Strong acids such as HCl, HNO_3, and H_2SO_4 and their salts, because of their abilities to enhance the mobility and transportation of metal ions in the soils, had commonly been used for leaching out the strongly bound metal ions from contaminated soils (Cline and Reed, 1995; Neale *et al.*, 1997; Van Benschoten *et al.*, 1997). However, these chemical solutions freely disturb the physical, chemical, and biological properties of the soil. Therefore, for less damaging washes compared to the those resulting from soil washing with strong mineral acids and their salts, the effectiveness of various chelating agents such as EDTA, NTA, DTPA (Hong and Pintauro, 1995, 1996) etc., in removing the adsorbed metal ions from contaminated soils has been shown.

Chelating agents contain varying numbers of functional groups or binding sites that are capable of complexing heavy metals. Farrah and Pickering (1978) examined the abilities of seventeen different chemical solutions to displace heavy metal ions (Pb^{2+}, Zn^{2+}, Cu^{2+}, and Cd^{2+} ions), presorbed on clay (kaolinite, illite, and montmorillionite) at pH 5 or 7. They reported that among the solutions, only EDTA (1 mM, pH 7.0) quantitatively removed all four ions from clays.

Hessling *et al.* (1990) washed contaminated soils obtained from battery-recycling facilities, to determine the effectiveness of several metal-removal solutions. Their results showed that an EDTA solution was far better than solutions. Davis and Singh (1995) applied several chemical washing procedures to a Zn-contaminated soil column (length = 0.03 m, diameter = 0.019 m) for the purpose of determining their metal-extraction efficiency. Specific extracts examined include acid solution, organic complexing agents such as EDTA and DTPA and chlorine, all at various concentrations. The most efficient washing occurred in response to organic complexing agents at the lowest flow rate. Removal of Zn ions from the soil increased with increasing EDTA concentration. Total zinc removal efficiency, however, increased to 79% with 1 mM EDTA extraction solution. The general trend of the washings with DTPA was the same as with EDTA

In studies involving extraction of lead from soil containing approximately 70% silt and clay, Peters and Shem (1996) removed 58-64% of the lead using EDTA over the entire pH range (4.9-11.3). The soil was spiked with lead nitrate solutions resulting in lead concentrations on 500 to 10000 mg kg^{-1} soil. The chelants studied included EDTA, NTA and water. The extraction of lead with EDTA was rapid, reaching equilibrium within a contact time of 1 h. The order of lead removal efficiency for the various extractive agents was as follows: EDTA > NTA > water (Peters and Shem, 1996).

Four chelators, NTA, EDTA, EGTA and DCYTA (1,2-Diaminocyclohexane-N,N,N'N'-tetraacetic Acid, Monohydrate), were tested for their abilities to remove metal ions from contaminated kaoline (Hong and Pintauro, 1995). All four chelators exhibited some desorption selectivity for Cd, Cu, and Pb ions adsorbed on kaoline, the observed order of metal chelation and dissolution was Cd> Cu> Pb (for EGTA), Cd> Pb> Cu (for EDTA and DCYTA), and Cu> Cd> Pb (for NTA).

Elliot and Brown (1989) compared the capabilities of EDTA and NTA to decontaminate a metal-polluted soil that had 2112.71, 665, 1383, and 332 mg kg-1 of Pb, Zn, Cu, and Cd ions, respectively. In general, increasing the concentrations of both chelators resulted in greater Pb solubilization. However, EDTA released 10-30% more Pb ions than NTA. As a hexadentate ligand, EDTA binds divalent cations like Pb^{2+} very strongly. NTA is quadridentate and leaves two coordination sites of Pb^{2+} ions available for interaction with the soil surface.

In addition to the chemical ligands, various other biological compounds such as carbohydrates (gluconic acid), amino acids (glycine), and organic acids (citrate, oxalate) etc., which are known for their chelating properties, have also been tested for their capabilities to extract metal ions from the contaminated soils (Francis *et al.*, 1992; Lo *et al.*, 1992; Neale *et al.*, 1997). Use of certain organic acids to extract heavy metal ions from contaminated soils has been demonstrated to have advantages over other existing ex-situ and in-situ washing solutions (Matsuyama *et al.*, 1996; Singh *et al.*, 1996; Wasay, 1998). Some of the advantages associated with the use of organic acids include i) lower cost, ii) biodegradability, iii) less destructive to soil structure, and iv) improve soil properties

(Wasay, 1998). The complexation between organic acids and metal ions in the natural ecosystem has already been studied (Ernst *et al.*, 1992; Wang *et al.*, 1992).

Farrah and Pickering (1978) demonstrated the ability of some organic acids to remediate clay soils, which were contaminated artificially with Pb, Zn, Cu, and Pb. Wasay (1998) compared the capacities of various organic acids and chelating agents to remove metal ions from three different types of soils. Ten weak organic acids and 2 chelating agents (EDTA and DTPA) were used for metal removal from the polluted soils. Among the extractants used, citric acid showed maximum removal capabilities.

Contaminated soils of different textures were also flushed in columns, namely citrate and tartarate or chelating agents such as EDTA and DTPA. Washing solutions, at optimum pH levels, were applied at the surface of the columns. The citrate and tartarate were quite effective at removing metal ions from the contaminated soils (Wasay, 1998)

2.11. Chemistry of Metal Extraction using Chelating agents

Complexation of metals by chelating agents is defined as the formation of ring compounds in which a metal is coordinately held by two or more electron donating groups of a ligand (Cline and Reed, 1995). When a chelating agent is placed in contact with the polyvalent metals in the soil, it releases protons or counterions in preference to metal ions. As a result, the soil-bound trace metals are removed and transformed into soluble metal complexes (Peters, 1999). In general, the larger the number of protons donated the stronger the stability of the metal-chelate complexes. However, chelating agents with more than 6 protons (e.g., DTPA) often form less stable complexes due to their large size ring formation. The partitioning of heavy metals between the soil surface groups and a soluble complexing ligand can be explained by the following reaction:

$$\text{Soil}^{n-}\text{-Metal}^{n+} + \text{Ligand}^{n-} \text{-----------}> \text{Ligand-Metal complex} + \text{Soil}^{n-}$$

2.12. Remediation of metal contaminated soils using natural chelating agent (soluble humic substances)

Humic substances are heterogeneous, high-molecular-weight organic substances that are composed of humic acid, fulvic acid, and humins, all of which have a different solubility in alkaline and acid solutions. Previous studies have indicated that both HA and FA have the capacity to bind metal ions (Benedetti *et al.*, 1996; Evangelou *et al.*, 2004). Humic substances have a complex structure with multiple functional groups that can interact with metal ions in several ways. One way is through electrostatic interactions between negatively charged humic substances and positively charged metal-ions (Evangelou and Marsi, 2001). The effects of humic acids on Pb and Cd were pH-dependent. The application of HA and FA effectively reduced Pb and Cd accumulation in tobacco leaves from the acidic red and paddy soils. As a result, humic acids could be used to reduce metal accumulation in plants growing in polluted acidic soils (Zhang *et al.*, 2013).

Humic substances (HS) play an important role in the behavior of heavy metals in the natural environment due to the formation of complexes that can modify the mobility of these ions (Christman and Christman, 1983; Stevenson and Fitch, 1986; Yin *et al.*, 1997). Among these, binding reaction to soil organic matter and, especially, to its humified fractions, for example humic acids (HAs), are known to play a key role (Sauve *et al.*, 2000; Tipping, 2002; Senesi and Leffredo, 2005). The capacity of humic substances to interact with metals is attributed to their high content of oxygen-containing functional groups, such as carboxyl, hydroxyl and carbonyl. The stable association of metals with humic substances may have significant influence on their mobilization in the soil. Varying contents of humic substances with different stability of their complexes with metals may result in varying capacity for metal sorption onto soil (Terbouche *et al.*, 2010). Soil organic matter has been of particular interest in studies of heavy metal retention in soils due to the tendency of transition metals to form stable complex with organic ligands (Chen, 1996). Contrasting results were found on the effect of OM on the availability of metals to plants. Natural soil OM (Romney *et al.*, 1977) and OM added to contaminated soils during remediation (Marquenie *et al.*, 1981) both decreased Zn availability to plants. Shuman (1999) found that organic amendments including an humic acid (HA) can lower the potential availability of metals by a redistribution into less available forms. Conversely, White and Chaney (1980) reported that organic matter was more effective in remediating the effects of toxic metals through plant uptake. Yuan and Lavkulich (1997) showed that the adsorption capacity of a soil for Zn was reduced by 72% when 11% of the organic C content was lost. Temminghoff *et al.* (1997) reported that dissolved organic carbon could effectively complex Cu from contaminated soils and the extent of complexation depended on soil pH.

Heavy metals such as Pb, Hg, Zn, Ni, Cu, Cr, and Cd, are often found in soils in high concentrations as a result of improper waste disposal practices. Heavy metals occur in soils in various forms and their retention has been generally found to increase with increase in soil pH, cation-exchange capacity, organic matter, clay content, and the metal oxide content of a soil. Awareness of the hazardous impact of heavy metal pollution led to the development of various pollution abatement measures. Thus, development of effective strategies aimed at preventing/ minimizing further addition of heavy metals into the environment and also at removal of those already polluting the environment become desirable.

Various soil remediation methods such as excavation, immobilization, electrokinetic, and phytoremediation etc. have been developed and used to remove metal ions from contaminated soils. Soil washing and flushing with various chelating agents has recently become a popular technique for remediating sites contaminated with heavy metals. Among various chelating agents, organic acids, have previously been shown as most suitable candidate for extracting metal ions from contaminated soils. Humic substances have shown tremendous capability of accumulating different metal ions from single-solute pure metal solutions.

Consequently, the aim of the present study was to compare the efficiency of different synthetic (salt solutions, mineral acid, chelating agents EDTA, DTPA) and natural (soluble humic and fulvic acids) extracting agents to extract Pb, Cd, Cr, Cu, Ni and Zn from strongly polluted acidic, calcareous and saline soils.

3. MATERIALS AND METHODS

The present research was carried out to evaluate different chemical technologies for remediation of metal contaminated soils in Bangladesh. The experiments were conducted in the Department of Soil, Water and Environment, University of Dhaka and Soil and Environment section, Biological Research Division, BCSIR Laboratories Dhaka during November 2012 to May 2017. For this study the followings steps were followed:

1. Selection and characterization of metal contaminated sites.
2. Categorization of metal contaminated soils according to the level and distribution of heavy metal.
3. Fractionation of heavy metals retained by different soil fractions.
4. Use of different chemical extractants for the remediation of metal contaminated soils.
5. Use of some natural extracting agent (humic substances) for metal removal from soil.
6. Observation of the combined effects of synthetic and natural extracting agents for the reclamation of metal contaminated soils.
7. Determination of the mobility and bioavailability of metals from remediated soil to plant using pot culture experiment.

3.1. Metal contaminated sites

A survey oriented work was carried out to find metal contaminated soils in Bangladesh. Location map, contaminated area, sampling point and other spatial data were done by GPS and ArcGIS 9.3.1 software. Samples were collected specially from different industrial areas according to types and level of metal contamination. Six representative sampling sites were selected (Table 3.1).

Table 3.1 Sampling sites of different contaminated areas.

SI No	Sampling Site	Designated Symbol
1.	Battery Industry, Madhupur Tract, Gazipur	MB
2.	Battery Industry, Ishurdi EPZ	IB
3.	Tannery Industrial area, Hazaribagh, Dhaka	HT
4.	Ship breaking yard, Sonaichhari, Shitakunda	SSy
5.	Cable and Metal Industries, Kushtia	KC
6.	Cable Industry, Chittagong	CC

3.1.1 Battery Industries, Zirani Bazar, Gazipur

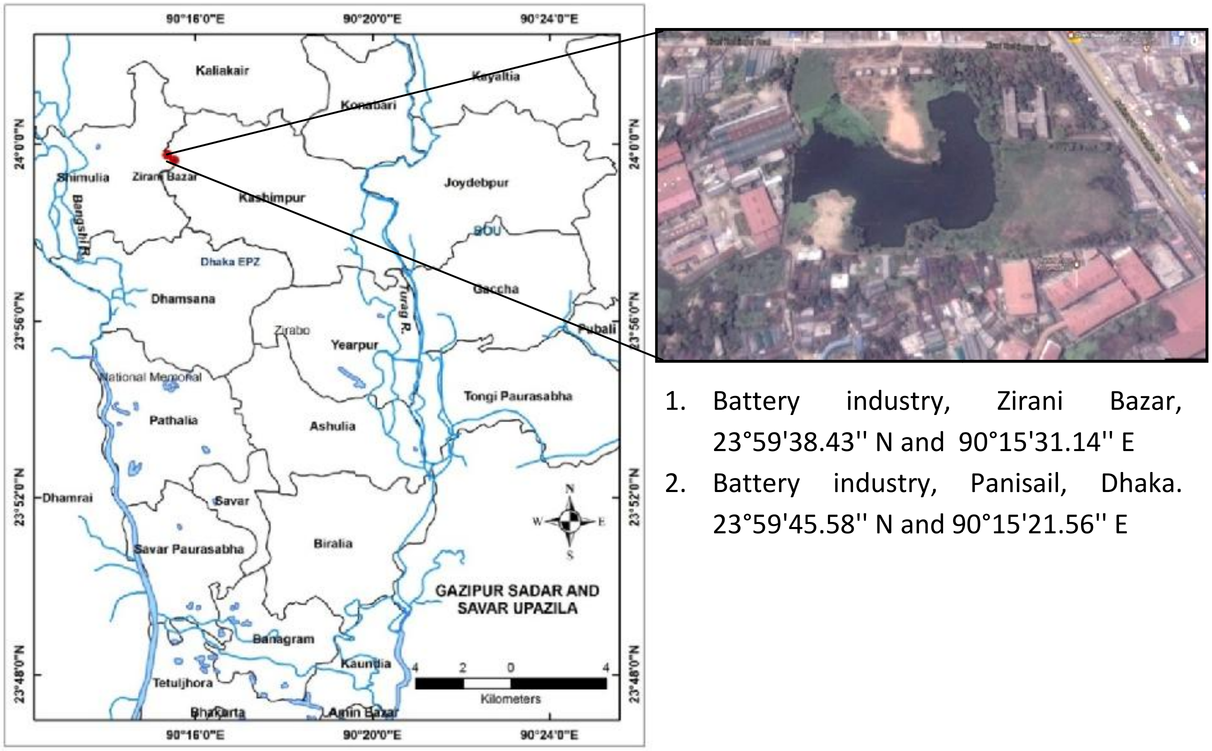
Two battery industries are located within 350 meter distance in Zirani bazar, Kashimpur Union, Gazipur Sadar Upazila. Samples were collected from the adjoining area of the battery industries. The soil is designated as MB soil in the text. The parent material of the soil is Madhupur clay of Pleistocene age, in general reddish brown in colour. Soils are acidic in reaction with low status of organic matter, low moisture holding capacity and low fertility level (Rahman *et al.*, 2012). The GPS-GIS based location map of the sampling site is shown in the Figure 3.1. The hydrology of the area is governed by the subtropical monsoon climate of Bangladesh with wide seasonal variations in rainfall, high temperatures and humidity (Banglapedia). The sampling areas of Madhupur tract soil are belonging to the Kashimpur soil series. A brief description of the Kashimpur soil series is given below:

Kashimpur Soil Series includes well drained soils developed in deeply weathered Madhupur clay on high terrace edges of the Madhupur tract. They occupy level to very gently sloping topography. They have red, friable, blocky clay subsoil which is distinctly mottled dark reddish brown and often black. This grades downward into a reticulately mottled red, light brown and black, friable clay substrutum which continues to great depth (Rahman, 2005). The general features of the soil series with classification are presented in the Table 3.2.

Table 3.2. General features of soil series with soil classification system.

Sample ID	Soil Series	Location	USDA Soil Taxonomy	Broad Physiography
MB	Kashimpur	Kashimpur Union, Gazipur Sadar Upazila	Typic Dystrudepts	Terrace (Madhupur tract)
IB	Manikdi	Pakshi Union, Ishwardi Upazila	Aeric Udorthents; Some are Typic Endoaquepts	Ganges River Floodplain
HT	Pagla	Sakta Union, Keraniganj Upazila	Typic Endoaquepts	Old Brahmaputra River Floodplain
SSy	Kumira	Sonaichhari Union, Sitakunda Upazila	Aeric Halaquepts; some are Typic Endoaquepts	Coastal Tidal Floodplain
KC	Gopalpur	Jagati Union, Kushtia Sadar Upazila	Aeric Endoaquepts	Ganges River Floodplain
CC	Patenga	Patenga Thana, Chittagong Metropolitan City	Aeric Endoaquepts; some are Aeric Halaquepts	Coastal Tidal Floodplain

(Rahman, 2005)



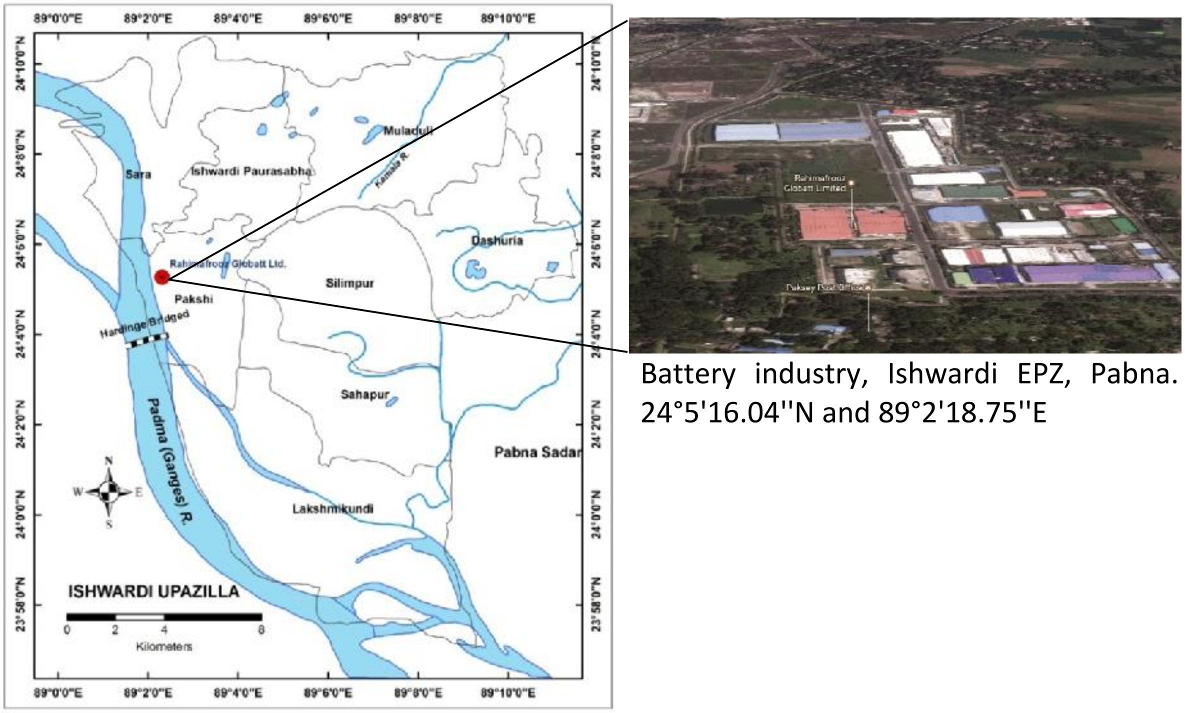
1. Battery industry, Zirani Bazar, 23°59'38.43'' N and 90°15'31.14'' E
2. Battery industry, Panisail, Dhaka. 23°59'45.58'' N and 90°15'21.56'' E

Fig. 3.1. Sampling area and satellite image of battery industries, Zirani bazar, Gazipur.

3.1.2. Battery Industry, Ishwardi

The battery industry located in Ishwardi EPZ, Pabna which was 900 meter away from Padma river. Soil samples were collected adjacent to battery industry. The soil is designated as IB soil in the text. This region occupies unstable alluvial land within and adjoining Ganges river. The area has complex mixtures of calcareous sandy, silty and clayey alluvium. The GPS-GIS based location map of the sampling site is shown in the Figure 3.2. The soils are belonging to the Manikdi soil series. A brief description of the Manikdi soil series is given below:

Manikdi series includes poorly drained, seasonally shallowly flooded soils developed in medium textured alluvium in the active and very young Ganges floodplain. They are olive-brown to olive-grey, stratified fine sandy barns and silt barns, overlying a stratified, usually medium to moderately fine textured substratum. They are calcareous throughout the profile (Rahman, 2005). General features of Manikdi soil series are given in Table 3.2.



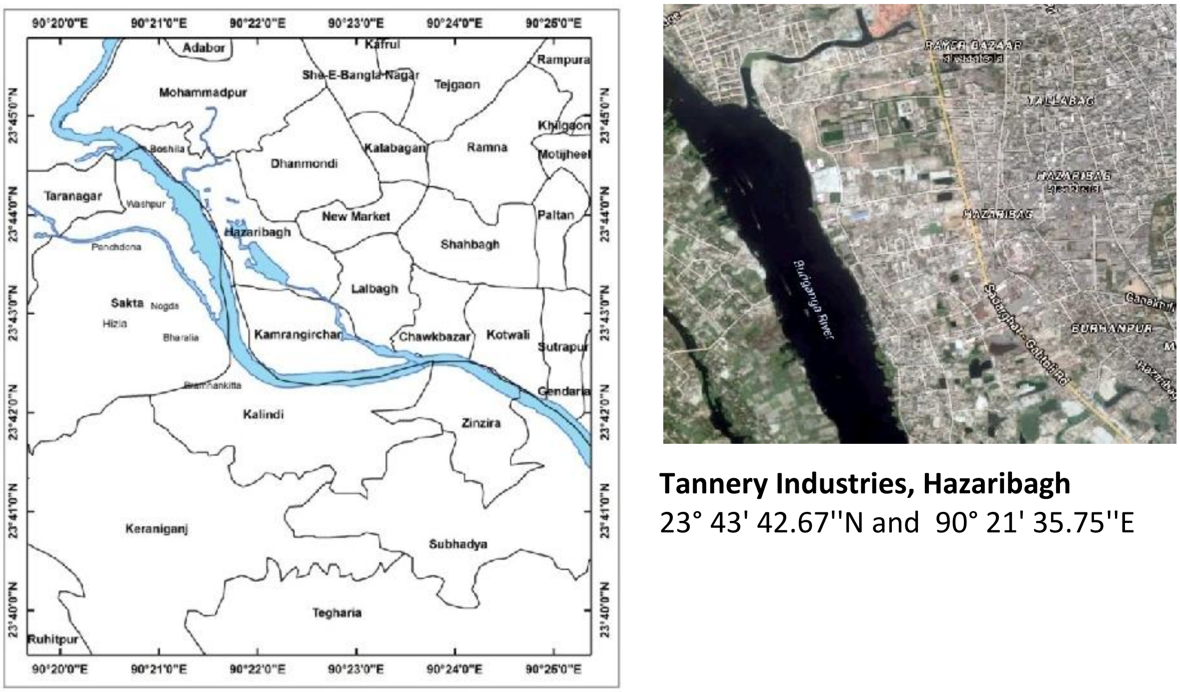
Battery industry, Ishwardi EPZ, Pabna. 24°5'16.04''N and 89°2'18.75''E

Fig. 3.2. Sampling area and satellite image of battery industry, Pakshi, Ishwardi.

3.1.3. Tannery Industrial area, Hazaribagh, Dhaka

Tannery industries were located in Hazaribagh Thana of Dhaka Metropolitan City along the Buriganga river. Soils were collected from the surrounding areas of the tannery industries. The soil is designated as HT soil in the text. The region comprises the area of Brahmaputra sediments. It has a complex relief of broad and narrow ridges, inter-ridge depressions, partially in filled cut-off channels and basin. This area is occupied by permeable silt loam soils on the ridges and impermeable clays in the basins, neutral to slightly acid in reaction. General soil types include predominantly grey floodplain soils. Organic matter content is low in ridges and moderate in basins. Soils are deficient in N, P, and S but the status of K and Zn are reasonable. The GPS-GIS based location map of the sampling site is shown in the Figure 3.3. The soils are belonging to the Pagla soil series. A brief description of the Pagla soil series is given below:

Pagla soil series comprises seasonally deeply flooded, poorly drained soils developed in fine textured alluvium on ridges on the young floodplain. They are grey, very firm, angular blocky clays with grey to dark grey coatings. No phases have been recorded (Rahman, 2005). General features of Pagla soil series with soil classification system are given in Table 3.2.



Tannery Industries, Hazaribagh
23° 43' 42.67''N and 90° 21' 35.75''E

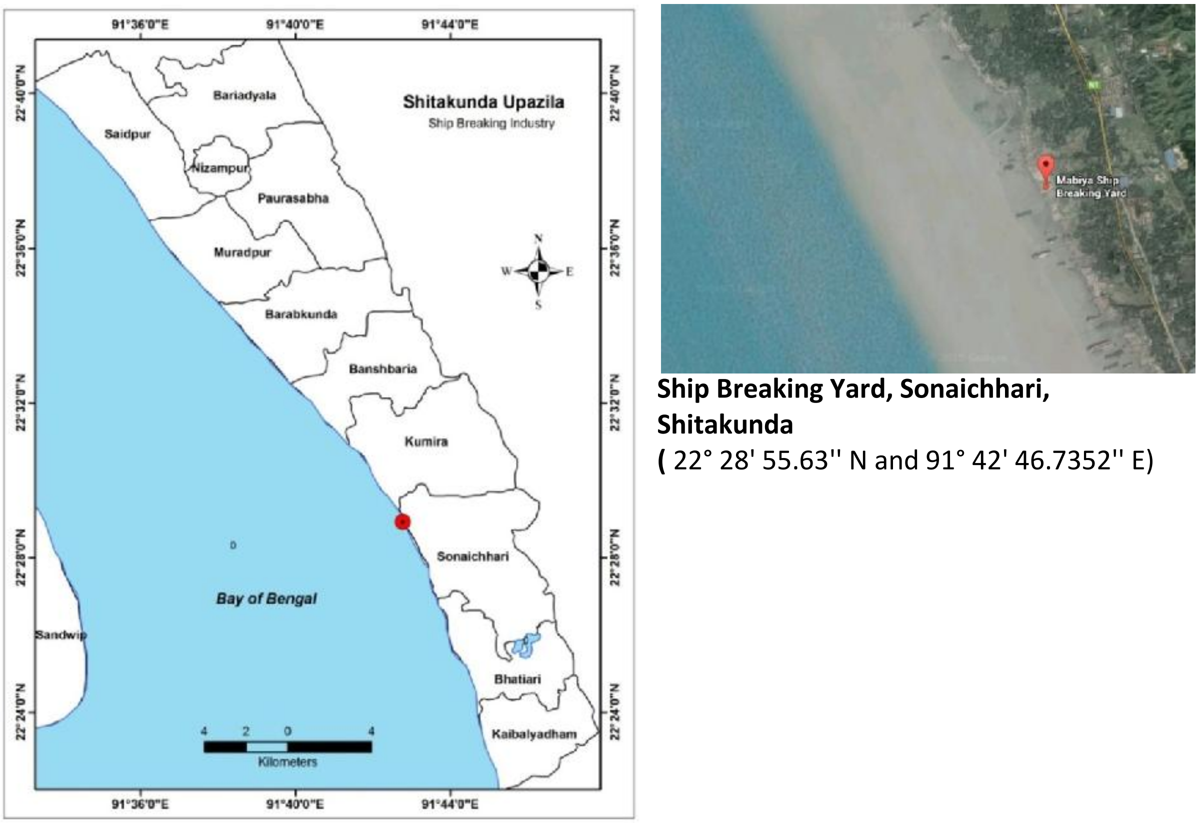
Fig. 3.3. Sampling area and satellite image in Tannery industrial area, Hazaribagh, Dhaka.

3.1.4. Ship breaking yard, Sonaichhari, Shitakunda

The selected ship breaking yard is located in Sonaichhari Union, Sitakunda Upazila, Chittagong along the Bay of Bengal. Soil samples were collected in and around the ship breaking yard. The soil is designated as SSy soil in the text. The GPS-GIS based location map of the sampling site is shown in the Figure 3.4.

This region occupies the plain land in Chittagong coastal plain. It is a compound unit of piedmont, tidal and estuarine floodplain landscapes. The major problem in these soils is high salinity during the dry season (October to May). General fertility level of the soils is medium, but N and K are limiting. Organic matter content is low to moderate. The soil is belonging to the Kumira soil series. A brief description of the Kumira soil series is given below:

Kumira series comprises seasonally flooded by rain-water, poorly drained, moderately fine textured soils developed in recent tidal sediments of coastal tidal plain. They occupy nearly level low ridges and shallow basins. They have a grey to olive-gray silt loam with moderate to strong prismatic and angular blocky structure in the B horizon. They have five phases: Non-saline, slightly saline, moderately saline, flood hazard and erosion hazard (Rahman, 2005). General features of Kumira soil series with soil classification system are given in Table 3.2.



Ship Breaking Yard, Sonaichhari, Shitakunda
(22° 28' 55.63'' N and 91° 42' 46.7352'' E)

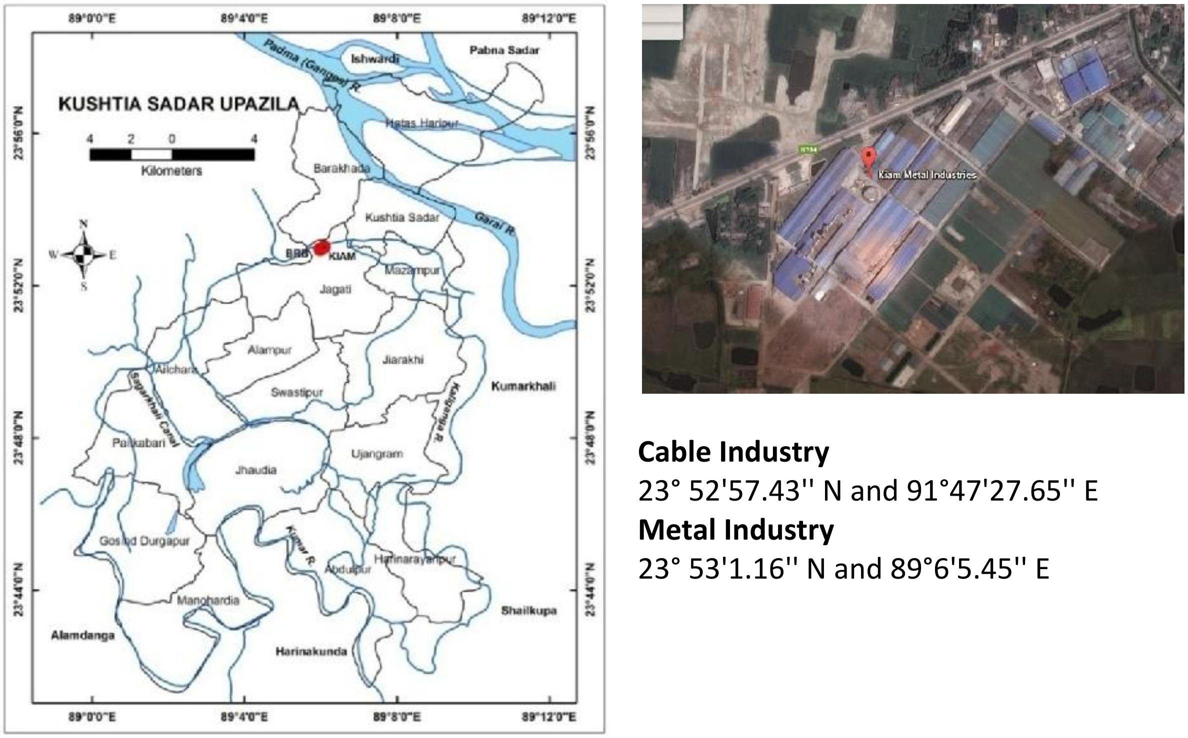
Fig. 3.4. Sampling area and satellite image of Ship breaking yard, Sonaichhari, Shitakunda.

3.1.5. Cable and Metal Industrial area of Kushtia

The cable and metal industries are located in Jagati Union, Kushtia Sadar Upazila. Soils were collected from surrounding areas of these industries. The GPS-GIS based location map of the sampling site is shown in the Figure 3.5. The soil is designated as KC soil in the text (Table 3.1).

This region includes the western part of the Ganges river floodplain which is predominantly highland and medium highland. Most areas have a complex relief of broad and narrow ridges and inter-ridge depressions. The upper parts of high ridges stand above normal flood level. Lower parts of ridges and basin margins are seasonally shallowly flooded. General soil types predominantly include Calcareous Dark Grey Floodplain soils and Calcareous Brown Floodplain soils. Soils are alkaline in reaction. General fertility level is low. The soils are belonging to the Gopalpur soil series. A brief description of the Gopalpur soil series is given below:

Gopalpur series are developed on the summits to upper slopes of gently undulating ridges. They are intermittently flooded, moderately poor to poorly drained, olive to olive-brown, friable, calcareous, silty-clay barns with moderate coarse and medium subangular blocky structure in the B horizon. They have five phases: Highland, smooth relief; highland, irregular relief; medium highland, smooth relief; medium highland, irregular relief and medium lowland, flood hazard (Rahman, 2005). General features of Gopalpur soil series with soil classification system are given in Table 3.2.

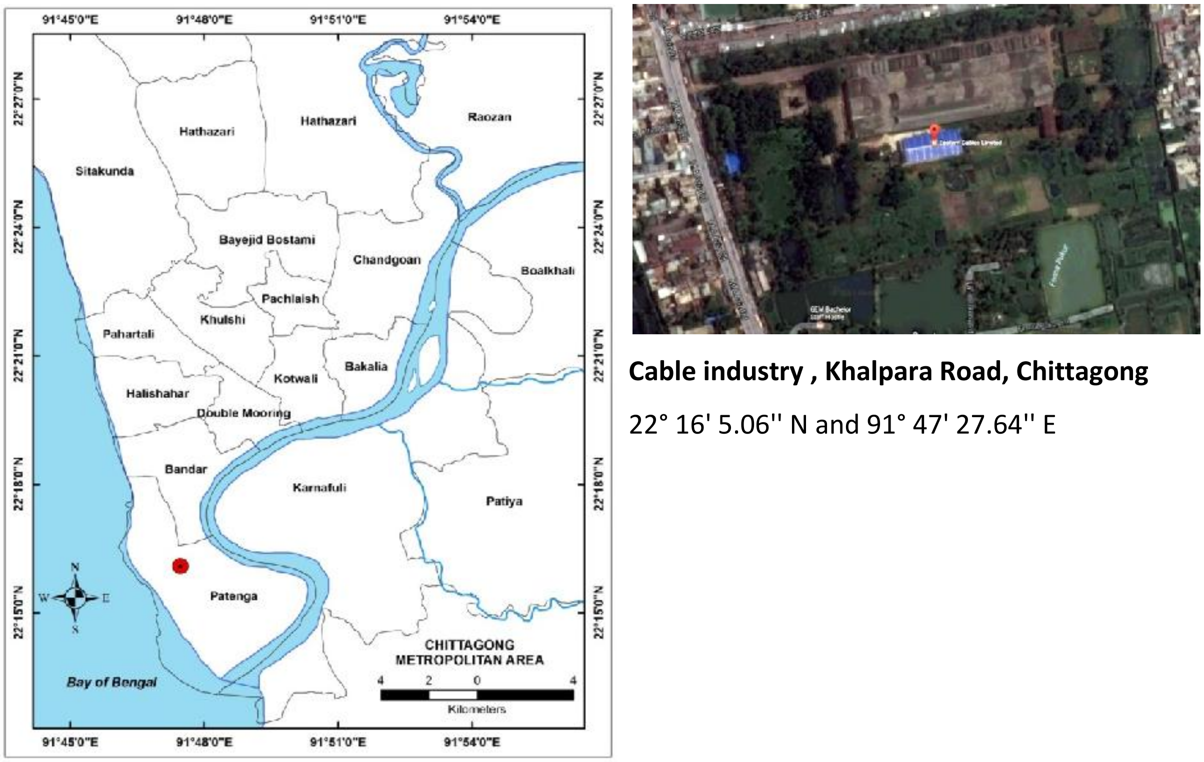


Cable Industry
23° 52'57.43'' N and 91°47'27.65'' E
Metal Industry
23° 53'1.16'' N and 89°6'5.45'' E

Fig. 3.5. Sampling area and satellite image of cable and metal industries, Kushtia.

3.1.6 Cable Industry, Chittagong

The Eastern Cable Industry is located in Patenga Thana, Chittagong Metropolitan City. Soils were collected from the surrounding areas of the metal industry. The soil is designated as CC soil in the text. The GPS-GIS based location map of the sampling site is shown in the Figure 3.6.



Cable industry , Khalpara Road, Chittagong

22° 16' 5.06'' N and 91° 47' 27.64'' E

Fig. 3.6. Sampling area and satellite image of cable industry, Chittagong.

The soils are belonging to the Patenga soil series. A brief description of the Patenga soil series is given below:

Patenga series comprises shallowly flooded, poorly drained, medium textured ridge soils developed in recent tidal deposits of the Chittagong coastal tidal floodplain. They have olive-grey silt loam subsoil with weak to moderate prismatic and angular blocky structure. Texture of the substrutum varies from silt loam to silty clay loam and occassionally stratified silt. They have four phases Non-saline flood hazard; slightly-saline, flood hazard; slightly saline, river erosion hazard; and moderately saline (Rahman, 2005). General features of Patenga soil series with soil classification system are given in Table 3.2.

3.2 Collection of soil samples

Soil samples were collected from the contaminated sites which are exposed to the different degrees of environmental pollution from industrial units. Soil samples were collected using an augar. The number of sampling points was selected according to the extension of contaminated area. The locations of sampling points were recorded with the help of a GPS. The locations are presented in map using ArcGIS 9.3.1 software. After collection of soil samples, each sample was taken in polyethylene plastic bags. Appropriate label with the name of the site of sample collection, depth and date were put both inside and outside the bags and the bags were tied securely with proper tag. The samples were carefully brought to the laboratory of Department of Soil, Water and Environment, University of Dhaka and Soil and Environment Section, Biological Research Division, BCSIR Laboratories, Dhaka for detail analysis.

3.3 Preparation of soil samples

The collected soil samples were dried in air by spreading in a thin layer on a clean piece of paper after being transported to the laboratory. Visible roots and debris were removed from the samples and discarded. To hasten the drying process, the samples were exposed to sunlight. After air-drying, a portion of the sample containing the larger aggregates was ground by gentle crushing with a wooden hammer. Ground samples were passed through a 2 mm sieve. The sieved samples were then mixed thoroughly and stored in labeled plastic containers until required for various physical, chemical and physicochemical analyses. Another portion of the soil sample (2 mm sieved) was further ground and passed through a 0.5 mm sieve. The sieved sample were mixed thoroughly and stored as above until required for chemical analyses.

3.4 Peat soil as a source of humic substances

Peat-basin soils occupy perennially wet basin or valley sites which are mainly deeply flooded in the rainy season. They occur extensively in the Gopalgonj-Khulna bills (Rahman, 2005). Gopalganj-Khulna Beel (2,247 sq km) region occupies extensive low-lying areas between the Ganges River Floodplain and the Ganges tidal floodplain. Soils of the area are grey, and dark grey, acidic, heavy clays overlay peat or muck at 25 - 100 cm. General soil types include mainly peat and Non-calcareous Dark Grey Floodplain soils (Banglapedia).

3.4.1 Collection of peat samples

Peat soils were collected from Khulna University campus (89°23’52.8”E & 22°52’8.4”N) and Gopalganj basin (89°54’3.6”E & 23°11’20.4”N). The GPS-GIS based location map of the sampling site is shown in the Figure 3.7. The soils of Khulna and Gopalgang regions are belonging to the Harta and Satla soil series, respectively. General features are presented in Table 3.7 and general description of the Harta and Satla soil series is given below.

Table 3.3. General features of Harta and Satla soil series with soil classification system.

Soil Series	Location	USDA Soil Taxonomy	General soil types
Harta Series	Khulna University	Typic Haplofibrists	Peat Basin
Satla Series	Gopalganj Basin	Typic Haplohemists	Peat Basin

Harta series includes seasonally flooded, poorly to very poorly drained, strongly gleyed which below a depth of 10-20 inches have a buried organic layer of 35 inches or more. Topsoil varies from clay to mucky clay in texture. Those soils usually remain waterlogged for 8 - 10 months in 6 - 10 feet deep water. They have two phases: Poorly drained; and very poorly drained (Rahman, 2005).

Satla series includes seasonally to almost perennially flooded, poorly to very poorly drained, very dark grey or black to very dark greyish brown, organic soils below the topsoil. These soils are developed in organic material derived from decomposed swamp grasses and reeds in the peat basin areas occupying low-lying basin depressions of Khulna-

Gopalgonj Bill areas. They have two phases poorly drained and very poorly drained (Rahman, 2005).

Collected samples were kept in paper bags with appropriate label and the bags were tied securely with tag. The samples were carefully brought to the laboratory of Department of Soil, Water and Environment, University of Dhaka and BCSIR for detail analysis.

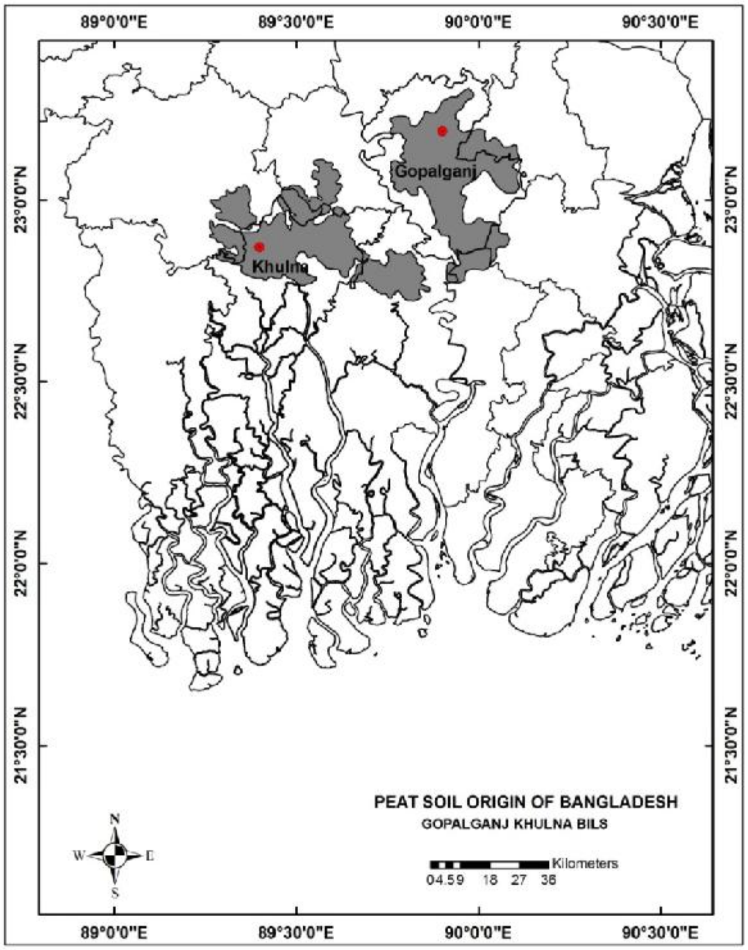


Fig. 3.7. Peat soil distribution in Khulna and Gopalganj district.

3.4.2. Preparation of peat samples

Peat soils were spread on separate sheet of thick coarse brown paper and dried in air. The air dried sample was oven dried and ground with morter and pestle. The ground sample was passed through a 0.5 mm sieve. The sieved samples were mixed thoroughly for making composite samples and preserved in the same way as was done for soil samples.

3.5. Incubation and leaching experiment

In order to determine the remediation techniques for metal contaminated soils, incubation studies were carried out in the laboratory of Department of Soil, Water and Environment, University of Dhaka and BCSIR Laboratories, Dhaka.

3.5.1. Incubation of soil samples

For the incubation study, 20 gm of 2 mm sieved air dried non-contaminated soil sample was taken in a beaker. The soil was artificially spiked with six metals: Pb, Cd, Cr, Ni, Cu and Zn. The metal salts used as sources of heavy metals are presented in Table 3.4.

Table 3.4. List of salts as source of heavy metals to prepare spiked soil.

Sl No	Heavy metals	Salts
01	Lead (Pb)	Lead Nitrate, $Pb(NO_3)_2$
02	Cadmium (Cd)	Cadmium Nitrate, $Cd(NO_3)_2.4H_2O$
03	Chromium (Cr)	Chromium Nitrate, $Cr(NO_3)_3.9H_2O$
04	Nickel (Ni)	Nickel Nitrate, $Ni(NO_3)_2.6H_20$
05	Copper (Cu)	Copper Sulfate, $CuSO_4.5H_2O$
06	Zinc (Zn)	Zinc Nitrate, $Zn(NO_3)_2.6H_2O$

Salt solutions were applied into soil to reach each metal concentration approximately 100 mg kg^{-1}. The pH of salt solutions was maintained at the pH levels of soils. During incubation soil was covered by parafilm to reduce moisture loss and moisture content was maintained at field capacity. Then soil containing beakers were kept for 2 weeks (after spiking) for partitioning of metals in different soil constituents. A control treatment (without adding metal salt solution) was also included. All the treatments were replicated thrice.

3.5.2 Soil washing experiment

In order to assess whether it is possible to remediate a metal contaminated soil using different extractant, *in situ* soil washing experiment was conducted. For conducting these experiments, incubated spiked soil samples were used. Then a leaching experiment was set up with different extracting agent. The incubation study and column leaching experiments was conducted in Soil and Environment Research Division, BCSIR Laboratories, Dhaka.

3.5.3 Soil washing through column leaching technique

The soil washing experiment was conducted in a column leaching process using different extractants. For this experiment, a leaching tube of 25 cm height was used. Twenty gram of spiked soil sample was kept in the leaching tube. Then successive leaching was conducted by different extractants. Each time 100 ml of liquid solution was used to leach the soil as shown in Figure 3.8. When the leaching of liquid through the soil was completed, the leachate was collected and digested to determine the metal concentration in the leachate by using Atomic Absorption Spectrophotometer (Shimadzu AA7000) following nitric acid (HNO_3) digestion (Hseu, 2004). The removal efficiency of heavy metals from each soil sample was then calculated based on its initial concentration of metal in the soil prior to leaching and its final concentration of the same metal in the leachate after column leaching experiment. All of the soil washing experiments was conducted in triplicate.

The percent of each metal removed/leached was calculated using an equation followed by Reddy and Chinthamreddy (2000) as:

Percent metal removed/leached (%) = $\frac{C_l V_l}{C_S m_s} \times 100$

Where C_l and Cs are the concentrations of metal in leachate (in mg/L) and soil (mg/kg), respectively; V_l is the volume of leachate (in L) and m_s is the dry mass of the soil (in kg).

Fig. 3.8. Soil washing through column leaching technique with different extractants.

3.6 Extraction of heavy metals by different extractants

The extractability of heavy metals from contaminated soils was compared by different single extraction and sequential extraction procedures. Single and sequential extractions of the contaminated, uncontaminated and spiked soils were done with different doses of extractants.

3.6.1 Single extraction

Eight different extractants were used for extraction of the heavy metals from different contaminated soils which are presented in Table 3.5. For extraction distilled water, mild acid solution, monovalent salt solution, divalent salt solution, two synthetic chelating agents (EDTA, DTPA) and two natural chelating agents (humic acid and fulvic acid) were used. Required amount of the extractant were prepared and specific ratio of soil to extractant was followed for each extractant. Extraction of contaminated soils with each single extractant was performed in triplicate. Metals present in extracts were determined by atomic absorption spectrometer (AAS).

Table 3.5. Single extraction procedures used for heavy metal removal from soil.

Extractant	**Pool or Phase extracted**	**Reference**
Water	Water soluble	Svete *et al.* 2000
0.1 M HCl	Weak acid extractable	CSTPA 1980
1 M Ammonium Acetate	Exchangeable, specifically sorbed and carbonate bound	Salbu *et al.* (1998)
0.1 M $CaCl_2$	Neutral salt soluble/exchangeable	Smilde *et al.* 1992
0.005 M DTPA	Chelating extractable	Wang *et al.* 2006, Gupta and Sinha 2006a
0.1 M EDTA	Chelating extractable	Lindsay and Norvell 1978
Humic acid	Natural Chelating extractable	--
Fulvic acid	Natural Chelating extractable	--

3.6.2 Sequential extraction

The technique which was successfully used to extract trace elements from various fractions of soils is as described by Tessier *et al.* (1979). Through this technique, elements are fractionated into water soluble (F1), exchangeable (F2), carbonate (F3), Fe + Mn oxides (F4), organic (F5) and residual (F6) fractions of the soil (Harrison, 1981). The water soluble, exchangeable, carbonate, oxide and organic fractions are also known as non-residual fraction and high percentage of recovery of elements from any of this fraction shows their high mobility in soils and availability to plants (Stephen *et al.*, 2008) whereas other fraction such as residual is considered to be highly immobile and high percentage of recovery of elements in this fraction represents low mobility and unavailable to plants (Singh and Singh., 1966; Clevenger and Mullins., 1982). Elements mobility in a soil was determined from mobility factor which is calculated from the following equation as described by Kabala and Singh (2001).

$$\text{Mobility Index/ Mobility Factor} = \frac{(F1+F2+F3)}{(F1+F2+F3+F4+F5+F6)} \times 100$$

The value of mobility index for elements provides information about their potential mobility in soils and availability to plants (Yusuf, 2007). The value of MF up to 10% for any element indicates that this element is immobile and unavailable for plants (Torri and Lavado, 2008). In addition to that, the value of mobility index from 10 to 50% or greater than 50 % for a particular element suggests that this element is highly mobile and available for plants (Ahumada *et al.*, 1999).

Although there are several sequential extraction procedure for heavy metals, the sequential extraction method of Salbu and his coworkers (Salbu *et al.*, 1998), modified from that of Tessier and his coworkers (Tessier *et al.*, 1979), was used to determine the solid-phase speciation of heavy metals Two grams of 2 mm sieved soil sample was placed in a 50 mL polycarbonate centrifuge tube and following extractions were performed sequentially. The extracted heavy metals were determined by Atomic Absorption Spectrophotometer (Shimadzu AA7000). The summary of the sequential extraction procedures used in this study are presented in Table 3.6. All tests were performed in triplicates and the results were presented as averages of the triplicate extracts.

Table 3.6. Sequential extraction scheme for determination of heavy metal speciation

Step	Fraction	Extractant	Reaction time	Device	Centrifuge/ Filtrate
1	F1: Water soluble	20 ml deionized water	1 h in 20^{0}C	Rolling table	10,000 rpm in 30 min
2	F2: Exchangeable	20 ml 1 M NH_4OAc (pH 7)	2 h in 20^0 C	Rolling table	10,000 rpm in 30 min
3	F3: Carbonate bound	20 ml 1 M NH_4OAc (pH 5)	2 h in 20^0 C	Rolling table	10,000 rpm in 30 min
4	F4: Fe and Mn oxide bound	20 ml 0.04 M $NH_2OH.HCl$ in 25% (v/v) Acetic acid (pH 3)	6 h in 80^0 C	Shaking water bath	10,000 rpm in 30 min
5	F5: Organically bound	15 ml 30% H_2O_2 (adj. pH 2), 5 ml 3.2 M NH_4OAc in 20% (v/v) HNO_3	5.5 h in 80^0 C, 0.5 h in 20^{0}C	Shaking water bath and Rolling table	10,000 rpm in 30 min
6	F6: Residual	7M HNO_3	6 h in 80^{0}C	Shaking water bath	Filtrate

Source: Salbu et al. (1998) modified from that of Tessier et al. (1979)

3.7 Extraction of heavy metals by natural chelating materials (humic substances)

Results from several studies indicated that humic substances (HS) form soluble complexes with many heavy metals. Humic substances have carboxylic, phenolic-OH and aliphatic-OH groups, which are the active groups forming soluble complexes with the heavy metals. As a natural source, humic substances (humic acid and fulvic acid) were employed for remediation of metal contaminated soils (Borggaard *et al.*, 2009).

Humic substances (humic and fulvic acid) were extracted from peat soils. These humic and fulvic acids were applied on spiked soils of six different regions to determine their metal extraction capacities from different types of soils. Different concentrations of humic substances were used for this experiment. The concentrations of humic and fulvic acids were gradually increased from 0.1 to 0.5 % and each time 100 ml of extractant was used for 20 gm spiked soil samples. A leaching tube of 25 cm height was used for this leacing experiment. Every extraction was done in triplicates. The leachates of humic substances were digested in microwave digester (Model: StartD, Minestone) using concentrated HNO_3 acid and H_2O_2 (Sastre *et al.*, 2002). The metal contents of the digest were determined using Atomic Absorption Spectrophotometer.

3.8 Combined natural and synthetic chelating materials for remediation of metal contaminated soils

Humic substances as natural chelator extracted metals from all soils. But humic substances may have some limitation. Humic substances in general extracted comparatively lower amount of metals from soils than that of synthetic chelating materials (EDTA). To improve the removal efficiency of metal from soils EDTA was combined with humic substances. Solutions of 0.3% or 0.4% of humic and fulvic acids were mixed with different concentrations of EDTA ranging from 0.02 to 0.1 M. The lists of combined extractants are given in Table 3.7 and 3.8. These combined HA + EDTA and FA + EDTA extractants were used for metal removal from spiked soils of different regions. The extractabilities of HA and FA were also compared with EDTA and combined extractants. Based on the preliminary experimental results the concentrations of humic and fulvic acids were selected for different soils used in these experiments. Maximum removals of metals were obtained with these concentrations.

Table 3.7. Combination of extractants consisting of humic acid and EDTA.

SI No	Combined extractant	
	For soils of Madhupur, Ishwardi and Kushtia	**For soils of Hazaribagh, Shitakunda and Chittagong**
01	0.3% Humic acid + 0.02 M EDTA	0.4% Humic acid + 0.02 M EDTA
02	0.3% Humic acid + 0.04 M EDTA	0.4% Humic acid + 0.04 M EDTA
03	0.3% Humic acid + 0.06 M EDTA	0.4% Humic acid + 0.06 M EDTA
04	0.3% Humic acid + 0.08 M EDTA	0.4% Humic acid + 0.08 M EDTA
05	0.3% Humic acid + 0.10 M EDTA	0.4% Humic acid + 0.10 M EDTA

Table 3.8. Combination of extractants consisting of fulvic acid and EDTA.

SI No.	Combined extractant for all soils
01	0.4% Fulvic acid + 0.02 M EDTA
02	0.4% Fulvic acid + 0.04 M EDTA
03	0.4% Fulvic acid + 0.06 M EDTA
04	0.4% Fulvic acid + 0.08 M EDTA
05	0.4% Fulvic acid + 0.10 M EDTA

The extractions of spiked soils of six selected regions by these combined extractants were performed through column leaching technique as mentioned in the section 3.5.3. Every treatment was practiced in triplicates. The leachates of extractants were digested in microwave digester (Model: StartD, Minestone) using concentrated HNO_3 acid and H_2O_2 (Sastre *et al.*, 2002). The metal contents of the digest were determined using Atomic Absorption Spectrophotometer.

3.9 Pot experiment

In order to study the mobility and bioavailability of metals from soil to plant, pot experiments were conducted using two crops. Pot experiments were performed with six different contaminated soils. Each contaminated soil was leached with different extractants at several times until very few or no metals were detected in leachate. The extractants humic acid, fulvic acid, EDTA and combined HA + EDTA and FA + EDTA were used for metal removal from soils. The experiment was setup in the net house of BCSIR. Heavy metal content of each soil was taken into consideration for particular area.

3.9.1 Sample collection and preparation

The bulk soil samples were collected for pot experiment. Samples were collected from six selected metal contaminated areas. Soils were air dried, cleared of debris and crushed to reduce the size of large clods. The crushed soil samples were screened through a 4 mm sieve.

3.9.2 Setup of experiment

For the pot culture experiment, an upland vegetable crop, commonly known as Red Amaranth (*Amaranthus gangeticus*) and a low land crop, Rice (*Oryza sativa*) - BRRI dhan 49 were used.

Soil types: Pot culture experiments were carried out with six contaminated soils viz.

1. Soil of Madhupur tract having battery industry.
2. Soil of Ishwardi having battery industry.
3. Soil of tannery industrial area, Hazaribagh.
4. Soil of ship breaking yard, Shitakunda.
5. Soil of cable and metal industries, Kushtia
6. Soil of cable industry, Chittagong.

Treatments: Seven treatments were applied for each contaminated soil as follows:

1. Non contaminated soil
2. Contaminated soil without extraction.
3. Contaminated soil treated with humic acid
4. Contaminated soil treated with fulvic Acid
5. Contaminated soil treated with combined humic acid and EDTA extractant
6. Contaminated soil extracted with combined fulvic acid and EDTA extractant
7. Contaminated soil extracted with only EDTA

The pot experiments were carried out following completely randomized design with three replications of each treatment.

3.9.3 Pot preparation: Air-dried, roots and debris free 4-mm sieved soil samples were used for pot experiments. Pots were filled up with 5 kg and 8 kg of soil, which were used to grow red amaranth and rice, respectively. The recommended doses of N, P and K (as urea, TSP and MOP) were estimated for each soil and plant as per fertilizer recommendation guide (BARC, 2005).

Fig. 3.9. Application of humic acid, fulvic acid and mixed suitable extractants to contaminated soil.

3.9.4 Soil washing and leachate collection

Contaminated soils were washed with different extractants. At first 1 liter of each extractant was applied on particular pot and then 500 ml of extractant was poured at every leaching treatment. Leachates were collected after each extraction (Fig. 3.10 and 3.11). Soil washing experiments were performed until metal concentration in leachate was decreased to minimum level.

Fig 3.10. Collection of leachate after extraction with humic acid and combined extractants.

Fig 3.11. Collection of leachate after extraction with fulvic acid and combined extractant

3.9.5 Sowing of seeds of red amaranth: Seeds of red amaranth (*Amaranthus gangeticus)* were obtained from Bangladesh Agricultural Development Corporation (BADC). Approximately 20 - 25 seeds of red amaranth were sown in each pot. Seeds in each pot were allowed to germinate and after 1 week the seeds were started to germinate. After germination, 15 healthy seedlings were kept in each pot and were allowed to grow. The pots were arranged in the net-house in a completely randomized design.

Transplantation of seedling of rice: Rice seedlings of BRRIdhan 49 were collected from Bangladesh Rice Research Institute (BRRI). Puddling of the treated and untreated soils was done properly. A total of three hills, each containing 3 - 4 seedling were transplanted in each pot. The pots were arranged in the net-house in a completely randomized design.

3.9.6 Culture of plant

Plants received water every day (especially rice). Sometimes watering of the pots was made twice daily during heavy sunshine and warm weather. Tap water was used for this purpose. One third of urea and basal doses of other fertilizers were added before transplantation of seedling (Fig. 3.12). Next one third of urea fertilizer was applied during active tillering stage and rest one third of urea was applied just before panicle initiation stage of rice plant. Intercultural operations were made whenever necessary. Weeds were removed manually. Positions of the pots were changed every alternate day to allow equal exposure of each of the pots to sunlight. Adequate plant protection measures were taken during the growing period. Visual observation like general growth, deficiency or toxicity symptoms, chlorosis, necrosis, pest or insect infestations etc. were noted during the growth periods of the plants.

Fig. 3.12. Pot preparation and sowing of rice plant.

3.9.7 Collection and processing of harvested plant samples: At harvest, the plants and soils were collected from each pot and processed and prepared for chemical analysis.

***Red amaranthus*:** Plants were allowed to grow for 45 days after emergence of the seedlings and then sampling of the plants was done. The plants were harvested manually by uprooting them carefully from the pots. The agronomic data such as plant height, leaf length, root length, fresh weight of plant were recorded for each pot. The roots of the harvested plants were washed first with tap water and then three times with distilled water to remove ions and to dislodge any particles adhering to the root surface. Aerial parts of the plants were also washed in the similar way. The wet samples were dried using paper towels.

Fig. 3.13. Growing stage of red amaranth plant

The plant samples were separated into three parts: roots and edible parts (shoots and leaves). The samples were first air-dried and then oven-dried at 70°C ± 5°C for 48 hours and the dry weights of the plant samples were measured. The dried plant samples were then ground to pass through a 0.2 mm sieve. The ground plant samples were mixed thoroughly and stored in container for further chemical analyses. After collection of plant samples, the soils from each pot were collected and processed.

Rice: Plants were harvested 110 days after seedling transplantation. The plants were harvested manually by removing the upper portions of the plants with scissors and then collecting the roots from the soil. The grains were also collected. The agronomic data such as plant weight, plant height, panicle length, 100 grain weight and total grain weight were recorded. The rice plants were separated into three parts: roots, straw and grains. The plant samples were washed and processed using the same procedure as for the *Red amaranth* plants. Plant samples were ground and stored in container for further analysis. After plant harvest soil samples were collected from each pot and then processed and stored.

Fig. 3.14. Harvesting stage of rice plant (BRRI dhan 49)

3.10 Physical, chemical and physico-chemical properties of soil

Various physical, chemical and physic-chemical parameters of soil samples were analyzed in the laboratory. The following analyses were carried out.

3.10.1. Physical properties

3.10.1.1. Particle size analysis

The particle size analysis (PSA) of the soil was carried out by hydrometer method (Bouyoucos, 1927). The textural classes were determined by Marshall's triangular co-ordinates as devised by the United States Department of Agriculture (USDA, 1951).

3.10.1.2. Moisture content

The moisture content of soil sample was determined by gravimetric method (Black, 1965)

3.10.1.3. Electrical conductivity (EC)

The electric conductivity of soil was estimated from saturated soil paste by using conductivity meter (HACH Senion 156) (Rhoades *et al.,* 1989)

3.10.1.4. Clay minerals analysis

The clay minerals of the soil samples were determined and interpreted with standard XRD interpretation procedures using X-ray diffractometer (Empyrean, PANalytical) (Moore and Reynolds, 1997).

3.10.2 Physico-chemical properties

3.10.2.1 Cation exchange capacity

The cation exchange capacity (CEC) of calcareous and non-calcareous soils was determined by using sodium acetate and ammonium acetate as described in METHOD 9081 (Chapman, 1965).

3.10.3 Chemical properties

3.10.3.1 Soil pH

Soil pH was measured electrochemically by using a glass electrode pH meter (Jenway 3305). The soil-water ratio was 1:2.5 and time of shaking was 30 min as outlined by Jackson (1958).

3.10.3.2 Total organic carbon

Total organic carbon of the sample was determined by wet oxidation method (Walkley and Black, 1934). Organic matter was calculated by multiplying the percent value of organic carbon by conventional van Bemmelen's factor of 1.724.

3.10.3.3. Total nitrogen

For determination of total nitrogen, the samples were digested by Kjeldahl's method as described by Jackson (1958). The distillation of digested sample was done with 40 % NaOH and the distillates were collected on a 4 % Boric acid and mixed indicator solution. The distillate was titrated against standard H_2SO_4.

3.10.3.4. Total phosphorus

The total phosphorus content of the samples ($HNO_3 : HClO_4 = 2:1$ digested) was determined by vanadomolybdophosphoric yellow color method using a spectrophotometer (SPCORD 205 AnalytikJena, Germany) as described by Jackson (1958).

3.10.3.5. Total potassium

Total potassium content of the nitric-perchloric acid digested samples was measured by flame photometer (Model: Jenway, PFP7) at 769 nm wavelength (Jackson, 1967).

3.10.3.6. Total calcium, magnesium, iron and manganese

Total content of calcium, magnesium, iron and manganese in the nitric-perchloric acid digest (AOAC, 1990) were determined using an Atomic Absorption Spectrophotometer (AAS) (Model: AA-7000, Shimadzu) followed by APHA 3111, which was calibrated using certified reference material (CRMs) (APHA, 1998)

3.10.3.7. Carbonate content

The carbonate content of soil was determined by titrimetric method followed by Rowell (1994).

3.10.4 Determination of heavy metals

3.10.4.1 Metal contents in soil

The soil samples were digested with concentrated HNO_3 for the determination of metals (Hseu, 2004). Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Copper (Cu) and Zinc

(Zn) contents of the soil digest were determined by using Atomic Absorption Spectrophotometer (Shimadzu AA-7000).

3.10.4.2 Metal contents in plant material

Plant materials were digested with aqua regia acid mixture as described by Sastre *et al.* (2002). Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Copper (Cu) and Zinc (Zn) contents of the digest were determined by using Atomic Absorption Spectrophotometer (Shimadzu AA-7000).

3.10.4.3. Metals contents in leachate

The collected leachates were digested in a microwave digester (Model: StartD, Minestone) using conc. HNO_3 acid and H_2O_2 (Sastre *et al.*, 2002). The heavy metals of the digested samples were determined by using Flame and Graphite Furnace Atomic Absorption Spectrophotometer (Shimadzu AA 7000).

3.10.5. Fraction of metals in soils samples

The fractionation of metals in soil samples before and after leaching with different extractants and soil after plant harvest was done by sequential extraction procedures (Salbu *et al.*, 1998).

3.10.6. Quality control and quality assurance (QC/QA)

The quality control and quality assurance (QC/QA) of analysis were ensured by using a reference sample and a spiked sample for every ten analysis.

3.10.7. Calibration and validation

The equipment flame photometer, UV-Visible Spectrophotometer and Atomic absorption spectrophotometer were calibrated for every six months and methods of analysis were validated by Bangladesh Accreditation Board (BAB) as per ISO/IEC 17025.

3.10.8. Statistical Analysis

The recorded data of different parameters and agronomic data of crops were subjected to statistical analysis. The analysis of variance for soil physical and chemical parameters, heavy metals contents, crop yields and other agronomic data was performed following ANOVA technique and the data were analyzed by Duncan's Multiple Range Test (DMRT) using SPSS Statistical software (SPSS version 20).

3.11. Extraction, quantification and characterization of humic substances

3.11.1. Extraction and quantification of humic substances

Extraction of Humic Acid and Fulvic Acid was done according to new standard method of International Humic Substances Society (Lamar *et al.,* 2014) [Method description Appendix: 01]

3.11.2. Characterization of humic substances

The humic acids and fulvic acids were characterized by various chemical techniques:

3.11.2.1. Optical density and E4/E6 Ratio

The optical density of humic substances and E4/E6 ratio were determined as described by Chen *et al.* (1977). The absorbance was measured at 465 and 665 nm after dissolving 2 mg of freeze-dried humic substances in 25 ml of $NaHCO_3$ (0.025M).

3.11.2.2. Elemental Analysis

The C, H, N and S contents of HAs and FAs were determined by using a CHNS analyzer, Perkin-Elmer 2400. Total ash contents were determined by the combustion method. The contents of Fe, and Al were determined using Atomic Absorption Spectrophotometer. The oxygen content was calculated by subtracting the C, H, N, S, and ash contents from the total weight. $Si0_2$ content was calculated by subtracting the Fe_20_3 and Al_20_3 contents from the total ash content. (Huang *et al.*, 2006)

3.11.3. Quantification of functional groups of humic substances

3.11.3.1. Fourier-Transform Infrared (FTIR) Spectroscopy

Functional groups of humic and fulvic acid were determined by FTIR (Perkin Elmer 1600) spectrophotometer using Infrared spectra from 4000 to 400 cm^{-1} range, recorded at a rate of 16 nm/s (Huang *et al.*, 2006)

3.11.3.2 Potentiometric Titration

Total acidity was determined by the titration method of Martin *et al.* (1963) modified by Tsutsuki and Kuwatsuka (1978a). Carboxyl group content was determined by the calcium acetate method of Blom *et al.* (1957). Total hydroxyl group content was determined by the acetylation method of DeWalt and Glenn (1952), modified by Tsutsuki and Kuwatsuka (l978a). Phenolic hydroxyl group content was estimated by subtracting the carboxyl group content from the total acidity. Alcoholic hydroxyl group content was estimated by subtracting the phenolic hydroxyl group content from the total hydroxyl group content. Carbonyl group content was determined by the oximation method of Schnitzer and Riffaldi (1972).

4. RESULTS AND DISCUSSION

4.1 Characteristics of soils

General soil properties of the contaminated soils were determined following standard methods. The physical, chemicals and physicochemical properties of soils of six different regions are presented in Table 4.1.1.

Table 4.1.1: The physical, chemical and physiochemical properties of soils.

Sl No.	Soil Properties	Soil of different regions					
		MB	IB	HT	SSy	KC	CC
1.	**pH**	4.72	8.11	5.94	7.85	8.19	7.17
2.	**Organic carbon (%)**	0.61	0.33	1.73	1.61	0.56	0.71
3.	**Organic matter (%)**	1.04	0.57	2.89	2.77	0.96	1.22
4.	**Sand (%)**	16.3	13.8	34.8	45.3	15.5	22.5
5.	**Silt (%)**	47.4	65.0	46.4	39.4	68.1	51.2
6.	**Clay (%)**	36.3	21.2	18.8	15.3	16.4	26.3
7.	**Textural Class**	Silty Clay Loam	Silt Loam	Loam	Loam	Silt Loam	Silt Loam
8.	**CEC (C molKg^{-1})**	22.25	13.82	7.49	9.94	14.19	20.74
9.	**Conductivity (µS cm^{-1})**	993	1320	3635	14850	1355	6855
10.	**Carbonate content (as $CaCO_3$) (%)**	ND	4.728	ND	0.438	4.932	0.072
11.	**Total Nitrogen (%)**	0.126	0.140	0.154	0.098	0.182	0.294
12.	**Total Phosphorus (%)**	0.017	0.058	0.051	0.038	0.076	0.079
13.	**Total Potassium (%)**	0.61	0.55	0.34	0.32	0.69	0.51
14.	**Total Calcium (%)**	0.114	3.959	0.517	1.014	4.104	0.303
15.	**Total Magnesium (%)**	0.32	0.94	0.59	0.61	1.05	0.65
16.	**Total Iron (%)**	3.78	2.26	1.92	4.86	2.12	3.42
17.	**Total Manganese (mg kg^{-1})**	712.92	373.32	264.38	770.53	525.12	407.83

MB = Soil of Madhupur tract having battery industry, IB = Soil of Ishwardi EPZ having battery industry, HT = Soil of Hazaribagh tannery industrial area, SSy = Soil of ship breaking yard, Shitakunda, KC = Soil of Kushtia having cable and metal industry and CC = Soil of Chittagong coastal plain having cable industry.

4.1.1 Soil of Madhupur tract having battery industry (MB)

The soil of Madhupur tract was located near battery industry, Zirani bazar, Gazipur. The soil is acidic in nature (pH 4.72). The soil contained high clay and textural class was silty clay loam. The organic matter content was low, CEC 22.25 cmol Kg^{-1} and EC 993 µS cm^{-1} which indicated the non sanile nature of soil and carbonate was not found in soil. The soil of Madhupur tract contained high amount of Fe and Mn of 3.78% and 712.92 mg kg^{-1}, respectively (Table 4.1.1) which indicated the presence of high quantities of Fe/Mn oxide compounds.

The clay mineralogy of the soil sample revealed the presence of koalinite, mica, Illite and vermiculite (Fig. 4.1.2 and Table 4.1.2). White (1985) reported that 20% Kaolinite, 20-50% Halloysite, 20% mica and 30-40% vermiculite present in some soil series of Madhupur Clay soil.

4.1.2 Soil of Ishwardi EPZ having battery industry (IB)

The soil of Active Ganges River Floodplain is alkaline in nature having the pH value 8.11. The CEC and EC of soil were recorded 13.82 cmol kg^{-1} and 1320 µS cm^{-1}, respectively. Total Ca and Mg contents were comparatively high and the values were 3.95% and 0.94%, respectively. Correspondingly the carbonate content of soil was high of 4.72%, because the soil was developed from calcareous parent material. The clay content of soil was 21.2%. The texture of the soil was silt loam (Table 4.1.1). The major minerals of soil were indentified i.e. quartz, mica, smectite, chlorite and gismondine (Table 4.1.2 and Fig 4.1.2).

4.1.3 Soil of Tannery Industrial areas, Hazaribagh (HT): The soil of Hazaribagh tannery industrial areas is medium acidic in nature (pH 5.94). The soil has high sand content (34.8%). The silt and clay contents were 46.4 and 18.8%, respectively. The textural class of the soil was sandy loam. Organic matter content was 2.89%, comparatively higher than other soils. But CEC of soil was relatively low 7.49 cmol kg^{-1}. Like Madhupur tract, the carbonate minerals were not indentified in soil of Hazaribagh due to its acidic nature (Table 4.1.1).

Major minerals i.e. quartz, mica, kaolinite, vermiculite, illite, chlorite were identified based on the relative peak intensities of the respective minerals using XRD from soil of Hazaribagh (Table 4.1.2 and Fig 4.1.2). Moslehuddin *et al.* (1999) observed that mica, vermiculite and kaolinite were the major minerals in this Active Brahmaputra-Jamuna Floodplain soil.

Table 4.1.2 Minerals content and clay mineralogical suite of Bangladesh.

Sample ID	Major minerals in soil (standard XRD interpretation procedure)	Clay Mineralogical Suite (Moslehuddin *et al.* 1999)
MB	Quartz, Koalinite, Mica, Illite,	Mica Mixed layer mineral Kaolinite Suite
IB	Quartz, mica, smectite, chlorite, gismondine	Mica-Smectite Suite
HT	Quartz, Mica, Kaolinite, Vermiculite, Chlorite, Illite (trace)	Mica-Vermiculite-Kaolinite Suite
SSy	Quartz, Mica, Kaolinite, Vermiculite, Periclase	Mica-Kaolinite-Verculite Suite
KC	Quartz, mica, smectite, chlorite, gismondine, calcite	Mica-Smectite Suite
CC	Quartz, Mica, Kaolinite, Vermiculite, Periclase Chlorite	Mica-Kaolinite-Verculite Suite

4.1.4 Soil of Ship breaking yard, Shitakunda (SSy): The soil of ship breaking yard of Shitakunda was slightly alkaline in nature (pH 7.85). Sand content was high about 45.3% and textural class was loam. The EC of soil was 14.85 mS cm^{-1}. Correspondingly Na content was also high of 3.21% which indicated the saline nature of the soil. The CEC of soil was 9.94 cmol kg^{-1}. The carbonate content of soil was 0.438 % as $CaCO_3$. The soil content 2.77% of organic matter (Table 4.1.1).

Major soil minerals were identified as quartz, mica, kaolinite, vermiculite, periclase etc. Among clay minerals the dominance of mica, kaolinite and vermiculite were also observed (Table 4.1.2 and Fig 4.1.2). Moslehuddin *et al.* (1999) observed the Mica-Kaolinite-Vermiculite suite in Chittagong Coastal Plain area (Fig. 4.1.1).

4.1.5 Soil of Cable industry, Kushtia (KC): The soil of the High Ganges River Floodplain was found to be alkaline in nature having the pH value 8.19. The EC of soil was 1355 µS cm^{-1}, indicating non-saline nature of the soil. The CEC of soil was 14.19 cmol kg^{-1}. Total Ca and Mg contents were relatively high 4.1% and 1.05%, respectively. Besides the carbonate content of soil was also found high (4.93%), because the soil was developed from calcareous parent material.

Silt content of the soil was high (67.5%). Clay and sand contents of soil were 25.1% and 7.5%, respectively (Table 4.1.1). Ripon *et al.* (2004) also reported high silt content in soil of same AEZ. The textural class of soil was silt loam similar to Ishurdi soil.

Based on XRD analysis major minerals of soil were indentified i.e. quartz, mica, smectite, chlorite and gismondine (Table 4.1.2 and Fig 4.1.2). Ripon *et al.* (2004) also reported that soil of the High Ganges River Floodplain was developed from the calcareous Ganges sediments, originally having good amounts of smectite in addition to mica.

4.1.6 Soil of Cable industry, Chittagong (CC): The soil belongs to Chittagong Coastal Plain AEZ. It was a compound unit of piedmont, river, tidal and estuarine floodplain landscapes. The pH of soil was neutral in nature (pH 7.17). Sand content of soil was 22.5%. Silt and clay contents were 51.2 and 26.3%, respectively. The textural class was silt loam. The EC of soil was 6.85 mS cm^{-1} which indicated the somewhat saline nature of the soil. The CEC of soil was comparatively high 20.74 cmol kg^{-1} (Table 4.1.1). General fertility level of the soils is medium according to the Bangladesh Soil Standard (BARC, 1997). Organic matter content was in low to moderate range (1.22%) (Table 4.1.1).

Based on the relative peak intensities of the respective minerals in XRD charts the major minerals such as quartz, mica, kaolinite, vermiculite, muscovite, chlorite and periclase were indentified in soil (Table 4.1.2 and Fig 4.1.2). White (1985) reported 30% kaolinite, 30% mica and 40% vermiculite present in soil series of Chittagong coastal plan alluvium.

4.2 Distribution of heavy metals in contaminated areas

A number of samples were collected from different geographic locations surrounding the selected industrial areas. Detail ArcGIS map of contaminated areas was prepared. The concentrations of metals were plotted in digitized GIS maps and generated a cartographic contour line map. Level and distribution of heavy metals in contaminated areas were determined using spacial analyst tools (spline, surface analysis, contour etc.) of ArcGIS 9.3.1. Representative contaminated and non contaminated point sources were also indentified.

4.2.1 Soil of Madhupur tract, Gazipur having battery industry (MB): Two battery industries of Rahimafrooz were located in Zirani bazar, Gazipur within 350 meter distance. Lead content of soil was observed high between two industrial areas. Solid wastes from different processing units of battery industries were dumped in nearby soil. There was also dry filling and lead recycling unit present in battery industries which exhibited lead containing dust into the environment. Highest lead content was recorded 450 mg kg^{-1} in soil near solid waste dumping area between two industries. About 1,40,000 m^2 area around those industries showed high concentration of lead 200 - 450 mg kg^{-1}. About 1.13 km^2 area surrounding industries has Pb concentration over 100 mg kg^{-1} (Fig. 4.2.1), which crossed the maximum allowable limit of 100 mg kg^{-1} (Table 4.2.1).

Contaminated soil was collected from geographic location of 23°59'42"N and 90°15'21.6"E, (Fig: 4.2.1) where Pb concentration was 245.21 mg kg^{-1} (Table 4.2.1). Soil was also collected from a non-contaminated point approximately 650 meter away from contaminated point source in the same region. Geographic location of that point was 24°0'0.3594"N and 90°15'17.9994"E (Fig: 4.2.1). Lead concentration in soil of non-contaminated point was 13.44 mg kg^{-1} (Table 4.2.2). Other heavy metals (Cd, Cr, Ni, Cu, Zn) were found at low concentrations and within safe limit.

4.2.2 Soil of Ishwardi EPZ having battery industry (IB): A battery industry is situated in Ishwardi EPZ area. Lead content was also found high in soil near battery industry. Solid wastes from different processing units of battery industry were dumped in soil behind the battery industry, which released lead into environment. The highest amount of Pb (164.23 mg kg^{-1}) was recorded in soil near solid waste dumping area (Fig: 4.2.2). About 1.3 km^2 area surrounding the industry has Pb concentration of 100 - 250 mg kg^{-1}, which crossed the maximum allowable concentration (Table 4.2.1). Contaminated soil was collected from geographic location 24°5'16.8"N and 89°2'9.6"E (Fig: 4.2.2) where Pb concentration was 164 mg kg^{-1} (Table 4.2.1).

Soil was also collected from an uncontaminated point source about 1.36 km away from contaminated point source in the same region. Geographic location of that point was 24°5'27.6"N and 89°2'56.4"E. Lead concentration of non-contaminated soil was 12.53 mg kg^{-1}. Other heavy metals (Cd, Cr, Ni, Cu, Zn) contents were presented within safe limits (Table 4.2.2).

Table 4.2.1. Heavy metals content (mg kg^{-1}) of soils in contaminated point source.

Sl No.	Heavy metals	Soil of different regions						Maximum allowable concentration*
		MB	IB	HT	SSy	KC	CC	
1.	Lead (Pb)	245.21	164.23	14.78	93.23	18.38	23.96	100.0
2.	Cadmium (Cd)	2.12	1.12	2.08	25.07	0.98	1.09	3.0
3.	Chromium (Cr)	3.98	4.21	256.13	71.12	13.65	35.65	100
4.	Nickel (Ni)	5.54	6.78	42.34	69.64	22.44	46.07	50
5.	Copper (Cu)	2.32	5.34	12.34	192.95	151.22	196.32	50
6.	Zinc (Zn)	13.65	18.46	65.65	845.12	24.31	42.23	300

**Kolke 1980 and Kabata Pendias 1995*

Table 4.2.2. Heavy metals content (mg kg^{-1}) of soils in non-contaminated point source.

Sl No.	Heavy metals	Soil of different regions					
		MB	IB	HT	SSy	KC	CC
1.	Lead (Pb)	13.44	12.53	10.16	18.45	10.23	9.22
2.	Cadmium (Cd)	0.78	0.58	0.88	1.02	0.68	0.85
3.	Chromium (Cr)	2.34	2.09	8.29	7.56	2.12	3.22
4.	Nickel (Ni)	4.12	3.34	6.09	6.74	3.62	3.03
5.	Copper (Cu)	2.12	2.34	4.12	5.23	6.14	7.07
6.	Zinc (Zn)	18.05	19.46	16.43	26.13	22.12	24.34

4.2.3 Soil of tannery industrial areas, Hazaribagh (HT): Most of the tannery industries were located in Hazaribagh area, Dhaka. These tannery industries used high amount of chromium salt in chrome tanning process and discarded effluent without proper treatment, from which Cr was entered into the surrounding environment. Chromium concentration was recorded high in soil of tannery industrial areas. The highest Cr was recorded (734 mg kg^{-1}) in soil near tannery industry. High concentrations of Chromium (400 - 700 mg kg^{-1}) was spreaded about 5.13 km^2 area surrounding tannery industries. Over 10.8 km^2 area surrounding tannery industry showed Cr concentration more than 100 mg kg^{-1} (maximum allowable concentration) (Fig: 4.2.3 and Table 4.2.1).

Contaminated soil was collected from geographic location of 23°43'26.4"N and 90°21'18"E. (Fig: 4.2.3) where Cr concentration was 256.13 mg kg^{-1} (Table 4.2.1). Soil sample was also collected from a non-contaminated point source about 1.26 km away from contaminated point source in the same region. Geographic location of that point was 23°43'1.1994"N and 90°20'16.7994"E (Fig 4.2.3). Chromium concentration of non-contaminated soil was 14.78 mg kg^{-1} (Table 4.2.2). Nickel concentration was also high (42.34 mg kg^{-1}) in tannery industrial area. But the value was within allowable limit (Table 4.2.1). Concentrations of other heavy metals in contaminated soil were found within the allowable limit (Table 4.2.1).

4.2.4 Soil of ship-breaking yard, Shitakunda: Ship breaking activities threaten the marine environment and the human health by potential release of many different organic and inorganic pollutants. Cutting, blasting, tank cleaning activities, bilge and ballast water discharges, asbestos removal, burning of electrical cables and plastic materials are examples of pollution sources in ship- breaking yards (Hossain and Islam, 2006). Due to reusing materials, the ship recycling industry generates a large amount of waste, including petroleum, persistent organic pollutants and heavy metals. Metals are of particular concern due to their toxicity to marine biota and persistence in the environment.

All heavy metal contents were very high in the study area (Ship braking yard, Sonaichhar). Metal concentrations were recorded Pb 80-113, Cd 3.33- 8.56, Cr 65-81, Ni 58-72, Cu 180-204 and Zn 900-1250 mg kg^{-1} within 1.56 km² areas around the ship breaking yard (Fig. 4.2.4 to 4.2.9). Over 9.11 km² areas surrounding the shipyard showed more than 300 mg kg^{-1} Zn (maximum allowable concentration) (Fig 4.2.9). The level of Cu concentration was higher than 50 mg kg^{-1} (maximum allowable limit) surrounding 10.45 km^{2} areas of the shipyard (Fig 4.2.8). Lead and chromium concentrations were recorded more than 50 mg kg^{-1} extended over 5.32 km^{2} and 3.78 km^{2} areas surrounding the ship breaking yard, respectively (Fig 4.2.5 and 4.2.6)

Contaminated soil was collected from geographic location of 22°28'37.2"N and 91°42'28.7994"E (Fig: 4.2.4) where heavy metal concentrations were Pb 93.23, Cd 25.7, Cr 71.12, Ni 69.64, Cu 192.95 and Zn 845.12 mg kg^{-1} (Table 4.2.1).

Soil was also collected from a non-contaminated point source that was 1.51 km away from contaminated point source. Geographic location of that point was 22°29'13.1994"N and 91°42'57.5994"E. Heavy metal concentrations of non-contaminated point source were Pb 18.45, Cd 1.02, Cr 7.56, Ni 6.74, Cu 5.23 and Zn 26.13 mg kg^{-1} (Table 4.2.2).

4.2.5 Soil of Kushtia having cable and metal industries (KC)

The cable industry generates two solid-phase wastes; slag and blow down slurry/sludge. Slag was generated during the smelting, converting, fire refining, and electrolytic refining stages. Slag contained 0.5 - 0.7% copper, which is discarded into nearby soil. Waste water produced from the process of copper smelting contained more than 100 mg/L copper. Near a copper smelter, soil contained high concentrations of copper from adjacent wastewater discharged point. There were four outlets present in BRB cable and Kiam metal industry. Wastewater discharged from these outlets is mixed with cannel water, which is used for irrigation. Copper content in soil adjacent to the outlets was very high. Copper content was also high in soil, which was irrigated with cannel water. Highest copper content 225 mg kg^{-1} was recorded in soil near effluent discharge outlets. About 570,000 m^2 areas surrounding the cable industry showed high Cu content of 150 to 220 mg kg^{-1}. Copper concentration exceeded the maximum allowable concentration (50 mg kg^{-1}) over 1.14 km^2 areas around the industry (Fig 4.2.10).

Contaminated soil was collected from Geographic location of 23°52'40.8"N and 89°5'56.4"E (Fig 4.2.10), where Cu concentration was 151.22 mg kg^{-1} (Table 4.2.1). Soil was also collected from a non-contaminated point source which was 1.11 km away from contaminated point source. Geographic location of that point was 23°52'30"N and 89°6'28.8"E (Fig 4.2.10). The concentration of Cu in the soil of non-contaminated point source was 6.14 mg kg^{-1} (Table 4.2.2).

4.2.6 Soil of Chittagong coastal plain having cable industry (CC)

The cable industry is situated near Khalpar road Chittagong. The industry generates sludge during smelting and other production processes. The sludge is dumped into nearby soil. Wastewater from copper smelting process discharged through outlets to adjacent soil and in some water reservoirs (fenna pukur). Discharged water is used for irrigation in crop land. Copper contents near solid waste dumping area and soil adjacent to outlet were very high (220 to 250 mg kg^{-1}) (Fig 4.2.11).

Copper content was also high in nearby irrigated agricultural land. About 152,230 m^2 areas surrounding the cable industry showed high Cu content from 110 to 230 mg kg^{-1}. Copper concentration exceeded the maximum allowable concentration (50 mg kg^{-1}) over 227,000 m^2 areas around the industry (Fig 4.2.11).

Contaminated soil was collected from Geographic location of 22°16'4.8"N and 91°47'31.2"E (Fig 4.2.9), where Cu concentration was 192.32 mg kg^{-1} (Table 4.2.1). Soil was also collected from a non-contaminated point source that was 814.65 m away from contaminated point source. Geographic location of that point was 22°15'46.8"N and 91°47'49.1994"E (Fig 4.2.11). The concentration of Cu in soil of non-contaminated point source was 7.07 mg kg^{-1} (Table 4.2.2).

4.3 Fractionation of heavy metals in contaminated soils

For a given element, the term speciation refers to its distribution amongst its chemical forms or species. It is now widely recognized that it is not the total amount or concentration of a given heavy metal which is most important for understanding its environmental behavior rather the key to the flux of a metallic element is its speciation. The partitioning of metals according to their association with the soil substrates is usually determined by the sequential extraction procedure (Kuo *et al.,* 2006). Heavy metals accumulate in soils in the forms of water soluble, exchangeable, carbonate, Fe-Mn oxides, organic and residual (Salomons and Forstner, 1980). Heavy metals present in these fractions have different remobilization behaviors under fluctuating environmental conditions (Forstner *et al.,* 1985).

Metals in water-soluble and exchangeable fractions are readily bioavailable to the environment whereas the metals in the residual fraction are tightly bound and may not be expected to be released under natural conditions (Clevenger and Mullins, 1982; Xian 1989). The fractions most amenable to metal removal by chemical leaching are: (1) exchangeable; (2) associated with carbonates; and (3) associated with reducible Fe-Mn oxides of soils (Finzgar and Lestan, 2006). However, extraction of metal bound to exchangeable and carbonate fractions was faster compared to extraction of metal bound to Fe-Mn oxides (Williford and Bricka, 2001). Organically bound metals can be extracted along with the target organic contaminants by the solvent extraction method. The removal efficiency of metals from the distinct fractions depends on the extracting reagents used. For instance, due to dissolution effects, certain acid leaching processes may partially remove metals from the crystalline lattice (Luttrell *et al.,* 2006).

The fractionation of heavy metals of six contaminated soils were done by sequential extraction procedure (Salbu *et al*., 1998) modified from that of Tessier *et al.* (1979) to observe the adsorption and/or absorption of metals by different soil constituents. The sequential extraction method involves treatment of a soil sample with a series of reagents in order to partition the trace element content (Table 3.6). The mobility of metals in soil profiles may be assessed on the basis of absolute and relative content of fractions weakly bound to soil components.

The mobility of metals in soil profiles may be assessed on the basis of absolute and relative content of fractions weakly bound to soil components. The relative index of metal mobility was calculated as mobility Index as described in materials and methods section (3.6.2).

The value of mobility index for elements provides information about their potential mobility in soils and availability to plants (Yusuf, 2007). The value of MF up to 10% for any element indicates that this element is immobile and unavailable for plants (Torri and Lavado, 2008). In addition to that, the value of mobility index from 10 to 50% or greater than 50 % for a particular element suggests that this element is highly mobile and available for plants (Ahumada *et al.*, 1999).

4.3.1 Soil of Madhupur tract having battery industry (MB)

The soil of contaminated point source belonged where lead content was recorded 245.21 mg kg^{-1}, which was 2.45 times higher than the maximum allowable concentration (Table 4.2.1). Among different soil fractions, water soluble form of Pb was generally found low of 5.2%. An amount of 37.05 mg kg^{-1} of Pb was associated with exchangeable fraction of soil (Table 4.3.1). Carbonate bound Pb was not detected due to acidic nature of the soil (Table 4.1.1). Therefore the mobility index of Pb was calculated as 17.87% in contaminated soil of Madhupur tract. In contrast a large quantity of Pb was occupied in non-labile fraction of soil. The highest amount of Pb (111 mg kg^{-1}) was associated with Fe/Mn oxides bound fraction which was accounted for 46.95% of total amount. The organic matter and residual fraction retained 18.63 and 16.54% of total Pb fraction, respectively (Table 4.3.1). Nogueira *et al*. (2010) reported that lead (Pb) was the element found associated to more stable Fe/Mn oxides and residual fractions and not to the exchangeable and OM fractions. Lead usually shows high affinity to the oxide and residual fractions, where it is strongly bound (Nogueira *et al*., 2008), which in fact decreases its mobility in soil (Sheppard and Thibault, 1992). Other heavy metals concentrations in contaminated soil were found low and within the maximum allowable concentrations (Table 4.2.1).

Table 4.3.1: Fractionation of Heavy metals (mg kg^{-1}) in contaminated soils.

Heavy metals	F1 (Water soluble)	F2 (Exchangeable)	F3 (Carbonate bound)	F4 (Fe/Mn oxides bound)	F5 (Organic bound)	F6 (Residual form)	Mobility index (%)
Soil of Madhupur Tract having battery industry (MB)							
Pb	**5.2**	**37.05**	**0.00**	**111.02**	**44.06**	**39.11**	**17.87**
%	2.20	15.67	0.00	46.95	18.63	16.54	
Soil of Ishwardi having battery industry (IB)							
Pb	**3.03**	**9.23**	**52.55**	**27.54**	**16.14**	**49.73**	**40.96**
%	1.92	5.83	33.21	17.41	10.20	31.43	
Soil of Hazaribagh tannery industrial area (HT)							
Cr	**5.71**	**19.96**	**0.00**	**53.81**	**121.21**	**45.48**	**10.45**
%	2.33	8.13	0.00	21.91	49.36	18.52	
Ni	**3.26**	**11.81**	**0.00**	**11.44**	**5.87**	**8.52**	**35.58**
%	7.70	27.89	0.00	27.01	13.86	20.12	
Zn	**3.38**	**9.81**	**0.00**	**23.32**	**6.33**	**21.54**	**20.09**
%	5.15	14.94	0.00	35.52	9.64	32.81	
Soil of ship breaking yard, Shitakunda (SSy)							
Pb	**1.98**	**10.22**	**13.45**	**29.12**	**13.22**	**19.02**	**29.48**
%	2.3	11.7	15.5	33.5	15.2	21.9	
Cd	**2.08**	**10.67**	**3.15**	**4.34**	**1.32**	**2.12**	**67.15**
%	8.8	45.1	13.3	18.3	5.6	9	
Cr	**1.96**	**4.02**	**6.45**	**16.98**	**19.87**	**17.44**	**18.63**
%	2.94	6.03	9.67	25.46	29.79	26.15	
Ni	**1.55**	**7.92**	**11.14**	**20.09**	**11.23**	**12.12**	**32.18**
%	2.59	13.21	18.59	33.52	18.74	20.22	
Cu	**4.45**	**19.02**	**39.01**	**63.23**	**31.01**	**29.67**	**33.52**
%	2.39	10.20	20.93	33.92	16.64	15.92	
Zn	**19.02**	**82.34**	**134.21**	**275.12**	**104.34**	**207.65**	**28.63**
%	2.32	10.04	16.36	33.54	12.72	25.32	
Soil of Kushtia cable industry							
Cu	**6.43**	**16.76**	**37.67**	**23.87**	**22.21**	**32.11**	**43.77**
%	4.6	12.1	27.1	17.2	16.0	23.1	
Soil of Chittagong having cable industry							
Ni	**0.45**	**6.78**	**10.73**	**11.62**	**5.48**	**11.01**	**38.98**
%	0.98	14.72	23.29	25.22	11.89	23.90	
Cu	**7.28**	**25.17**	**18.84**	**62.15**	**36.89**	**31.21**	**28.39**
%	3.98	13.75	10.29	33.95	20.15	17.05	

4.3.2. Soil of Ishwardi EPZ having battery industry (IB)

Total Pb content was found high (158.23 mg kg^{-1}) in contaminated soil of Ishwardi EPZ having battery industry, which also exceeded the maximum allowable concentration (Table 4.2.1). The fractions of contaminated soil indicated that very low amount of Pb was presented in water soluble and exchangeable forms and the values were 1.92% and 5.83% of total fraction, respectively. The carbonate fraction of soil occupied a major portion of Pb of 33.2% (Table 4.3.1). Consequently the mobility index of Pb was found high (40.96%) in contaminated soil. Pulford *et al.* (2009) also found that more than 70% of Pb was associated with carbonate bound fraction of the lead contaminated calcareous sediments. In contrast contaminated soil of Ishwardi also contained a portion of Pb in non-labile fraction. The Fe/Mn oxides and organic matter of soil retained 17.41 and 10.20% of total Pb fractions, respectively (Table 4.3.1). Siddiqui and Khattak (2010) reported that the percentage recovery of Pb in exchangeable, organic and oxide bound fractions was found low, however, carbonate adsorbed high amount of Pb in calcareous soils of Peshawar, Pakistan. The residual fraction of soil retained 31.4% of Pb (Table 4.3.1). Concentrations of other heavy metals in contaminated soil of Ishwardi were negligible and within the maximum allowable limit (Table 4.2.1).

4.3.3 Soil of Hazaribagh tannery industrial area

The soil of tannery industrial area of Hazaribah contained 256.13 mg kg^{-1} of total Cr, which was more than 2.5 times higher than that of maximum allowable concentration (Table 4.2.1). The fractions of contaminated soil of Hazaribagh indicated that the water soluble and exchangeable forms of Cr amounted 2.33 and 8.13% of total content, respectively. Therefore the mobility index of Cr was recorded low (10.45%) in contaminated soil (Table 4.3.1).

Most abundant portion of Cr (121.2 mg kg^{-1}) was retained in organic matter bound form, which was 49.36% of total amount. Fraction bound to Fe/Mn oxides accounted for 18.27% of total Cr. The residual fraction was 14.8% in contaminated soil (Table 4.3.1). The quantities of other metals were within the maximum allowable concentrations.

Allué *et al.* (2014) found that the major portion of Cr was found to associate with organic matter in tannery sludge-amended soil and to a lesser extent, with Fe and Mn oxides. Kalembkiewicz and Soèo (2005) observed that the chromium content in particular fractions using sequential extraction methods was in order exchangeable fraction < carbonate < oxide < organic.

4.3.4 Soil of ship breaking industry, Shitakunda (SSy)

Heavy metal contents were found very high in soil of ship-breaking industry of Shitakunda. Total Pb, Cd, Cr, Ni, Cu and Zn contents of soil were 93.23, 25.07, 71.12, 69.64, 192.95 and 845.12 mg kg^{-1}, respectively. It was found that water soluble fraction contained very low amount of metals and the values were 1.98, 2.08, 1.96, 1.45, 4.45 and 19.02 mg kg^{-1} for Pb, Cd, Cr, Ni, Cu and Zn, respectively, which were accounted for average 3.6% of total fractions of metal. Among heavy metals, a major portion of Cd (45.1%) was associated with exchangeable fraction of soil (Table 4.3.1). Hickey and Kittrick (1984) reported more than 37% of Cd was present in exchangeable form a cadmium containing soil. Li and Thornton (2001) observed that the first three fractions (exchangeable, Fe-Mn oxide and carbonate bound) accounted for more than 70% Cd and the exchangeable cadmium alone was up to 52% of the total content. The very low amount of residual cadmium in soil showed that cadmium in soil was highly mobile and the mobility factor of Cd was 67.15% in contaminated soil of Shitakunda (Table 4.3.1).

The exchangeable fraction of soil contained 10.22, 10.67, 4.02, 5.82, 19.02 and 82.34 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively, which were on an average 16.1% of total content of metals. Among metals, Cr was found in lowest quantity as exchangeable forms (6.03%). Carbonate fraction adsorbed 15.5, 13.3, 9.67, 15.4, 20.93 and 16.36% of Pb, Cd, Cr, Ni, Cu and Zn of total fraction of metals in contaminated soil, respectively. Consequently the mobility indexes were recorded 29.48, 67.15, 18.63, 27.53, 33.52 and 28.63% for Pb, Cd, Cr, Ni, Cu and Zn in contaminated soil of ship-breaking yard, respectively (Table 4.3.1).

A portion of the metals were also associated with non-labile fractions of contaminated soil. Metals bound to Fe/Mn oxides accounted for a major portion such as Pb 21.9%, Cd 9.0 %, Cr 26.15%, Ni 20.22%, Cu 15.92% and Zn 25.32% of total amount of metals in soil (Table 4.3.1). Organic matter bound Cr was as high as 29.79%. Other organic bound heavy metals Pb, Cd, Ni, Cu and Zn were calculated as 15.2, 5.6, 18.74, 16.64 and 12.72% of total content, respectively (Table 4.3.1).

A portion of the metals were retained within lattice of silicate minerals as residual fraction. About 21.9, 9.0, 26.15, 20.22, 15.92 and 25.32 % of Pb, Cd, Cr, Ni, Cu and Zn were held in residual fraction, respectively. Among the metals Cd content in the residual fraction was the lowest of 9% (Table 4.3.1).

4.3.5 Soil of cable and metal industrial areas, Kushtia

Total copper content was high (151.22 mg kg^{-1}) in contaminated point source located near the cable and metal industries of Kushtia. The Cu concentration was 3.0 times higher than maximum allowable concentration (Table 4.2.1). Among soil fractions, water soluble forms of Cu was generally low (4.6%) and exchangeable form of Cu amounted 12.1% of total Cu fraction. The carbonate fraction of soil accounted for a major portion of Cu of 27.1%. Therefore the mobility index of Cu was recorded 43.77% in contaminated soil of Kushtia. Soil also retained a portion of metal in non-labile fraction. The Fe/Mn oxides and organic matter of soil bound 17.2 and 16.0% of total Cu, respectively. The residual fraction was found to be 23.1% (Table 4.3.1). Other heavy metals concentration in KC soil was negligible and within the maximum allowable concentration.

Siddiqui and Khattak, (2010) reported that greater concentration of Cu was found in carbonate bound fraction than other fractions in calcareous soils of Peshawar, Pakistan and the percentage of Cu in this fraction was 30% in Entisols and increased to 56% in Alfisols and Inceptisols. The greater amount of Cu bound with carbonate in this study is related with the greater percentage of CO_3^{2-} in soils. This also suggests that CO_3^{2-} in calcareous soils can be a dominant Cu^{2+} sink.

The result of this study is also in agreement with the findings of Mengel and Kirkby (2001) who found that Cu was strongly associated in carbonate fraction of calcareous soils. Similar result was reported by Adamo *et al.* (1996).

4.3.6 Soil of Chittagong cable industry area

The copper content was very high in contaminated soil of Chittagong having cable industry. Total concentration of copper was 196 mg kg^{-1} in contaminated point source, which was almost 4.0 times higher than maximum allowable concentration (Table 4.3.1). The fraction of contaminated soil indicated that, the water soluble form of Cu was low (3.98%). The exchangeable fraction of soil was 13.75 % of total Cu content. The carbonate of soil bound 18.84 mg kg^{-1} of Cu in soil. Consequently the mobility index of Cu was recorded 28.39% in contaminated soil. But the largest portion of Cu (62.15 mg kg^{-1}) was retained in Fe/Mn oxides bound fraction which was accounted for 33.95% of total Cu present in soil. The organic matter and residual fractions retained 20.15 and 17.05% of Cu of total content (Table 4.3.1). Concentrations of other heavy metals in contaminated CC soil were found within the maximum allowable concentration (Table 4.2.1). Graf *et al.* (2007) studied the fractionation of Cu in young and old alluvial soils and found that greater percentage of Cu was associated with Fe/Mn oxide and carbonate bound fraction.

According to Tessier *et al.* (1979) the order of mobility of the metals considering their abundance in the fractions is: exchangeable > bound to carbonate > bound to oxides > bound to organics > residual.

4.4 Remediation of metal contaminated soil by chemical extractants

Remediation of heavy metal polluted soils is a serious problem and a great challenge because heavy metals are non-degradable and strongly bonded to organic and inorganic soil solids (Heil *et al.*, 1999; Barona *et al.*, 2001; Sun *et al.*, 2001; Finˇzgar and Leˇstan, 2006; Kirkham, 2006; Di Palma *et al.*, 2007; Evangelou *et al.*, 2007). Metal removal efficiency by the chemical extraction process depends on the soil geochemistry (e.g., soil texture, cation exchange capacity, buffer capacity, and organic matter content); metal contamination characteristics (type, concentration, fraction or speciation of metals); dosage and chemistry of extracting agent; and processing conditions (solution pH, residence time, number of successive extraction steps, mode of reagent addition, liquid/solid ratio, among others) (Ayejuyo *et al.,* 2012).

Factors that may limit the applicability and effectiveness of the chemical process include: (1) high clay/silt content; (2) high humic content; (3) high content of Fe and Ca; (4) high calcite content or high buffering capacity; (5) simultaneous contamination of heavy metals; (6) high heterogeneity of soil; and (7) metals associated with residual soil fraction, imbedded in the mineral lattices, or discrete particle forms (Ayejuyo *et al.*, 2012).

In this study 100 mg kg^{-1} of each metal was spiked in non-contaminated soils collected from different regions, which were used to examine the extractability of different extractants. Initially five extractants: distilled water, buffered salt solution (NH_4OAc), salt of divalent cation ($CaCl_2$), HCl acid solution, chelating agents EDTA and DTPA were used to observe the metal removal efficiency.

4.4.1 Metal removal from spiked soil of Madhupur tract by chemical extractants

In order to assess the efficacy of different extractants, the redistribution patterns of metals in spiked soils were determined using the Tessier *et al.* (1979) sequential extraction procedure before and after treatment.

The fractions of metal spiked soil of Madhupur tract (MB) indicated that most of the metals hitherto associated with the Fe/Mn oxides bound fractions. This fraction occurred 46.48, 28.5, 33.82, 34.95, 32.7 and 35.02 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn which were accounted for 36.5 % of total amount. The water soluble fraction of soil contained 3 to 5 % of metals. The spiked MB soil adsorbed the highest 50.53 % of Cd and the lowest 9.36 % of Cr in exchangeable forms. Chromium was found to exit in different forms, of these the maximum quantity was in non-labile metal pool of soil. Other four metals (Pb, Ni, Cu and Zn) were accounted for average 22.6% as exchangeable forms of total content (Fig 4.4.1). Carbonate bound metals were not detected due to acidic nature of Madhupur soil (pH < 5.0) (Fig. 4.4.1). The mobility indexes of metals were 18.85, 56.50, 12.19, 31.84, 27.37 and 31.21 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked MB soil, respectively (Table 4.4.2). The organic matter and residual forms of soil retained 14 and 19 % metals of total amount (Fig. 4.4.1).

Table 4.4.1 The Removal efficiencies (%) of metals from spiked soil of Madhupur tract by different extractants.

Extractant	**Pb**	**Cd**	**Cr**	**Ni**	**Cu**	**Zn**
H_2O	5.44e	5.88c	3.19d	7.53e	4.62e	8.52f
1 M NH_4OAc	21.84c	55.82b	9.41c	29.78d	18.21b	39.69d
0.1 M $CaCl_2$	14.21d	52.74b	7.56d	27.51d	10.61d	29.61e
0.1 M HCl	55.04a	63.05a	15.38b	50.58a	53.06a	60.22a
0.1 M EDTA	56.78a	62.93a	22.84a	51.16a	51.68a	52.67b
0.005 M DTPA	38.42b	57.75b	15.56b	38.84b	42.26b	41.5c

In was observed that H_2O extracted only 3 to 5% metals from spiked MB soil. Among the salt solutions, 1 M NH_4OAc removed 21.84, 55.82, 9.41, 29.78, 18.21 and 39.69 % of Pb, Cd, Cr, Ni, Cu and Zn from soil (Table 4.4.1). The extractant NH_4OAc removed average 94.78 % of water soluble and 84.93 % of exchangeable forms of metals. Whereas, the 0.1 M $CaCl_2$ solution leached average 93.15 % and 65.3 % of metals from above respective forms (Fig. 4.4.1). Consequently the removal efficiency of metals of 1 M NH_4OAc was higher than that of 0.1 M $CaCl_2$. The mobility indexes of metals were 4.25 to 17.21% with an average 9.93% in soil after extraction with $CaCl_{2.}$ In contrast NH_4OAc caused the mobility indexes 2.61 to 11.12% with an average 5.28% in spiked MB soil (Table 4.4.2).

Table 4.4.2 Mobility indexes (%) of metals in spiked soil of Madhupur tract before and after extraction with different extractants.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
Unwashed Soil	18.85±0.95	56.50±2.81	12.19±0.62	31.84±1.6	27.37±1.35	31.21±1.53
H_2O	14.89±0.73	54.11±2.73	9.96±0.51	28.73±1.42	23.24±1.14	26.24±1.33
1 M NH_4OAc	2.65±0.14	11.12±0.54	3.16±0.15	2.61±0.14	7.88±0.37	4.26±0.22
0.1 M $CaCl_2$	9.80±0.48	14.78±0.73	4.25±0.22	5.80±0.27	17.21±0.85	7.76±0.37
0.1 M HCl	1.09±0.06	2.60±0.14	0.91±0.06	1.03±0.05	1.16±0.07	1.31±0.08
0.1 M EDTA	1.20±0.05	2.41±0.11	0.93±0.05	1.23±0.07	1.05±0.06	1.10±0.05
0.005 M DTPA	1.63±0.09	4.12±0.22	1.09±0.06	1.60±0.09	1.37±0.07	2.06±0.1

The extractants 0.1 M HCl and 0.1 M EDTA leached significant amount of metals from spiked MB soil. The HCl solution removed 55.04, 63.05, 15.38, 50.58, 53.06 and 60.22% of Pb, Cd, Cr, Ni, Cu and Zn from soil, respectively. In contrast chelating agent EDTA removed 56.78, 62.93, 22.84, 51.16, 51.68 and 52.67% of above respective metals from spiked soil (Table 4.4.1). Both HCl and EDTA extracted move than 94 and 97% of water soluble and exchangeable forms of metals from soil. In addition HCl and EDTA removed 53.65 and 51.3% metals from Fe/Mn oxides bound fraction of soil, respectively (Fig 4.4.1). The average mobility indexes of metals were 1.35 and 1.32% in spiked MB soil after extraction with HCl and EDTA, respectively (Table 4.4.2), which indicated the metals in spiked soil were entirely immobile and unavailable to plant (Torri and Lavado, 2008) after extraction.

The extractant 0.005M DTPA removed 38.42, 57.75, 15.56, 38.84, 42.26 and 41.5% of Pb, Cd, Cr, Ni, Cu and Zn from the spiked soil, respectively (Table 4.4.1). DTPA extracted over 90% of water soluble, 96% of exchangeable forms of metals. The extractant DTPA also removed 27.24% of metals from Fe/Mn oxide bound fractions (Fig. 4.4.1). Therefore, the extraction capacity of metals of DTPA was higher than NH_4OAc and $CaCl_2$ but lower than that of HCl and EDTA solution. The average mobility index of metals was 1.98% in spiked MB soil after extraction with DTPA (Table 4.4.2).

4.4.2 Metal removal from spiked soil of Ishwardi by chemical extractants

Soils of Ishwardi EPZ are complex mixtures of calcareous silty and clayey alluvium. The soil belongs to the Active Ganges River Floodplain was alkaline in nature. The textural class was silt loam. The Ca and Mg contents were high, because the soils were developed from calcareous parent material (Table 4.1.1).

Fractionation study of spiked calcareous soil of Ishwardi (IB) indicated that very small amount of metals were found in water soluble forms (1.36 to 7.2 mg kg^{-1}). The exchangeable fraction of soil contained 5.11, 31.34, 2.55, 6.36, 6.83 and 5.35 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively. It was observed that Cd was found in high quantity as water soluble and exchangeable forms. Other metals occupied of an average 1.63 and 5.28 % of water soluble and exchangeable forms of total content, respectively. The carbonate bound fraction of soil adsorbed 32.36, 23.35, 14.79, 19.19, 22.56 and 41.08 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively, which were accounted for 25.77% of total amount (Fig. 4.4.2). Metals were mostly bound to carbonates and under alkaline conditions precipitation of metal carbonate was favoured in calcareous soil (Lo and Yang, 1999). Consequently the mobility indexes of metals were recorded high in spiked soil of Ishwardi and the values were 39.61, 62.80, 19.10, 27.45, 31.39 and 47.65 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively (Table 4.4.4). According to Ahumada *et al.* (1999) the metals in calcareous spiked soil of Ishwardi were highly mobile.

The soil also retained a part of metals in non-labile fractions. The Fe/Mn oxides of soil bound 17.63, 21.72, 19.1, 20.81, 20.52 and 21.9 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn which accounted for 20.45% of total metal content. The organic matter and residual fractions of soil hold 15.96 and 25.57% of metals, respectively (Fig. 4.4.2).

Table 4.4.3. Removal efficiencies (%) of metals of different extracting agents from spiked calcareous soil of Ishwardi.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
H_2O	1.94d	5.21f	1.44d	0.72e	2.04d	1.36d
1 M NH_4OAc	6.56c	27.77d	5.21c	8.23c	7.57c	6.49c
0.1 M $CaCl_2$	3.28cd	20.46e	5.05c	6.39d	6.95c	6.76c
0.1 M HCl	3.14cd	38.32c	6.37c	8.55c	7.58c	6.31c
0.1 M EDTA	37.21a	62.04a	24.21a	35.68a	35.01a	43.85a
0.005 M DTPA	25.17b	48.38b	13.46b	20.34b	25.54b	30.55b

The extractant H_2O removed very small amount of metals (average 2.5 %) from spiked soil of Ishwardi (IB). Among salt solutions 0.1 M $CaCl_2$ removed 3.28, 20.46, 5.05, 6.39, 6.95 and 6.76 % and 1 M NH_4OAc removed 6.56, 27.77, 5.21, 8.23, 7.57 and 6.49 % of Pb, Cd, Cr, Ni, Cu and Zn from soil, respectively. The 0.1 M HCl solution extracted 3.14, 38.32, 6.37, 8.55, 7.58 and 6.31 % of above respective metals from soil (Table 4.4.3). The extractants $CaCl_{2,}$ NH_4OAc and HCl removed 89.77, 90.89 and 92.85 % of water soluble and 84.29, 79.38 and 79.99% of exchangeable forms of metals from soil, respectively. These extractants were unable to extract much metal from carbonate bound forms of soil. The NH_4OAc, $CaCl_2$ and HCl extracted only 14.87, 6.88 and 19.27 % of metals from carbonate adsorbed fraction, respectively (Fig. 4.4.2). Therefore among three extractants, NH_4OAc and HCl showed better removal efficiency of metals from IB soil than that of $CaCl_2$. The average mobility indexes of metals were 28.91, 31.21 and 28.42 % after extraction with NH_4OAc, $CaCl_{2,}$ and HCl, respectively (Table 4.4.4). The high mobility indexes (>10) indicated that the metals were yet mobile in soil after extraction and available for plants (Ahumada *et al.*, 1999)

Table 4.4.4. Mobility indexes (%) of metals in spiked calcareous soil of Ishwardi before and after extraction with different extractants**.**

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
Unwashed Soil	39.61±1.98	62.8±3.14	19.1±0.96	27.45±1.37	31.39±1.57	47.65±2.38
H_2O	37.61±1.88	60.48±3.02	15.33±0.77	26.14±1.31	29.94±1.50	46.48±2.32
1 M NH_4OAc	35.8±1.79	44.89±2.24	10.3±0.52	17.28±0.86	23.72±1.19	41.46±2.07
0.1 M $CaCl_2$	35.4±1.77	51.18±2.56	12.68±0.63	21.05±1.05	24.74±1.24	42.18±2.11
0.1 M HCl	34.8±1.74	40.27±2.01	10.47±0.52	18.44±0.92	24.54±1.23	42.02±2.10
0.1 M EDTA	17.61±0.88	18.57±0.93	8.92±0.45	8.95±0.45	16.66±0.83	15.3±0.77
0.005 M DTPA	20.87±1.04	20.92±1.05	10.5±0.53	9.95±0.50	15.03±0.75	25.07±1.25

The extractant 0.1 M EDTA leached the highest amount of metals from spiked calcareous soil of Ishwardi and the values were 37.21, 62.04, 24.21, 35.68, 35.01 and 43.85 % for Pb, Cd, Cr, Ni, Cu and Zn in soil, respectively (Table 4.4.3). The extractant EDTA removed over 92% of metals from water soluble and exchangeable forms. In addition EDTA extracted 68.3 and 35.2% of metals from carbonate and Fe/Mn oxides bound fractions of soil, respectively (Fig. 4.4.2). Tandy *et al.* (2004) and Borggaard *et al.* (2009) also reported that EDTA removed most of the exchangeable and carbonate-bound fractions and also part of the residual fraction of Cd, Ni and Cu from calcareous soil. The mobility indexes were found lowest in calcareous IB soil after extraction with EDTA and the values were 17.61, 18.57, 8.92, 8.95, 16.66 and 15.3 % for Pb. Cd, Cr, Ni, Cu and Zn, respectively. These values of mobility index indicated that Cr and Ni were immobile (MI<10) in soil (Torri and Lavado, 2008) after extraction with EDTA, but the Pb, Cd, Cu and Zn were yet mobile (MI>10) in soil (Ahumada *et al.*, 1999).

Next to EDTA, 0.005 M DTPA removed 25.17, 48.38, 13.46, 20.34, 25.54 and 30.55 % of Pb, Cd, Cr, Ni, Cu and Zn from IB soil, respectively (Table 4.4.3). The DTPA extracted 91.3, 88.3 and 56.6 % of metals from water soluble, exchangeable and carbonate bound fractions of soil, respectively. DTPA also extracted small amount of metals (9.73 %) from Fe/Mn oxide bound fractions of spiked IB soil (Fig. 4.4.2). The average mobility index of metals was 17.06 % in spiked IB soil after extraction with DTPA (Table 4.4.4), which was lower than NH_4OAC, $CaCl_2$ and HCl treatment, but higher than that of EDTA extraction.

All together 0.1 M EDTA showed the highest removal efficiency of metals from spiked calcareous soil of Ishwardi.

4.4.3 Metal removal from spiked soil of Hazaribagh by chemical extractants

The soil of Hazaribagh was slightly acidic in nature. The soil has high sand content (34.8%). The textural class of soil was loam (Table 4.1.1). It was found from the fractionation of metal spiked soil of Hazaribagh that water soluble forms of soil amounted on an average 6.5% of metals. The soil contained 15.58, 39.83, 12.21, 28.84, 23.31 and 17.2 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn in exchangeable form. Metals were not found as carbonate bound form possibly due to acidic nature of Hazaribagh soil (pH 5.92). Among the metals most of the Cd was distributed in labile fractions. Contrary to that Cr was detected as the lowest amount in labile forms. Therefore the mobility indexes of Pb, Cd, Cr, Ni, Cu and Zn were recorded 21.18, 55.93, 15.13, 38.42, 28.21 and 21.07 % in spiked HT soil, respectively. The soil also contained considerable amount of metals in non-labile fractions. The Fe/Mn oxides of soil bound 39.37, 24.72, 26.1, 24.99, 28.15 and 39.5 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn which were accounted for 30.92% of total metal content. The average organic matter bound metals was 19.28%. Among the metals, the maximum amount of Cr was found to exit in organic matter bound forms (41.44%). Average 20.12 % of metals were remained in residual form of soil (Fig. 4.4.3).

Table 4.4.5. Removal efficiencies (%) of metals of different extracting agents from spiked soil of Hazaribagh.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
H_2O	3.76e	15.84e	2.35d	7.34d	3.26e	5.17d
1 M NH_4OAc	16.95d	47.04d	6.59c	31.34c	19.12d	16.95c
0.1 M $CaCl_2$	5.63e	44.52d	6.65c	29.85c	11.64de	8.65d
0.1 M HCl	54.37b	72.06a	30.48a	60.01a	65.25a	60.11a
0.1 M EDTA	63.51a	70.29a	31.09a	59.51a	59.03b	58.41a
0.005 M DTPA	40.25c	56.61c	19.38b	45.19b	32.61c	43.65b

It was observed from the extraction efficiency that H_2O removed the lowest amount of metals (average 5.96 %) from spiked soil of Hazaribagh (HT). Among the salt solutions, 0.1 M $CaCl_2$ leached 5.63, 44.52, 6.65, 29.85, 11.64 and 8.65 % and 1 M NH_4OAc removed 16.95, 47.04, 6.59, 31.34, 19.12 and 16.95 % of Pb, Cd, Cr, Ni, Cu and Zn from spike HT soil, respectively (Table 4.4.5). The extractants $CaCl_2$ and NH_4OAc removed over 93 and 95 % of water soluble and 60 and 84 % of exchangeable forms of metals from soil, respectively (Fig. 4.4.3).

Therefore the metal extraction capacity of $CaCl_2$ was lower than that of NH_4OAc. The average mobility indexes of metals were 10.58 and 4.77 % after extraction with $CaCl_2$ and NH_4OAc, respectively (Table 4.4.6).

Table 4.4.6 Mobility indexes (%) of metals in spiked soil of Hazaribagh before and after extraction with different extractants.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
Unwashed Soil	21.18±1.04	55.93±2.78	15.13±0.75	38.42±1.93	28.21±1.42	21.07±1.04
H_2O	16.94±0.82	47.99±2.41	13.19±0.65	32.51±1.61	24.64±1.22	16.42±0.83
1 M NH_4OAc	2.53±0.14	8.68±0.42	4.95±0.23	4.17±0.22	4.69±0.24	3.59±0.19
0.1 M $CaCl_2$	11.33±0.55	14.66±0.74	5.43±0.28	8.86±0.42	12.68±0.62	10.52±0.52
0.1 M HCl	1.76±0.08	5.38±0.25	1.07±0.05	1.90±0.11	0.90±0.06	1.11±0.07
0.1 M EDTA	1.67±0.09	5.42±0.26	1.15±0.07	1.55±0.08	0.66±0.04	0.77±0.05
0.005 M DTPA	1.85±0.11	5.75±0.28	1.46±0.08	2.84±0.13	1.25±0.07	1.77±0.08

The extractants 0.1 M HCl and 0.1 M EDTA leached significant amount of metals from spiked HT soil. The HCl removed the highest amount of Ni, Cu and Zn of 60.01, 65.25 and 60.11 % and chelating agent EDTA removed the highest amount of Pb, Cd and Cr of 63.51, 70.29 and 31.09 % from HT soil, respectively (Table 4.4.5). The extractants HCl and EDTA extracted more than 95 and 97% of water soluble and exchangeable forms of metals, respectively. Consequently the mobility indexes of metals were 0.9 to 5.38 % and 0.66 to 5.42 % in spiked soil of Hazaribagh after extraction with HCl and EDTA, respectively (Table 4.4.6). This indicated a complete reduction of metal mobility in soil after extraction according to Ahumada *et al.* (1999).

In addition HCl and EDTA removed metals from non-labile fractions. About 72 and 68 % of metals of Fe/Mn oxides bound fraction were leached by HCl and EDTA, respectively. The extractant EDTA also solubilized a portion of metals from organic matter bound fraction (Fig. 4.4.3). Li and Shuman (1996) also found that EDTA solubilized metals from the organic matter bound fraction by forming soluble complexes.

Next to HCl and EDTA, 0.005 M DTPA removed 40.25, 56.61, 19.38, 45.19, 32.61 and 43.65 % of Pb, Cd, Cr, Ni, Cu and Zn from HT soil, respectively (Table 4.4.5). The DTPA reclaimed average 93.98, 95.47 and 34.88 % of metals from water soluble, exchangeable and Fe/Mn oxides bound fractions of HT soil, respectively (Fig. 4.4.3). So the removal efficiency of metals of DTPA was higher than NH_4OAc and $CaCl_2$ but lower than that of HCl and EDTA. The average mobility index of metals was 2.49 % in spiked HT soil after extraction with DTPA (Table 4.4.6). Both 0.1 M HCl and EDTA exhibited the maximum extraction capacity of metals from spiked soil of Hazarizabh.

4.4.4 Metal removal from spiked soil of Shitakunda (SSy) by chemical extractants

The soil of Shitakunda (SSy) contained high amount of sand and the textural class was loam. The fractions of metal spiked soil of Shitakunda indicated very low quantities of metals in water soluble form. The exchangeable fraction of soil contained 13.65, 38.68, 7.58, 16.81, 20.47 and 18.99 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively, which were accounted for 19.4% of total amount. The carbonate of the soil adsorbed 15.15, 18.82, 11.4, 22.02, 13.13 and 18.48 mg kg^{-1} of above respective metals and this fraction occupied 16.53% of total amount (Fig. 4.4.4). The mobility indexes were 33.64, 63.51, 21.05, 41.48, 37.32 and 40.98 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked SSy soil, respectively (Table 4.4.8).

The soil also contained a large quantity of metals in non-labile fractions. Among these, most of the metals were associated with the Fe/Mn oxides bound fraction and the values were 27.39, 20.34, 24.18, 24.01, 23.79 and 24.59 mg kg^{-1} for Pb, Cd, Cr, Ni, Cu and Zn, respectively, which were 24.1% of total amount. The organic matter and residual forms retained 14.47 and 21.77% of metals of total amount in spiked SSy Soil, respectively (Fig. 4.4.4).

Table 4.4.7. Removal efficiencies (%) of metals of different extracting agents from spiked soil of Shitakunda.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
H_2O	2.32e	5.02e	1.32d	3.45d	4.54f	5.23d
1 M NH_4OAc	21.51d	40.32c	6.74c	17.26c	17.48d	14.13c
0.1 M $CaCl_2$	18.21d	21.63d	5.68c	16.10c	11.34e	13.14c
0.1 M HCl	52.33b	70.27a	36.08a	64.23a	66.26a	56.05a
0.1 M EDTA	65.16a	71.90a	34.16a	63.49a	60.45b	56.44a
0.005 M DTPA	38.65c	58.10b	16.68b	39.59b	30.61c	34.43b

The extractant H_2O leached very small quantities of metals (0.12 to 5.2%) from SSy soil. Among the salt solutions, 1 M NH_4OAc extracted 21.51, 40.32, 6.74, 17.26, 17.48 and 14.13 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked SSy soil, respectively. In contrast 0.1 M $CaCl_2$ removed 18.21, 21.63, 5.68, 16.10, 11.34 and 13.14 % of above respective metals from the soil (Table 4.4.7). The extractants NH_4OAc and $CaCl_2$ removed over 93 and 83% of water soluble and 92 and 71% of exchangeable forms of metals from SSy soil, respectively (Fig. 4.4.4). But these extractants were not able to extract much metal from other fractions. Consequently the extraction capacity of metals of NH_4OAc was higher than that of $CaCl_2$. The mobility indexes of metals were within the range of 12.78 to 32.82% and 14.86 to 48.33% after extraction with NH_4OAc and $CaCl_2$, respectively (Table 4.4.8). These high mobility index values indicated that metals were mobile in soil and available to plants after extraction.

Table 4.4.8 Mobility indexes (%) of metals in spiked soil of Shitakunda before and after extraction with different extractants.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
Unwashed Soil	33.64±1.65	63.51±3.15	21.05±1.02	41.48±2.02	37.32±1.76	40.98±2.01
H_2O	30.83±1.56	61.17±3.03	19.58±0.93	36.88±1.81	30.96±1.53	37.70±1.92
1 M NH_4OAc	12.78±0.65	32.82±1.61	12.86±0.64	25.01±1.23	15.1±0.78	27.16±1.33
0.1 M $CaCl_2$	16.42±0.82	48.33±2.42	14.86±0.73	25.44±1.25	20.62±1.05	29.76±1.45
0.1 M HCl	1.29±0.07	6.13±0.34	2.08±0.11	1.36±0.08	1.13±0.07	3.49±0.15
0.1 M EDTA	1.44±0.0	5.9±0.28	3.9±0.22	1.99±0.11	1.88±0.11	7.46±0.41
0.005 M DTPA	6.49±0.31	10.9±0.57	4.83±0.23	14.14±0.84	9.76±0.44	13.84±0.65

Among the extractants both 0.1 M HCl and 0.1 M EDTA removed significant amount of metals from spiked SSy soil. Among them HCl extracted the highest amount of Cr, Ni and Cu of 36.08, 64.23 and 66.26 %, whereas EDTA extracted the highest amount of Pb, Cd and Zn of 65.16, 71.9 and 56.44 % from SSy soil, respectively (Table 4.4.7). Both HCl and EDTA removed more than 95 and 97 % of metals from water soluble and exchangeable fractions, respectively. In addition HCl and EDTA leached 93 to 99% and 80 to 98% of metals from carbonate bound fraction, respectively (Fig. 4.4.4). The mobility indexes of metals were considerably lowered in spiked SSy soil after extraction with HCl and EDTA and the values were 1.13 to 6.13% and 1.44 to 7.46%, respectively (Table 4.4.8), which indicated a entire reduction of metal mobility in soil after extraction.

The extractant HCl and EDTA also released metals from non-labile fraction. More than 66% of Fe/Mn oxides bound metals were removed by these extractant. EDTA also solubilized metals from organic matter bound fraction (Fig. 4.4.4). Petruzzelli (1989) indicated that EDTA released the metals complexed or adsorbed by organic substances.

Next to EDTA and HCl, 0.005M DTPA extracted significant amount of metals from SSy soil. The extractant DTPA reclaimed 38.65, 58.10, 16.68, 39.59, 30.61 and 34.43 % of Pb, Cd, Cr, Ni, Cu and Zn from soil, respectively (Table 4.4.7). From fractionation study it was revealed that DTPA removed more than 93, 97 and 67% of metals from water soluble, exchangeable and carbonate adsorbed fractions of SSy soil, respectively (Fig. 4.4.4). The mobility indexes of metals were 6.49, 10.9, 4.83, 14.14, 9.76 and 13.84 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked SSy soil after extraction with DTPA, respectively (Table 4.4.8). However DTPA was unable to extract much metal from non-labile fraction. The removal efficiency of metals of DTPA from SSy soil was higher than NH_4OAc and $CaCl_2$ but lower than that of HCl and EDTA treatment (Fig. 4.4.4). All at once both 0.1 M EDTA and HCl exhibited the maximum extraction efficiency of metals from spiked soil of Shitakunda compared to other extractant.

4.4.5 Metal removal from spiked soil of Kushtia by chemical extractants

Soil of Kushtia (KC) belongs to High Ganges River Floodplain which was found to be alkaline in nature. The calcium carbonate of soil was found high, resulting from the calcareous nature of parent material. The textural class of the soil was silt loam (Table 4.1.1). The fractions of spiked calcareous soil of Kushtia (KC) indicated that very small amount of metals were present in water soluble forms (average 3.22 %). The exchangeable fraction of soil amounted 6.58, 25.78, 3.51, 6.59, 9.59 and 6.78 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively which were occupied 10% of total amount. However, most of the metals hitherto were associated with carbonate fraction.

The carbonate of soil bound 31.58, 23.98, 16.69, 21.48, 24.12 and 42.5 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively which were amounted 27.3% of total content (Fig. 4.4.5). Cavallaro and McBride (1978) reported that calcareous soil contaminated with metal showed evidence of being saturated with respect to carbonate precipitation. Jensen *et al.* (2013) also found from the sequential extraction of calcareous soil that Zn was strongly attached to the carbonate-bound fraction. Consequently the high mobility indexes of metals indicated a high metal mobility in calcareous spiked KC soil and the values were 40.93, 60.41, 22.81, 31.24, 36.03 and 51.49 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively (Table 4.4.10).

Table 4.4.9. Removal efficiencies (%) of metals of different extracting agents from spiked soil of Kushtia.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
H_2O	1.78d	4.05e	1.34d	1.72e	2.22e	3.33d
1 M NH_4OAc	7.49c	24.44c	6.22c	10.32c	11.84c	11.32c
0.1 M $CaCl_2$	4.28cd	17.27d	5.65c	6.67d	6.74d	5.28d
0.1 M HCl	4.93cd	26.03c	6.44c	11.47c	11.23c	5.03d
0.1 M EDTA	36.77a	60.24a	25.11a	34.41a	35.45a	42.14a
0.005 M DTPA	26.84b	45.92b	15.36b	20.93b	19.29b	29.95b

The non-labile fractions of soil also contained a large portion of metals (60.6%). The Fe/Mn oxides retained 16.66, 19.76, 16.37, 19.88, 18.12 and 21.25 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn which were occupied 19.07 % of total metal. The organic matter bound and residual forms of soil were 15.3 and 24.8 % of metals of total amount, respectively (Fig. 4.4.5).

The extractant H_2O removed very low quantity of metals from spiked soil of Kushtia (KC). Between the salt solutions 1 M NH_4OAc extracted 7.49, 24.44, 6.22, 10.32, 11.84 and 11.32 % and the 0.1 M $CaCl_2$ solution extracted 4.28, 17.27, 5.65, 6.67, 6.74 and 5.28 % of Pb, Cd, Cr, Ni, Cu and Zn from same soil, respectively. In contrast 0.1 M HCl solution removed 4.93, 26.03, 6.44, 11.47, 11.23 and 5.03 % of above respective metals from the soil (Table 4.4.9). The extractants $CaCl_2$, NH_4OAc and HCl removed above 90, 91 and 87 % of water soluble and 91, 86 and 87% of exchangeable forms of metals from soil, respectively. But these extractants dissolved only 17, 2.5 and 15.8% of metals from carbonate bound fraction of soil, respectively (Fig. 4.4.5). Therefore from above three extractants, the NH_4OAc showed better removal efficiency of metals in calcareous KC soil. The mobility indexes of metals were recorded high after extraction with NH_4OAc, $CaCl_2$, and HCl, and the average values were 28.85, 32.85 and 30.07%, respectively (Table 4.4.10). The extractant 0.1 M EDTA showed the highest removal efficiencies of metals from spiked calcareous soil of Kushtia (KC) and the values were 36.77, 60.24, 25.11, 34.41, 35.45 and 42.14% for Pb, Cd, Cr, Ni, Cu and Zn, respectively (Table 4.4.9). The extractant EDTA removed more than 93 and 96 % of metals from water soluble and exchangeable fractions, respectively. EDTA also removed above 69% of metals from carbonate bound forms in calcareous soil (Fig. 4.4.5). Miller *et al.* (1986b) observed that 0.1 M EDTA (pH 7.0) was effective to dissolve carbonates by complexation and thereby mobilize the metal occluded in them. Moreover EDTA extracted mean 31.28 % of metals from Fe/Mn oxides bound fraction (Fig. 4.4.5). The mobility indexes of metals were recorded lowest in spiked calcareous KC soil after extraction with EDTA and the values were 17.7, 21.77, 8.16, 8.76, 15.18 and 19.92 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively (Table 4.4.10). The values of mobility index indicated that Cr and Ni were found immobile (MI<10) in soil and unavailable to plant (Torri and Lavado, 2008), and the metals Pb, Cd, Cu and Zn were remained mobile (MI>10) in soil and available for plant (Ahumada *et al.*, 1999) after extraction with EDTA.

Table 4.4.10. Mobility indexes (%) of metals in spiked soil of Kushtia before and after extraction with different extractants.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
Unwashed Soil	40.93±2.42	60.41±3.57	22.81±1.35	31.24±1.85	36.03±2.14	51.49±3.05
H_2O	39.78±2.31	55.52±3.31	22.0±1.33	29.66±1.75	34.0±2.01	48.95±2.91
1 M NH_4OAc	32.58±1.98	41.25±2.51	13.66±0.85	19.95±1.21	24.2±1.52	41.44±2.53
0.1 M $CaCl_2$	36.84±2.16	46.68±2.82	16.62±0.98	24.14±1.42	27.03±1.64	45.77±2.71
0.1 M HCl	35.46±2.11	43.2±2.56	14.48±0.88	20.69±1.26	21.55±1.24	45.03±2.65
0.1 M EDTA	17.7±1.02	21.77±1.33	8.16±0.52	8.76±0.55	15.18±0.94	19.92±1.16
0.005 M DTPA	22.72±1.31	29.83±1.72	10.53±0.65	10.1±0.63	18.37±1.08	26.63±1.53

Next to it 0.005 M DTPA solution showed higher removal efficiencies of metals. The extractant DTPA reclaimed 26.84, 45.92, 15.36, 20.93, 19.29 and 29.95 % of Pb, Cd, Cr, Ni, Cu and Zn from KC soil, respectively (Table 4.4.9). The DTPA extracted over 91, 96 and 51 %

of metals from water soluble, exchangeable and carbonate bound fractions of soil, respectively. DTPA also extracted small amount of metals (9.73%) from Fe/Mn oxide bound fractions of soil (Fig. 4.4.5). The mobility indexes of metals were recorded 10.1 to 29.8 % with an average 19.7% in spiked KC soil after extraction with DTPA (Table 4.4.10). Therefore, among the extractants 0.1 M EDTA showed the highest removal efficiency of metals from spiked calcareous soil of Kushtia.

4.4.6 Metal removal from spiked soil of Chittagong by chemical extractants

The soil belongs to Chittagong Coastal Plain. The area was a compound unit of piedmont, river, tidal and estuarine floodplain landscapes. The pH of soil was neutral in nature (Table 4.1.1). The fractions of metals of spiked Chittagong (CC) soil indicated that about 2 to 5% of metals were found in water soluble forms. The exchangeable fraction of soil amounted 15.42, 37.98, 6.45, 18.37, 15.01 and 16.2 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively which were occurred 18.6% of total amount. Among metals Cd was found in highest amount and Cr was detected in lowest amount as exchangeable forms of soil. The carbonate of soil bound 18.69, 13.56, 4.18, 13.75, 10.52 and 14.17 mg kg^{-1} of above respective metals and this fraction occupied 12.7% of total metal content (Fig. 4.4.6). Therefore the mobility indexes were 36.49, 56.80, 15.67, 35.55, 28.98 and 34.24 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked CC soil, respectively (Table 4.4.12). The higher values of mobility indexes indicated that metals in soil were highly mobile and available to plants.

The soil also retained a large amount of metals in non-labile fractions. Among these fractions, the Fe/Mn oxides of soil bound the highest amount of metals. This fraction bound 25.78, 24.49, 27.57, 27.62, 28.6 and 28.9 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively which were accounted for 27.65 % of total amount. The organic matter and residual fractions retained 14 and 23.4% of metals in spiked SSy Soil, respectively (Fig.4.4.6).

Table 4.4.11. Removal efficiencies (%) of metals of different extracting agents from spiked soil of Chittagong.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
H_2O	2.54f	4.67e	3.12e	1.12d	2.21d	3.52d
1 M NH_4OAc	21.63d	53.14c	6.65d	26.60c	19.42c	21.67c
0.1 M $CaCl_2$	8.88e	46.88d	7.05d	28.22c	18.34c	16.60c
0.1 M HCl	42.77b	63.87a	14.72c	54.32a	54.08a	51.03a
0.1 M EDTA	57.12a	62.59a	25.28a	53.26a	56.76a	50.01a
0.005 M DTPA	32.02c	56.97b	18.62b	34.42b	38.70b	35.93b

The extractant H_2O removed very small amount of metals (3.6%) from CC soil. Among salt solutions, 1 M NH_4OAc extracted 21.63, 53.14, 6.65, 26.6, 19.42 and 21.67 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked SSy soil, respectively. Contrary to that 0.1 M $CaCl_2$ removed 8.88, 46.88, 7.05, 28.22, 18.34 and 16.6 % of above respective metals from soil (Table 4.4.11).

It was observed that NH_4OAc extracted more than 94, 92 and 42 % of metals from water soluble, exchangeable and carbonate bound fractions, respectively, whereas $CaCl_2$ extracted over 93, 83 and 25 % of metals from same respective fractions of soil. But these extractants were not found to extract metals from other fractions (Fig. 4.4.6). The metal extraction capacity of NH_4OAc was higher than that of $CaCl_2$. The average mobility indexes of metals were 10.25 and 15.07% in soil after extraction with NH_4OAc and $CaCl_2$, respectively (Table 4.4.12).

Table 4.4.12. Mobility indexes (%) of metals in spiked soil of Chittagong before and after extraction with different extractants.

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
Unwashed Soil	36.49±1.78	56.80±2.82	15.67±0.76	35.55±1.76	28.98±1.43	34.24±1.72
H_2O	34.31±1.71	53.64±2.64	11.76±0.61	33.47±1.63	26.45±1.31	30.57±1.52
1 M NH_4OAc	15.10±0.78	10.96±0.56	5.77±0.32	8.35±0.44	10.55±0.52	10.75±0.54
0.1 M $CaCl_2$	28.16±1.42	21.49±1.02	5.99±0.31	7.54±0.39	11.08±0.57	16.13±0.82
0.1 M HCl	9.60±0.46	5.77±0.32	1.40±0.06	1.44±0.06	1.67±0.07	1.74±0.08
0.1 M EDTA	5.71±0.32	6.06±0.31	1.48±0.08	1.62±0.09	1.58±0.08	1.43±0.06
0.005 M DTPA	9.74±0.47	13.57±0.66	2.24±0.13	14.26±0.73	7.65±0.35	10.39±0.53

The extractants 0.1 M HCl and 0.1 M EDTA removed significant amount of metals from spiked soil of Chittagong. Among them HCl extracted the highest amount of Cd, Ni and Zn of 63.87, 54.32 and 51.03 % and EDTA extracted the highest amount of Pb, Cr and Cu of 57.12, 25.28 and 56.76 % from CC soil, respectively (Table 4.4.11). Both HCl and EDTA removed more than 95 and 97 % of metals from water soluble and exchangeable fractions. In addition HCl extracted above 89 and 48 % and EDTA extracted above 91 and 57 % of metals from carbonate and Fe/Mn oxides bound fractions of soil, respectively (Fig. 4.4.6). The average mobility indexes of metals were marked reduced in spiked CC soil after extraction with HCl and EDTA and the values were 3.6 and 2.98 %, respectively (Table 4.4.12). These values were indicated a complete reduction of metal mobility in soil after extraction.

Next to these extractants 0.005 M DTPA leached significant amount of metals from CC soil. The extractant DTPA reclaimed 32.02, 56.97, 18.62, 34.42, 38.7 and 35.93 % of Pb, Cd, Cr, Ni, Cu and Zn from CC soil, respectively (Table 4.4.11). From fractionation study it was observed that DTPA removed more than 94, 98 and 55 % of metals from water soluble, exchangeable and carbonate bound fractions of CC soil, respectively. DTPA also reclaimed over 29 % of metals from Fe/Mn oxides bound fraction (Fig. 4.4.6). Consequently the extraction efficiency of metals of DTPA from CC soil was comparatively higher than NH_4OAc and $CaCl_2$ but lower than that of HCl and EDTA solutions. The mobility indexes of metals were 2.24 to 14.26 % with an average 9.64 % in spiked CC soil after extraction with DTPA (Table 4.4.12). All together both 0.1 M EDTA and 0.1 M HCl showed the maximum extraction efficiency of metals from spiked soil of Chittagong compare to other extractants.

From the above findings it can be concluded that among the metals, most of the Cd was presented in labile fractions of different soils. Consequently the highest amount of Cd was leached from soils by all extractants. Elliott and Shastri (1999) reported that a significantly greater proportion of Cd, about 78% was released by EDTA treatment. Karaca *et al.* (1944) observed that, in metal-amended soils, Cd in all extraction effectively increased with EDTA in both fine and coarse-textured soil.

It was observed that high amount of Cd was also released even from spiked calcareous soil by chelating agents (Table 4.4.3 and 4.3.9). Aydinalp and Katkat (2004) found a high recovery of Cd (57%) with DTPA extraction in calcareous soils. The high extraction percentages of Cd with EDTA and DTPA are closely related to the chelating capacity of the solution while maintaining the soil pH at slight alkaline values to optimize cation extraction (Mahler and Ryan, 1988). It was also found that the extraction capacity of Cd was higher in coarse textured soil than that of light textured soil. Karaca *et al.* (1944) noted that extractable Cd was higher in the coarse-texture soil than in the fine textured soil due to its high CEC and complexing ability.

Contrary to that Cr was distributed mostly in non-labile fraction of soils. Among them organic matter bound the highest amount of Cr (30 to 40%). Next to it, Fe/Mn oxides and residual fractions retained considerable amount of Cr in soils (Fig. 4.4.1 to 4.4.6). Aydinalp and Katkat, (2004) also reported the low solubility of Cr consistent with the very low amounts (0.1 to 6.0%) of Cr in the soluble-exchangeable pool of soil. Cifuentes *et al.* (1996) found that ionic Cr^{3+} species precipitate being favored by the presence of Fe in soil at slightly acidic to alkaline pH values. Therefore the removal efficiencies of Cr by different extractants were comparatively lower compare to other metals. Maximum 30 to 36% of Cr was reclaimed by HCl and EDTA from light textured HT and SSy soil (Table 4.4.5 and 4.4.7).

The extraction capacities of Ni, Cu, Pb and Zn were comparatively higher than Cr, but lower than that of Cd for all soils.

4.4.7 Soil pH after extraction with different extractant

The extraction of soil with 1 M NH_4OAC and 0.1 M $CaCl_2$ salt solution did not affect the pH of soil. The pH of soils was within the range of 7.01 to 8.07 after extraction with chelating agents 0.1 M EDTA and 0.005M DTPA solution. But the 0.1 M HCl solution markedly reduced pH particularly in MB, HT, SSy and CC soil and the values were 1.66, 1.93, 3.47 and 2.88, respectively. The extractant 0.1 M HCl also reduced the pH of calcareous IB and KC Soils (Table 4.4.13).

Table 4.4.13: Soil pH after extraction with different extractant.

Extractant	MB	IB	HT	SSy	KC	CC
Back ground	4.52	8.45	5.94	7.81	8.15	7.77
H_2O	4.58	8.35	5.72	7.72	8.21	7.69
1 M NH_4OAc	4.68	8.18	5.62	8.04	8.06	7.02
0.1 M $CaCl_2$	5.05	7.92	5.61	7.97	7.88	7.08
0.1 M HCl	1.66	6.16	1.93	3.47	6.13	2.88
0.1 M EDTA	7.01	7.88	7.03	8.07	7.83	7.47
0.005 M DTPA	7.15	7.72	7.29	7.72	7.94	7.16

4.4.8 Release of other cations during the extraction of heavy metals by different extractant

The release of other cations i.e. Ca, Mg, Fe, Mn and K were determined with the extraction of heavy metals from different spiked soils.

Calcium: The highest amount of Ca was found in leachate of soils after extraction with 0.1 M $CaCl_2$ because the extractant itself contained high amount of Ca. Next to it, significant amount of Ca was leached by 0.1 M HCl solution. The high amounts of Ca (1.64 and 1.67%) were recorded in leachate of calcareous IB and KC soils after extraction with 0.1 M HCl solution, respectively (Table 4.4.14).

Magnesium: The extractant 0.1 M HCl not only leached the highest amount of Mg from metal spiked soils of Madhupur, Hazaribagh, Shitakunda and Chittagong but also from the calcareous soils of Ishwardi and Kushtia. Next to it the extractant 0.1 M $CaCl_2$ and EDTA removed significant amount of Mg from all soils (Table 4.4.14).

Iron: Among the extractants, 0.1 M HCl released the highest amount of Fe from MB, HT, SSy and CC soils and the values were recorded 681.96, 461.04, 360.42 and 281.96 mg L^{-1} in leachate of respective soil, whereas EDTA extracted significant amount of Fe from calcareous IB and KC soils, the values were 62.25 and 105.22 mg L^{-1}, respectively (Table 4.4.14).

Table 4.4.14. Release of other cations during extraction of heavy metals by different extractant

Extractant	**MB**	**IB**	**HT**	**SSy**	**KC**	**CC**
			Calcium (Ca) %			
H_2O	0.0056d	0.0124e	0.014d	0.0096e	0.0072f	0.0075e
1 M NH_4OAc	0.111c	0.248d	0.228c	0.193d	0.142e	0.149d
0.1 M $CaCl_2$	1.516a	2.057a	1.653a	1.9701a	2.667a	1.745a
0.1 M HCl	0.213b	1.647b	0.312b	0.421b	1.676b	0.209b
0.1 M EDTA	0.107c	0.924c	0.241c	0.325c	0.906c	0.188c
0.005 M DTPA	0.101c	0.284d	0.305b	0.314c	0.249d	0.187c
			Magnesium (Mg) (mg kg^{-1})			
H_2O	13.87e	22.57d	13.27d	24.41e	21.77e	27.07c
1 M NH_4OAc	277.32b	461.35b	265.33b	488.12c	435.45c	541.35a
0.1 M $CaCl_2$	370.25a	474.61b	431.05a	563.93b	503.45b	581.45a
0.1 M HCl	380.13a	583.25a	435.45a	676.22a	594.31a	587.55a
0.1 M EDTA	236.95c	487.05b	209.31c	583.95b	511.32b	560.53a
0.005 M DTPA	175.92d	346.95c	219.12c	418.75d	361.45d	339.22b
			Potassium (K) (mg kg^{-1})			
H_2O	32.22d	71.12d	20.65e	32.22e	38.89e	36.66d
1 M NH_4OAc	1242.10a	1033.12a	1684.65a	555.35a	1226.91a	1797.60a
0.1 M $CaCl_2$	810.90b	733.26b	1033.20b	522.17b	890.80b	1221.90b
0.1 M HCl	382.21c	362.20c	299.98c	382.19c	388.85c	346.63c
0.1 M EDTA	353.32c	377.76c	288.87c	367.75c	363.30d	372.20c
0.005 M DTPA	108.88d	133.32d	126.65d	244.42d	228.86d	211.09cd
			Iron (Fe) (mg kg^{-1})			
H_2O	0.44d	0.59c	0.50d	0.44d	0.57c	1.19c
1 M NH_4OAc	0.33d	0.55c	0.30d	0.10d	0.55c	1.61c
0.1 M $CaCl_2$	0.54d	0.63c	0.69d	0.77d	0.58c	0.76c
0.1 M HCl	681.96a	0.62c	411.04a	360.42a	8.39b	281.96a
0.1 M EDTA	347.11b	62.25a	107.95b	60.76b	105.22a	247.11a
0.005 M DTPA	252.36c	11.91b	35.71c	11.19c	37.20bc	152.36b
			Manganese (Mn) (mg kg^{-1})			
H_2O	5.90e	0.02d	2.38e	0.05c	0.02d	6.99d
1 M NH_4OAc	118.06c	0.34d	47.62d	0.90c	0.42d	139.71c
0.1 M $CaCl_2$	109.85cd	1.65cd	60.22c	3.20c	1.93d	142.76c
0.1 M HCl	286.16a	37.91b	121.25a	154.31a	67.44b	168.51a
0.1 M EDTA	136.71b	93.47a	69.93b	70.66b	156.14a	166.62ab
0.005 M DTPA	103.74d	6.10c	59.61c	6.60c	20.36c	169.80b

Manganese: The extractant 0.1 M HCl also removed the highest amount of Mn from MB, HT, SSy and CC soils and the values were 286.16, 121.25, 154.31 and 168.51 mg L^{-1}, respectively. The 0.1 M EDTA solution leached significant amount of Mn from calcareous IB and KC soils of 93.47 and 156.14 mg L^{-1}, respectively (Table 4.4.14).

Potassium: The highest amount of K was recorded in 1 M NH_4OAc extraction for all soils. The second highest K extraction was observed in 0.1 M $CaCl_2$ solution. Next to these extractants, 0.1 M HCl and 0.1 M EDTA removed significant amount of K from all soils (Table 4.4.14).

Discussions: The removal efficiencies of heavy metals (HMs) in light texture soil (HT and SSy) were higher than those in heavy texture soil (MB, IB and KC), when the same extracting agent was used. It was likely to be related to the soil properties. The high clay and silt content in soil inhibit the metals migration to other media (Lo and Yang, 1999). Generally, the higher the proportion of the clay and silt in soil, the harder the metal to extract, because extracted heavy metals could easily be adsorbed by iron-manganese oxides located on the surface of those soil particles (Zhuang *et al.*, 2000). Karaca *et al.* (1944) evaluated that extractable Zn was higher in the coarse-texture soil than in the fine-texture soil. Fine-textured soils reduced metal mobility in soil solution. High clay content was assumed to result in stronger adsorption of metals on soil particles.

Among the salt solutions, the metal extraction capacity of NH_4OAc from different soils was comparatively higher than that of $CaCl_2$, because the buffered NH_4OAc were able to extract higher amount of metals from different soil fractions compare to $CaCl_2$. Forstner *et al.* (1981) reported that 1 M ammonium acetate effectively extracted the "ion-exchangeable" trace metals. Shuman (1979) reported that ammonium acetate dissolves oxide coatings in the hydrous oxide fraction. Gibbs (1977) reported that 1 M ammonium acetate effectively dissolved transition metals from surface coatings of soil particles. However, the removal efficiencies of metals by 1 M NH_4OAc and 0.1 M $CaCl_2$ solutions was comparatively lower than that of HCl, EDTA and DTPA extractants.

The 0.1 M HCl acid solution removed significantly high amounts of metals from spiked soils of Madhupur tract, Hazaribaagh, Shitakunda and Chittagong. Lim *et al.* (2004) reported that the extractability of most heavy metals increased when pH of the solution decreased. Soil pH plays a major function in the sorption of heavy metals as it directly controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates. It also influences ion-pair formation, solubility of organic matter, as well as surface charge of Fe, Mn and Al–oxides, organic matter and clay edges.

The fractionation study of spiked soils indicated that HCl was not only extracted metals from labile forms but also dissolved metals from non-labile forms of soil. Ahmad (2009) reported that extraction of heavy metals from contaminated soils with acid proceeds different processes involving protonation of oxide surface sites and/or exchange with sorbed metals on the permanent charge sites to dissolution process in which the reactive surface of the oxides and chlorides are dissolved. Sobrinho *et al.* (2009) reported that the 0.1 mol L^{-1} HCl was used to extract exchangeable or adsorbed cations, has demonstrated its ability to displace Cd and Zn adsorbed on Fe and Mn oxides, carbonates or organic matter fraction. Washing of metal-contaminated soils with 0.1 M HCI showed some promise as a remediation technique as it rapidly and effectively removes some heavy metals (e.g. Cd, Pb, Zn and Ni) from contaminated soils (Ahmad 2009; Devis and Hotha, 1998).

But the extraction of heavy metals with 0.1 M HCl solution showed some difficulties. The acid solution markedly reduced the pH of soils. Besides at low pH, the sorption of mineral elements on soil surface decreases and the solubilization of elements in soil solution increases. It was found from Table 4.4.15 that the extractant HCl leached the highest amount of Ca, Mg, Fe and Mn from different soils. So HCl solution not only removed a large quantity of heavy metals but also dissolved a considerable amount of beneficial elements from soil. Palma and Mecozzi (2007) observed Fe dissolution up to about the 39% of the total Fe content in the sediment under acid treatment. It seems that the removal of heavy metals from the soils should increase with decreasing pH, but this is offset by an increase in the extraction of Fe and Ca (Tandy *et al.*, 2004). Yu and Klarup (1994) reported that strong acids attack and degrade the soil crystalline structure at extended contact times.

Moreover, the 0.1 M HCl solution could not extract sufficient amount of metals from calcareous soils of Ishwardi and Kushtia. This might be due to that the HCl solution becomes neutralized by reacting with calcareous minerals of soil.

For less damaging washes, organic acids and chelating agents are often suggested as alternatives to straight mineral acid use (Yu and Klarup, 1994). Thus metal dissolution by chelating agents is likely to be more representative of a mobile metal fraction that is available to biota (Labanowski *et al.*, 2008).

The extractant 0.005 M DTPA removed considerable amounts of metals from all soils. Schalscha *et al.* (1980) reported that DTPA seems to be a better extractant for the majority of the soils, probably due to its capacity to dissolve metal precipitates. Shuman (1988) stated that addition of soluble ligands in organic matter could decrease adsorption and increase leaching.

Among the chelating agents, EDTA removed comparatively higher amount of metals than that of DTPA. Huang *et al.* (1997) found that the order of effectiveness in increasing metal desorption from the soil was (ethylene- diaminetriacetic acid (EDTA) > diethylenetriaminepentaacetic acid (DTPA).

The 0.1 M EDTA extracted significantly high amount of heavy metals from above six soils. The extractant EDTA removed the maximum amount of metals from labile fraction even though the soil was calcareous. Peters (1999) noted that the chelating organic acid EDTA was able to dislodge the exchangeable, carbonate and reducible fractions of heavy metals by washing procedures. Consequently the mobility indexes of metals were found lowest in all soils after extraction with EDTA.

The fractionation of metal from spiked soils indicated that EDTA dissolved a considerable amount of metals from carbonate, Fe/Mn oxides and organic matter bound fractions of soils beside the removal of entire amount of water soluble and exchangeable forms (Fig.

4.4.1 to 4.4.6). Stumm (1992) reported that EDTA promotes dissolution of heavy metals in hydrous oxides. Carboxylic groups of EDTA can form inner-sphere ring-type surface complexes with Fe. A resulting shift of electron density toward the central Fe ions weakens the link between the Fe and the solid lattice, thus promoting detachment of Fe and bound heavy metals into solution. EDTA is also effective in displacing organically bound metals present in soils (Pickering, 1986). Stumm (1992) proposed that the ligand of EDTA catalyzed dissolution of Fe/Mn-oxides can be described by three steps: (1) a fast ligand adsorption step, (2) a slow metal detachment step, and (3) rapid protonation of the surface restoring the original surface configuration. Thus, the exchangeable, dilute acid extractable, oxide bound and organic matter bound fractions of soil were likely available for release by the EDTA.

Elliott and Brown (1989) reported that even for relatively modest chelant dosages (EDTA : Pb molar ratio of 1:1), 70% Pb recovery was achieved from the Indiana soil. Karaca *et al.*, (1944) found that extractable metals concentration increased with increasing amounts of EDTA in both natural and metal-amended soils because of an increase in metal solubility by forming soluble metal chelates.

EDTA has been widely proposed and studied, because of its attributes of high efficiency of metal extraction, weak adsorption on soils, and effective recovery and reuse (Lestan *et al.*, 2008; Barona *et al.*, 2001). Leaching heavy metals from the polluted soil using a chelating agent is the most appealing because it generally creates less surface damage, requires a minimal amount of facilities and reduces the potential for human exposure (Ayejuyo *et al.,* 2012).

The highest degree of metal decontamination was achieved by soil washing with 0.1 M EDTA. All together EDTA was the most effective extractant for the reclamation of metals from different types of soils by chemical technology.

4.5 Humic substances as natural extracting agent for the remediation of metal contaminated soils

Since heavy metals are generally strongly bonded to soil solids, removal of heavy metals requires harsh chemicals such as strong mineral acids or powerful complexants; e.g. synthetic ethylene diaminetetraacetic acid (EDTA) (Adriano, 2001; Meers *et al.*, 2005; Kirkham, 2006; Dermont *et al.*, 2008). The use of such chemicals is not only problematic due to replacement of one pollutant by another pollutant but also because strong acids are soil destructive and complexants such as EDTA is toxic and rather persistent in the environment (Barona *et al.*, 2001; Bianchi *et al.*, 2008; Dermont *et al.*, 2008; Les`tan *et al.*, 2008). Furthermore, addition of EDTA and other strong ligands to speed up extraction (enhanced phytoextraction) may also increase heavy metal leaching from the topsoil and cause water pollution (Kos and Leˇstan, 2003; Chen *et al.*, 2004; Meers *et al.*, 2005; Evangelou *et al.*, 2007). Despite its potential, use of EDTA as a soil washing agent has disadvantages including high costs, slow biodegradability, ecosystem toxicity, replacement of one kind of chemical (heavy metals) with another (EDTA) and deterioration of soil structure (Heil *et al.*, 1999; Barona *et al.*, 2001; Kos and Leˇstan, 2003; Meers *et al.*, 2005; Evangelou *et al.*, 2007). To circumvent the disadvantages by using synthetic chemicals such as mineral acid and EDTA, it might be attractive to use soluble natural humic substances (HS) as suggested by Conte *et al.* (2005) for washing soils contaminated by heavy metals. Therefore, it is environmentally attractive if these synthetic chemicals can be replaced by cheap naturally occurring compounds as soil washing agents (Les`tan *et al.*, 2008). Due to their natural ubiquity and their capacity to form complexes with heavy metals by means of the carboxylic acid and phenolic groups (Strobel *et al.*, 2001; Weng *et al.*, 2002; Zhao *et al.*, 2007; Bianchi *et al.*, 2008; Borggaard *et al.*, 2009), it seems obvious to test soluble humic substances (HS) as cleaning agents.

4.5.1 Characterization of humic substances

In this experiment two peat soils were collected, one from Khulna University campus and another from Gopalganj basin. Humic substances were extracted from peat soils. The characterizations of humic substances were done by various parameters and evaluated their maturity. The detail analysis of optical density, E4:E6 ratio and coagulation threshold of humic substances were describe in Appendix - 8.2. It was found that humic substances (HS) of Khulna region showed higher maturity due to higher optical density, lower E4:E6 ratio and coagulation thresholds values due to intense humification process compare to that of humic substances in Gopalganj region.

4.5.2 Quantification of functional groups

The quantifications of functional groups of humic substances were obtained from FTIR spectroscopy and potentiometric titration. The detail analysis of FTIR spectra of humic substances were described in Appendix - 8.2. As revealed in the functional group analysis, the total acidity, carboxyl (-COOH), phenolic (-OH), aliphatic (-OH) and carbonyl groups of humic substances were found higher in peat of Khulna region due to intense humification processes than that of Gopalganj peat. Therefore HS of Khulna region have more functional groups compare to HS of Gopalganj region (Table 4.5.1).

Table 4.5.1: Functional groups of humic and fulvic acids in peat soils of Gopalganj and Khulna regions.

Sources	Humic Substances	Total Acidity (meq g^{-1})	Carboxyl COOH (meq g^{-1})	Phenolic[a] OH (meq g^{-1})	Aliphatic[b] OH (meq g^{-1})	Total OH (meq g^{-1})	Carbonyl (meq g^{-1})
Gopalganj Peat Soil (Satla)	Humic acid	5.05	3.12	1.93	2.58	4.51	3.12
	Fulvic acid	5.96	4.64	1.32	2.12	3.44	4.16
Khulna Peat Soil (Harta)	Humic acid	6.28	4.07	2.21	2.97	5.18	3.75
	Fulvic acid	7.42	5.41	2.01	2.34	4.35	4.76

[a] *Estimated by subtraction of carboxyl group content from total acidity.* [b] *Estimated by subtraction of Phenoli OH content from total OH content*

4.5.3 Yield of humic substances: The increased yields of humic substances (HS) were observed in peat of Khulna region possibly due to higher humification process compare to that of Gopanganj peat (Table 4.5.2). In general, younger peat contains lower levels of HS as compared to mature one (Chefetz *et al.*, 1981; Inbar *et al.*, 1990). This trend was also observed in the present study. The yields of humic and fulvic acids from mature peat of Khulna region were 2.48 and 2.29 folds higher than that of Gopalganj peat, respectively (Table 4.5.2).

Table 4.5.2 Yields of humic acid and fulvic acid from peat of Gopalganj and Khulna region.

Sources	Humic Substances	Yield (%)
Peat of Gopalganj region (Satla soil series)	Humic acid	3.14
	Fulvic acid	2.09
Peat of Khulna region (Harta soil series)	Humic acid	7.81
	Fulvic acid	4.78

4.5.4 Metal binding capacity of different humic substances

The complexing capacities of humic substances with metal ions depend on its maturity, molecular dimension and presence of functional groups. Humic substances showed comparatively higher binding capacity with Cu and Ni. Maximum 70 and 68% of Cu and Ni were bound with HS of Khulna region. Zinc, cadmium and lead also had affinity to form complex with humic substances. But humic substances showed relatively low attraction to form complex with Cr ion (Table 4.5.3).

Humic and fulvic acids of Khulna region absorbed more heavy metals ions. It was found that HS of Khulna region absorbed 1.6 to 2.0 times higher amount of metals from spiked solution than that of HS of Gopalganj region (Table 4.5.3). The humic substances of mature Khulna peat contained more functional groups, which increased its metal holding capacity. So the metal reclamation capacity of HS of Khulna peat was higher than that of Gopalganj peat.

Table 4.5.3: Absorption of Pb, Cd, Cr, Ni, Cu, Zn (%) from 100 ppm solution with different sources of humic substances (at 0.25% Conc.) passing through DAX-8 adsorbent resin containing leaching tube.

Sources	**Humic Substances**	**Absorption (%)**					
		Pb	**Cd**	**Cr**	**Ni**	**Cu**	**Zn**
Gopalganj Peat Soil (Satla)	Humic acid	26.54	31.23	24.11	38.76	42.76	31.25
	Fulvic acid	29.11	35.18	22.87	40.19	42.11	35.65
Khulna Peat Soil (Harta)	Humic acid	52.98	59.12	38.54	67.54	68.32	62.34
	Fulvic acid	57.34	62.98	35.98	68.22	70.23	63.76

Therefore, the humic substances of Khulna region were selected for further investigation.

4.5.5. Extraction capacity of humic substances from different soils

The present investigation is focused on the suitability of using soluble humic substances instead of mineral acid (HCl) and synthetic chelating agents EDTA as a washing agent for metal contaminated soil. Several studies have demonstrated that humic substances (HS) can form soluble complexes with many heavy metal ions, thus influencing their extraction efficiencies from soils (Chang *et al.*, 2003). Due to the content of carboxylic acid and phenolic groups, humic substances possess ability to form complexes with di- and trivalent metal cations (Christensen and Christensen, 1999; Weng *et al.*, 2002; Hruˇska *et al.*, 2003). The efficiency of the humic substances as heavy metal extractants is in fair agreement with the comparable contents of carboxylic acid groups and phenolic groups, which are the active groups forming soluble complexes with the heavy metals (Strobel *et al.*, 2001; Weng *et al.*, 2002; Zhao *et al.*, 2007; Borggaard *et al.*, 2009). Accordingly, a substantial capacity of soluble humic substances to dissolve (extract) heavy metals through complex formation has been demonstrated in many studies (Raulund-Rasmussen *et al.*, 1998; Jensen *et al.*, 2000; Strobel *et al.*, 2001a, 2005; Zhao *et al.*, 2007).

Therefore, humic substances rich materials are adequate for remediation of heavy metal polluted soils; it has been developed as a valuable soil reclamation material because of its low cost and few by-products (Clemente *et al.*, 2003.)

In this study, humic substances (humic acid and fulvic acid) were extracted from peat soils. Different concentrations of humic acid and fulvic acids were applied on spiked soil of six different regions. An amount of approximately 100 mg kg^{-1} of six metal ions (Pb, Cd, Cr, Ni, Cu and Zn) were used in the spiked soils. The concentrations of humic substances were gradually increased from 0.1 to 0.5 %, from which the significant doses of humic substances were determined for the maximum extraction of metals from particular soil.

Effect of pH on metal extraction from spiked soils with humic substances

The extraction capacities of humic substances were largely depend on pH. Giannis *et al.* (2007) reported that metal ion-humic fraction complexes at pH 3 were much weaker than the complexes formed at pH 6. Based on chemical equilibrium considerations, the strength of metal-organic complexes is related to the concentration of the free metal-ions in solution. The lower the free metal-ion concentration in solution under equilibrium conditions, the stronger the complex is, and the higher the free metal-ion concentration in the solution under equilibrium conditions, the weaker the complex is (Giannis *et al.,* 2007).

The extraction capacities of humic substances largely varied with the pH of soil and solution (Fig. 4.4.4). The extraction of metals from spiked soil of Madhupur tract (MB) by humic substances was increased with increasing the pH of extractant from 4.0 to 6.0. Humic substances at the concentration of 0.3 % extracted the maximum quantity of metals from spiked soil of Madhupur tract at pH 6.0. The spiked soil of Hazaribagh (HT) showed the similar pattern of extraction by humic substances at different pH. The maximum extraction of metals from spiked HT soil was executed by humic substances at pH 6.0 to 7.0. The metal extraction yields of humic substances from spiked soils of Madhupur tract and Hazaribagh were again decreased at solution pH of 8.0 (Fig.4.5.4).

Contrary to that the extraction capacities of metals from spiked calcareous soils of Isheardi and Kushtia were increased with increasing pH of humic substances from 4.0 to 8.0. The maximum extraction of metals from calcareous soils was recorded with humic substances at pH 7 to 8 (Fig. 4.5.1). Gueguen *et al.* (2003) found that the tendency of CO_3^{2-} to the form complex in solution with metal in the pH range ($7<pH<9$). Thus, the change of pH in the range from 7 to 9 may strongly influence metal complexation with HS in calcareous soil.

The highest removal efficiencies of metals from spiked soils of Shitakunda and Chittagong were observed with humic substances when the pH of solution was 7.0 (Fig. 4.5.1).

It was appeared from the above findings that the extraction capacity of metals were highly depends on the pH of soil. The soil of Madhupur and Hazaribagh were slightly acidic in nature. Consequently the maximum extraction of metals from MB and HT soil were observed with humic substances at pH 6.0 to 7.0. In contrast calcareous soils were alkaline in nature. So that humic acid removed the maximum amount of metals from calcareous soil at high pH of solution.

The binding of metal cations with humic substances is strongly affected by pH, firstly due to the competition between protons and metal cations for humic binding sites, and secondly because of cation hydrolysis (Tipping, 2002). The complex reaction of HS and heavy metal ions may be caused by the dissociation of protons from the carboxyl group of HS which starts at pH 6. This association increases with increasing pH (Allard and Arsenie, 1991).

In this experiment, the pH of humic and fulvic acid were maintained 6.5 for slightly acidic soil of Madhupur and Hazaribagh. And the pH of humic substance were maintained at 7.5 for the extraction of calcareous IB, KC and saline SSy and CC soils.

4.5.5.1 Reclamation of metal spiked Madhupur tract soil (MB) by humic substances

The red soil of Madhupur tract (MB) has high clay content and textural class was silty clay loam. The soil contained high amount of Fe/Mn oxides compound. Among clay minerals, koalinite, fine grained mica and Illite were dominant in soil of Madhupur tract (Table 4.1.1 and 4.1.2).

Extraction of metals from spiked soil of Madhupur tract (MB) by humic substances indicated that the amount of metals in the leachate increased with increasing concentrations of humic substances. Borggaard *et al.* (2009) showed that at increasing solution to soil ratio and HS concentration, the proportion of activated HS increases, leaving more HS available for complexation and extraction of heavy metal cations.

Humic acid at the concentration of 0.3 % extracted maximum 22 % Pb, 45 % Cd, 20.5 % Cr, 36.42 % Ni, 34.85 % Cu and 28.39 % Zn from heavy textured MB soil at the concentration of 0.3 %. In contrasts fulvic acid removed 43.68 % Pb, 64.32 % Cd, 32.64 % Cr, 48.04 % Ni, 45.02 % Cu and 43.03 % Zn from MB soil at 0.4 % concentration (Table 4.5.4).

It was observed that humic substances extracted high amount of Cd from the spiked soil, because most of the Cd was amounted in water soluble and exchangeable forms of soil (Fig 4.5.2). Li and Thornton (2001) observed that the first three fractions (exchangeable, Fe/Mn oxides and carbonate bound) accounted for more than 70 % Cd and the exchangeable cadmium alone was up to 52 % of the total content. In contrast humic substances extracted relatively lower amount of Cr from soil. This was possibly due to that chromium was found to distribute mostly in non-labile fraction of the soil of Madhupur tract (Fig. 4.5.2).

Fractionation study of spiked MB soil indicated that humic acids removed 93 - 97 % of metals from water soluble and exchangeable fractions. Fulvic acid extracted 95 - 98 % of metals from water soluble and exchangeable forms. No metals were detected in carbonate bound fractions (Fig. 4.5.2) as the pH of Madhupur soil was acidic in nature (Table 4.1.1). Consequently the mobility indexes of metals were considerably lowered to 1.17, 7.38, 2.2, 3.24, 2.61 and 2.34 % for Pb, Cd, Cr, Ni, Cu and Zn after extraction with 0.3 % humic acid, respectively. In contrast extraction of soil with fulvic acid at the concentration of 0.4 % caused the mobility indexes of 0.42, 1.6, 0.93, 1.17, 0.61 and 0.53 % for above respective metals in spiked MB soil (Table 4.5.5). Results of several experiments showed that the addition of humic substances lowered the free and labile metal concentrations of the soil (Yu *et al.*, 2002; Li *et al.*, 2003; Jiang *et al.*, 2005; Gao *et al.*, 2009; Janos *et al.*, 2010).

It was observed that humic substances also leached part of metals from non-labile fractions of MB soil. Humic acid removed 16.29, 4.15, 19.1, 23.11, 32.29 and 13.28 % of Pb, Cd, Cr, Ni, Cu and Zn from Fe/Mn oxides bound fraction, whereas fulvic acid extracted 54.95, 47.31, 41.4, 59.28, 65.38 and 48 % of above respective metals from the same fraction of spiked soil. Of the metals, humic substances extracted highest amount of Cu from Fe/Mn oxide bound fractions of soil (Fig. 4.5.2). Sawada *et al.* (2003) reported that copper-oxinate could be easily dissolved by humic substances from kaolinite and removed by electro-osmotic flow.

Between the humic substances, fulvic acid extracted comparatively higher amount of metals from Fe/Mn oxides bound fractions than that of humic acid. Lehmann *et al.* (1987) reported that fulvic acids have electron donating substituents on the aromatic ring - when added to soils containing Fe and Mn oxides are chemically reduced, releasing Fe^{2+} and Mn^{2+} as soluble products. Dissolution can be assisted or enabled by redox reactions in which fulvic acids reduce Fe (Ill) at the surface (Goodman and Cheshire, 1987). Fulvic acid also dissolves the Mn-oxide surfaces following same reaction (Stone and Morgan, 1984a, 1984b).

All together, the metals removal efficiency of fulvic acid was considerably higher than that of humic acid in acidic red soil of Madhupur tract. Zhang *et al.* (2013) reported that fulvic acid reduced Pb and Cd uptake by plant more efficiently than humic acid in acidic red and paddy soil and they found that the addition of 4 g kg^{-1} HA decreased the tobacco leaf Cd concentration by 8.74 % and 11.03 %, while FA decreased leaf Cd concentration by 13.58 % and 32.84 % in the acidic red and paddy soils, respectively.

Organic matter bound metals were, however, increased with increasing concentrations of humic substances. Humic acid at 0.3 % concentration increased 7.1 to 15.71 % metals and fulvic acid increased 4.22 to 14.03 % metals at 0.4 % concentration in organic matter bound fractions of soil (Fig. 4.5.2). Consequently the metal extraction capacities of HA and FA from spiked MB soil were slightly decreased after 0.3 and 0.4 % of concentration, respectively possibly due to the immobilization effect of HS at high concentration.

4.5.5.2. Reclamation of metal spiked calcareous soil of Ishwardi (IB) by humic substances

Soil of Ishwardi EPZ belongs to the active Ganges River Floodplain. The soil was alkaline in nature. The textural class was silt loam. The carbonate content was found high (Table 4.1.1), because the soil was developed from calcareous parent material.

Humic acid removed maximum 15.02, 38.83, 13.78, 20.64, 21.43 and 18.95 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked calcareous soil of Ishwardi (IB) at the concentration of 0.3 %, respectively. In contrast fulvic acid leached the highest 25.85, 48.13, 20.44, 27.06, 28.56 and 26.31 % of above respective metals from same soil at the concentration of 0.4 % (Table 4.5.6).

The fractions of spiked calcareous soil of Ishwardi (IB) indicated that very low amounts of metals were present in water soluble and exchangeable forms and the values were 3.13 and 9.64 % of total amount, respectively. Humic and fulvic acids each removed 94 - 97 % of metals from these water soluble and exchangeable fractions of soil (Fig 4.5.3).

A major portion of metals (25.77 %) were associated with carbonate adsorbed fraction in IB soil (Fig 4.5.3). In calcareous soils metals were mostly bound to carbonates and under alkaline conditions precipitation of metal carbonates were favoured (Lo and Yang, 1999). Humic acid removed maximum 21.56, 12.89, 23.28, 12.75, 32.9 and 28.94 % of Pb, Cd, Cr, Ni, Cu and Zn from carbonate bound fraction, whereas fulvic acid extracted maximum 37.08, 35.93, 36.26, 10.16, 39.5 and 36.8 % of above respective metals from carbonate adsorbed fraction of soil (Fig. 4.5.3). The mobility indexes of metals were 30.05, 40.7, 13.7, 17.92, 19.99 and 37.16 % for Pb, Cd, Cr, Ni, Cu and Zn after extraction with 0.3 % humic acid, respectively. On the contrary the mobility indexes were 26.73, 35.7, 12.15, 14.61, 19.87 and 35.4 % of above respective metals in spiked soil after extraction with 0.4 % fulvic acid (Table 4.5.7). Therefore, these indexes of metals were yet high after extraction because, humic substances were not able to extract much metal from calcareous soils. During extractions, the active, complex-forming carboxylate and phenolate groups of HS (Christensen and Christensen, 1999; Strobel *et al.*, 2001b, 2005; Weng *et al.*, 2002) are inactivated by bonding to calcium from $CaCO_3$ and other major di- and trivalent cations of calcareous soil (Zhao *et al.*, 2007). Borggaard *et al.* (2009) extracted only 27 % Cd from contaminated calcareous soil.

Moreover humic substances extracted small amount of metals from non-labile fraction of IB soil. Among them, humic and fulvic acids removed 7 to 38 % and 17 to 51 % of metals from Fe/Mn oxides bound fraction, respectively (Fig 4.5.3).

It is evident from the data that the extraction efficiencies of metals of fulvic acid were relatively higher than that of humic acid from spiked calcareous soil of Ishwardi (Table 4.5.6). Borggaard (2011) reported that the HA is unsuited for cleaning Pb contaminated soils at least if the soil was calcareous. Zhang *et al.* (2013) reported that fulvic acid reduced Pb and Cd uptake by plant more efficiently than humic acid in calcareous paddy soil. Zhang *et al.* (2013) reported that FA decreased the leaf Pb concentration by 62.65 % from soil having 2.48 g kg^{-1} $CaCO_3$ while Pb concentration increased slightly in the presence of HA.

Like the acidic soil of Madhupur tract humic substances gradually increased organic matter bound metals with increasing concentrations. Humic and fulvic acids increased 8 and 10 % metals in organic matter bound fractions of IB soil at the concentration of 0.3 and 0.4 %, respectively (Fig. 4.5.3). As a result the metal extraction capacities of HA and FA from spiked IB soil were slightly decreased after 0.3 and 0.4 % concentration (Table 4.5.6), respectively, possibly due to the formation of stable HS-metal complex at high concentration of HS.

4.5.5.3. Reclamation of metal spiked soil of Hazaribagh (HT) by humic substances

The soil of Hazaribagh areas was found to be slightly acidic in nature. The textural class of soil was loam (Table 4.1.1). Humic acid extracted maximum 44.18, 67.02, 30.71, 50.23, 52.01 and 44.46 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked soil of Hazaribagh (HT) at the concentration of 0.4 %. In contrasts fulvic acid removed 43.08, 66.36, 27.67, 49.32, 51.04 and 42.14 % of above respective metals from HT soil at the same concentration (Table 4.5.8).

The fractions of light textured spiked soil of Hazaribagh (HT) indicated that both humic and fulvic acids removed 94 - 98 % of metals from water soluble and exchangeable forms of spiked soil. Metals were not detected in carbonated bound fractions possibly due to slightly acidic nature of Hazaribagh soil (Fig. 4.5.4). As a result the mobility indexes of metals were considerably reduced after extraction with humic substances. The mobility indexes were 1.2, 1.12, 1.09, 0.99, 1.05 and 1.16 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked HT soil after extraction with 0.4 % humic acid, respectively. In contrast the mobility indexes were 1.25, 1.26, 1.12, 1.09, 1.25 and 1.26 % for above respective metals in spiked HT soil after extraction with the same concentration of fulvic acid (Table 4.5.9).

Humic substances leached high amount of Cd, because a large amount of Cd (55.92 %) was presented in labile fractions of spiked HT soil (Fig 4.5.4). Li and Thornton (2001) observed that the first three fractions (exchangeable, Fe/Mn oxide and carbonate bound) accounted for more than 70 % of cadmium. Next to it humic substances extracted high amount of Ni and Cu from soil (Table 4.5.8). McBride (1989) reported that Ni^{2+} and Cu^{2+} ions bonded more strongly on humic substances. Contrary to that humic substances removed relatively lower amount of Cr from spiked HT soil (Table 4.5.11) because most of the chromium (84.4 %) were retained in non-labile fraction of soil (Fig. 4.5.4).

It was found that humic substances also leached a portion of metals from non-labile fraction of soil. Humic acid at the concentration of 0.4 % extracted 64.21, 62.5, 51.5, 59.67, 75.73 and 59.85 % of Pb, Cd, Cr, Ni, Cu and Zn from Fe/Mn oxides bound fraction and fulvic acid at the concentration of 0.4 % removed 63.68, 62.06, 46.44, 55.17, 75.36 and 53.14 % of above respective metals from same fractions of spiked HT soil (Fig. 4.5.4). Humic substances with the capability to form complex with metals can potentially increase concentrations of these metals in soil solution by dissolution reactions at mineral surfaces (Jorgensen, 1976; Manley and Evans, 1986; Pohlman and McColl, 1986). Goodman and Cheshire (1982) reported an enhanced reduction of Fe/Mn oxide surfaces by humic acid.

All at once humic substances extracted considerable amount of metals from spiked light textured soil of Hazaribagh. Among the humic substances, humic acid extracted somewhat higher amounts of metals than that of fulvic acid. This was possibly due to that larger molecular sized humic acid got more contact time with soil particles during percolation through light textured soil than that of fulvic acid. It was found from the experiment that fulvic acid quickly percolated the light textured soil compare to humic acid.

However organic matter bound metals were increased with increasing concentrations of humic substances. Humic and fulvic acids increased 6.95 and 6.53 % metals in organic matter bound fractions of soil at the concentration of 0.4 % (Fig. 4.5.4). Consequently the metal removal efficiencies of humic substances decreased with increasing concentration above 0.4 % possibly due to the immobilization effect of HS at high concentration.

4.5.5.4. Reclamation of metal spiked soil of Shitakunda (SSy) by humic substances

The soil of Shitakunda was slightly alkaline in nature. Sand content was high and textural class was loam (Table 4.1.1). Humic substances extracted considerable amount of metals from spiked soils of Shitakunda. Humic acid at the concentration of 0.4 % removed maximum 43.33, 68.82, 31.12, 46.05, 46.71 and 42.28 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked SSy soil. On the other hand fulvic acid extracted 41.88, 66.12, 30.14, 45.12, 45.84 and 41.09 % of above respective metals from SSy soil at the same concentration (Table 4.5.10).

Fractionation study of spiked soil of Shitakunda indicated that very low amounts of metals (2 - 5 %) were found in water soluble forms. The soil adsorbed 19 % of metals in exchangeable form. Among them, humic acid and fulvic acids at the concentration of 0.4 % removed 93 - 97 % of metals from water soluble and exchangeable fractions of spiked SSy soil (Fig. 4.5.5).

Carbonate fraction of soil adsorbed 16.53 % metals of total amount. Humic acid and fulvic acids leached 73.3 % and fulvic acid extracted 69.56 % of metals from above carbonate bound fraction (Fig. 4.5.5). The mobility indexes of metals were 6.16, 18.96, 5.97, 12.42, 4.75 and 10.85 % for Pb, Cd, Cr, Ni, Cu and Zn after extraction with 0.4 % humic acid, respectively. In contrast the mobility indexes were 6.4, 19.28, 6.11, 13.03, 5.69 and 11.87 % of above respective metals in spiked SSy soil after extraction with 0.4 % fulvic acid (Table 4.5.11). Therefore mobility indexes of Cd, Ni and Zn were yet high (>10) after extraction with HS, which indicated the mobility of these metals in soil after extraction with HS.

It was found that humic substances also removed a portion of metals from non-labile fraction. Humic acid extracted 34.89 to 67.23 % and fulvic acid extracted 37.9 to 65.94 % of metals from Fe/Mn oxides bound fraction of spiked SSy soil at the concentration of 0.4 % (Fig. 4.5.5).

Therefore humic substances extracted significant amount of metals from spiked soil of Shitakunda. Among the humic substances humic acid extracted fairly higher amount of metals form the light textured soil of Shitakunda than that of fulvic acid.

However organic matter bound metals were increased with increasing concentrations of humic substances. Humic and fulvic acids increased 3 to 11 % metals in organic matter bound fractions of soil at the concentration of 0.4 % (Fig. 4.5.5). It was also observed that metal removal efficiencies of humic substances decreased with increasing concentration of HS above 0.4 % (Table 4.5.10) possibly due to the immobilization effect of HS at high concentration (Fig. 4.5.5).

4.5.5.5. Reclamation of metal spiked calcareous soil of Kushtia (IB) by humic substances

Soil of Kushtia was a complex mixture of calcareous silty and clayey alluvium. The soil belongs to the High Ganges River Floodplain which was found to be alkaline in nature. The textural class of soil was silt loam. The carbonate content was recorded high in soil of Kushtia (Table 4.1.1), because it was developed from the calcareous parent material.

Humic acid at the concentration of 0.3 % extracted the maximum 14.74, 36.76, 12.61, 20.57, 21.63 and 17.54 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked calcareous soil of Kushtia (KC), respectively. Contrary to that fulvic acid removed the higher amount of 24.07, 46.02, 17.41, 26.87, 27.23 and 25.41 % of above respective metals from soil at the same concentration (Table 4.5.12).

It was observed that humic substances extracted relatively lower amount of metals from calcareous soils compare to other soils. This might be due to that during extractions, the active, complex-forming carboxylate and phenolate groups of HS (Christensen and Christensen, 1999; Strobel *et al.*, 2001b, 2005; Weng *et al.*, 2002) are inactivated by bonding to calcium from $CaCO_3$ of calcareous soil (Zhao *et al.*, 2007). Borggaard *et al.* (2011) extracted only 17 % of total Ni from calcareous soil by 600 mM HS.

The obtained fractions of spiked calcareous soil of Kushtia (KC) indicated that very small amounts of metals were associated with water soluble and exchangeable forms and the values were 3.22 and 10 % of total amount, respectively. Humic acid at the concentration of 0.3% removed 90 to 93% and 90 to 94% of metals from water soluble and exchangeable forms of soil, respectively. In contrast FA at the concentration of 0.4% leached 90 to 95% and 90 to 97% of metals from water soluble and exchangeable forms of soil, respectively (Fig 4.5.6).

It was also found that 27.3 % of metals were associated with carbonate bound fraction of spiked soil of Khustia (Fig. 4.5.6). Humic acid removed maximum 20.42, 23.52, 32.67, 35.07, 40.6 and 23.62 % and fulvic acid extracted maximum 37.01, 39.7, 38.23, 48.1, 58.02 and 39.13 % of Pb, Cd, Cr, Ni, Cu and Zn from this carbonate adsorbed fraction, respectively.

All together HA and FA dissolved average 29.32 and 43.35 % of metals from carbonate bound fraction of KC soil, respectively (Fig 4.5.6). The mobility indexes of metals were recorded lowest in soil after extraction with fulvic acid at the concentration 0.4 % and the values were 26.68, 30.68, 13.76, 17.06, 15.55 and 35.32 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively. Whereas 0.3% humic acid lowered the mobility indexes minimum 31.91, 34.39, 14.21, 19.59, 19.84 and 39.61 % for above respective metals in the same soil (Table 4.5.13). Therefore, mobility indexes of metals were hitherto high after extraction with humic substances. The high values of mobility index indicated that humic substances were not that much effective to extract metal from calcareous soils.

The non-labile fractions of soil also retained a large quantity of metals in spiked KC soil. Among them the Fe/Mn oxides of soil binded 19.07 % of metal of total amount (Fig. 4.5.6). Humic substances dissolved small amount of metals from these oxide fractions. Humic and fulvic acids at the concentration of 0.3 % and 0.4 % extracted 4 to 28 % and 10 to 39 % of metals from Fe/Mn oxides bound fraction, respectively (Fig 4.5.6).

Fulvic acid at the concentration of 0.4% leached 27.85 % of metals and 0.3% humic acid removed 20.64 % of metals of total amount from KC soil. Therefore the metal removal efficiency of fulvic acid from spiked calcareous soil of Kushtia was comparatively higher than that of humic acid.

Like investigated soils humic substances accumulated metals in organic matter bound fraction with increasing concentrations. Humic and fulvic acids increased 3 to 16 % and 3 to 21 % metals in this fraction at the concentration of 0.3 and 0.4 %, respectively (Fig. 4.5.6). Consequently the extraction capacities of metals of HA and FA from spiked KC soil were gradually decreased above concentrations of 0.3 and 0.4 %, respectively (Table 4.5.12), possibly due to the formation of immobile HS-metal complex at high concentration (Fig 4.5.6).

4.5.5.6. Reclamation of metal spiked soil of Chittagong (CC) by humic substances

The soil belongs to Chittagong Coastal Plain AEZ. The pH of soil was neutral in nature. The textural class was silt loam (Table 4.1.1). Humic substances extracted considerable amounts of metals from the spiked soil of Chittagong. Humic acid at the concentration of 0.4% removed maximum 26.45, 52.83, 24.14, 40.01, 38.22, and 32.71 % of Pb, Cd, Cr, Ni, Cu and Zn from the soil. Whereas fulvic acid at the same concentration extracted maximum 29.26, 57.41, 25.02, 39.83, 37.24 and 34.36 % of above respective metals from the spiked soil of Chittagong (Table 4.5.14).

From the fractionation of spiked CC soil, it was observed that 0.4 % humic acid removed 90 to 98 % and 95 to 99 % of metals from water soluble and exchangeable forms. Whereas fulvic acid at same concentration removed 93 to 99 % and 96 to 99% of metals from the same respective forms of soil. The carbonate fraction of soil adsorbed 12.7 % of metals of total content in soil. Humic and fulvic acids at the concentration of 0.4 % leached 40 to 60 % and 45 to 59% of metals from carbonate bound forms which were 6.31 and 6.3 % of total amount, respectively (Fig 4.5.7).

The mobility indexes of metals were reduced after extraction with humic substances. The mobility indexes were 16.01, 15.54, 4.01, 10.05, 6.97 and 7.69 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked CC soil after extraction with 0.4 % humic acid, respectively. In contrast the mobility indexes of spiked CC soil were 14.98, 13.84, 3.46, 10.21, 7.71 and 6.57 % for above respective metals after extraction with same concentration of fulvic acid (Table 4.5.15).

The soil retained 54.43 % of metals in non-labile fractions. Among them the Fe/Mn oxides of soil bound the highest amount of metals (27.64 %). It was found that humic and fulvic acids at the concentration of 0.4 % dissolved 39.26 and 42.25 % of metals from these oxides fraction, respectively (Fig 4.5.7). All at once fulvic acid at the concentration of 0.4 % extracted somewhat higher amount of metals from spiked soil of Chittagong than that of humic acid at the same concentration.

Like previous soil humic substances increased metal contents in organic matter bound fraction with increasing concentrations of humic substances. Humic and fulvic acids increased 16.33 and 14.02 % metals in organic matter bound fractions of soil at the

concentration of 0.4 % (Fig. 4.5.7). The metal extraction capacities of humic substances decreased with increasing concentration of HS above 0.4 %.

From the above findings it can be concluded that among the metals, most of the Cd were distributed in labile fraction, which occupied 52 to 63 % of total content in soil. Consequently the Cd removal efficiencies of humic substances were observed high in all soils. The highest 65 to 70 % of Cd was removed by humic substances from spiked soils of Hazaribagh and Shitakunda.

Contrary to that humic substances extracted considerably lower amount of Cr from spiked soils. The $Cr(H_20)^{6+}$ is an aquo complex with very slow H_20 exchange rates and extremely sluggish exchange with ligands. The unusually large crystal-field stabilization energy of Cr presents an energy barrier to ligand exchange because the exchange process necessitates that the six-coordinate complex be distorted (McBride, 1989; Lames *et al.*, 1983). Besides, most of the Cr was found in non-labile fraction of soil. The Fe/Mn oxides and organic matter of soil retained 50 to 65 % of Cr. Cifuentes *et al.* (1996) reported that Cr^{3+} species precipitated in presence of Fe and occluded into them.

Humic substances extracted significant amount of Cu from all soils. Extraction of Cu increased with increasing doses of humic substances. Kochany and Smith (2001) reported that the removal efficiency of copper increased with increasing dose of humate solution and up to 94 % of Cu was removed from an artificial sewage by the addition of 2000 mg L^{-1} humate. It was found that humic acid removed maximum 48 - 52 % of Cu from light textured HT and SSy soils. Borggaard *et al.* (2011) also extracted up to 54 % Cu from a light textured soil by HS at 600 mM concentration. Boyd *et al.* (1981a) found that the Cu^{2+}-humic acid complexes has fairly high Cu^{2+} sorption levels of chelates and Cu^{2+} at high levels in humic acid is bonded to carboxylate via a monodentate bond. Therefore humic substances removed comparatively higher amount of Cu than that of other metals like Cr, Ni, Zn and Pb even from calcareous soil. Borggaard *et al.* (2009) reported that the extraction of Cu from the calcareous soil increased from 29 % to 41 % when the concentration was increased from 25 mM to 100 mM DOC. In general, metals within the same group in the periodic table and having the same charge tend to have more rapid rates of exchange as their ionic radii increase. Copper has a rapid exchange rate for ligands relative to the other first-row transition metals (Cotton and Wilkinson, 1980).

Humic substances also leached significant amount of Ni from soils. This was because Ni^{2+} ion would gain the stability to form complex more strongly to amines groups of humic substances (McBride, 1989).

Humic substances removed considerable amount of Pb and Zn from soils. But the average extraction capacities of Pb and Zn were lower than that of Cu and Ni. Marquenie *et al.* (1981) reported that humic substances decreased Zn availability to plants when added to contaminated soils for remediation. Yuan and Lavkulich (1997) showed that the adsorption capacity of a soil for Zn was reduced by 72 % when 11 % of the organic carbon content was lost.

It was commonly found that humic substances extracted considerably higher amount of metals from light textured soil than that of heavy textured soil. The effect of humic substances on the extraction of heavy metals from soils is strongly dependent on the inherent soil properties and the source of heavy metal contamination in soil. The stimulative effect of HS is probably related to the content of extractable fraction of metals in contaminated soils. Heavy metals in clay are more difficult to be extracted out than those in silt/sand probably because extracted heavy metals could easily be readsorbed by iron-manganese oxides located on the surface of clay particles, and the combination is too strong to make the metals migrate to other media (Xia *et al.*, 2009).

It was also found that among the humic substances, humic acid removed comparatively lower amount of metals from heavy textured soil of Madhupur, Ishwardi and Kushtia than that of fulvic acid. This was probably due to that higher molecular structure of HA take longer time to penetrate heavy textured soil and form immobile HS-metal complex at high concentration. Zhang and Lo (2006) and Mulligan *et al.* (1999) reported that the fine-grained soils reduced chemical extraction efficiency when required longer contact times. On the other hand the lower molecular structure and higher functional groups containing fulvic acid can easily penetrate and extract more metals from those heavy textured soils.

On the other hand fulvic acid extracted somewhat lower amount of metals from light textured soil of Hazaribagh and Shitakunda compare to humic acid. This might be due to that fulvic acid has a lower molecular structure, which percolated the light textured soil with a very short period of time (2-3 hours) compared to humic acid (more than 6 hours). Chao *et al.* (1998) reported that a common washing time of 6 h was required for complexation of these divalent metal ions by chelants which was found to be almost effective. Consequently higher molecular sized humic acid got more contact time with soil particles and extracted to some extent higher amount of metals from spiked soil.

All at once both humic substances extracted relatively lower amount of metals from calcareous soil. The corresponding major cations of Ca may interfere with chelating process of HS in calcareous soil. High calcite content and high buffering capacity of calcareous soil may decrease the leaching efficiency of humic substances (Mulligan *et al.* 1999; Tejowulan and Hendershot 1998).

Therefore humic substances can extract the most bioavailable and leachable heavy metal fractions, it seems to be a suitable substitute for the environmentally problematic EDTA as soil cleaning agent (Borggaaed *et al.*, 2009).

In addition, HS seems very effective in removal of organic pollutants in soils (Conte *et al.*, 2005), indicating that HS can be used in remediation of soil contaminated by a mixture of organic and inorganic pollutants. On the other hand, the possibility of using processed HS isolated from organic wastes and peat soils has economic as well as environmental advantages. Instead it might be possible to produce substantial quantities of cheap soluble HS by subjecting various organic waste products (Stevenson and Cole, 1999)

The next step in development of the cleaning technique must deal with handling of the extracts obtained by extraction of the heavy metal polluted soil by humic substances. These extracts were aqueous solutions containing heavy metals, HS and may be organic pollutants, i.e. the extracts were polluted water and should be treated as such. This is an advantage as more possibilities exist for handling the pollution when in water than in soil. Of these possibilities the following can be mentioned (Borggaard *et al.*, 2009):

(i) water evaporation and burning of organic matter may turn the heavy metals into slag that might be used in metal refinement or disposed of as concentrated inorganic waste residue;
(ii) precipitation of the heavy metals for example by adding a strong base;
(iii) regaining of the metals by electrolysis;

(iv) oxidative decomposition (mineralization) of the ligand (HS) by a strong oxidizing agent such as ozone (O_3) will lead to heavy metal precipitation and removal of the organic compounds.

Therefore, humic substances might be suggested for environmentally friendly remediation of heavy metal contaminated soils.

4.6Interaction among natural and synthetic chelating materials for the remediation of metal contaminated soils

A comparison study of metal removal efficiencies of humic substances and EDTA is presented in this section. EDTA (0.1 M) extracted 37 - 65 % Pb, 60 - 71 % Cd, 24 - 34 % Cr, 35 - 63 % Ni, 35 - 60 % Cu and 43 - 58 % Zn of total amount from different types of spiked soils (Table 4.4.1, 4.4.3, 4.4.5, 4.4.7, 4.4.9 and 4.4.11). Whereas, humic substances extracted maximum 16 - 45 % Pb, 44 - 70 % Cd, 16 - 33 % Cr, 22 - 51 % Ni, 24 - 52 % Cu and 21 - 45 % Zn of total amount from different soils (Table 4.5.4, 4.5.6, 4.5.8, 4.5.10, 4.5.12 and 4.5.14). EDTA in general extracted comparatively higher amount of metals from soils than that of humic substances. The difference between HS and EDTA efficiency may be explained by the considerably higher content of carboxylic acid groups in EDTA compared with HS for the same carbon concentration (Borggaard *et al.*, 2011). With a concentration of 25 mM DOC, EDTA contributes 10 mmol COOH, whereas the HS samples contribute 1.8 - 2.5 mmol COOH (Borggaard *et al.*, 2011). The content of nearly 100 mmol COOH/mol C in HS may, however, be compared with the 400 mmol COOH/mol C in EDTA indicating that EDTA has a stronger ligand than HS (Borggaard *et al.*, 2009). Consequently, EDTA is expected to be a more efficient cleaning agent of heavy metal polluted soils than HS.

Even though humic substances extracted less heavy metal than EDTA, the HS-extracted metal may comprise the most soluble, and hence most mobile, fractions as indicated by sequential extraction of metal from the residues resulting from several extractions with HS and EDTA (Borggaard *et al.*, 2009). Both extractants almost eliminated the most soluble fractions including exchangeable, carbonate- and oxide-bound (Borggaard *et al.*, 2009). Furthermore, the amounts of heavy metals extracted by HS increased with increasing HS concentration. Consequently, HS can be considered a possible and environmentally friendly alternative to the environmentally problematic EDTA as washing agents for metal contaminated soils in accordance with previous studies (Bianchi *et al.*, 2008; Borggaard *et al.*, 2009; Soleimani *et al.*, 2010).

Humic substances may also have some limitation. Humic substances were less suited for cleaning metals from contaminated calcareous soil. During extractions, the active, complex-forming carboxylate and phenolate groups of HS (Christensen and Christensen, 1999; Strobel *et al.*, 2001b, 2005; Weng *et al.*, 2002) are inactivated by bonding to calcium from $CaCO_3$ and other major di- and trivalent cations of calcareous soil (Zhao *et al.*, 2007). In addition humic acid could not extract sufficient amount of metals from heavy textured soil due to its large molecular structure. To solve the above difficulties of humic substances to extract metals from calcareous and heavy textured soils and to improve its efficiency powerful extractants like EDTA combined with HS were used to remediate metal contaminated soils.

4.6.1. Remediation of metal spiked soil of Madhupur tract (MB) by different combinations of humic substances and EDTA

Interaction among humic acid and EDTA has shown that the significant amounts of metals were leached from spiked soil of Madhupur (MB) by 0.3 % HA in combination with 0.06 M EDTA solution (Table 4.6.1). This combined extractant removed 57.54, 63.03, 25.52, 53.65, 53.32 and 53.54 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked soil of Madhupur tract (MB) by using 40 % less EDTA which were also significantly similar as extracted by 0.1 M EDTA solution (Table 4.6.1).

Table 4.6.1. Metal extraction efficiencies (%) of humic substances, EDTA and combined extractants from spiked soil of Madhupur tract (MB).

Extractants	Pb	Cd	Cr	Ni	Cu	Zn
0.3 % HA	22.33d	45.37c	20.54b	36.42d	34.85c	28.18d
0.1 M EDTA	56.78a	62.93a	24.84a	51.16ab	51.68a	52.67a
0.3 % HA + 0.02 M EDTA	35.76c	54.15b	22.33b	42.02c	39.63c	36.21c
0.3 % HA + 0.04 M EDTA	48.89b	60.45a	25.11a	49.45b	46.67b	47.85b
0.3 % HA + 0.06 M EDTA	57.54a	63.03a	25.52a	53.65ab	53.32a	53.54a
0.3 % HA + 0.08 M EDTA	58.94a	64.44a	26.57a	54.78a	54.18a	55.22a
0.3 % HA + 0.10 M EDTA	59.03a	65.34a	26.88a	55.22a	54.87a	55.67a
0.4 % FA	43.68c	64.32b	32.64c	48.04c	45.02d	43.03c
0.1M EDTA	56.78a	62.93b	24.84d	51.16bc	51.68bc	52.67ab
0.4 % FA + 0.02 M EDTA	50.76b	64.06b	33.54bc	50.27bc	49.74cd	49.01b
0.4 % FA + 0.04 M EDTA	59.05a	69.96a	36.43ab	54.45ab	55.76ab	53.32ab
0.4 % FA + 0.06 M EDTA	60.17a	70.59a	37.65a	56.93a	58.22a	57.02a
0.4 % FA + 0.08 M EDTA	61.32a	70.78a	38.05a	57.44a	58.34a	57.58a
0.4 % FA + 0.10 M EDTA	62.56a	70.22a	38.87a	58.21a	59.39a	58.25a

The fractionation of spiked MB soil indicated that the mixed 0.3 % HA + 0.06 M EDTA extractant removed 94 to 98% and 96 to 99 % of metals from water soluble and exchangeable forms which were 2-4 % higher than only HA treatment (Fig. 4.6.1). The mobility indexes were 1.09, 1.36, 0.68, 0.8, 0.79 and 0.72 % for Pb, Cd, Cr, Ni, Cu and Zn in soil after extraction with combined extractant, respectively. Consequently this extractant reduced average mobility index of metals from 29.12 to 0.91 % in spiked soil of Madhupur which was lower than individual HA treatment (Table 4.6.2). This combined extractant also removed 59.23 % of metals from Fe/Mn oxides fraction of soil which was 40.8 % higher than that of only HA treatment (Fig. 4.6.1). All together the combined 0.3 % HA + 0.06 M EDTA extractant removed 35.21, 17.66, 4.98, 17.23, 18.47 and 25.36 % more Pb, Cd, Cr, Ni, Cu and Zn that of individual HA extractant, respectively (Table 4.6.1).

The combined effects of fulvic acid and EDTA indicated that, 0.4 % FA removed significant amounts of metals from spiked MB soil when mixed with 0.04 M EDTA solution which were more or less similar as extracted by 0.1 M EDTA solution. The combined 0.4 % FA + 0.04 M EDTA extractant leached 59.05, 69.96, 36.43, 54.45, 55.76 and 53.32 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked soil of Madhupur tract (MB) with using 60 % less EDTA than that of only 0.1 M EDTA extractant (Table 4.6.1).

Table 4.6.2. Mobility indexes (%) of metals in spiked soil of Madhupur tract (MB) after treated with humic substances, EDTA and combined extractants.

Treatments	Pb	Cd	Cr	Ni	Cu	Zn
Contaminated soil	19.36±0.95	53.04±2.25	12.19±0.63	31.84±1.6	27.08±1.3	31.21±1.56
0.3 % HA	1.42±0.08	3.48±0.16	1.01±0.06	1.63±0.09	1.62±0.07	1.79±0.09
0.4 % FA	1.14±0.07	2.49±0.11	0.75±0.04	1.19±0.06	0.82±0.04	0.53±0.03
0.1M EDTA	1.20±0.06	2.41±0.12	0.93±0.05	1.23±0.07	1.05±0.06	1.10±0.06
0.3 % HA + 0.06M EDTA	1.09±0.06	1.36±0.08	0.68±0.04	0.80±0.05	0.79±0.03	0.72±0.04
0.4 % FA + 0.04M EDTA	0.84±0.03	1.12±0.05	0.57±0.03	0.77±0.04	0.68±0.03	0.52±0.03

The fraction of MB soil indicated that the combined FA + EDTA extractant leached 95 to 98 % and 97 to 99 % of metals from water soluble and exchangeable forms of soil. In addition combined extractant removed 62 to 81 % of metals from Fe/Mn oxides bound fraction of soil which was on an average 16.37 % higher than that of only FA extractant. All together this combined extractant removed 15.37, 5.64, 3.79, 6.41, 10.74 and 10.29 %

more Pb, Cd, Cr, Ni, Cu and Zn than that of individual FA treatment, respectively. This combined extractant also reduced metals in organic matter bound fraction of spiked MB soil (Fig. 4.6.1). The mobility index of metals was found lowest in spiked MB soil after extraction with the combined FA + EDTA extractant and the values were 0.84, 1.12, 0.57, 0.77, 0.68 and 0.52 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively (Table 4.6.2)

By comparing the removal efficiencies of different extractants it might be concluded that the combined 0.4 % FA + 0.04 M EDTA extractant removed the highest amount of metals from different soil fractions of Madhupur tract by using the lowest amount of EDTA.

4.6.2. Remediation of metal spiked calcareous soil of Ishwardi (IB) by different combinations of humic substances and EDTA

The reclamation of metal from spiked calcareous soil of Ishwardi by combined humic substances and EDTA indicated that the combined extractant significantly increased metal extraction from soil than that of individual HS treatment. Humic acid at the concentration of 0.3 % in combination with 0.06 M EDTA leached 37.92, 62.18 25.02, 35.23, 36.26 and 44.12 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked soil of Ishwardi (IB) which were statistically similar as done by only 0.1 M EDTA solution (Table 4.6.3).

Table 4.6.3. Metal extraction efficiencies (%) of humic substances, EDTA and combined extractants from spiked calcareous soil of Ishwardi (IB).

Extractant	**Pb**	**Cd**	**Cr**	**Ni**	**Cu**	**Zn**
0.3 % HA	15.02d	38.83d	13.78d	20.64d	21.43d	18.95d
0.1 M EDTA	37.31a	62.04ab	24.21ab	35.68a	35.01ab	43.85a
0.3 % HA + 0.02 M EDTA	26.43c	47.76c	19.16c	28.21c	25.51c	28.75c
0.3 % HA + 0.04 M EDTA	33.76b	53.45bc	22.89b	31.97b	30.15b	38.34b
0.3 % HA + 0.06 M EDTA	37.92a	62.18ab	25.02ab	35.23a	36.26ab	44.12a
0.3 % HA + 0.08 M EDTA	38.52a	64.08a	25.81a	36.06a	38.12a	45.56a
0.3 % HA + 0.10 M EDTA	39.16a	64.82a	26.02a	36.76a	38.81a	46.12a
0.4 % FA	24.45c	48.13c	19.44b	27.06c	28.06b	26.31c
0.1M EDTA	37.31ab	62.04ab	24.21b	35.68ab	35.01b	43.85ab
0.4 % FA + 0.02 M EDTA	30.54b	54.88b	26.21b	32.67b	34.21b	36.34b
0.4 % FA + 0.04 M EDTA	38.93ab	63.91ab	29.21a	35.45ab	39.15a	43.12ab
0.4 % FA + 0.06 M EDTA	40.83a	65.67a	30.33a	38.03a	41.12a	47.97a
0.4 % FA + 0.08 M EDTA	41.45a	66.92a	30.67a	38.35a	41.93a	48.03a
0.4 % FA + 0.10 M EDTA	41.67a	67.17a	31.79a	39.05a	42.23a	48.74a

From the fractionation of spiked IB soil it was found that the combined HA + EDTA extractant removed 87 to 98 % and 90 to 99 % of metals from water soluble and exchangeable forms of soil, respectively. This combined extractant extracted 68 to 78% of metals from carbonate adsorbed fraction which was on an average 49.41 % higher than that of individual 0.3 % HA treatment. Therefore the mobility indexes of metals were 13.12, 18.5, 7.01, 7.41, 9.59 and 16.63 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively after extraction with the combined extractant (Table 4.6.4), which were much lower than that of individual 0.3% HA extractant (Table 4.5.7). The values of mobility index indicated that the metals Cd, Cr and Ni were found immobile and metals Pb, Cu and Zn were remained mobile in spiked soil after extraction. Moreover this combined extractant removed 45.15 % of metals from Fe/Mn oxides bound fraction, which was average 18.48 % higher than only 0.4 % HA treatment (Fig. 4.6.2). Therefore the mixed 0.3 % HA + 0.06 M EDTA extractant reclaimed 22.9, 23.35, 11.24, 14.59, 14.83 and 25.17 % more Pb, Cd, Cr, Ni, Cu and Zn from that of only HA extractant, respectively (Table 4.6.3).

On the other hand fulvic acid at the concentration of 0.4 % leached significant amount of metals from spiked soil of Ishwardi (IB) when mixed with 0.04 M EDTA solution. This mixed extractant removed 38.93, 63.91, 29.21, 35.45, 39.15 and 43.12 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked IB soil by using 60 % less amount of EDTA, which were also similar as extracted by individual 0.1 M EDTA extractant (Table 4.6.3).

It was appeared from fractionation of spiked IB soil that the mixed FA + EDTA extractant leached more than 93, 97 and 79 % of metals from water soluble, exchangeable and carbonate bound fractions of soil, respectively (Fig. 4.6.2). Therefore the mobility indexes were lowered after extraction with the combined FA + EDTA extractant and the values were 9.88, 15.50, 4.78, 7.04, 8.83 and 17.87 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked IB soil, respectively (Table 4.6.4). The values of mobility index indicated that the metals Pb, Cr, Ni and Cu were found immobile and the metals Cd and Zn were observed mobile in calcareous IB soil after extraction.

This combined extractant also removed 51.14 % of metals from non-labile Fe/Mn oxides bound fraction which was 11% higher than only 0.4 % FA treatment (Fig. 4.6.2).

Simultaneously the combined FA + EDTA extractant removed 14.48, 15.78, 9.77, 8.39, 11.09 and 16.81 % additional Pb, Cd, Cr, Ni, Cu and Zn, respectively, than that of only 0.4 % FA extractant (Table 4.6.3).

Table 4.6.4. Mobility indexes (%) of metals in spiked soil of Ishwardi (IB) after treated with humic substances, EDTA and combined extractants.

Treatments	Pb	Cd	Cr	Ni	Cu	Zn
Control	39.61±1.98	62.8±3.14	19.1±0.96	27.59±1.36	31.58±1.57	47.65±2.38
0.3 % HA	29.36±1.47	43.51±2.15	12.67±0.65	17.92±0.87	19.99±0.96	37.16±1.84
0.4 % FA	23.44±1.17	26.14±1.31	12.3±0.61	14.61±0.73	19.65±0.94	35.5±1.75
0.1M EDTA	17.47±0.87	18.57±0.91	8.92±0.43	9.09±0.44	16.66±0.84	15.3±0.73
0.3 % HA + 0.06 M EDTA	13.12±0.64	18.5±0.93	7.01±0.35	7.41±0.35	9.59±0.47	16.63±0.83
0.4 % FA + 0.04 M EDTA	9.88±0.48	15.5±0.76	4.78±0.22	7.04±0.34	8.83±0.43	17.87±0.85

It was observed that the combined 0.3 % HA + 0.06 M EDTA and 0.4 % FA + 0.04 M EDTA extractants removed more or less similar amount of metals from spiked soil by using 40 and 60 % less amount EDTA as extracted by individual 0.1 M EDTA extractant, respectively.

4.6.3. Remediation of metal spiked soil of Hazaribagh (HT) by different combinations of humic substances and EDTA

The effects of humic substances in combination with EDTA on metals reclamation from spiked soil of Hazaribagh are given in Table 4.6.5. It was observed that 0.4 % humic acid leached significant amounts of metals from spiked HT soil when combined with 0.04 M EDTA solution. This combined extractant released 63.18, 75.32, 36.45, 61.45, 61.91 and 57.89 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked HT soil which were statistically similar as extracted by 0.1 M EDTA and higher combined HA + EDTA extractants (Table 4.6.5).

Table 4.6.5. Metal extraction efficiencies (%) of humic substances, EDTA and combined extractants from spiked soil of Hazaribagh (HT).

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
0.4 % HA	44.18d	67.02b	30.71b	50.23c	52.01b	44.46c
0.1M EDTA	63.51ab	70.29b	31.09b	60.51ab	59.03ab	58.41a
0.4 % HA + 0.02M EDTA	54.43c	72.67ab	32.21b	57.51b	54.53b	51.13b
0.4 % HA + 0.04M EDTA	63.18ab	75.32a	36.45a	61.45ab	61.91ab	57.89a
0.4 % HA + 0.06M EDTA	66.34a	75.65a	36.77a	62.23ab	64.98a	61.19a
0.4 % HA + 0.08M EDTA	67.07a	75.93a	37.11a	64.37a	65.11a	61.87a
0.4 % HA + 0.10M EDTA	67.33a	76.33a	37.21a	64.55a	65.33a	62.24a
0.4 % FA	43.08d	66.36b	27.67c	49.32c	51.04c	42.14c
0.1M EDTA	63.51ab	70.29ab	31.09b	60.51ab	59.03ab	58.41a
0.4 % FA + 0.02M EDTA	54.58c	69.87ab	30.71b	55.34b	56.92bc	49.67b
0.4 % FA + 0.04M EDTA	62.34ab	75.35a	34.78a	62.45a	60.43ab	55.89a
0.4 % FA + 0.06M EDTA	65.39a	76.44a	34.87a	63.26a	63.38a	60.23a
0.4 % FA + 0.08M EDTA	66.49a	76.12a	35.08a	64.75a	63.82a	61.24a
0.4 % FA + 0.10M EDTA	67.02a	76.62a	36.13a	65.06a	64.01a	61.89a

The fractionation study of spiked HT soil indicated that combined 0.4 % HA + 0.4 M EDTA extractant removed 95 to 99 % and 97 to 99% of metals from water soluble and exchangeable forms. The combined extractant also dissolved 66 to 92 % of metals from Fe/Mn oxides bound fraction of soil which was on an average 18.73 % higher than that of individual HA treatment (Fig. 4.6.3). All together the HA + EDTA mixed extractant removed 19.0, 8.3, 5.7, 11.2, 9.9 and 13.4 % more Pb, Cd, Cr, Ni, Cu and Zn from only 0.4 % HA treatment, respectively (Table 4.6.5). The mobility indexes were 1.01, 1.16, 0.73, 0.75, 1.21 and 0.36 % for Pb, Cd, Cr, Ni, Cu and Zn after extraction with this mixed extractant, respectively. Consequently the average mobility was also reduced from 31.21 to 1.17 % after extraction in spiked HT soil (Table 4.6.6).

The combine effects of fulvic acid and EDTA indicated that fulvic acid at the concentration of 0.4 % significantly increased metals extraction from spiked soil of Hazaribagh when incorporated with 0.04M EDTA solution. The combined 0.4 % FA + 0.04 M EDTA solution removed 62.34, 75.35, 34.78, 62.45, 60.43 and 55.89 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked HT soil which were statistically similar as extracted by 0.1 M EDTA and higher FA + EDTA combinations (Table 4.6.5). The mixed 0.4 % FA + 0.04 M EDTA extractant also

lowered the mobility indexes and the values were 1.29, 1.52, 0.67, 0.66, 0.95 and 0.33 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked HT soil, respectively (Table 4.6.6).

Table 4.6.6. Mobility indexes (%) of metals in spiked soil of Hazaribagh (HT) after treated with humic substances, EDTA and combined extractants.

Treatments	Pb	Cd	Cr	Ni	Cu	Zn
Control	21.2±1.26	55.9±3.36	15.6±0.93	38.4±2.34	28.7±1.72	27.4±1.65
0.3 % HA	1.20±0.06	1.12±0.06	1.09±0.06	1.04±0.05	1.05±0.06	0.70±0.04
0.4 % FA	1.25±0.07	1.26±0.08	1.12±0.07	1.09±0.06	1.25±0.08	0.79±0.05
0.1M EDTA	1.67±0.11	1.57±0.08	1.15±0.08	1.55±0.09	0.66±0.04	0.39±0.02
0.3 % HA + 0.06M EDTA	1.01±0.06	1.16±0.07	0.73±0.06	0.75±0.05	1.21±0.07	0.36±0.04
0.4 % FA + 0.04M EDTA	1.29±0.07	1.52±0.09	0.67±0.04	0.66±0.04	0.95±0.06	0.33±0.03

The fraction of spiked soil of Hazaribagh indicated that the 0.4 % FA + 0.04 M EDTA mixed extractant leached 95 to 99% and 97 to 99 % of metals from water soluble and exchangeable forms. Additionally combined extractant removed 65 to 91% of metals from Fe/Mn oxides bound fraction which was on an average 37.47 % higher than that of individual 0.4 % FA treatment (Fig. 4.6.3). The FA + EDTA mixed solution extracted 19.26, 8.99, 7.11, 13.13, 9.39 and 13.75 % additional Pb, Cd, Cr, Ni, Cu and Zn over that of only 0.4 % FA extractant, respectively (Table 4.6.5). The average mobility factor of metals was reduced from 31.21 to 0.9 % in spiked HT soil after extraction with the mixed FA + EDTA solution which was lower than that of only FA treatment (Table 4.6.6).

The extraction capacities of humic substances were significantly increased when incorporated with small amount of EDTA. It might be concluded that both humic and fulvic acids at the concentration of 0.4 % removed significant amount of metals from spiked soil of Hazaribagh when mixed with 0.04 M EDTA solution.

4.6.4. Remediation of metal spiked soil of Shitakunda (SSy) by different combinations of humic substances and EDTA

The combined effects of HS and EDTA on metal spiked soil of Shitakunda (SSy) indicated that the metal removal efficiencies of humic substances increased when incorporated with EDTA solution. Humic acid at the concentration of 0.4 % removed significant amounts of metals from the soil when mixed with 0.04 M EDTA solution. This extractant reclaimed 62.67 % Pb, 74.76 % Cd, 38.07 % Cr, 63.05 % Ni, 60.89 % Cu and 55.56 % Zn from spiked SSy soil which were significantly similar as extracted by 0.1M EDTA solution alone (Table 4.6.7).

Table 4.6.7. Metal extraction efficiencies (%) of humic substances, EDTA and combined extractants from spiked soil of Shitakunda (SSy).

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
0.4 % HA	43.33c	68.82b	31.21b	46.05c	46.71c	42.28c
0.1M EDTA	65.16a	71.90ab	34.16b	63.49a	60.45ab	56.44a
0.4 % HA + 0.02 M EDTA	51.87b	68.94b	35.76ab	56.56b	54.67b	49.45b
0.4 % HA + 0.04 M EDTA	62.67ab	74.76a	38.07a	63.05a	60.89ab	55.56a
0.4 % HA + 0.06 M EDTA	67.89a	75.08a	38.27a	65.78a	63.78a	58.89a
0.4 % HA + 0.08 M EDTA	68.98a	75.28a	38.86a	66.45a	64.32a	59.29a
0.4 % HA + 0.10 M EDTA	69.34a	75.89a	39.02a	67.21a	65.04a	60.76a
0.4 % FA	41.88c	66.12b	30.14c	45.12c	45.84c	41.09d
0.1M EDTA	65.16a	71.90ab	34.16bc	63.49a	60.45a	56.44a
0.4 % FA + 0.02 M EDTA	52.67b	70.45ab	35.18ab	54.67b	53.12b	48.11c
0.4 % FA + 0.04 M EDTA	63.45ab	74.23a	37.86a	62.67a	59.98a	55.87a
0.4 % FA + 0.06 M EDTA	65.17a	75.07a	38.07a	64.32a	63.08a	58.69a
0.4 % FA + 0.08 M EDTA	67.78a	75.13a	38.18a	65.77a	63.58a	59.67a
0.4 % FA + 0.10 M EDTA	68.12a	75.83a	38.97a	66.89a	64.02a	60.11a

From the fractionation study of spiked soil of Shitakunda it was observed that combined 0.4 % HA + 0.04 M EDTA extractant removed 90 to 97 % and 97 to 99 % of metals from water soluble and exchangeable forms. This mixed extractant also removed 87 to 97 % and 62 to 86 % of metals from carbonate and Fe/Mn oxides bound fractions of spiked SSy soil, which were 22 and 18 % higher than that of individual 0.4 % HA treatment. Therefore, mixed 0.4 % HA + 0.04 M EDTA extractant leached 19.34, 5.94, 6.86, 17.0, 14.18 and 13.28 % more Pb, Cd, Cr, Ni, Cu and Zn over that of individual 0.4 % HA extractant, respectively (Fig. 4.6.4). The mobility indexes of metals were considerably lowered after extraction with the mixed extractant and the values were 2.08, 7.85, 2.50, 2.89, 1.73 and 6.16 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked SSy soil, respectively (Table 4.6.8).

Table 4.6.8. Mobility indexes (%) of metals in spiked soil of Shitakunda (SSy) after treated with humic substances, EDTA and combined extractants.

Treatments	Pb	Cd	Cr	Ni	Cu	Zn
Control	29.88±1.79	63.51±3.81	21.52±1.08	41.48±2.07	36.69±1.83	40.98±2.05
0.3 % HA	6.16±0.37	18.96±1.14	5.97±0.30	12.42±0.62	8.87±0.44	10.85±0.54
0.4 % FA	6.40±0.38	19.28±1.16	6.11±0.31	13.03±0.65	10.39±0.52	11.87±0.59
0.1M EDTA	2.30±0.14	5.89±0.35	3.90±0.20	2.23±0.11	1.88±0.09	7.46±0.37
0.3 % HA + 0.06 M EDTA	2.08±0.12	7.85±0.47	2.50±0.13	2.89±0.14	1.73±0.09	6.16±0.31
0.4 % FA + 0.04 M EDTA	2.20±0.13	7.47±0.45	2.73±0.14	2.45±0.12	1.93±0.10	7.25±0.36

Fulvic acid also increased the removal efficiencies of metals from the spiked soil when mixed with EDTA. It was found that, 0.4 % fulvic acid extracted significant amount of metals from spiked soil of Shitakunda when 0.04 M EDTA solution was incorporated with it. The mixed 0.4 % FA + 0.04 M EDTA extractant leached 63.45, 74.23, 37.86, 62.67, 59.98 and 55.87 % of Pb, Cd, Cr, Ni, Cu and Zn from spiked SSy soil which were similar as extracted by 0.1 M EDTA alone (Table 4.6.7).

The fractionation study of spiked SSy soil indicated that the combined 0.4 % FA + 0.04 M EDTA solution extracted 93 to 96 % of water soluble, 95 to 99 % of exchangeable and 84 to 97 % of carbonate bound forms of metals from spiked SSy soil (Fig. 4.6.4). Therefore the average mobility index of metals was reduced from 39.01 to 4.01 % after extraction, which was lower than that of only 0.4% FA treatment (Table 4.6.8). In addition this combined extractant leached 58 to 95 % of metals from Fe/Mn oxides bound fraction of soil (Fig. 4.6.4). All together the combined 0.4 % FA + 0.04 M EDTA solution extracted 21.57, 8.11, 7.72, 17.55, 14.14 and 14.78 % more Pb, Cd, Cr, Ni, Cu and Zn from spiked SSy soil as done by 0.4 % FA treatment alone, respectively (Table 4.6.7).

The metal extraction capacities of humic substances from spiked soil of Shitakunda were increased when incorporated with EDTA solution. Both humic and fulvic acid at the concentration of 0.4 % removed significant amount of metals from spiked SSy soil when mixed with 0.04 M EDTA solution, and also saved 60 % EDTA compared to that of only 0.1 M EDTA treatment.

4.6.5. Remediation of metal spiked calcareous soil of Kushtia (KC) by different combinations of humic substances and EDTA

The combined effects of humic substances and EDTA for the reclamation of metal from spiked calcareous soil of Kushtia (KC) are presented in Table 4.6.9. It was found that 0.3 % humic acid in combination with 0.06 M EDTA removed significant amount of metals from spiked soil of Kushtia (KC) and the values were 35.03, 60.12, 26.19, 35.03, 35.06 and 41.32 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively, which were similar as extracted by individual 0.1 M EDTA solution (Table 4.6.9).

Table 4.6.9. Metal extraction efficiencies (%) of humic substances, EDTA and combined extractants from spiked soil of Kushtia (KC).

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
0.3 % HA	14.74d	36.76d	12.61d	20.57d	21.63d	17.54d
0.1 M EDTA	36.77a	60.24a	25.11a	34.41a	35.45a	42.14a
0.3 % HA + 0.02 M EDTA	20.89c	41.43c	16.14c	25.46c	24.32c	24.19c
0.3 % HA + 0.04 M EDTA	28.67b	52.95b	20.01b	30.56b	29.82b	33.76b
0.3 % HA + 0.06 M EDTA	35.03a	60.12a	26.19a	35.03a	35.06a	41.32a
0.3 % HA + 0.08 M EDTA	37.06a	62.32a	26.74a	36.48a	36.11a	42.67a
0.3 % HA + 0.10 M EDTA	37.75a	62.89a	27.08a	36.98a	36.65a	43.03a
0.4 % FA	24.07d	46.02d	17.41c	26.87c	27.23c	25.41d
0.1M EDTA	36.77ab	60.24b	25.11ab	34.41ab	35.45ab	42.14ab
0.4 % FA + 0.02 M EDTA	30.67c	52.15c	22.56b	30.89b	30.23bc	32.67c
0.4 % FA + 0.04 M EDTA	36.07ab	59.34b	24.02ab	35.67ab	35.08ab	39.98b
0.4 % FA + 0.06 M EDTA	40.12a	62.45ab	26.89a	38.88a	37.98a	44.67a
0.4 % FA + 0.08 M EDTA	41.85a	64.29a	27.56a	39.26a	38.23a	48.79a
0.4 % FA + 0.10 M EDTA	42.01a	64.92a	27.87a	40.12a	38.77a	49.23a

From the fractionation study of spiked soil it was observed that this HA + EDTA mixed extractant leached 90 to 97%, 93 to 99% and 66 to 76 % of metals from the labile forms including water soluble, exchangeable and carbonate bound fractions, respectively, which were higher than that of only 0.3 % HA extractant. This mixed extractant also removed 19 to 48 % of metals from Fe/Mn oxides bound fractions of soil which was on an average 19.48 % higher than that of only 0.4 % HA extractant, respectively (Fig. 4.6.5). All together the combined 0.3 % HA + 0.06 M EDTA extractant removed 20.29, 23.36, 13.58, 14.46, 13.43 and 23.78 % additional Pb, Cd, Cr, Ni, Cu and Zn from that of only 0.3 % HA extractant, respectively (Table 4.6.9). The mobility indexes were 17.7, 23.8, 7.7, 10, 11.6 and 18.95 % after extraction with combined HA + EDTA extractant in calcareous KC soil (Table 4.6.10).

Table 4.6.10. Mobility indexes (%) of metals in spiked soil of Kushtia (KC) after treated with humic substances, EDTA and combined extractants.

Treatments	Pb	Cd	Cr	Ni	Cu	Zn
Control	40.93±2.46	60.47±3.63	22.81±1.37	31.24±1.87	36.03±2.16	51.49±3.09
0.3 % HA	31.91±1.91	34.39±2.06	14.21±0.85	19.59±1.18	19.84±1.19	39.61±2.38
0.4 % FA	26.67±1.60	30.68±1.84	13.76±0.83	17.06±1.02	15.55±0.93	35.32±2.12
0.1M EDTA	17.70±1.06	21.77±1.31	8.16±0.49	8.76±0.53	15.18±0.91	19.92±1.20
0.3 % HA + 0.06M EDTA	17.70±1.06	23.81±1.43	7.70±0.46	10.00±0.60	11.58±0.69	18.89±1.13
0.4 % FA + 0.04M EDTA	15.90±0.95	20.98±1.26	6.42±0.39	9.62±0.58	11.09±0.67	17.30±1.04

The combined fulvic acid and EDTA treatment considerably increased the removal efficiencies of metals from spiked soil of Kushtia. The 0.4 % fulvic acid with 0.04 M EDTA leached significant amount of metals from the spiked soil and the values were 36.07, 59.34, 24.02, 35.67, 35.08 and 39.98 % for Pb, Cd, Cr, Ni, Cu and Zn, respectively. This extractant removed statistically similar amount of metals by using 60 % less amount of EDTA from spiked KC soil as obtained by only 0.1 M EDTA treatment (Table 4.6.9).

The fractionation study of spiked soil indicated that the combined 0.4 % FA + 0.04 M EDTA extractant leached 87 to 98 %, 95 to 99% and 71 to 78 % of metals from the first three labile fractions of soil, respectively. This mixed extractant also removed 18 to 51 % of metals from non-labile Fe/Mn oxides bound fraction, which was on an average 14.63 % higher than that of only 0.4 % FA treatment (Fig. 4.6.5). All together the combined 0.4 % FA + 0.04 M EDTA solution extracted 12.0, 13.3, 6.6, 8.8, 7.9 and 14.6 % more Pb, Cd, Cr, Ni, Cu and Zn over that of only 0.4 % FA extractant, respectively (Table 4.6.9). The mobility indexes were reduced after extraction with this combined extractant and the values were 15.90, 20.98, 6.42, 9.62, 11.09 and 17.3 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked KC soil, respectively, which were lower than that of only 0.4% FA treatment (Table 4.6.10).

By comparing the extraction capacities of different combined extractants it was found that the combined 0.4 % FA + 0.04 M EDTA solution extracted relatively higher amount of metals form calcareous spiked soil of Kushtia using the lowest amount of EDTA.

4.6.6. Remediation of metal spiked soil of Chittagong (SSy) by different combinations of humic substances and EDTA

The extraction capacity of humic substances increased in combined with EDTA solution. It was observed that humic acid at the concentration of 0.4 % leached statistically high amounts of metals from spiked soil of Chittagong (CC) when mixed with 0.04 M or 0.06 M EDTA solution.

The combined 0.4 % HA + 0.04 M extractant leached 62.21 % Cd, 29.52 % Cr, 51.23 % Ni and the mixed 0.4 % HA + 0.06 M extractant leached 58.17 % Pb, 57.01 % Cu and 51.49 % Zn which were more or less similar as extracted by only 0.1 M EDTA extractant (Table 4.6.11).

Table 4.6.11. Metal extraction efficiencies (%) of humic substances, EDTA and combined extractants from spiked soil of Chittagong (CC).

Extractant	Pb	Cd	Cr	Ni	Cu	Zn
0.4 % HA	26.45d	52.83c	24.14b	40.01c	38.22d	32.71d
0.1M EDTA	57.12ab	62.59ab	25.28b	53.26a	56.76a	50.01ab
0.4 % HA + 0.02 M EDTA	36.34c	56.89bc	26.14b	47.56b	44.45c	40.34c
0.4 % HA + 0.04 M EDTA	45.87b	62.21ab	29.52a	51.23ab	50.56b	48.01b
0.4 % HA + 0.06 M EDTA	58.17a	63.77a	29.67a	54.78a	57.01a	51.49ab
0.4 % HA + 0.08 M EDTA	58.97a	63.87a	30.19a	55.77a	58.05a	53.78a
0.4 % HA + 0.10 M EDTA	60.89a	63.93a	30.82a	56.34a	59.56a	54.88a
0.4 % FA	29.26d	57.41b	25.02b	39.83c	37.24d	34.36d
0.1M EDTA	57.12a	62.59ab	25.28b	53.26a	56.76a	50.01b
0.4 % FA + 0.02 M EDTA	39.78c	58.65b	26.18ab	44.67b	43.65c	41.78c
0.4 % FA + 0.04 M EDTA	48.34b	64.05ab	28.19a	50.76ab	49.34b	49.11b
0.4 % FA + 0.06 M EDTA	58.89a	69.91a	28.57a	53.89a	57.02a	53.95a
0.4 % FA + 0.08 M EDTA	59.86a	70.78a	28.92a	55.45a	58.14a	55.11a
0.4 % FA + 0.10 M EDTA	60.08a	71.09a	29.01a	55.97a	58.89a	55.22a

The fractionation study of metal spiked soil of Chittagong indicated that the mixed HA + EDTA extractant removed 91 to 97 %, 98 to 99 % and 67 to 97 % of metals from the water soluble, exchangeable and carbonate bound forms of soil, respectively. These combined extractants also removed 49 to 82 % of metals from non-labile Fe/Mn oxides bound fraction of soil which were on an average 26.44 % higher than that of only 0.4 % HA treatment (Fig. 4.6.6).

Consequently combined application of 0.4 % HA + 0.04 M EDTA removed 9.38, 5.38 and 11.22 % more Cd, Cr and Ni and the combined 0.4 % HA + 0.06 M EDTA extractant removed 31.72, 18.79 and 18.78 % more Pb, Cu and Zn from that of single 0.4 % HA treatment, respectively (Table 4.6.11). The mobility indexes of metals were also lowered by these combined extractants and the values were 5.1, 13.98, 1.28, 1.48, 1.49 and 2.77 % for Pb, Cd, Cr, Ni, Cu and Zn in spiked CC soil, respectively (Table 4.6.12).

Table 4.6.12. Mobility indexes of metals (%) in spiked soil of Chittagong after treated with humic substances, EDTA and combined extractants.

Treatments	Pb	Cd	Cr	Ni	Cu	Zn
Control	36.5±2.03	56.8±3.12	15.7±0.87	35.5±1.94	28.9±1.56	34.5±1.92
0.3 % HA	16.01±0.86	17.75±0.96	4.01±0.24	10.05±0.53	6.97±0.37	11.58±0.63
0.4 % FA	14.98±0.81	18.58±1.04	3.46±0.32	10.21±0.54	7.71±0.41	10.38±0.54
0.1M EDTA	5.71±0.33	14.31±0.76	1.48±0.11	1.62±0.08	1.58±0.09	2.94±0.18
0.3 % HA + 0.06M EDTA	5.10±0.24	13.98±0.72	1.28±0.07	1.48±0.09	1.48±0.07	2.77±0.17
0.4 % FA + 0.04M EDTA	3.16±0.15	11.45±0.64	0.96±0.06	1.54±0.07	1.59±0.08	2.87±0.19

Fulvic acid also increased the removal efficiencies of metals while applied with EDTA solution. It was observed that 0.4 % fulvic acid extracted considerable amounts of metals from spiked soil of Chittagong when mixed with 0.04 M or 0.06 M EDTA solution. The combined 0.4 % FA + 0.06 M EDTA extractant leached 58.89 % Pb, 57.02 % Cu and 53.95 % Zn and the combined 0.4 % FA + 0.04 M EDTA extracted 64.05 % Cd, 28.18 % Cr and 50.76 % Ni from spiked SSy soil which were more or less similar as extracted by only 0.1 M EDTA solution (Table 4.6.11).

The fractions of metal spiked CC soil indicated that the combined FA + EDTA solution removed 95 to 99 % of water soluble, 98 to 99% of exchangeable and 76 to 97% of carbonate bound metals from soil. In addition combined extractant dissolved 61 to 81 % of metals from Fe/Mn oxides bound fraction (Fig. 4.6.6). The FA + EDTA mixture extracted 21.57, 8.11, 7.72, 17.55, 14.14 and 14.78 % more Pb, Cd, Cr, Ni, Cu and Zn from spiked CC soil as extracted by 0.4 % FA solution, respectively (Table 4.6.11). The average mobility indexes of metals were reduced from 34.67 to 3.59 % after extraction with the combined

FA + EDTA extractants, which were also lower than that of individual 0.1 M EDTA treatment (Table 4.6.12).

It was found from the metal extraction capacities of different extractant that humic substances at the concentration of 0.4 % removed significant amount of Cd, Cr and Ni when combined with 0.04 M EDTA and extracted significant amount of Pb, Cu and Zn while combined with 0.06 M EDTA solution from spiked soil of Chittagong.

The extraction capacities of humic substances were significantly increased when incorporated with small amount of EDTA. It was found from previous chapter that fulvic acid effectively extracted metals from heavy textured soil of Madhupur tract. Consequently fulvic acid extracted similar amount of metals from soil as extracted by 0.1 M EDTA solution, by mixing with only 0.4 M EDTA. Humic acid also positively influenced the extraction capacity of metals by mixing with EDTA solution, but HA required more EDTA (0.06 M EDTA) to removed significantly similar amount of metals as extracted by combined FA + EDTA extractant.

Humic substances were unable to extract considerable amount of metals from calcareous soils, but humic substances in combination with EDTA increased the extraction efficiency of metals even from calcareous soil. Among the humic substances, fulvic acid removed comparatively higher amount of metals from calcareous soil of Ishwardi and Kushtia than that of humic acid. Therefore the combined 0.4 % FA + 0.04 M EDTA required lowest amount of EDTA to remove significantly similar amount of metals from calcareous soil as done by individual 0.1 M EDTA and mixed 0.3 % HA and 0.06 M EDTA extractant.

Consequently combined fulvic acid and EDTA extractant more effectively removed metals from above three heavy textured soils using lower amount of EDTA compared to combined humic acid and EDTA extractant.

It was observed that humic substances extracted comparatively higher amounts of metals from light textured soils of Hazaribagh and Shitakunda. Consequently both humic and fulvic acids considerably increased the extraction efficiencies of metals by mixing with low amount of EDTA (0.04 M) which were similar as extracted by 0.1 M EDTA solution.

In spiked soil of Chittagong, humic substances significantly increased the removal efficiency of metals by mixing with both 0.04 and 0.06 M EDTA solutions which were similar as extracted by 0.1 M EDTA solution only.

Xia *et al.* (2009) found that the extraction efficiency of Pb using EDTA was increased with the increasing concentration of humic acid and the concentrations of Pb in the extractable fraction of HA-EDTA treated soils were higher than those in control soil. They reported that the Pb removal efficiency increased from 46.97 % to 79.74 % by EDTA extraction with elevated dose of humic acid in heavy and light textured soils, respectively.

Xia *et al.* (2009) reported that the Cu extraction capacity increased from 19.5 to 57.81 % in 0.05 M EDTA with elevated dose of HA in light textured soil than single 0.05 M EDTA extraction. Copper removal efficiency also increased up to 22.63 % by EDTA extraction with increasing dose of humic acid in heavy textured soil.

4.7. Pot experiments

Pot experiments were conducted using two crops, an upland vegetable crop, red amaranth (*Amaranthus gangeticus*) and a low land crop, rice (*Oryza sativa*) BRRI dhan 49. Six separate pot experiments were carried out with six contaminated soils. Each contaminated soil was washed with different extractants until no metal was detected in leachate. The extractants were humic acid, fulvic acid, EDTA and combined HA + EDTA and FA + EDTA solutions.

Seven treatments were applied for each contaminated soil. One treatment contained only contaminated soil without treatment represented as the control. Five other treatments included treatment of contaminated soils with humic acid, fulvic acid, 0.1 M EDTA, and combined HA + EDTA and FA + EDTA solutions. There was also treatment containing non-contaminated soil of the same region. Plants were grown on different treated and untreated contaminated soils to determine the phyto-availability of metals from soils. The best effective extractants were selected by comparing the metal removal efficiency and reduction of uptake of metals by plants.

4.7.1. Experiment with contaminated soil of Madhupur tract

The soil was collected from an area of Madhupur tract where two battery industries were located. Lead concentration was recorded very high (245 mg kg^{-1}) in adjoining area of these industries (Table 4.2.1). The properties of the soil are described in section 4.4.1.

Experiment with red amaranth: The vegetative growth (plant height, root and leaf length) and yield of *Amaranthus gangeticus* growing in untreated and treated contaminated and non-contaminated soil of Madhupur tract are shown in Table 4.7.1. The plant height, root and leaf length and dry weight were found lowest in contaminated soil. The values were significantly higher in remediated pots treated with humic substances and EDTA. High metal concentrations in soil caused injury to plant and consequently reduced the vegetative growth and yield of red amaranth. Visual differences of growth of red amaranth are shown in Figure 4.7.1. Zhang *et al.* (2013) found that 500 mg kg^{-1} Pb caused injurious effects on tobacco growth, and the plant biomass of the acidic red and paddy soils decreased by 20.25 and 20.43 %, respectively. Soil used in the present experiment contained 245 mg kg^{-1} Pb.

Table 4.7.1. Vegetative growth and yield attributes of *Amaranthus gangeticus* grown in treated and untreated contaminated soils and non-contaminated soil of Madhupur tract.

Treatment	Plant height (cm)	Leaf length (cm)	Root length (cm)	Fresh weight (g plant^{-1})	Yield (Dry weight) (g pot^{-1})
Contaminated	7.23f	3.39d	3.02d	4.93d	4.77e
0.3 % HA	14.19d	4.89b	4.79b	8.84b	9.18d
0.4 % FA	14.49d	5.66a	5.39a	8.98b	11.63c
0.3 %HA + EDTA 0.06M	18.89b	5.49a	5.41a	9.16b	12.64b
0.4 % FA + EDTA 0.04M	20.78a	5.63a	5.54a	10.57a	13.58a
0.1 M EDTA	11.89e	4.45c	4.32c	6.78c	8.62d
Non-contaminated	17.04c	4.92b	4.83b	9.21b	12.73b

Fig. 4.7.1. Photograph of *Amaranthus gangeticus* grown in treated and untreated contaminated soils of Madhupur tract.

The plant height, leaf and root length and yield (fresh and dry weights) of red amaranth increased in pots over contaminated soil where the soils were treated with humic substances, EDTA and different combinations of HS + EDTA extractants. These extractants removed toxic metals from soil and thereby created effects on increased growth and yield of plant. The highest yield (dry weight) of red amaranth was observed where combined FA + EDTA extractant was used for metal removal from soils, which increased plant yield of 184 % over that of contaminated soil. Next to it combined HA + EDTA extractant increased plant yield which was similar to plant grown in non-contaminated soil and also 164 % higher than that of contaminated soil. It was also observed that the yield (dry weight) of red amaranth in fulvic acid treated soil was higher than that each of humic acid and EDTA treatment (Table 4.7.1). The positive effect of humic substances on the growth of numerous plants is well documented (Visser, 1986; Chen and Aviad, 1990). Several authors demonstrated that the addition of humic substances can favor the growth of both the root and the aerial parts of the vegetable plant (Verlinden, 2009). Sharif *et al.* (2002) found that the addition of 50 and 100 mg kg^{-1} of humic substances caused a significant increase of 20 and 23 % in shoot and 39 and 32 % in root dry weight of maize, respectively.

Experiment with rice: The growth parameters (plant weight and height, panicle length, 100 grain weight and total grain yield per pot) of rice growing in treated and untreated contaminated soil and non-contaminated soil are shown in Table 4.7.2. The vegetative growth and grain yields of rice plant were observed lowest in contaminated soil possibly due to plant injury from heavy metal toxicity (Table 4.7.2 and Figure 4.7.2).

Table 4.7.2. Vegetative growth and yield attributes of *Oryza sativa* grown in treated and untreated contaminated soils and non-contaminated soil of Madhupur tract.

Treatment	Plant Weight (g pot^{-1})	Plant Height (cm)	Panicle Length (cm)	100 grain weight (gm)	Total grain weight (g pot^{-1})
Contaminated	206.21d	86.36b	17.18d	1.27e	13.74d
0.3 % HA	249.34b	90.12ab	20.8bc	1.74c	18.06c
0.4 % FA	266.19ab	93.12ab	20.15c	1.98b	22.11a
0.3 % HA + 0.06M EDTA	257.54ab	91.62ab	22.21b	1.89b	20.51b
0.4 % FA + 0.04M EDTA	270.22a	96.52a	24.02a	2.19a	22.69a
0.1M EDTA	228.29c	90.44ab	21.74bc	1.52d	17.34c
Non contaminated	252.72ab	91.23ab	20.42c	1.72c	20.57b

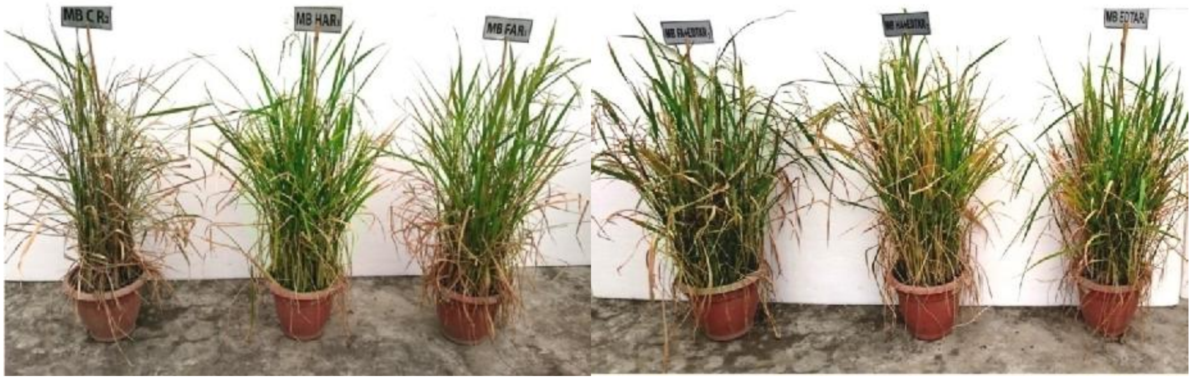


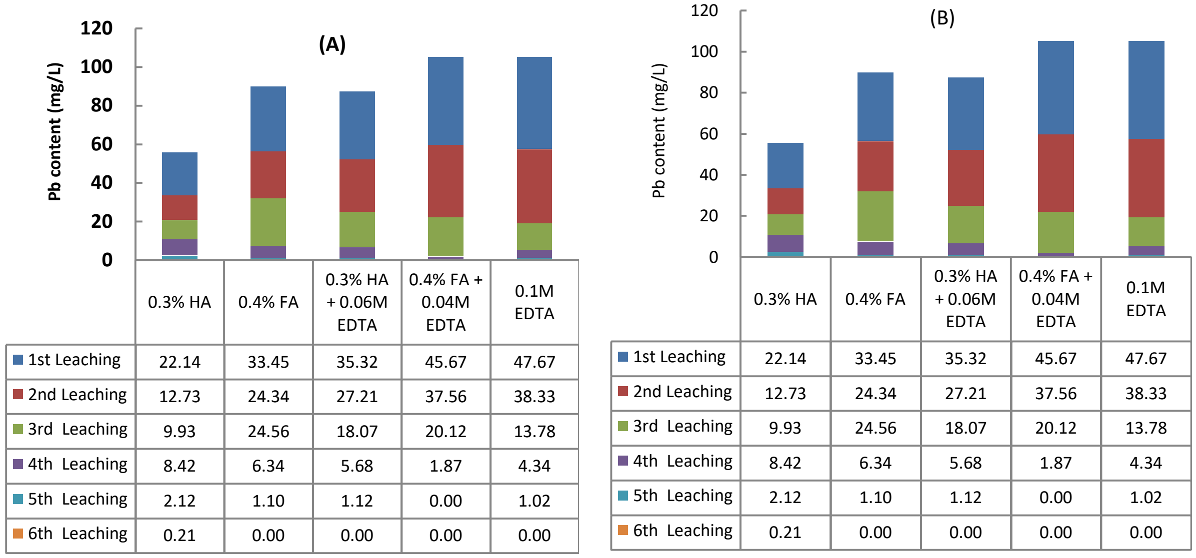
Fig. 4.7.2. Photograph of *Oryza sativa* (BRRI dhan 49) plant grown in treated and untreated contaminated soils of Madhupur tract.

Treatments of soil with humic substances and combined HS + EDTA extractants significantly created effects on increased vegetative growth and grain yields of rice plant over the yield of plants grown in contaminated soil which were more or less similar as plant grown in non-contaminated soil. The maximum grain yield of rice was observed in soil treated with combined 0.4 % FA + 0.04 M EDTA and individual 0.4 % FA extractant which increased total grain yield 65.1 and 60.8 % from untreated contaminated soil. Next to it combined treatment with HA + EDTA increased 49.3 % grain yield over that of contaminated soil, which was also similar to the grain yield of plant grown in non-contaminated soil The grain yields obtained with combined HS + EDTA and only 0.4 % FA treated pots were higher than that of only 0.1 M EDTA treated soil (Table 4.7.2).

Plants grown in soil which was treated with only 0.3 % HA also showed 31.4 % increased grain yield from that of contaminated soils (Table 4.7.2). Treatment with humic substances created stimulating effect on plant growth in terms of increasing plant height and dry or fresh weight (Lee and Bartlett, 1976; Mylonas and McCants, 1980a; Mylonas and McCants, 1980b). Stimulation of root and shoot growth by humic acid was also reported in corn plant (Lee and Bartlett, 1976). They reported an increase in branching and root hair development of corn when plants were grown in a nutrient solution containing humic acids.

The fractionation study of contaminated soil of Madhupur tract (MB) indicated that largest amount of Pb were associated with Fe/Mn oxides bound fraction (Fig. 4.7.4) and the mobility indexes of Pb were recorded 20% in contaminated soils after plant harvest (Table 4.7.3).

The extractants humic substances (HS), EDTA and combined HS + EDTA leached considerable amount of Pb from contaminated soil. The concentrations of Pb in leachate were found high from first four extractions and then concentrations were sharply decreased with increasing number of leaching. Lead was not detected in leachate after 5th and/or 6th extractions (Fig. 4.7.3).



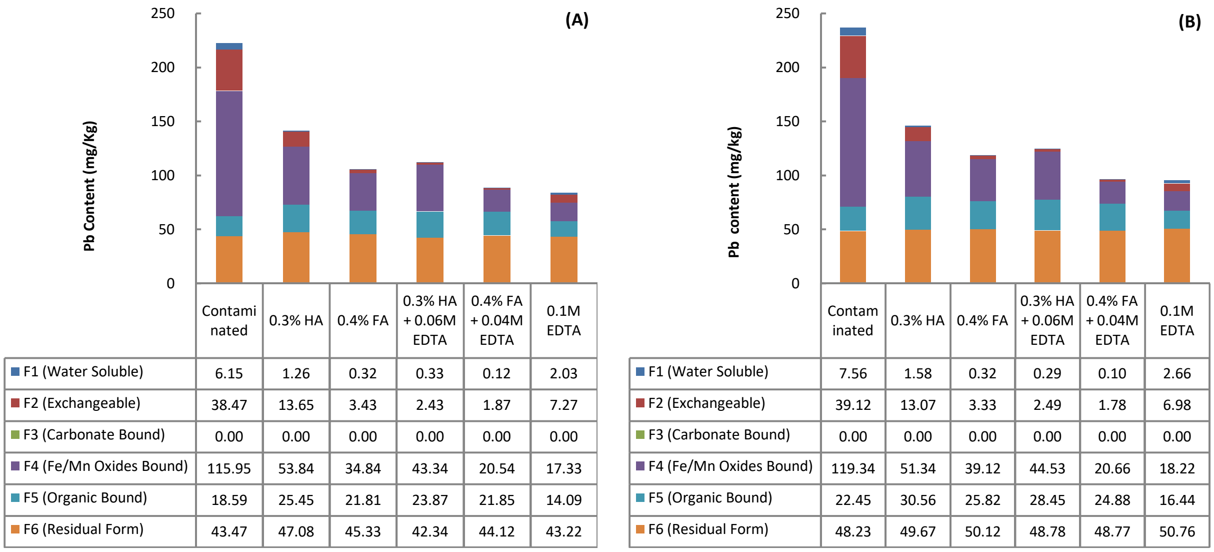
	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	22.14	33.45	35.32	45.67	47.67
🟥 2nd Leaching	12.73	24.34	27.21	37.56	38.33
🟩 3rd Leaching	9.93	24.56	18.07	20.12	13.78
🟪 4th Leaching	8.42	6.34	5.68	1.87	4.34
🟦 5th Leaching	2.12	1.10	1.12	0.00	1.02
🟧 6th Leaching	0.21	0.00	0.00	0.00	0.00

	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	22.14	33.45	35.32	45.67	47.67
🟥 2nd Leaching	12.73	24.34	27.21	37.56	38.33
🟩 3rd Leaching	9.93	24.56	18.07	20.12	13.78
🟪 4th Leaching	8.42	6.34	5.68	1.87	4.34
🟦 5th Leaching	2.12	1.10	1.12	0.00	1.02
🟧 6th Leaching	0.21	0.00	0.00	0.00	0.00

Fig. 4.7.3. Concentration of Pb (mg L^{-1}) in leachate after extraction of contaminated MB soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.

Humic acid at the concentration of 0.3% leached 7.45 to 24.13 mg L^{-1} of Pb, whereas fulvic acid leached 6.33 to 35.22 mg L^{-1} of Pb at the concentration of 0.4% during first four extractions. Among the humic substances, fulvic acid extracted comparatively higher amount of Pb from contaminated soils than that of humic acid (Fig. 4.7.3). Lower molecular sized FA easily penetrated the heavy textured soil of Madhupur tract and extracted more Pb compared to that of humic acid.

It was observed from fractionation study that the soil remediated with humic acid retained less than 21 and 35 % of Pb in water soluble and exchangeable forms, respectively. In contrast soil treated fulvic acid hold less than 5 and 10 % of Pb in the above respective forms. Humic acid could not extract sufficient amount of Pb from exchangeable forms (Fig. 4.7.4). Therefore mobility indexes of Pb were recorded 10.02 to 10.55% and 3.0 to 3.5 % in humic and fulvic acids treated soils after plant harvest, respectively (Table 4.7.3). Humic substances increased Pb concentration in organic matter bound fraction of soil (Fig. 4.7.4).



(A)

	Contaminated	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	6.15	1.26	0.32	0.33	0.12	2.03
🟥 F2 (Exchangeable)	38.47	13.65	3.43	2.43	1.87	7.27
🟩 F3 (Carbonate Bound)	0.00	0.00	0.00	0.00	0.00	0.00
🟪 F4 (Fe/Mn Oxides Bound)	115.95	53.84	34.84	43.34	20.54	17.33
🟦 F5 (Organic Bound)	18.59	25.45	21.81	23.87	21.85	14.09
🟧 F6 (Residual Form)	43.47	47.08	45.33	42.34	44.12	43.22

(B)

	Contaminated	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	7.56	1.58	0.32	0.29	0.10	2.66
🟥 F2 (Exchangeable)	39.12	13.07	3.33	2.49	1.78	6.98
🟩 F3 (Carbonate Bound)	0.00	0.00	0.00	0.00	0.00	0.00
🟪 F4 (Fe/Mn Oxides Bound)	119.34	51.34	39.12	44.53	20.66	18.22
🟦 F5 (Organic Bound)	22.45	30.56	25.82	28.45	24.88	16.44
🟧 F6 (Residual Form)	48.23	49.67	50.12	48.78	48.77	50.76

Fig. 4.7.4. Fractionation of Pb (mg kg^{-1}) in treated and untreated contaminated soils of Madhupur tract after harvest of *Amaranthus gangeticus* and *Oryza sativa* plants.

The combined 0.4 % FA + 0.04 M EDTA and 0.3 % HA + 0.06 M EDTA extractants increased the removal efficiencies of Pb from contaminated soil over that of only 0.3 % HA treatment (Fig. 4.7.3). Here EDTA in combined solution increased Pb extractability from different soil fractions. The removal efficiency of combined FA + EDTA extractant was similar to that of only 0.1 M EDTA solution (Fig. 4.7.3).

Table 4.7.3. Mobility indexes of Pb (%) in treated and untreated contaminated MB soils after plant harvest.

Extractants	Soil of Red Amaranth plant	Soil of Rice plant
Contaminated	20.04±1.1	19.72±0.98
0.3 % HA	10.55±0.13	10.02±0.13
0.4 % FA	3.54±0.14	3.07±0.11
0.3 % HA + 0.06M EDTA	2.46±0.15	2.23±0.10
0.4 % FA + 0.04M EDTA	2.25±0.11	1.95±0.09
0.1M EDTA	11.08±0.54	10.14±0.51

The treatment of soil with combined HA + EDTA retained less than 5 and 7 % of Pb in water soluble and exchangeable forms, respectively. Whereas combined FA + EDTA extractant retained only 2 to 5% of Pb in same respective fractions. The mobility indexes of Pb were found 2.2 to 2.5% and 1.9 to 2.2% in soils where HA + EDTA and FA + EDTA extractants were used for metal removal, respectively. In addition combined HA + EDTA and FA + EDTA extractants dissolved 62 and 82 % of Pb from Fe/Mn oxide bound fractions, respectively. Combined extractants increased 27 and 14 % of Pb in organic matter bound fractions, respectively.

It was observed that the extractant 0.1 M EDTA removed the highest amount of Pb (84 to 85 %) from Fe/Mn oxides bound fractions in soil where red amaranth and rice plants were grown, respectively. In addition 0.1 M EDTA dissolved 24 to 27 % Pb from organic matter bound fraction of soil. But more than 32 % of water soluble and 18 % of exchangeable forms of Pb were remained in EDTA treated soils after plant harvest. Therefore the mobility indexes of Pb were recorded above 10 % in EDTA treated soils, which were higher than soils treated with combined FA + EDTA, HA + EDTA and only FA treatments (Table 4.7.3).

Lead content in plant: The red amaranth plant obtained significantly higher amount of Pb from contaminated soil. The concentrations of Pb in root, stem and leaves of plant were 70.61, 22.52 and 26.54 mg kg^{-1}, respectively, grown in untreated contaminated MB soil. Similarly the highest Pb concentration in rice plant was also observed in contaminated MB soil. The root of rice contained the highest amount of Pb of 79.12 mg kg^{-1}. The stem and grain of rice plant contained 13.78 and 0.85 mg kg^{-1} of Pb, respectively (Table 4.7.4).

Table 4.7.4. Concentration of Pb (mg kg^{-1}) in different parts of *Amaranthus gangeticus* and *Oryza sativa* plants grown in treated and untreated contaminated and non-contaminated soils of Madhupur tract (MB).

Treatment	***Amaranthus gangeticus***			***Oryza sativa***		
	Root	**Stem**	**Leave**	**Root**	**Stem**	**Grain**
Contaminated	70.61a	22.52a	26.54a	79.12a	13.78a	0.85a
0.3 % HA	12.17c	6.74c	7.53c	11.21c	5.11c	0.31c
0.4 % FA	3.21d	2.07d	2.03d	2.06d	2.34d	0.00d
0.3 % HA + 0.06 M EDTA	3.23d	2.12d	2.12d	3.23d	2.03de	0.00d
0.4 % FA + 0.04 M EDTA	2.82de	1.86d	1.81d	2.04d	1.71e	0.00d
0.1 M EDTA	26.12b	8.54b	10.09b	25.87b	4.45b	0.55b
Non-contaminated	1.32e	0.32e	0.00e	2.09d	0.26f	0.00d

The contaminated soils treated with humic substances significantly reduced the concentration of Pb in plant materials compared to contaminated soils (Table 4.7.4). Kwiatkowska and Maciejewska (2006) reported that the availability of Pb in soils and their accumulation in plants decreased with the addition of humic substances. Kruatrachue *et al.*

(2002) found that a high concentration of HS (160 mg L^{-1}) decreased Pb uptake by *Lemna minor* and brought about increases in total chlorophyll content, growth rate and multiplication rate compared to control.

Among the humic substances, fulvic acid in general reduced the concentration of Pb in plants which was significantly lower than plant grown in soil treated with humic acid (Table 4.7.4). Zhang *et al.* (2013) also found that fulvic acid reduced Pb uptake by plant more efficiently than humic acid in acidic red and paddy soil. They reported that the leaf Pb concentration decreased by 17.78 and 30.22 % for HA and 19.44 and 48.32 % for FA when plants were grown in the acidic red and paddy soils, respectively.

The combined HA + EDTA and FA + EDTA solutions reduced the Pb content in plants which were comparatively lower than that of 0.3% HA treatment. Only humic acid did not extract sufficient amount of Pb from labile fractions. Among all extractants, the combined FA + EDTA treated soil exhibited the lowest concentration of Pb in both plants.

The treatment of soil with 0.1 M EDTA alone significantly reduced Pb content in plants from that of contaminated soil but the concentration was higher than plants grown in only HS and combined HS + EDTA treated soils (Table 4.7.4). This might be due to that excess EDTA in soil continued to release Pb from non-labile to labile fractions, which increased the concentration of Pb in plants. Grˇcman *et al.* (2001) reported that EDTA in the soil increased from 3 to 10 mmol kg^{-1}, the concentrations of Pb in the leaves of cabbage plant were increased 6.3 times. EDTA caused uncontrolled metal dissolution when persisted in soil. The Pb-EDTA in soil increased with the increase in rates of EDTA and as a consequence uptake of Pb by plant increased (Cunninghan and Ow, 1996; Blaylock *et al.*, 1997, Chen *et al.*, 2004, Komárek *et al.*, 2007b).

On the other hand remaining humic substances in soil after extraction with HS and combined HS + EDTA extractants, however, reduced the mobility of metals in soil by forming immobile metal-HS complexes. That’s why organic matter bounds Pb were increased in the range of 14 to 36 % in soil after extraction with these extractants. Results of some experiments have shown that the additional humic substances lowered the free and labile metal concentrations of the soil (Yu *et al.*, 2002; Jiang *et al.*, 2005, Li *et al.*, 2003; Gao *et al.*, 2009; Janos *et al.*, 2010) and inhibited plant metal accumulation (Li *et al.*, 2003; Kalis *et al.*, 2006). As a result HS and combined HS + EDTA extractants reduced Pb uptake by plants more efficiently than EDTA treated soil.

Therefore the combined FA + EDTA, HA + EDTA and only FA extractants considerably reduced the mobility indexes of Pb in soils and created effects on the minimum concentration of Pb in plants. Among them the use of only 0.4% fulvic acid is recommended for metal removal from contaminated soil of Maduhupur tract without using EDTA.

4.7.2 Experiment with contaminated soil of Ishwardi (IB)

A bulk amount of soil was collected adjacent to a batter industry located in Ishwardi EPZ where Pb content was recorded 164 mg kg^{-1} (Table 4.2.1). The properties of the contaminated calcareous soil of Ishwardi are described in section 4.1.2.

Experiment with *Amaranthus gangeticus*: The growth and yield of red amaranth grown in treated and untreated contaminated soil and non-contaminated soil are presented in Table 4.7.5. The plant grown in contaminated soil showed the lowest vegetative growth (plant height, root and leaf length) and yield (fresh and dry weight) compare to other treatments. High concentration of Pb in contaminated soil caused plant injury, which negatively influenced the growth of red amaranth. Zhang *et al.* (2013) reported that 500 mg kg^{-1} Pb caused injurious effects on tobacco growth, and the plant biomass grown in calcareous soil decreased by 20.18 %, compared with that of the unpolluted controls soil. Soil used in this present experiment contained 164 mg kg^{-1} (Table 4.2.1). Visual observation of the treatment effects on the growth of red amaranth in contaminated soil are presented in Figure 4.7.5.

Table 4.7.5. Vegetative growth and yield attributes of *Amaranthus gangeticus* grown in treated and untreated contaminated soils and non-contaminated soil of Ishwardi.

Treatment	Plant height (cm)	Leaf length (cm)	Root length (cm)	Fresh weight (g $plant^{-1}$)	Yield (Dry weight) (g pot^{-1})
Contaminated	7.04e	3.54d	3.21c	5.57c	4.85e
0.3 % HA	16.78d	5.12bc	4.94b	7.56b	9.17c
0.4 % FA	21.19bc	5.38bc	5.48a	8.23b	11.03b
0.3 % HA + 0.06M EDTA	22.34b	5.47b	5.39a	8.19b	10.83b
0.4 % FA + 0.04M EDTA	25.34a	5.88a	5.63a	9.79a	12.96a
0.1 M EDTA	15.36d	5.03c	4.69b	8.19b	7.35d
Non-contaminated	20.12c	5.95a	5.55a	9.48a	10.74b

Fig. 4.7.5. Photograph of *Amaranthus gangeticus* grown in treated and untreated contaminated soils of Ishwardi (IB).

The extraction of contaminated soils with humic substances, EDTA and combined HS + EDTA solutions increased the plant height, leaf and root length, and yield (fresh and dry weight) of *Amarantus gangeticus* (Table 4.7.5). These extractants removed Pb from contaminated soil, thereby increased plant growth and yield. The highest yield of red amaranth was observed in soil treated with combined 0.4 % FA + 0.04 M EDTA extractant which increased plant yield (dry weight) of 167.2 % over untreated contaminated soil. Next to it soils leached with only 0.4 % FA and combined 0.3 % HA + 0.06 M EDTA extractants also increased plant yield (dry weight) of 127.4 and 123.3 % from that of untreated contaminated soil, respectively. These yield values were similar to plant grown in non-contaminated soil (Table 4.7.5). Tufencki *et al.* (2006) applied increasing doses of humic substances, varying from 500 to 2000 mg kg^{-1} and observed that application of humic substances had positive impacts on the vegetative growth of lettuce plants with a short growing period. Moreover the yields of red amaranth grown in soils treated with humic substances were higher than that of plant grown in 0.1 M EDTA treated soil (Table 4.7.5).

Experiment with rice (*Oryza sativa*): The vegetative growth, panicle length and grain yield of rice plants grown in untreated and treated contaminated and non-contaminated pots are presented in Table 4.7.6 and Figure 4.7.6. The lowest vegetative growth and grain yield of rice plant was recorded in contaminated soil of Ishwardi possibly due to metal toxicity, which negatively influenced biochemical processes and growth of rice plant.

Table 4.7.6. Vegetative growth and yield attributes of *Oryza sativa* grown in treated and untreated contaminated soils and non-contaminated soil of Ishwardi.

Treatment	Plant dry weight (g pot^{-1})	Plant height (cm)	Panicle length (cm)	100 grain weight (gm)	Total grain weight (g pot^{-1})
Contaminated	218.72d	90.27c	18.53e	1.64d	17.9c
0.3 % HA	267.67b	96.01bc	23.48bcd	1.89c	20.88b
0.4 % FA	285.45ab	101.5ab	23.99b	2.33ab	23.23a
0.3 % HA + 0.06M EDTA	276.43ab	98.76ab	21.76d	1.93c	21.14b
0.4 % FA + 0.04M EDTA	289.76a	104.24a	25.92a	2.37a	23.87a
0.1 M EDTA	247.55c	95.76bc	22.06cd	2.01c	21.07b
Non-contaminated	275.76ab	97.21abc	23.55bc	2.19b	23.01a

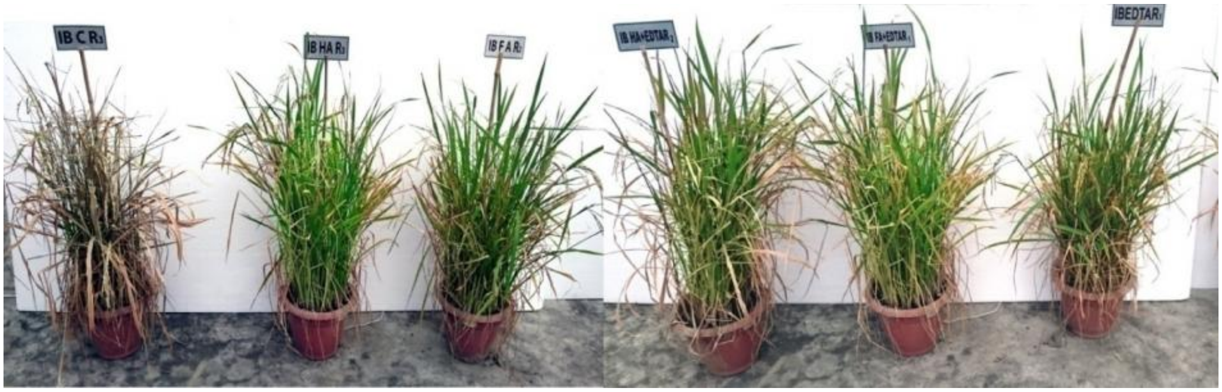


Fig. 4.7.6. Photograph of *Oryza sativa* (BRRI dhan 49) grown in treated and untreated contaminated soils of Ishwardi (IB).

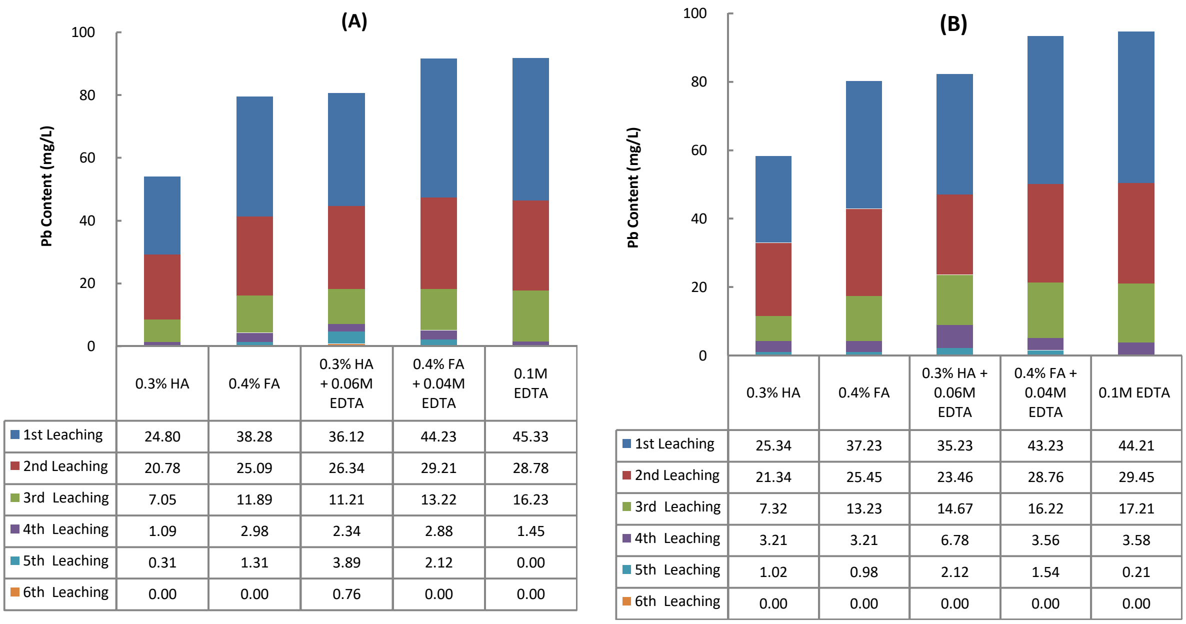
Treatment of soil with humic substances and combined HS + EDTA extractants significantly increased plant dry weight, plant height, panicle length and grain yield of rice from that of plants grown in contaminated soil. The maximum grain yields of rice plant were found in soils where combined 0.4 % FA + 0.04 M EDTA and individual 0.4% FA extractants were used for metal removal from soils, which were 33.4 and 29.8 % over that of contaminated soil, respectively. These yield values were also higher than that of plant grown in non-contaminated soil. Next to these extractants, combined 0.3 % HA + 0.06 M EDTA and only 0.3 % HA extractants increased grain yields of 18.1 and 16.6 % from the yield obtained in contaminated soil, respectively (Table 4.7.6). Eyheraguibel (2004) detected that humic substances accelerated both vegetative and reproductive growth of maize plants and thus stimulated optimal production of plant biomass (shoot and cobs). Root growth was stimulated as well with more fine lateral and secondary roots in the pots where humic substances were used for metal contamination remediation. In line with these results of Sharif *et al.* (2002) who reported a yield increase of 20 to 23 % in shoot dry weight and 32 to 39 % in root dry weight of crop plants by applying humic substances in a pot experiment.

Humic substances could not extract sufficient amount of Pb from contaminated calcareous soil of Ishwardi (Fig. 4.7.7). Because the complex-forming groups of HS are inactivated by bonding to calcium from $CaCO_3$ of calcareous soil (Zhao *et al.,* 2007). But humic substances in combination with EDTA increased the extractability of Pb from contaminated IB soil than that of only HS treatment.

Among the extractants, humic acid leached the lowest amount of Pb from contaminated soil and the concentration of Pb in leachate was recorded 7.05 to 25.34 mg L^{-1} from first three extractions. Fulvic acid extracted 11.89 to 38.28 mg L^{-1} of Pb from first three leaching of soil which were comparatively higher than that of humic acid. Therefore combined 0.4 % FA + 0.04 M EDTA extractant leached the maximum amount of Pb from IB soil and the concentrations of Pb in leachate were recorded 13.22 to 44.23 mg L^{-1} during first three extractions, which was similar as extracted by 0.1 M EDTA solution alone (Fig. 4.7.7).

After three extractions, the concentrations Pb in leachate were sharply decline with increasing number of leaching. The Pb contents in leachate from 5th and 6th extractions were found very low in amount (Fig. 4.7.7).

The combined FA + EDTA extractant leached the maximum amount of Pb from contaminated calcareous soil and the values were 13.22 to 44.23 mg L^{-1} Pb during first three extractions which was also similar as extracted by 0.1 M EDTA alone. Next to it combined HA + EDTA extractant leached 11.21 to 36.12 mg L^{-1} of Pb during same extractions of soil.



(A)

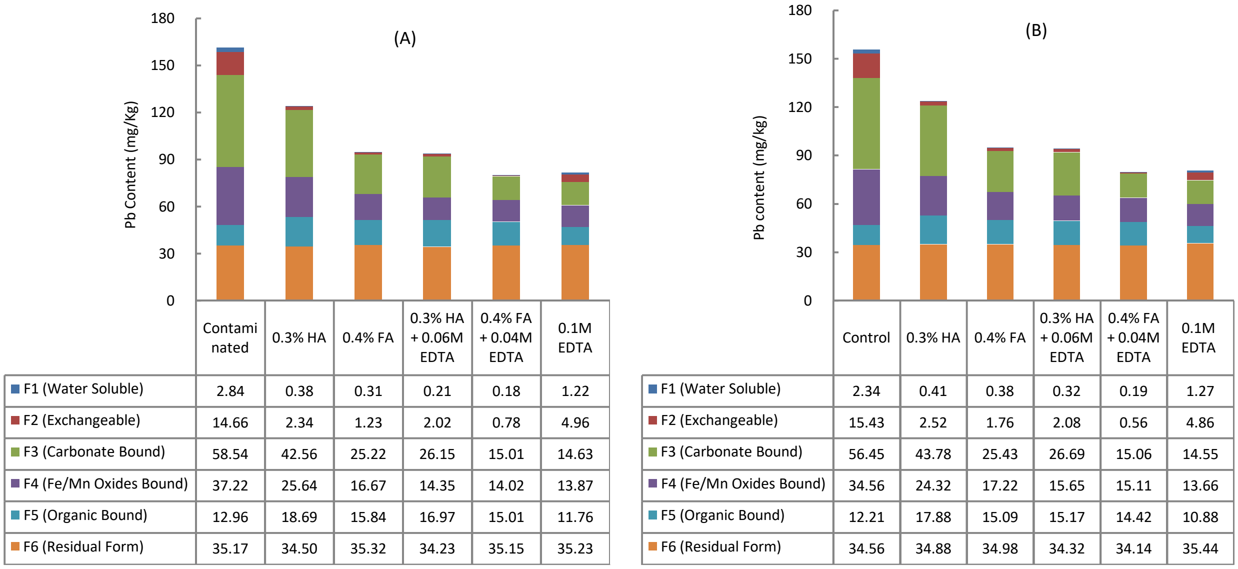
	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	24.80	38.28	36.12	44.23	45.33
🟥 2nd Leaching	20.78	25.09	26.34	29.21	28.78
🟩 3rd Leaching	7.05	11.89	11.21	13.22	16.23
🟪 4th Leaching	1.09	2.98	2.34	2.88	1.45
■ 5th Leaching	0.31	1.31	3.89	2.12	0.00
🟧 6th Leaching	0.00	0.00	0.76	0.00	0.00

(B)

	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	25.34	37.23	35.23	43.23	44.21
🟥 2nd Leaching	21.34	25.45	23.46	28.76	29.45
🟩 3rd Leaching	7.32	13.23	14.67	16.22	17.21
🟪 4th Leaching	3.21	3.21	6.78	3.56	3.58
■ 5th Leaching	1.02	0.98	2.12	1.54	0.21
🟧 6th Leaching	0.00	0.00	0.00	0.00	0.00

Fig. 4.7.7. Concentration of Pb (mg L^{-1}) in leachate after extraction of contaminated IB soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.

The fractionation study indicated that mobility indexes of Pb were 47.71 and 46.98 % in contaminated soil after harvest of red amaranth and rice plants, respectively (Table 4.4.7). A range of 14 to 18 % and 16 to 17 % of Pb were found in water soluble and exchangeable forms where 0.3% humic acid was used for metal removal from soil, respectively. In contrast soil treated with fulvic acid retained 11 to 16% of Pb in water soluble and 8 to 12 % of Pb in exchangeable forms. Soils leached with humic and fulvic acids also retained 73 to 78% and 43 to 45% of Pb in carbonate bond fractions, respectively (Fig 4.7.8). Therefore the mobility indexes of Pb were 36.5 to 37.7% and 28.3 to 29 % in soils remediated with humic and fulvic acids, respectively (Fig. 4.7.7). Humic and fulvic acids retained 44 to 46 and 22 to 24 % of Pb in organic matter bound fraction over that of contaminated soil (Fig. 4.7.8).



(A)

	Contaminated	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	2.84	0.38	0.31	0.21	0.18	1.22
🟥 F2 (Exchangeable)	14.66	2.34	1.23	2.02	0.78	4.96
🟩 F3 (Carbonate Bound)	58.54	42.56	25.22	26.15	15.01	14.63
🟪 F4 (Fe/Mn Oxides Bound)	37.22	25.64	16.67	14.35	14.02	13.87
🟦 F5 (Organic Bound)	12.96	18.69	15.84	16.97	15.01	11.76
🟧 F6 (Residual Form)	35.17	34.50	35.32	34.23	35.15	35.23

(B)

	Control	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	2.34	0.41	0.38	0.32	0.19	1.27
🟥 F2 (Exchangeable)	15.43	2.52	1.76	2.08	0.56	4.86
🟩 F3 (Carbonate Bound)	56.45	43.78	25.43	26.69	15.06	14.55
🟪 F4 (Fe/Mn Oxides Bound)	34.56	24.32	17.22	15.65	15.11	13.66
🟦 F5 (Organic Bound)	12.21	17.88	15.09	15.17	14.42	10.88
🟧 F6 (Residual Form)	34.56	34.88	34.98	34.32	34.14	35.44

Fig. 4.7.8. Fractionation of Pb (mg kg^{-1}) in contaminated soil of Ishwardi after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.

The treatment of soil with combined HA + EDTA extractant hold 7 to 14 %, 13 to 14% and 45 to 47 % of Pb in water soluble, exchangeable and carbonate bound fractions. In contrast soil remediated with combined FA + EDTA extractant retained only 6 to 8 %, 4 to 5 % and 24 to 27% of Pb in first three labile fractions (Fig. 4.7.8). Therefore the mobility indexes of Pb were 30.2 to 30.9% and 18.9 to 19.9% in soil treated with combined HA + EDTA and FA + EDTA extractants, respectively (Table 4.7.7). However these combined extractants increased 24 to 30 % and 16 to 18% of Pb in organic matter bound fraction over that of contaminated soil, respectively.

After plant harvest the fractionation of 0.1 M EDTA treated soil indicated that 43 to 54 %, 32 to 34% and 25 to 26% of Pb were found in water soluble, exchangeable and carbonate bound fractions, respectively. Among the extractants, the water soluble and exchangeable forms of Pb were found highest where only EDTA was used for reclamation of Pb from soil (Fig. 4.7.8). Free EDTA in soil continued to dissolve metals from non-labile to labile fractions. The mobility index of Pb was recorded 25.4 to 25.6 % in soil treated with only EDTA, which were higher than combined FA + EDTA treatment (Table 4.7.7). Lai and chen (2004) found that the formation of Pb-EDTA increased with increased rates of EDTA in soils due to the high bond strength of Pb-EDTA.

Table 4.7.7. Mobility indexes of Pb (%) in treated and untreated contaminated IB soils after plant harvest.

Extractants	Soil of Red Amaranth plant	Soil of Rice plant
Contaminated	46.98±2.81	47.71±2.81
0.3 % HA	36.48±2.11	37.73±2.27
0.4 % FA	28.29±1.73	29.06±1.71
0.3 % HA + 0.06M EDTA	30.21±1.84	30.87±1.88
0.4 % FA + 0.04M EDTA	18.91±1.12	19.89±1.11
0.1M EDTA	25.48±1.56	25.64±1.58

Pb content in plants: The highest concentrations of Pb were observed in root, stem and leaf of red amaranth grown in contaminated soil of Ishwardi and the values were 76.14, 20.84 and 29.94 mg kg^{-1}, respectively. Similarly rice plant accumulated the maximum amount of Pb from contaminated soil. The root of rice plant contained 83.45 mg kg^{-1} of Pb, followed by stem and grain of rice, the values were only 16.34 and 0.87 mg kg^{-1} of Pb, respectively (Table 4.7.8).

Table 4.7.8. Concentration of Pb (mg kg^{-1}) in different parts of *Amaranthus gangeticus* and *Oryza sativa* plants grown in treated and untreated contaminated and non-contaminated soils of Ishwardi (IB).

Treatment	*Amaranthus gangeticus*			*Oryza sativa*		
	Root	Stem	Leave	Root	Stem	Grain
Contaminated	76.14a	20.84a	29.94a	83.45a	16.34a	0.87a
0.3 % HA	18.87c	11.25b	9.53bc	16.85c	9.03c	0.45c
0.4 % FA	12.02d	8.98c	8.82c	14.65d	6.26d	0.33d
0.3 % HA + 0.06 M EDTA	13.23d	10.98b	6.65d	17.67c	6.09d	0.41c
0.4 % FA + 0.04 M EDTA	5.12e	4.78d	2.03e	3.03e	1.69e	0.00e
0.1 M EDTA	24.21b	11.23b	11.89b	27.32b	12.45b	0.53b
Non Contaminated	1.87f	0.13e	0.56f	2.09f	0.26f	0.00e

The extractants humic acid, fulvic acid, EDTA and combined solutions significantly reduced the concentration of Pb in plant materials. Among the humic substances, fulvic acid reduced the concentration of Pb in both red amaranth and rice plants which were relatively lower than that of humic acid treatment (Table 4.7.8). Zhang *et al.* (2013) also reported that fulvic acid reduced metal uptake by plant more efficiently than humic acid from calcareous soils.

The lowest concentrations of Pb in plant materials were observed in soil treated with combined 0.4 % FA + 0.04 M EDTA solution. Next to it 0.4 % fulvic acid and combined 0.3 %

HA + 0.06 M EDTA extractants reduced the concentration of Pb which were comparatively lower than the values obtained with only 0.3 % HA (Fig.4.7.8).

Although 0.1 M EDTA extracted the highest amount of Pb from contaminated soil of Ishwardi, due to the presence of higher amount of Pb in water soluble and exchangeable fractions, EDTA caused the higher Pb concentration in plants than plant grown in combined FA + EDTA treated soil (Table 4.7.8). Huang and Cuningham (1997) found that Pb accumulation in shoot enhanced 1 week after transplanting of plant with increasing dose of EDTA in soil.

It was found from the above results that combined FA + EDTA extractant caused the lowest mobility index of Pb in soil and thereby caused the minimum Pb concentration in plants grown in contaminated calcareous soils of Ishwardi.

4.7.3. Experiment with soil of Hazaribagh tannery industrial area (HT)

Soils of tannery industrial areas are highly contaminated with chromium. A bulk amount of soil was collected from a contaminated point source of industrial area where Cr concentration was 256.13 mg kg^{-1} (Table 4.2.1). The detail properties of soil were described in section 4.1.3.

Experiment with red amaranth: The vegetative growth and yield of red amaranth plant grown in untreated and treated contaminated soil and non-contaminated soil are illustrated in Table 4.7.9. The lowest values of plant height, root and leaf length were recorded in contaminated soil. Consequently the yield (fresh and dry weight) of *Amaranthus gangeticus* was also observed minimum in untreated contaminated soil of Hazaribagh (Table 4.7.9), possibly due to plant injury from Cr toxicity. A visual observation of the treatment effects on the growth of red amaranth in presented in Figure 4.7.9.

Table 4.7.9. Vegetative growth and yield attributes of *Amaranthus gangeticus* grown in treated and untreated contaminated soils and non-contaminated soil of Hazaribagh.

Treatment	Plant height (cm)	Leaf length (cm)	Root length (cm)	Fresh weight (g plant^{-1})	Yield (Dry weight) (g pot^{-1})
Contaminated	8.04d	2.79c	3.27c	5.67d	5.95d
0.4 % HA	21.09ab	5.45a	5.44ab	9.03b	11.89b
0.4 % FA	20.17b	5.37a	5.41ab	9.12b	11.37b
0.4 % HA + EDTA 0.04 M	22.27a	5.72a	5.76a	10.34a	14.11a
0.4 % FA + EDTA 0.04 M	21.89a	5.66a	5.68a	10.11a	13.93a
0.1 M EDTA	18.07c	4.98b	5.19b	8.04c	9.82c
Non-contaminated	21.76a	5.57a	5.49ab	10.23a	13.28a

Fig. 4.7.9. Photograph of *Amaranthus gangeticus* grown in treated and untreated contaminated soils of Harazibagh (HT).

The soil treated with humic substances, EDTA and mixed HS + EDTA extractants significantly increased plant height, root and leaf length and thereby increased fresh and dry yield of red amaranth than that of contaminated soil. The maximum yields (fresh and dry weight) of plant were observed in soils where mixed HA + EDTA and FA + EDTA extractants were used for metal removal from soil which were also significantly similar to plant grown in non-contaminated soil. The above combined extractants increased plant yield (dry weight) of 137.1 and 134.1 % over that of contaminated soil. The treatment of soils with humic and fulvic acids were also favored the increased yields (fresh and dry weight) of plant, which were higher than that of only 0.1 M EDTA treatment (Table 4.7.9). The growth promoting results of humic substances were reported for a wide number of plant species (Visser, 1986; Chen and Aviad, 1990). Verlinden *et al.* (2009) reported that Humifirst had a positive effect on total tuber yield (+ 17 % compared to control). Anonymous (2002) also found positive effects of Humifirst on potato yield at Gembloux (+ 25 %) and Geer (+ 11 %) located in the southern part of Belgium.

Experiment with rice: The vegetative growth, panicle length and grain yields of rice plant grown in different treated and untreated contaminated and non-contaminated soils are presented in Table 4.7.10. The vegetative growth and grain yield of rice plant were also recorded lowest in contaminated soil of Hazaribagh. A visual observation of the treatment effects of humic substances and EDTA on the growth and grain yield of rice plant are shown in Figure 4.7.10.

Table 4.7.10. Vegetative growth and yield attributes of *Oryza sativa* grown in treated and untreated contaminated soils and non-contaminated soil of Hazaribagh.

Treatment	**Plant dry weight (g Pot^{-1})**	**Plant height (cm)**	**Panicle length (cm)**	**100 grain weight (g)**	**Total grain weight (g pot^{-1})**
Contaminated	158.23e	73.66d	17.78f	1.506c	16.81c
0.4 % HA	209.45bc	91.44abc	22.23bc	2.193a	20.88ab
0.4 % FA	202.29cd	88.9bc	20.96cd	2.213a	20.65ab
0.4 % HA + 0.04M EDTA	228.43a	96.52a	25.4a	2.252a	22.21a
0.4 % FA + 0.04M EDTA	226.49a	91.44abc	22.86b	2.223a	22.06a
0.1M EDTA	188.39d	86.36c	19.05ef	1.891b	19.55b
Non contaminated	224.26ab	93.98ab	20.32de	2.197a	21.32a

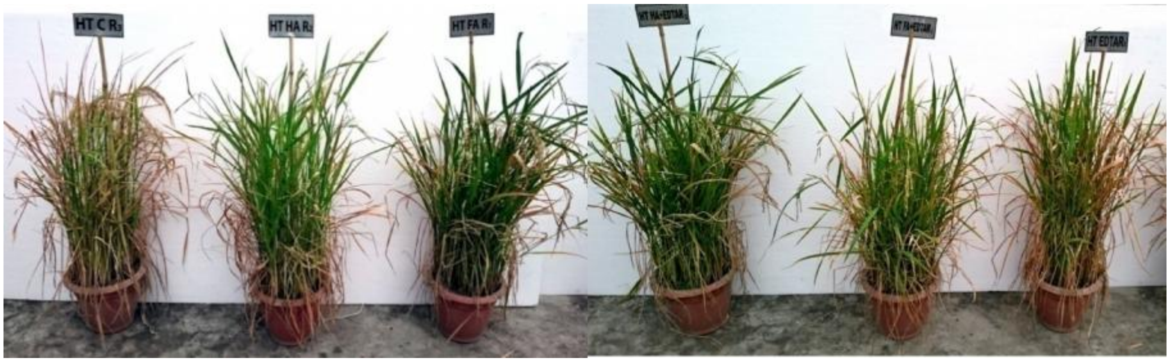
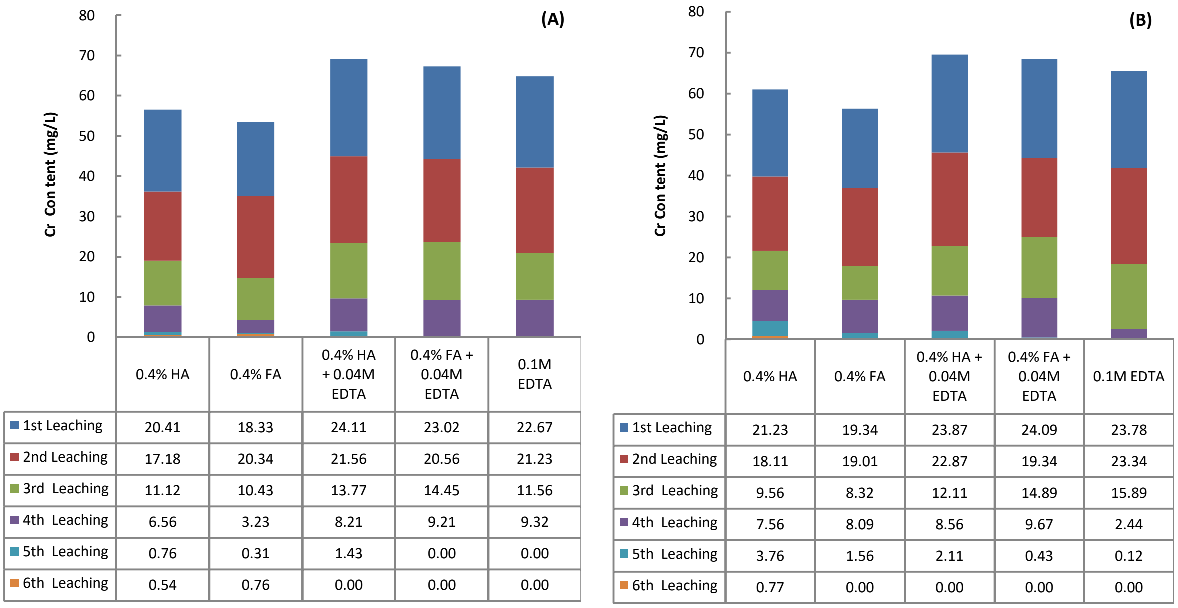


Fig. 4.7.10. Photograph of *Oryza sativa* (BRRI dhan 49) grown in treated and untreated contaminated soils of Harazibagh (HT).

The extraction of contaminated soil of Hazaribagh area by humic substances, EDTA and combined extractant increased the vegetative growth (plant height and dry mass) and grain yield of rice plant. It was found that the maximum grain yield of rice was recorded in soils treated with combined 0.4 % HA + 0.04 M EDTA and 0.4 % FA + 0.04 M EDTA solutions which were 32.1 and 31.2 % higher than that of contaminated soil, respectively. The grain yields of combined extractants were significantly similar to plant grown in non-contaminated soil. The individual humic and fulvic acids treatment also enhanced the growth and grain yield of rice plant. Cooper *et al.* (1998) found that the incorporation of humic substances in the soil stimulated 45 % more root mass of crop plant at the 0 to 10 cm depth and increased crop yield by 22 %.

The fraction of contaminated soil of Hazahibagh after plant harvest indicated that the maximum amount of Cr was retained in non-labile pools of soils. The organic matter fraction retained the largest amount of Cr (Fig. 4.7.12). The mobility index of Cr was recorded 11.6 to 12.5 % in untreated contaminated HT soil used for pot experiments (Table 4.7.11).

Humic substances, EDTA and combined extractants leached considerable amounts of Cr up to four extractions. The concentrations of Cr in leachate were found very low in amount at 5th and 6th extractions for all extractants (Fig. 4.7.11).



(A)	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	20.41	18.33	24.11	23.02	22.67
🟥 2nd Leaching	17.18	20.34	21.56	20.56	21.23
🟩 3rd Leaching	11.12	10.43	13.77	14.45	11.56
🟪 4th Leaching	6.56	3.23	8.21	9.21	9.32
🟦 5th Leaching	0.76	0.31	1.43	0.00	0.00
🟧 6th Leaching	0.54	0.76	0.00	0.00	0.00

(B)	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	21.23	19.34	23.87	24.09	23.78
🟥 2nd Leaching	18.11	19.01	22.87	19.34	23.34
🟩 3rd Leaching	9.56	8.32	12.11	14.89	15.89
🟪 4th Leaching	7.56	8.09	8.56	9.67	2.44
🟦 5th Leaching	3.76	1.56	2.11	0.43	0.12
🟧 6th Leaching	0.77	0.00	0.00	0.00	0.00

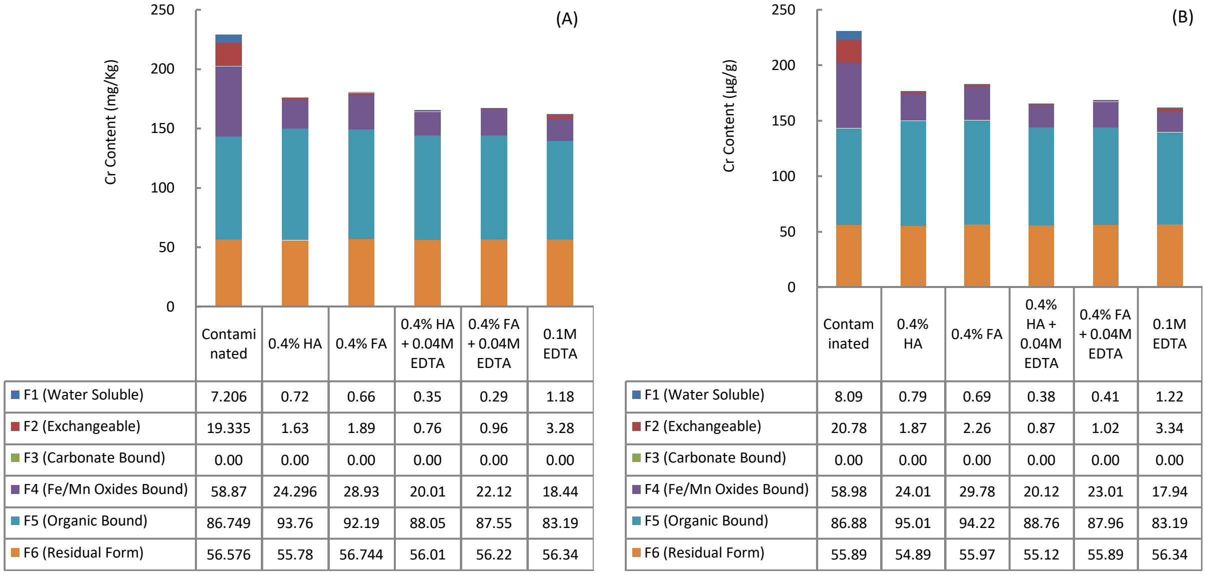
Fig. 4.7.11. Concentration of Cr (mg L^{-1}) in leachate after extraction of contaminated HT soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.

It was observed that humic and fulvic acids at the concentration of 0.4 % leached 6.56 to 21.23 mg L^{-1} and 3.23 to 19.34 mg L^{-1} of Cr during first four extractions, respectively. The metal removal efficiencies of humic acid from contaminated SSy soil were somewhat higher than that of fulvic acid (Fig 4.7.11).

The combined 0.4 % HA + 0.04 M EDTA and 0.4 % FA + 0.04 M EDTA extractants leached 8.21 to 24.11 mg L^{-1} and 9.67 to 24.09 mg L^{-1} of Cr during first four extractions in contaminated soil, respectively. Consequently the extraction yields of combined HS and EDTA were higher than that of HS extractant alone. The extractant 0.1 M EDTA leached 2.44 to 23.78 mg L^{-1} of Cr during first four extractions (Fig. 4.7.11).

From fractionation study of contaminated HT soil after plant harvest it was found that humic and fulvic acids at the concentration of 0.4 % retained less than 10 % of Cr in water soluble and exchangeable forms (Fig. 4.7.12). These respective humic substances also hold 41 to 42 % and 50 to 51 % of metals in Fe/Mn oxides bound fractions. Therefore the mobility indexes of Cr were found 1.33 to 1.51 % and 1.41 to 1.61 % in soils remediated with humic and fulvic acids after plant harvest, respectively. However, humic substances increased 6 to 9 % of Cr in organic matter bound fraction over that of contaminated soil. Humic substances formed immobile HS-metal complex at high concentration.

The treatment of soils with combined 0.4 % HA + 0.04 M EDTA and 0.4 % FA + 0.04M EDTA extractants retained 4 to 6% of Cr in water soluble and exchangeable forms after plant harvest. These combined extractants removed comparatively higher amount of Cr (60 - 66 %) from Fe/Mn oxides bound fractions than that of only HS treatments. The mobility indexes of Cr were found lowest (below 1 %) in soils where combined extractants were used for metal remediation. Combined extractants increased 2 to 3 % of Cr in organic matter bound fractions.



	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	7.206	0.72	0.66	0.35	0.29	1.18
🟥 F2 (Exchangeable)	19.335	1.63	1.89	0.76	0.96	3.28
🟩 F3 (Carbonate Bound)	0.00	0.00	0.00	0.00	0.00	0.00
🟪 F4 (Fe/Mn Oxides Bound)	58.87	24.296	28.93	20.01	22.12	18.44
🟦 F5 (Organic Bound)	86.749	93.76	92.19	88.05	87.55	83.19
🟧 F6 (Residual Form)	56.576	55.78	56.744	56.01	56.22	56.34

	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	8.09	0.79	0.69	0.38	0.41	1.22
🟥 F2 (Exchangeable)	20.78	1.87	2.26	0.87	1.02	3.34
🟩 F3 (Carbonate Bound)	0.00	0.00	0.00	0.00	0.00	0.00
🟪 F4 (Fe/Mn Oxides Bound)	58.98	24.01	29.78	20.12	23.01	17.94
🟦 F5 (Organic Bound)	86.88	95.01	94.22	88.76	87.96	83.19
🟧 F6 (Residual Form)	55.89	54.89	55.97	55.12	55.89	56.34

Fig. 4.7.12. Fractionation of Cr (mg kg^{-1}) in treated and untreated contaminated soils of Hazaribagh after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.

From fractionation study it was observed that the extractant EDTA removed the highest amount of Cr (68 to 69 %) from Fe/Mn oxides bound fraction and also removed 4 to 5 % of Cr from organic matter bound fractions. But the soil treated with 0.1 M EDTA retained more than 17 % of Cr in water soluble and exchangeable forms which were comparatively higher than that of combined HS + EDTA and only HS treatments. It was found from many studies that excess EDTA in soil caused further dissolution of metal from non-labile to labile fraction in soil (Chen and Cutright, 2001). Therefore the mobility index of Cr was recorded 2.75 to 2.81% in EDTA treated soil after plant harvest (Table 4.7.11).

Table 4.7.11. Mobility indexes of Cr (%) in treated and untreated contaminated HT soils after plant harvest.

Extractants	Soil of Amaranthus plant	Soil of Rice plant
Contaminated	11.60±0.55	12.52±0.62
0.4 % HA	1.33±0.05	1.51±0.07
0.4 % FA	1.41±0.06	1.61±0.06
0.4 % HA + 0.04M EDTA	0.67±0.05	0.76±0.05
0.4 % FA + 0.04M EDTA	0.75±0.04	0.85±0.07
0.1M EDTA	2.75±0.13	2.81±0.15

Concentration of Cr in plants: The root, stem and leaf of red amaranth assimilated the highest amount of Cr when grown in contaminated HT soil and the concentrations of Cr were 45.54, 8.07 and 12.37 mg kg^{-1}, respectively (Table 4.7.12). Rice root contained the highest amount of Cr of 94.34 mg kg^{-1}. Whereas, stem and grain of rice contained 2.07 and 0.72 mg kg^{-1} of Cr, respectively.

Table 4.7.12. Concentration of Cr (mg kg^{-1}) in different parts of *Amaranthus gangeticus* and *Oryza sativa* plants grown in treated and untreated contaminated and non-contaminated soils of Hazaribagh (HT).

Treatment	*Amaranthus gangeticus*			*Oryza sativa*		
	Root	Stem	Leave	Root	Stem	Grain
Contaminated	45.54a	8.07a	12.37a	94.34a	2.07a	0.72a
0.4 % HA	4.92c	0.34cd	0.00c	7.23cd	0.52d	0.00c
0.4 % FA	5.34c	0.42c	0.00c	9.12c	0.67c	0.00c
0.4 % HA + 0.04 M EDTA	3.65cd	0.21d	0.00c	6.23d	0.34e	0.00c
0.4 % FA + 0.04 M EDTA	4.98c	0.51c	0.00c	7.68cd	0.49d	0.00c
0.1 M EDTA	14.27b	2.54b	1.88b	16.54b	1.76b	0.25b
Non-contaminated	1.65d	0.00e	0.00c	2.03e	0.00f	0.00c

Chromium content reduced in plants when grown in soil treated with humic substances, EDTA and combined extractants. The lowest Cr content in plants was observed in soils washed with combined 0.4 % HA + 0.04 M EDTA extractant.

Alone 0.1 M EDTA extractant also reduced the Cr content in plants but the values were comparatively higher than individual HS and combined HS + EDTA treatments. It was found from fractionation that EDTA in soil increased the available forms of chromium with time and thereby increased Cr concentration in plants (Fig. 4.7.12 and Table 4.7.12). Chen and Cutright (2001) reported that mobile fraction of Cr significantly increased up to 30 mg kg^{-1} with excess EDTA application in soil. On the other hand, residual humic substances in soil after extraction with humic substances and combined HS + EDTA extractants formed insoluble metal-HS complex with free and available metal ions and inhibited metals uptake by plants (Fig. 4.7.12 and Table 4.7.12).

By comparing the above extractants, it can be concluded that only humic and fulvic acids and combined HS + EDTA extractants were equally effective to reduce Cr concentration in plants grown in contaminated soil of Hazaribagh compared to that of only EDTA treatment. Therefore the use of only 0.4% HA or 0.4% FA is recommended for metal removal from soil without using EDTA

4.7.4. Experiment with soil of ship breaking yard, Shitakunda (SSy)

In soil of ship breaking yard (SSy) all heavy metal contents were found very high in amount (Figure 4.2.5 to 4.2.9). The soil was saline in nature. The detail properties of soil were described in section 4.1.4.

Experiment with red amaranth: The growth parameters (plant height, root and leaf length, total plant biomass and yield) of *Amaranthus gangeticus* grown in untreated and treated contaminated soil and also non-contaminated soil of same region are presented in Table 4.7.13. It was found that contaminated soil of Shitakunda exhibited the lowest growth and yield (dry weight) of *Amaranthus gangeticus*. Most of the plants were found damaged in contaminated soil containing pots possibly due to high metal toxicity. A visual observation of the treatment effects of humic substances and EDTA on the growth of red amaranth is presented in Figure 4.7.13.

Table 4.7.13. Vegetative growth and yield attributes of *Amaranthus gangeticus* grown in treated and untreated contaminated soils and non-contaminated soil of ship breaking yard, Shitakunda (SSy)

Treatment	Plant height (cm)	Leaf length (cm)	Root length (cm)	Fresh weight (g plant^{-1})	Yield (Dry weight) (g pot^{-1})
Contaminated	6.09d	2.76d	2.98d	4.32d	3.98e
0.4 % HA	22.23b	6.02ab	5.74ab	11.32bc	13.48ab
0.4 % FA	19.67c	5.27c	5.48b	11.06bc	12.52c
0.4 %HA + EDTA 0.04M	24.14a	6.12a	6.02a	12.43a	14.26a
0.4 % FA + EDTA 0.04M	22.96ab	5.73b	5.34bc	11.67ab	13.53ab
0.1M EDTA	19.45c	5.19c	5.09c	10.64c	11.11d
Non contaminated	20.09c	5.87ab	5.67ab	11.07bc	12.73bc

Fig. 4.7.13. Photograph of *Amaranthus gangeticus* plants grown in treated and untreated contaminated soils of ship breaking yard, Shitakunda (SSy).

The contaminated soils treated with humic substances, EDTA and combined extractants reduced metal toxicity and increased the growth and yield of *Amaranthus gangeticus*. The highest plant height, leaf and root length and yield (fresh and dry weight) of red amaranth were recorded in soil treated with the combined 0.4 % HA + 0.04 M EDTA extractant. This extractant favored plant yield (dry weight) by 2.58 times higher than that of contaminated soil. Next to it combined 0.4 % FA + 0.04 M EDTA and individual 0.4 % HA extractant increased plant yield (dry weight), which were 2.39 and 2.38 times higher than that of plant grown in contaminated soil. The plant yields (dry weight) were also higher than that of non-contaminated soil (Table 4.7.13). Humic substances have stimulating effect on plant growth and yield by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (Vaughan and Malcolm, 1979; Albuzio, 1986; Concheri *et al.*, 1994; Chen, 2004). Chen and Aviad (1990) reported the promoted growth and nutrient uptake of plants by the addition of humic substances in contaminated saline soil due to better-developed root systems.

Experiment with rice: The vegetative growth, total plant biomass, panicle length and total grain yield of rice growing in different treated and untreated contaminated and non contaminated soils are given in Table 4.7.14. The vegetative growth and grain yield of plant was found lowest when grown in untreated contaminated soil of Shitakunda due to plant damage from metal toxicity in soil. The treatment of soils with humic substances, EDTA and combined extractants created impacts on increased dry weight, height, panicle length and grain yield of rice. Humic and fulvic acids remediated soils favored the increased grain yield of 89 and 87 % over that of plant grown in contaminated soil, respectively (Table 4.7.14). Khaled and Fawy (2011) observed that the dry weight and yield of corn were found higher by the application of humic substances in contaminated saline soil. Visual differences of growth of rice plant are shown in Figure 4.7.14.

Therefore the highest plant dry weight, height, panicle length and grain yield were found in soil where combined 0.4 % HA + 0.04 M EDTA extractant was used for metal removal. Plant grown in soil treated with this combined extractant created impacts on increased grain yield of 112.8 % over that of plant grown in contaminated soil. Next to it combined 0.4 %

FA + 0.04 M EDTA extractant treated soil also favored the grain yield of rice plant, which was 94.7 % higher than that of contaminated soil (Table 4.7.14).

Table 4.7.14. Vegetative growth and yield attributes of *Oryza sativa* grown in treated and untreated contaminated soils and non-contaminated soil of ship breaking yard, Shitakunda.

Treatment	**Plant dry weight (g pot^{-1})**	**Plant height (cm)**	**Panicle length (cm)**	**100 grain weight (g)**	**Total grain weight (g pot^{-1})**
Contaminated	147.62d	75.44c	17.15d	1.221e	10.28d
0.4 % HA	186.24ab	87.78b	20.38b	1.828cd	19.44b
0.4 % FA	181.11b	80.65c	19.72bc	1.922bc	19.28b
0.4 % HA + 0.04 M EDTA	198.54a	96.52a	22.86a	2.094a	21.38a
0.4 % FA + 0.04 M EDTA	194.33ab	91.44ab	21.23b	2.078a	20.02b
0.1 M EDTA	164.73c	87.11b	18.22cd	1.745d	17.42c
Non-contaminated	186.23ab	89.28b	20.94b	1.952ab	19.82b

Fig. 4.7.14. Photograph of *Oryza sativa* (BRRI dhan 49) plant grown in treated and untreated contaminated soils of ship breaking yard, Shitakunda (SSy).

The vegetative growths and grain yields of rice grown in soil treated with only humic substances and combined HS + EDTA treatments were more or less similar to plant grown in non-contaminated soil (Table 4.7.14). Duplessis and Mackenzie (1983) found that the grain yield of several crops increased by the use of humic substances. Humic acids were added to an alkaline soil with fertilizer to wheat grown in field trials. It was observed that yield was increased by 25 % (Wang, 1995).

The soils of ship breaking yard were highly toxic due to presence of heavy metals. The soil used for pot experiment contained 93.23, 25.07, 71.12, 69.64, 192.95 and 845.12 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively (Table 4.2.1).

From the fractionation study of contaminated soil of Shitakunda after plant harvest it was found that the mobility indexes of metals were 29.38, 18.75, 27.47, 33.3 and 28.19 % for Pb, Cr, Ni, Cu and Zn in soil, respectively (Table 4.7.16).

Table 4.7.15. Fractionation of metals (mg kg^{-1}) in contaminated soil of ship breaking yard (SSy) after plants harvest.

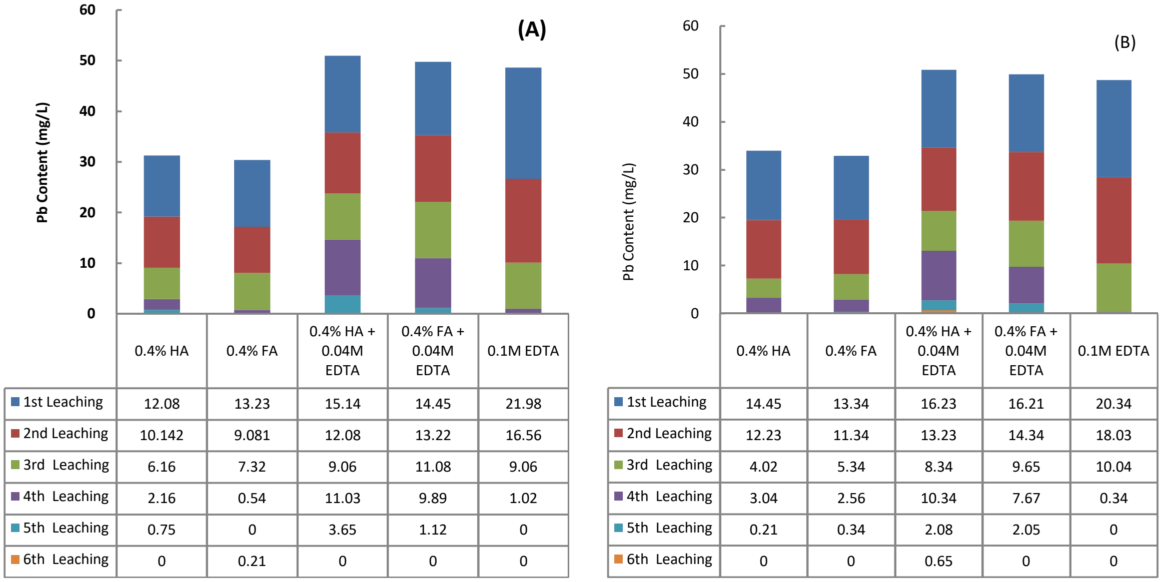
Metals	F1 (Water Soluble)	F2 (Exchangeable)	F3 (Carbonate Bound)	F4 (Fe/Mn Oxides Bound)	F5 (Organic Bound)	F6 (Residual Form)	Mobility Index (%)
Pb	2.40	9.36	13.25	28.77	13.00	18.34	29.38
%	2.81	10.99	15.57	33.80	15.28	21.54	
Cr	2.21	3.85	6.36	17.08	19.45	17.27	18.75
%	3.34	5.81	9.61	25.79	29.36	26.08	
Ni	1.68	5.58	9.00	19.84	10.62	12.48	27.47
%	2.83	9.43	15.21	33.51	17.95	21.08	
Cu	5.0	18.7	38.6	64.0	30.9	29.7	33.33
%	2.7	10.0	20.6	34.2	16.5	15.9	
Zn	18.7	80.0	131.1	274.4	105.1	208.6	28.10
%	2.3	9.8	16.0	33.6	12.9	25.5	

Extraction: Humic substances, EDTA and combined treatments leached considerable amount of metals from contaminated SSy soils. The concentrations of metals in leachate were observed high up to four extractions. Among the metals, Cr and Ni concentrations were found high up to 2nd leaching and the Zn, Cu and Pb concentrations were recorded high in leachate up to 4th extractions. Either very low or negligible amount of metals was detected in leachate at 6th extraction (Fig. 4.7.15 to 4.7.19).

It was observed that humic substances leached 7.01 to 9.12 mg L^{-1} of Cr and 6.01 to 10.32 mg L^{-1} of Ni during first two extractions and 2.16 to 14.45 mg L^{-1} of Pb, 4.32 to 29.56 mg L^{-1} of Cu and 18.56 to 72.34 mg L^{-1} of Zn at the time of first four extractions. The metal extraction capacities of humic substances from contaminated SSy soil were somewhat higher than that of fulvic acid (Fig. 4.7.15 to 4.7.19).

The combined 0.4 % HA + 0.04 M EDTA and 0.4 % FA + 0.04 M EDTA extractants leached 7.01 to 11.02 mg L^{-1} of Cr and 9.23 to 12.34 mg L^{-1} of Ni at the time of first two extractions and 7.67 to 16.23 mg L^{-1} of Pb, 3.8 to 32.34 mg L^{-1} of Cu and 20.45 to 75.45 mg L^{-1} of Zn during first four extractions of contaminated soil. It was observed that the extraction yields of combined HS and EDTA were higher than that of individual HS extractants. The extractant 0.1 M EDTA leached 6.08 to 8.32 mg L^{-1} of Cr and 9.68 to 11.02 mg L^{-1} of Ni during first two extractions and 0.34 to 21.98 mg L^{-1} of Pb, 0.83 to 34.56 mg L^{-1} of Cu and 15.67 to 75.45 mg L^{-1} of Zn at the time of first four extractions (Fig. 4.7.15 to 4.7.19).

The removal efficiency of combined HA + EDTA extractant was fairly higher than that of combined FA + EDTA and individual EDTA extractants.



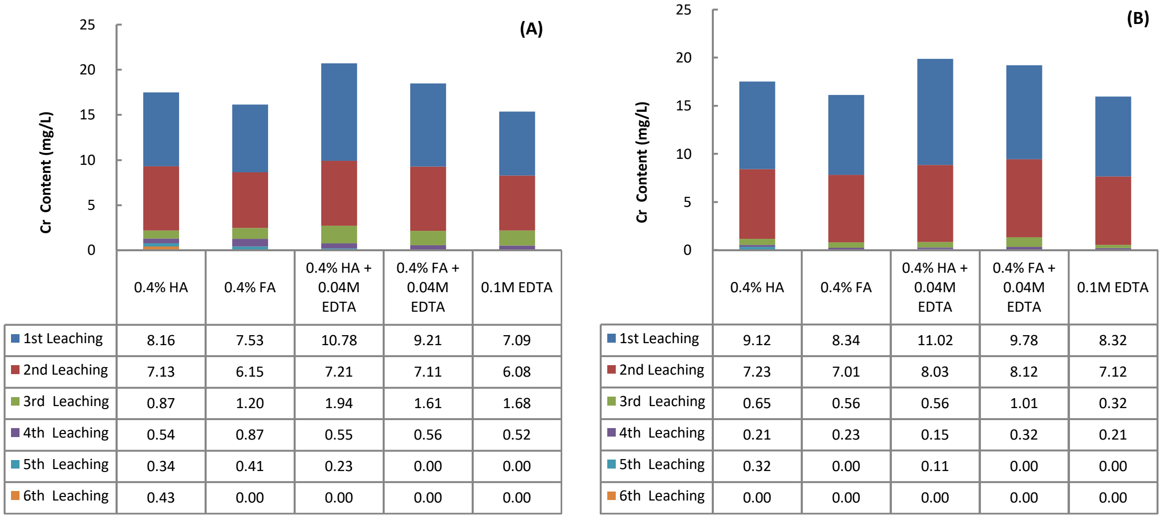
(A)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	12.08	13.23	15.14	14.45	21.98
🟥 2nd Leaching	10.142	9.081	12.08	13.22	16.56
🟩 3rd Leaching	6.16	7.32	9.06	11.08	9.06
🟪 4th Leaching	2.16	0.54	11.03	9.89	1.02
🟦 5th Leaching	0.75	0	3.65	1.12	0
🟧 6th Leaching	0	0.21	0	0	0

(B)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	14.45	13.34	16.23	16.21	20.34
🟥 2nd Leaching	12.23	11.34	13.23	14.34	18.03
🟩 3rd Leaching	4.02	5.34	8.34	9.65	10.04
🟪 4th Leaching	3.04	2.56	10.34	7.67	0.34
🟦 5th Leaching	0.21	0.34	2.08	2.05	0
🟧 6th Leaching	0	0	0.65	0	0

Fig. 4.7.15. Concentration of Pb (mg L^{-1}) in leachate after extraction of contaminated SSy soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.



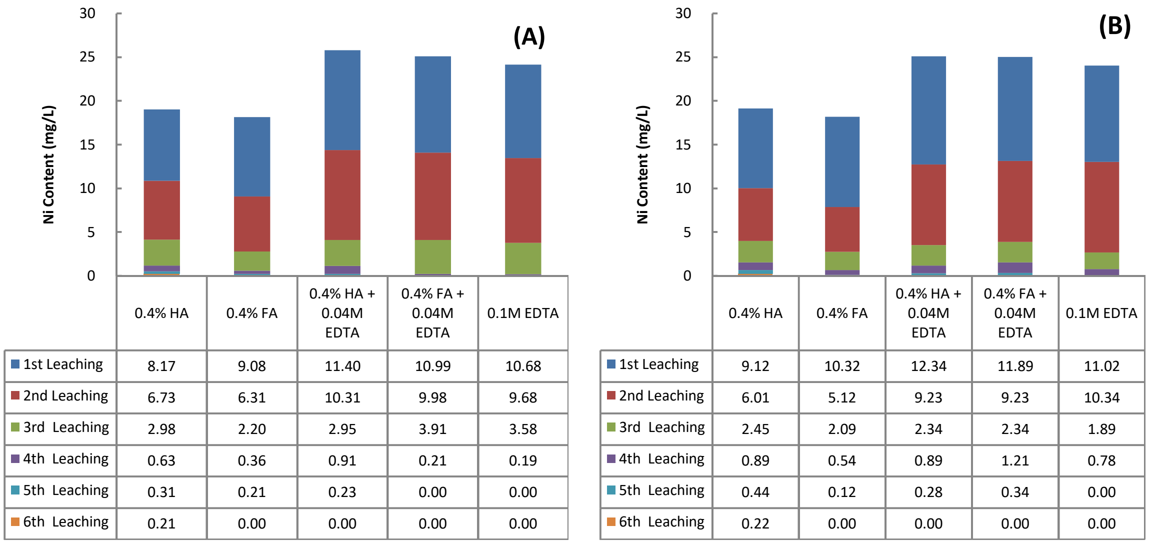
(A)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	8.16	7.53	10.78	9.21	7.09
🟥 2nd Leaching	7.13	6.15	7.21	7.11	6.08
🟩 3rd Leaching	0.87	1.20	1.94	1.61	1.68
🟪 4th Leaching	0.54	0.87	0.55	0.56	0.52
🟦 5th Leaching	0.34	0.41	0.23	0.00	0.00
🟧 6th Leaching	0.43	0.00	0.00	0.00	0.00

(B)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	9.12	8.34	11.02	9.78	8.32
🟥 2nd Leaching	7.23	7.01	8.03	8.12	7.12
🟩 3rd Leaching	0.65	0.56	0.56	1.01	0.32
🟪 4th Leaching	0.21	0.23	0.15	0.32	0.21
🟦 5th Leaching	0.32	0.00	0.11	0.00	0.00
🟧 6th Leaching	0.00	0.00	0.00	0.00	0.00

Fig. 4.7.16. Concentration of Cr (mg L^{-1}) in leachate after extraction of contaminated SSy soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.



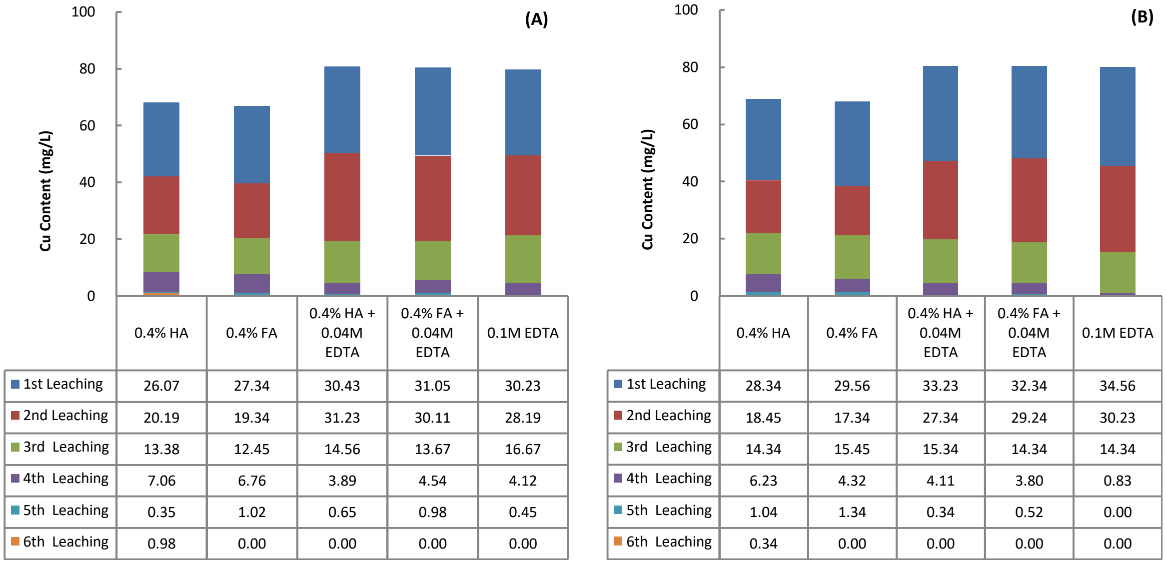
(A)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	8.17	9.08	11.40	10.99	10.68
🟥 2nd Leaching	6.73	6.31	10.31	9.98	9.68
🟩 3rd Leaching	2.98	2.20	2.95	3.91	3.58
🟪 4th Leaching	0.63	0.36	0.91	0.21	0.19
🟦 5th Leaching	0.31	0.21	0.23	0.00	0.00
🟧 6th Leaching	0.21	0.00	0.00	0.00	0.00

(B)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	9.12	10.32	12.34	11.89	11.02
🟥 2nd Leaching	6.01	5.12	9.23	9.23	10.34
🟩 3rd Leaching	2.45	2.09	2.34	2.34	1.89
🟪 4th Leaching	0.89	0.54	0.89	1.21	0.78
🟦 5th Leaching	0.44	0.12	0.28	0.34	0.00
🟧 6th Leaching	0.22	0.00	0.00	0.00	0.00

Fig 4.7.17. Concentration of Ni (mg L^{-1}) in leachate after extraction of contaminated SSy soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.



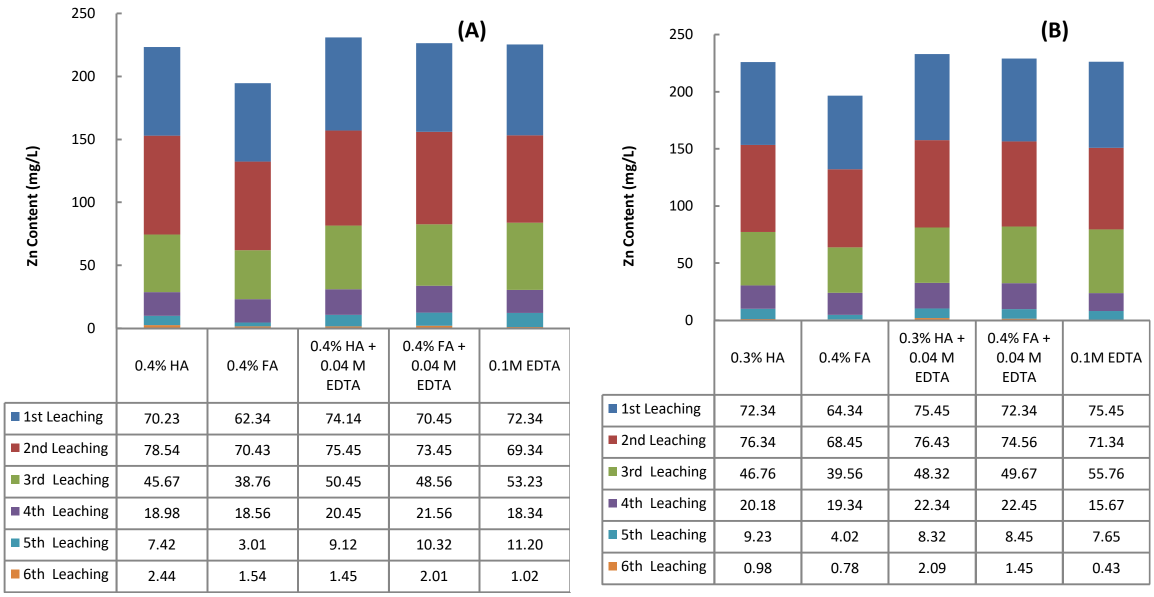
(A)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	26.07	27.34	30.43	31.05	30.23
🟥 2nd Leaching	20.19	19.34	31.23	30.11	28.19
🟩 3rd Leaching	13.38	12.45	14.56	13.67	16.67
🟪 4th Leaching	7.06	6.76	3.89	4.54	4.12
🟦 5th Leaching	0.35	1.02	0.65	0.98	0.45
🟧 6th Leaching	0.98	0.00	0.00	0.00	0.00

(B)

	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	28.34	29.56	33.23	32.34	34.56
🟥 2nd Leaching	18.45	17.34	27.34	29.24	30.23
🟩 3rd Leaching	14.34	15.45	15.34	14.34	14.34
🟪 4th Leaching	6.23	4.32	4.11	3.80	0.83
🟦 5th Leaching	1.04	1.34	0.34	0.52	0.00
🟧 6th Leaching	0.34	0.00	0.00	0.00	0.00

Fig. 4.7.18. Concentration of Cu (mg L^{-1}) in leachate after extraction of contaminated SSy soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.



(A)

	0.4% HA	0.4% FA	0.4% HA + 0.04 M EDTA	0.4% FA + 0.04 M EDTA	0.1M EDTA
🟦 1st Leaching	70.23	62.34	74.14	70.45	72.34
🟥 2nd Leaching	78.54	70.43	75.45	73.45	69.34
🟩 3rd Leaching	45.67	38.76	50.45	48.56	53.23
🟪 4th Leaching	18.98	18.56	20.45	21.56	18.34
🟦 5th Leaching	7.42	3.01	9.12	10.32	11.20
🟧 6th Leaching	2.44	1.54	1.45	2.01	1.02

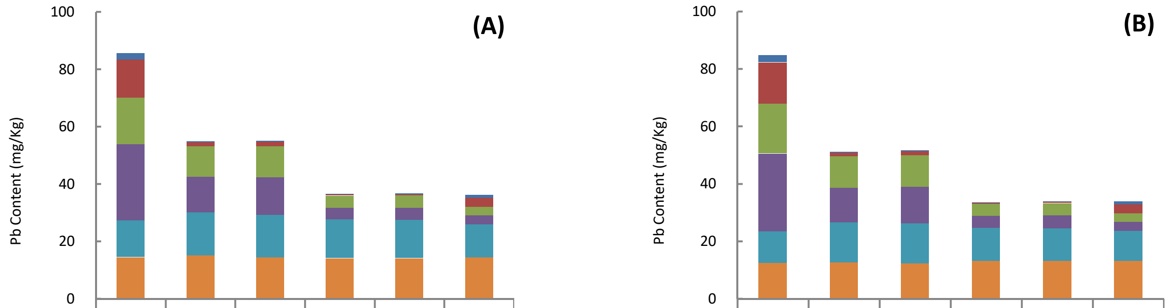
(B)

	0.3% HA	0.4% FA	0.3% HA + 0.04 M EDTA	0.4% FA + 0.04 M EDTA	0.1M EDTA
🟦 1st Leaching	72.34	64.34	75.45	72.34	75.45
🟥 2nd Leaching	76.34	68.45	76.43	74.56	71.34
🟩 3rd Leaching	46.76	39.56	48.32	49.67	55.76
🟪 4th Leaching	20.18	19.34	22.34	22.45	15.67
🟦 5th Leaching	9.23	4.02	8.32	8.45	7.65
🟧 6th Leaching	0.98	0.78	2.09	1.45	0.43

Fig. 4.7.19. Concentration of Zn (mg L^{-1}) in leachate after extraction of contaminated SSy soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.

Fractionation: It was observed that soil treated with 0.4 % humic acid retained 7 to 15 %, 5 to 12% and 35 to 78% of metals in water soluble, exchangeable and carbonate bound fraction. In contrast 0.4% fulvic acid remediated soils hold 8 to 15%, 7 to 13 % and 36 to 79% of metals in above respective labile fractions (Fig. 4.7.20 to 4.7.24). Therefore the mobility indexes of metals were 8.23 to 20.07% and 7.84 to 20.44% in soils where humic and fulvic acids were used for metal remediation, respectively (Table 4.7.16). Soils treated with humic substances increased 15 to 20 % of metals in organic matter bound fractions possibly due to the formation of HS-metal complex at high concentration of HS (Fig. 4.7.20 to 4.7.24).

The soil retained 23 to 59 %, 15 to 32 % and 19 to 59% of metals in water soluble, exchangeable and carbonate adsorbed forms where 0.1 M EDTA was used for metal removal from soil after plant harvest (Fig. 4.7.20 to 4.7.24). Therefore the mobility indexes were recorded 14.3 to 15.9%, 9 to 11%, 8.9 to 9.3% 11 to 12.7% and 16.4 to 16.9% of Pb, Cr, Ni, Cu and Zn in 0.1 M EDTA remediated soil, respectively (Table 4.7.16). It was observed that the water soluble and exchangeable forms of metals were found high where soil was treated with only 0.1 M EDTA. Excess EDTA in soil enhanced further dissolution of metals from non-labile to labile fractions.



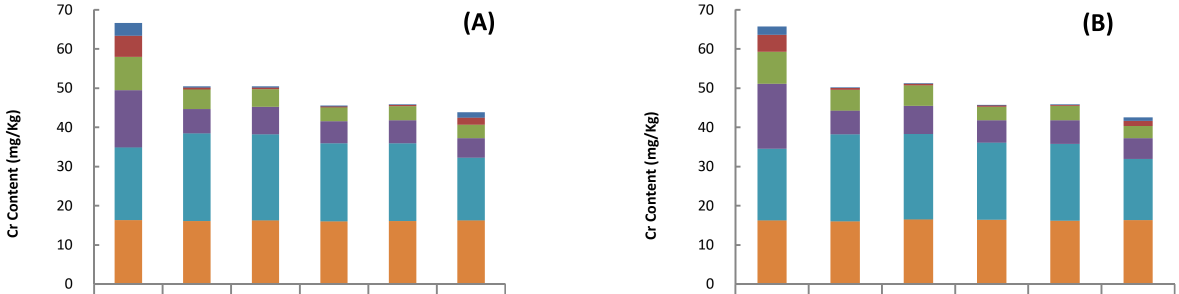
(A)

	Contami nated	0.4% HA	0.4% FA	0.4% HA + 0.06M EDTA	0.4% FA + 0.06M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	2.34	0.37	0.42	0.21	0.25	0.98
🟥 F2 (Exchangeable)	13.36	1.43	1.56	0.43	0.48	3.23
🟩 F3 (Carbonate Bound)	16.16	10.61	10.85	4.09	4.34	3.04
🟪 F4 (Fe/Mn Oxides Bound)	26.44	12.41	13.04	4.19	4.23	3.06
🟦 F5 (Organic Bound)	12.92	15.01	14.98	13.48	13.35	11.52
🟧 F6 (Residual Form)	14.33	14.97	14.23	14.06	14.02	14.27

(B)

	Contami nated	0.4% HA	0.4% FA	0.4% HA + 0.06M EDTA	0.4% FA + 0.06M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	2.45	0.48	0.47	0.10	0.12	1.01
🟥 F2 (Exchangeable)	14.36	1.26	1.35	0.53	0.56	3.33
🟩 F3 (Carbonate Bound)	17.34	10.86	11.02	4.19	4.26	3.01
🟪 F4 (Fe/Mn Oxides Bound)	27.09	12.09	12.77	4.12	4.56	3.11
🟦 F5 (Organic Bound)	11.09	14.08	13.88	11.58	11.47	10.48
🟧 F6 (Stable Form)	12.34	12.43	12.27	13.02	12.98	13.01

Fig. 4.7.20. Fractionation of Pb (mg kg^{-1}) in treated and untreated contaminated SSy soil after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.



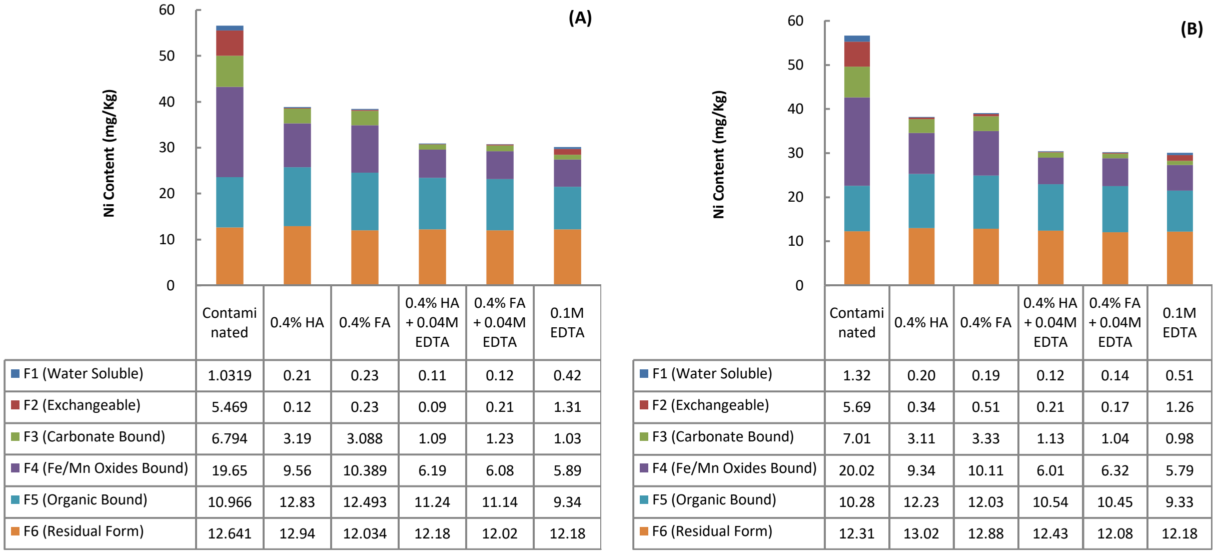
(A)

	Contami nated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	3.292	0.32	0.27	0.17	0.19	1.33
🟥 F2 (Exchangeable)	5.378	0.48	0.46	0.31	0.34	1.82
🟩 F3 (Carbonate Bound)	8.495	5.01	4.44	3.49	3.61	3.41
🟪 F4 (Fe/Mn Oxides Bound)	14.591	6.21	7.09	5.61	5.81	5.01
🟦 F5 (Organic Bound)	18.57	22.34	21.95	19.94	19.85	16.04
🟧 F6 (Residual Form)	16.316	16.12	16.24	16.02	16.12	16.22

(B)

	Contamin ated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	2.13	0.21	0.19	0.15	0.13	0.87
🟥 F2 (Exchangeable)	4.32	0.43	0.40	0.31	0.29	1.37
🟩 F3 (Carbonate Bound)	8.23	5.34	5.19	3.39	3.68	3.09
🟪 F4 (Fe/Mn Oxides Bound)	16.56	6.08	7.23	5.73	6.03	5.31
🟦 F5 (Organic Bound)	18.32	22.17	21.75	19.69	19.62	15.59
🟧 F6 (Residual Form)	16.23	16.02	16.54	16.44	16.15	16.33

Fig. 4.7.21. Fractionation of Cr (mg kg^{-1}) in treated and untreated contaminated SSy soil after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.



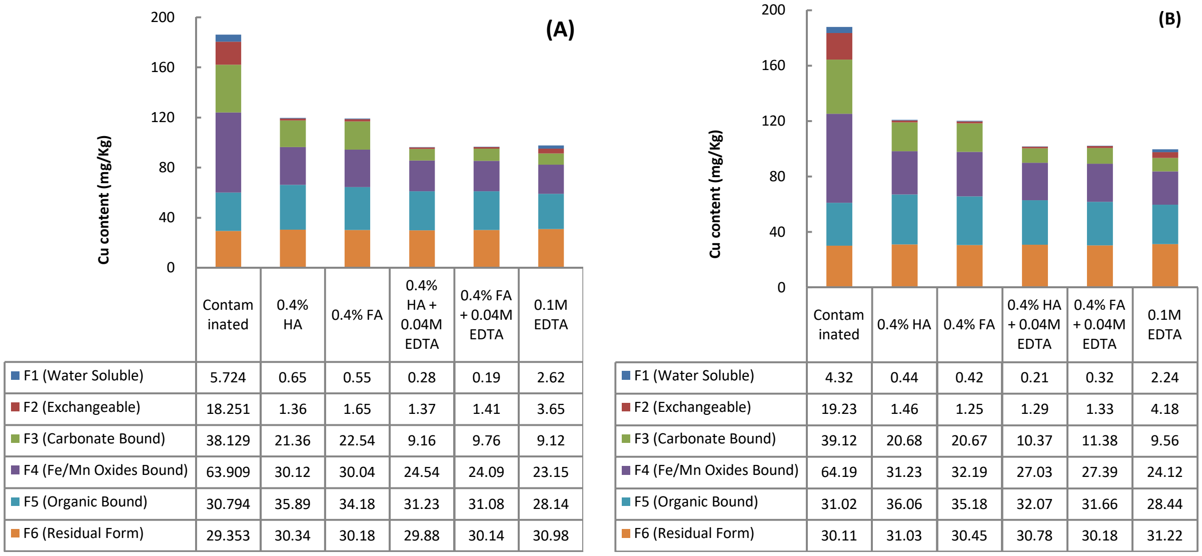
(A)

	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	1.0319	0.21	0.23	0.11	0.12	0.42
🟥 F2 (Exchangeable)	5.469	0.12	0.23	0.09	0.21	1.31
🟩 F3 (Carbonate Bound)	6.794	3.19	3.088	1.09	1.23	1.03
🟪 F4 (Fe/Mn Oxides Bound)	19.65	9.56	10.389	6.19	6.08	5.89
🟦 F5 (Organic Bound)	10.966	12.83	12.493	11.24	11.14	9.34
🟧 F6 (Residual Form)	12.641	12.94	12.034	12.18	12.02	12.18

(B)

	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	1.32	0.20	0.19	0.12	0.14	0.51
🟥 F2 (Exchangeable)	5.69	0.34	0.51	0.21	0.17	1.26
🟩 F3 (Carbonate Bound)	7.01	3.11	3.33	1.13	1.04	0.98
🟪 F4 (Fe/Mn Oxides Bound)	20.02	9.34	10.11	6.01	6.32	5.79
🟦 F5 (Organic Bound)	10.28	12.23	12.03	10.54	10.45	9.33
🟧 F6 (Residual Form)	12.31	13.02	12.88	12.43	12.08	12.18

Fig. 4.7.22. Fractionation of Ni (mg kg^{-1}) in treated and untreated contaminated SSy soil after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.



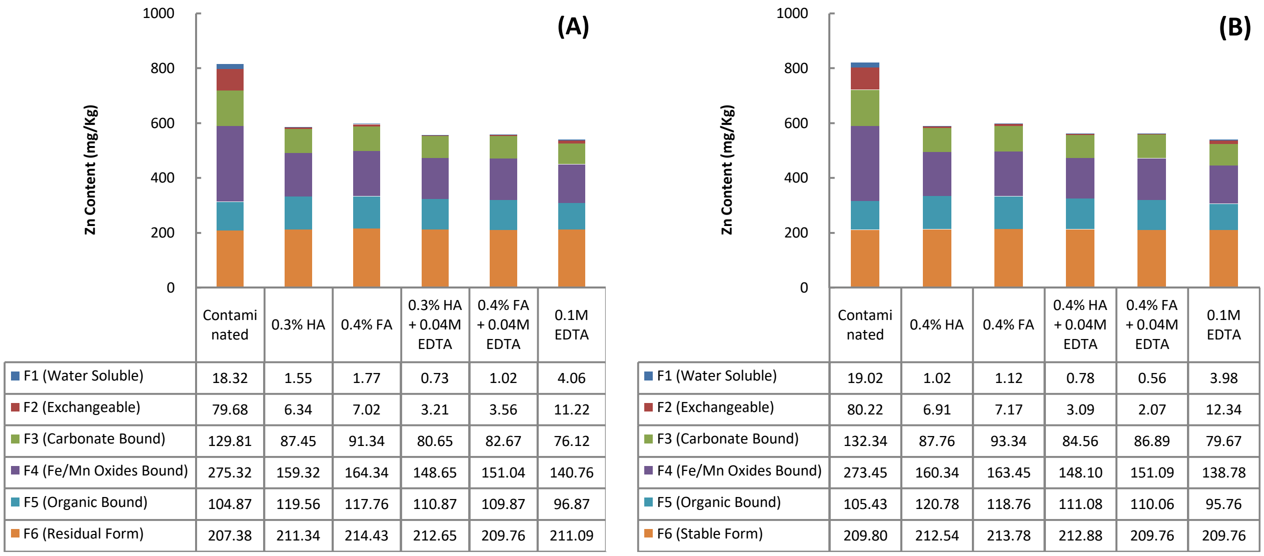
(A)

	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	5.724	0.65	0.55	0.28	0.19	2.62
🟥 F2 (Exchangeable)	18.251	1.36	1.65	1.37	1.41	3.65
🟩 F3 (Carbonate Bound)	38.129	21.36	22.54	9.16	9.76	9.12
🟪 F4 (Fe/Mn Oxides Bound)	63.909	30.12	30.04	24.54	24.09	23.15
🟦 F5 (Organic Bound)	30.794	35.89	34.18	31.23	31.08	28.14
🟧 F6 (Residual Form)	29.353	30.34	30.18	29.88	30.14	30.98

(B)

	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	4.32	0.44	0.42	0.21	0.32	2.24
🟥 F2 (Exchangeable)	19.23	1.46	1.25	1.29	1.33	4.18
🟩 F3 (Carbonate Bound)	39.12	20.68	20.67	10.37	11.38	9.56
🟪 F4 (Fe/Mn Oxides Bound)	64.19	31.23	32.19	27.03	27.39	24.12
🟦 F5 (Organic Bound)	31.02	36.06	35.18	32.07	31.66	28.44
🟧 F6 (Residual Form)	30.11	31.03	30.45	30.78	30.18	31.22

Fig. 4.7.23. Fractionation of Cu (mg kg^{-1}) in treated and untreated contaminated SSy soil after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.



(A)

	Contaminated	0.3% HA	0.4% FA	0.3% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	18.32	1.55	1.77	0.73	1.02	4.06
🟥 F2 (Exchangeable)	79.68	6.34	7.02	3.21	3.56	11.22
🟩 F3 (Carbonate Bound)	129.81	87.45	91.34	80.65	82.67	76.12
🟪 F4 (Fe/Mn Oxides Bound)	275.32	159.32	164.34	148.65	151.04	140.76
🟦 F5 (Organic Bound)	104.87	119.56	117.76	110.87	109.87	96.87
🟧 F6 (Residual Form)	207.38	211.34	214.43	212.65	209.76	211.09

(B)

	Contaminated	0.4% HA	0.4% FA	0.4% HA + 0.04M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 F1 (Water Soluble)	19.02	1.02	1.12	0.78	0.56	3.98
🟥 F2 (Exchangeable)	80.22	6.91	7.17	3.09	2.07	12.34
🟩 F3 (Carbonate Bound)	132.34	87.76	93.34	84.56	86.89	79.67
🟪 F4 (Fe/Mn Oxides Bound)	273.45	160.34	163.45	148.10	151.09	138.78
🟦 F5 (Organic Bound)	105.43	120.78	118.76	111.08	110.06	95.76
🟧 F6 (Stable Form)	209.80	212.54	213.78	212.88	209.76	209.76

Fig. 4.7.24. Fractionation of Zn (mg kg^{-1}) in treated and untreated contaminated SSy soil after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.

The combined HA + EDTA and FA + EDTA treated soils hold only 5 to 10% of water soluble and exchangeable forms after plant harvest. These combined extractants retained 13 to 63 % and 14 to 65% of metals in carbonate bound fraction, respectively (Fig 4.7.20 to 4.7.24). Therefore combined HA + EDTA and FA + EDTA extractants caused the lowest mobility indexes of metals in soil and the values were 4.17 to 15.61% and 4.47 to 15.73%, respectively. It was also observed that mobility indexes of metals in combined HS + EDTA treated soils were lower than that of only 0.1 M EDTA remediated soil (Table 4.7.16).

Metal concentrations in plants: The contaminated SSy soil containing pot showed the highest metal content in different plant parts. The roots contained the highest amount of metals and the values were 102.5, 67.9, 69.8, 140.8, 148.8 mg kg^{-1} for red amaranth and 107.2, 108.9, 98.7, 176.3 and 266.3 mg kg^{-1} for rice of Pb, Cr, Ni, Cu and Zn grown in contaminated soil of ship breaking yard, respectively. So the metal concentrations in root of rice were higher than that of red amaranth. Other parts of red amaranth contained relatively higher amount of metals compare to rice plant. The grain of rice plant contained the lowest amount of metals (Table 4.7.17).

Table 4.7.17. Concentration of metals (mg kg^{-1}) in different parts of *Amaranthus gangeticus* and *Oryza sativa* plants grown in treated and untreated contaminated and non contaminated soils of Shitakunda.

Treatment	*Amaranthus gangeticus*			*Oryza sativa*		
	Root	**Stem**	**Leave**	**Root**	**Stem**	**Grain**
			Pb (mg kg^{-1})			
Contaminated	102.58a	64.33a	125.85a	107.23a	9.38a	1.93a
0.4 % HA	12.76c	8.32c	10.02c	15.08c	2.14c	0.00c
0.4 % FA	13.21c	8.98c	11.51c	17.02c	2.78c	0.00c
0.4 % HA + 0.04 M EDTA	6.32d	3.76d	5.23d	6.11d	0.56d	0.00c
0.4 % FA + 0.04 M EDTA	7.07d	4.54d	4.87d	8.87d	0.98d	0.00c
0.1 M EDTA	18.22b	13.76b	14.29b	22.01b	6.44b	0.21b
Non contaminated	1.65e	0.87e	1.04e	2.03e	0.89d	0.00c
			Cr (mg kg^{-1})			
Contaminated	67.96a	28.17a	33.13a	108.98a	2.13a	1.48a
0.4 % HA	5.54c	2.13c	0.23c	9.13c	0.38c	0.00c
0.4 % FA	6.02c	2.02c	0.45c	10.22c	0.48c	0.00c
0.4 % HA + 0.04 M EDTA	2.01d	0.56d	0.00d	5.71d	0.00d	0.00c
0.4 % FA + 0.04 M EDTA	2.44d	0.64d	0.00d	6.87d	0.00d	0.00c
0.1 M EDTA	9.02b	5.21b	4.56b	18.78b	2.31b	0.92b
Non contaminated	0.45e	0.00e	0.00d	1.09e	0.00d	0.00c
			Ni (mg kg^{-1})			
Contaminated	69.82a	12.42a	14.09a	98.71a	6.72a	4.69a
0.4 % HA	5.76c	2.07c	1.67c	8.01c	1.04c	0.38c
0.4 % FA	6.45c	2.13c	1.92c	8.43c	1.29c	0.39c
0.4 % HA + 0.04 M EDTA	2.01d	0.34d	0.00e	3.91d	0.29d	0.00d
0.4 % FA + 0.04 M EDTA	2.23d	0.00e	0.18d	4.06d	0.33d	0.00d
0.1 M EDTA	10.01b	6.51b	5.21b	13.64b	2.51b	1.09b
Non contaminated	0.56e	0.00e	0.00e	1.78e	0.00e	0.00d
			Cu (mg kg^{-1})			
Contaminated	140.87a	22.68a	31.65a	176.34a	18.32a	2.69a
0.4 % HA	11.42c	3.45c	4.11c	14.21c	5.28c	0.89c
0.4 % FA	13.21c	4.67c	4.32c	15.22c	5.02c	0.93c
0.4 % HA + 0.04 M EDTA	6.89d	1.58d	1.79d	7.01d	3.02d	0.49c
0.4 % FA + 0.04 M EDTA	6.54d	1.88d	1.69d	6.96d	3.56d	0.65c
0.1 M EDTA	28.91b	12.09b	8.18b	27.23b	7.37b	1.64b
Non contaminated	3.19e	2.05e	1.54e	4.89e	2.11e	0.33d
			Zn (mg kg^{-1})			
Contaminated	148.8a	38.2a	112.9a	266.3a	137.4a	39.7a
0.4 % HA	16.4c	14.3c	24.5c	46.4c	25.7c	12.1c
0.4 % FA	17.8c	16.6c	23.7c	48.8c	27.2c	12.1c
0.4 % HA + 0.04 M EDTA	11.5d	10.7d	18.1d	32.1d	20.5d	9.9cd
0.4 % FA + 0.04 M EDTA	13.9cd	11.8d	20.2d	33.3d	21.1d	10.2cd
0.1 M EDTA	32.3b	21.9b	36.8b	81.7b	42.1b	17.0b
Non contaminated	10.9d	9.9d	14.5e	22.3e	20.1d	6.4d

It was observed that plants grown in soil remediated with humic substances and combined extractants markedly reduced the concentration of metals in red amaranth and rice plants (Table 4.7.17). Kruatrachue *et al.* (2002) reported that humic acids decreased lead uptake by *Lemna minor* and brought about increases in total chlorophyll content, growth rate and multiplication rate compared with contaminated soil. Bunluesin *et al.* (2007) reported that the accumulation of zinc to *Ceratophyllum demersum*, was significantly reduced in presence of humic acids. Wang *et al.* (2010) found that humic acids decreased copper accumulation 44–77 and 35–62.7 % in roots and shoots of *Vallisneria spiralis*, respectively growing in copper contaminated sediments. Several studies also reported the decreases in Cu uptake and toxicity caused by humic substances (Inaba and Takenaka 2005; Remon *et al.* 2005).

The treatment of soil with only 0.1 M EDTA considerably reduced the concentration of metal in plants but the metal content of plants in EDTA treated soil was generally higher than plant grown in only HS and combined HS + EDTA treated soils (Table 4.7.17). Grˇcman *et al.* (2001) found that EDTA in the soil increased the uptake of Zn in leaves of cabbage plant 3.2 fold when EDTA concentration in soil increased from 3 mmol/kg to 10 mmol/kg. Consequently EDTA caused uncontrolled metal dissolution when persisted in soil. Chen and Cutright (2001) found that mobile fraction of Ni increased more than 2 fold with increasing level of EDTA from 0.5 to 1 M in soil. Bennett *et al.* (1998) found that high amount of EDTA in soil enhanced Ni uptake in *B. coddii*, despite causing an increase in the concentration of soluble nickel.

But the residual humic substances in soils reduced free and available metal by forming stable HS-metal complex. As a result organic matter bound fractions of metal increased 3 - 8 % after extraction with combined HS + EDTA solutions which inhabited the uptake of metal by plants (Fig. 4.7.20 to 4.7.24). Gueguen *et al.* (2003) found that Cd and Zn free ion concentration decreased markedly in the presence of humic substances. Bunluesin *et al.* (2007) reported that 2 mg L^{-1} solution of HA significantly reduced soluble Zn accumulation from 2,167 to 803 mg kg^{-1} in *Ceratophyllum demersum* plant. Several studies reported on the decreases in metal uptake and toxicity caused by the addition of humic acid. Koukal *et* *al.* (2003) found that in presence of humic acid in solution markedly decreased Cd and Zn bioavailability and toxicity to *Pseudokirchnerriella subcapitata* plant.

It was found that soils treated with combined HA + EDTA and FA + EDTA extractants caused the lowest mobility indexes of metal in soils (Table 4.7.16). Thus the combined extractants reduced the concentration of metals in plant materials which were also significantly lower than that of only HA and FA treatments. It can be concluded that for highly contaminated soils of Shitakunda, the use of only HA and FA was not enough to reduce metals concentration in soil and plants. Therefore combined HA + EDTA and FA + EDTA extractants were required to detoxified metal contaminated soils of ship breaking yard.

4.7.5. Experiment with soil of cable and metal industry, Kushtia

Soil located near the cable and metal industries of Kushtia (KC) belongs to High Ganges River Floodplain where carbonate content was found high, resulting from the calcareous parent material. The detail properties of soil were described in section 4.4.5. A bulk amount soil was collected from a contaminated point near the outlets of the industries where Cu content was 151 mg kg^{-1} (Table 4.2.1).

Experiment with *Amaranthus gangeticus*: The growth and yield attributes data of red amaranth grown in untreated and treated contaminated soil and non-contaminated soil of same region are given in Table 4.7.18. Plants grown in pot containing contaminated soil exhibited the minimum plant height, leaf and root length and yield (fresh and dry weight). Visual differences of growth of red amaranth are shown in Figure 4.7.25.

Table 4.7.18. Vegetative growth and yield attributes of *Amaranthus gangeticus* grown in treated and untreated contaminated soils and non-contaminated soil of Kushtia (KC).

Treatment	Plant height (cm)	Leaf length (cm)	Root length (cm)	Fresh weight (g $plant^{-1}$)	Yield (Dry weight) (g pot^{-1})
Contaminated	12.02d	4.24c	4.29c	4.78d	5.06d
0.3 % HA	15.43c	4.89b	5.05b	7.61c	8.73c
0.4 % FA	21.13b	5.23b	5.36ab	8.41ab	12.51a
0.3 % HA + 0.06 M EDTA	21.23b	5.17b	5.41ab	8.37ab	11.44b
0.4 % FA + 0.04 M EDTA	24.21a	5.79a	5.56a	8.65a	13.15a
0.1M EDTA	19.76b	5.65a	5.39ab	7.89bc	8.48c
Non contaminated	21.09b	5.69a	5.61a	8.55a	12.39a

Fig. 4.7.25. Photograph of *Amaranthus gangeticus* plants grown in treated and untreated contaminated soils of Kushtia.

The soils treated with humic substances, EDTA and combined extractants favored the increased vegetative growth and yield (fresh and dry weight) of red amaranth. Among the humic substances, fulvic acid treated soil created impact on yield (dry weight) which was higher than that of plant grown in soil treated with humic acid. However the maximum yield (dry weight) of red amaranth was observed in soil where combined 0.4 % FA + 0.04 M EDTA and only 0.4 % fulvic acid extractants were used for metal removal. The treatment of soils with these extractants favored the increased yield (dry weight of plant) of 159.9 and 147.2 % over that obtained with plants grown in contaminated soil and the values were also statistically similar to plant grown in non-contaminated soil. Next to these extractants, the combined 0.3 % HA + 0.06 M EDTA also created positive impact on plant yield (dry weight) which was 126.1 % higher than plant grown in contaminated soil (Table 4.7.18). Studies on the effects of humic substances on plant growth, showed improved effects on growth and yield of many plants (Chen and Aviad, 1990; Dursun, 1999; Aydin, 1999).

Experiment with Rice (*Oryza sativa*). The vegetative growth and grain yield of rice plant were found lowest in contaminated KC soil possibly due to plant injury from metal toxicity (Table 4.7.19). A visual observation of the treatment effects on the growth of rice plant is shown in Figure 4.7.26.

Table 4.7.19. Vegetative growth and yield attributes of *Oryza sativa* grown in treated and untreated contaminated soils and non-contaminated soil of Kushtia (KC).

Treatment	Plant dry weight (g pot^{-1})	Plant Height (cm)	Panicle Length (cm)	100 grain weight (g)	Total grain weight (g pot^{-1})
Contaminated	164.21d	78.74c	19.56b	2.053d	14.41c
0.3 % HA	200.23c	81.28c	20.32b	2.0955cd	20.06b
0.4 % FA	228.53ab	92.08ab	23.18a	2.3985ab	21.76a
0.3 % HA + 0.06 M EDTA	219.22abc	87.63abc	22.23ab	2.2883b	21.17ab
0.4 % FA + 0.04 M EDTA	238.23a	96.52a	24.13a	2.481a	22.28a
0.1M EDTA	214.25bc	85.41bc	21.75ab	2.2401bc	20.89ab
Non-contaminated	228.56ab	92.08ab	23.18a	2.3846ab	21.73a

The plant dry weight, plant height, penicle length and grain yield of rice were found increased in contaminated soil treated with humic substances, EDTA and combined extractants. The maximum vegetative growth and grain yield of rice plant was observed in soil where combined 0.4 % FA + 0.04 M EDTA and individual 0.4 % FA were used for metal remediation. These extractants created impacts on grain yield of 54.6 and 51.0 % over that of contaminated soil, respectively, and these yields (dry weight) were also statistically similar to plant grown in non contaminated soil. Next to these extractants, combined 0.3 % HA + 0.06 M EDTA and individual 0.3 % HA also benefited the plants in increasing grain yield of rice of 46.9 and 39.2 % over that of contaminated soil, respectively (Table 4.7.19). Several reports have showed that humic substances act as a plant growth enhancer in soil. Lee and Bartlette (1976) reported that the addition of humates to a hydroponic solution stimulated both root and shoot development, resulting in an increase of 87 % in corn yield.

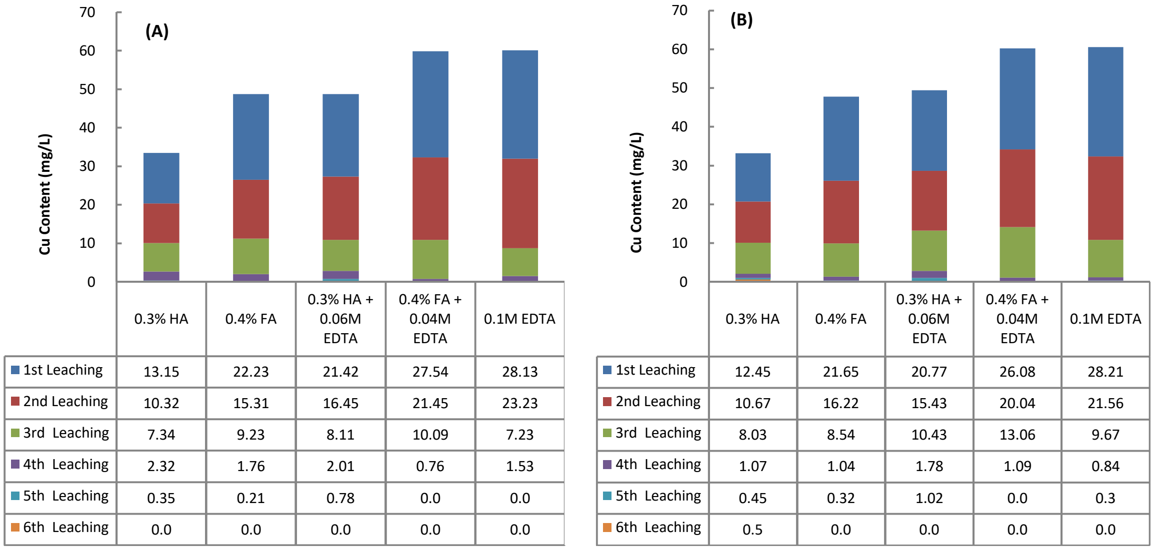
Fig. 4.7.26. Photograph of *Oryza sativa* (BRRI dhan 49) plants grown in treated and untreated contaminated calcareous soils of Kushtia (KC).

Although humic substances did not extract sufficient amount of metals from calcareous soil due to inactivation of its functional groups by bonding with calcium from $CaCO_3$, in combination with small amount of EDTA, humic substances increased the extraction capacities of Cu from calcareous soil (Fig. 4.7.27).

Among the extractants 0.4% humic acid leached the minimum amount of Cu from contaminated soil and the concentration was 7.34 to 13.15 mg L^{-1} in leachate from first three extractions. Fulvic acid at the concentration of 0.4% extracted comparatively higher amount of Cu (8.54 to 22.23 mg L^{-1}) found in first three leachate compare to humic acid. However the combined 0.4 % FA + 0.04 M EDTA and individual 0.1 M EDTA extractants leached the maximum amount of Cu of 10.09 to 27.54 mg L^{-1} and 7.23 to 28.13 mg L^{-1}

during first three extractions of contaminated soil. After three extractions, the concentrations Cu in leachate of all extractants were sharply dropped with increasing number of leaching treatment. The Cu contents were found very low in amount in 5th and 6th leachate (Fig. 4.7.27).

It was observed from Figure 4.7.27 that fulvic acid and combined FA + EDTA extractants showed better removal efficiencies of Cu from contaminated soil than that of Individual HA and combined HA + EDTA extractants.



(A)

	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	13.15	22.23	21.42	27.54	28.13
🟥 2nd Leaching	10.32	15.31	16.45	21.45	23.23
🟩 3rd Leaching	7.34	9.23	8.11	10.09	7.23
🟪 4th Leaching	2.32	1.76	2.01	0.76	1.53
🟦 5th Leaching	0.35	0.21	0.78	0.0	0.0
🟧 6th Leaching	0.0	0.0	0.0	0.0	0.0

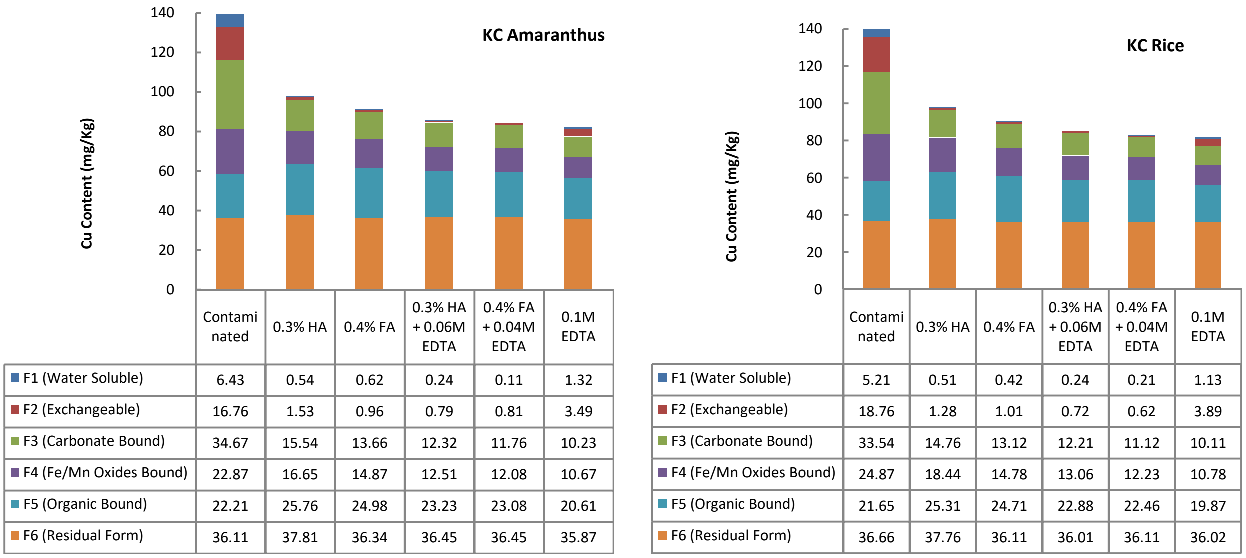
(B)

	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
🟦 1st Leaching	12.45	21.65	20.77	26.08	28.21
🟥 2nd Leaching	10.67	16.22	15.43	20.04	21.56
🟩 3rd Leaching	8.03	8.54	10.43	13.06	9.67
🟪 4th Leaching	1.07	1.04	1.78	1.09	0.84
🟦 5th Leaching	0.45	0.32	1.02	0.0	0.3
🟧 6th Leaching	0.5	0.0	0.0	0.0	0.0

Fig. 4.7.27. Concentration of Cu (mg L^{-1}) in leachate after extraction of contaminated KC soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.

From the fractionation study of contaminated calcareous soil of Kushtia after plant harvest it was found that the mobility index of Cu was 40.88 to 41.61 % due to presence of high amount of Cu in carbonate bound fraction (Fig. 4.7.28 and Table 4.7.20).

It was found that soil retained 12 to 16%, 10 to 12% and 76 to 78% of Cu in labile fractions of water soluble, exchangeable and carbonate bound forms after extraction with 0.3 % humic acid. In contrast soil treated with fulvic acid at the concentration of 0.4% hold 13 to 14%, 8 to 10% and 49 to 53% of Cu in above respective labile fractions. Consequently the mobility indexes of Cu were 24 to 25 % and 19 to 21 % in soil after extraction with humic and fulvic acids, respectively (Fig. 4.7.28). The Cu extraction capacity of fulvic acid was comparatively higher than that of humic acid in contaminated KC soil. It was found from many studies that fulvic acid effectively dissolves metals from soil surface (Stone and Morgan, 1984a, 1984b; Goodman and Cheshire, 1987) which increased its metal extraction capacity from soils. However organic matter bound Cu increased 17 to 25% and 12 to 14% over that of contaminated soil after extraction with humic acid fulvic acids, respectively (Fig. 4.7.28).



	Contaminated	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
F1 (Water Soluble)	6.43	0.54	0.62	0.24	0.11	1.32
F2 (Exchangeable)	16.76	1.53	0.96	0.79	0.81	3.49
F3 (Carbonate Bound)	34.67	15.54	13.66	12.32	11.76	10.23
F4 (Fe/Mn Oxides Bound)	22.87	16.65	14.87	12.51	12.08	10.67
F5 (Organic Bound)	22.21	25.76	24.98	23.23	23.08	20.61
F6 (Residual Form)	36.11	37.81	36.34	36.45	36.45	35.87

	Contaminated	0.3% HA	0.4% FA	0.3% HA + 0.06M EDTA	0.4% FA + 0.04M EDTA	0.1M EDTA
F1 (Water Soluble)	5.21	0.51	0.42	0.24	0.21	1.13
F2 (Exchangeable)	18.76	1.28	1.01	0.72	0.62	3.89
F3 (Carbonate Bound)	33.54	14.76	13.12	12.21	11.12	10.11
F4 (Fe/Mn Oxides Bound)	24.87	18.44	14.78	13.06	12.23	10.78
F5 (Organic Bound)	21.65	25.31	24.71	22.88	22.46	19.87
F6 (Residual Form)	36.66	37.76	36.11	36.01	36.11	36.02

Fig. 4.7.28. Fractionation of Cu (mg kg^{-1}) in treated and untreated contaminated soil of Kushtia after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.

The highest amount of copper was leached from contaminated soil of Kushtia due to extraction with combined 0.4 % FA + 0.04 M EDTA extractant which was similar to that extracted by only 0.1 M EDTA solution (Fig. 4.7.27). From fractionation of KC soil after plant harvest it was found that soil treated with mixed FA + EDTA extractant retained 5 to 10 %, 6 to 8 % and 27 to 29 % of Cu in water soluble, exchangeable and carbonate bound fractions. Whereas soil remediated with combined 0.3 % HA + 0.06 M EDTA hold 7 to 11%, 9 to 10% and 55 to 56% of metals in above three labile fractions, respectively (Fig. 4.7.28). So the Cu removal efficiencies of combined FA + EDTA extractant from different soil fractions was comparatively higher than that of combined HA + EDTA extractant. Therefore the mobility indexes of Cu (13.2 to 13.9%) in soil treated with combined FA + EDTA extractant was lower than that of soil treated with mixed HA + EDTA extractant (Table

4.7.20). However, these extractants increased 3 to 10 % of Cu in organic matter bound fractions in contaminated KC soil (Fig. 4.7.28).

Table. 4.7.20. Mobility indexes of Cu (%) in treated and untreated contaminated KC soils after plant harvest.

Extractants	Soil of Amaranthus plant	Soil of Rice plant
Contaminated	41.61±2.53	40.88±2.48
0.3 % HA	25.55±1.54	24.75±1.41
0.4 % FA	21.38±1.25	19.63±1.19
0.3 % HA + 0.06 M EDTA	21.25±1.26	21.67±1.32
0.4 % FA + 0.04 M EDTA	13.92±0.82	13.18±0.72
0.1 M EDTA	19.42±1.13	18.37±1.15

It was found that a range of 29 to 33 % of waters soluble and 23 to 27 % of exchangeable and 27 to 29 % of carbonated bound forms of Cu were remained in soil where only 0.1 M EDTA was used for metal removal during plant harvest (Fig. 4.7.28). It was found from several studies that excess EDTA in soil caused further dissolution of metal from non-labile to labile forms. The mobility index of Cu was recorded 18.4 to 19.4 % in EDTA treated soils which were higher than that found with combined HS + EDTA treatments (Table 4.7.20).

Copper concentration in plants: The root, stem and leaf of red amaranth obtained the maximum amounts of Cu when grown in contaminated KC soil and the values were 83.43, 26.03 and 40.81 mg kg^{-1}, respectively. Similarly the root, stem and grain of rice plant contained the highest amount of Cu of 123.5, 20.3 and 10.91 mg kg^{-1} of Cu when grown in contaminated soil (Table 4.7.21).

Table 4.7.21. Concentration of Cu (mg kg^{-1}) in different parts of *Amaranthus gangeticus* and *Oryza sativa* plants grown in treated and untreated contaminated and non-contaminated soils of Kushtia (KC).

Treatment	*Amaranthus gangeticus*			*Oryza sativa*		
	Root	**Stem**	**Leave**	**Root**	**Stem**	**Grain**
Contaminated	83.43a	26.03a	40.81a	123.5a	20.3a	10.91a
0.3 % HA	18.78c	16.21c	19.34c	21.54c	13.22b	5.34b
0.4 % FA	16.65d	15.93c	16.33d	16.67d	12.93b	4.03c
0.3 % HA + 0.06 M EDTA	15.12d	13.11d	18.43c	17.12d	10.78c	3.93c
0.4 % FA + 0.04 M EDTA	8.43e	5.34e	7.19e	9.89e	7.43d	1.06d
0.1 M EDTA	29.21b	21.33b	26.67b	35.56b	13.11b	5.03b
Non Contaminated	3.88f	1.54f	1.21f	4.56f	2.11e	0.97d

The treatment of soil with humic substances, EDTA and combined extractants considerably reduced the concentration of Cu in different plant parts though their removal effects of metals from soil. Therefore the lowest Cu concentration in plants was recorded in soil treated with combined 0.4 % FA + 0.04 M EDTA solution. Next to it combined HA + EDTA extractant declined the Cu content in plants but the values were significantly higher than that of combined FA + EDTA treatment (Table 4.7.21). Zhang *et al.* (2013) reported that fulvic acid reduced metal uptake by plant more efficiently than humic acid from calcareous soils. Goodman and Cheshire, (1987) reported that fulvic acids effectively reduced Fe (III) at the surface. Fulvic acid also dissolves the Mn-oxide surfaces (Stone and Morgan, 1984a, 1984b).

Though 0.1 M EDTA remediated soil significantly reduced the concentrations of Cu in plants but the values were higher than that of plant grown in only FA and combined FA + EDTA treated soils. Soil extracted with high amount of EDTA (0.1 M) caused further dissolution of Cu from non-labile fraction and increased plant available forms, which enhanced Cu uptake by both plants (Table 4.7.21). Besides, the remaining humic substances in soil after extraction with HS and combined HS + EDTA solutions reduced free and bio-available forms of metals in soil by forming immobile HS-metal complex, which inhibited metal uptake by plant. Fan *et al.* (2007) also reported that the bioavailability of copper and cadmium in sediments was reduced in the presence of humic substances.

Although EDTA extracted the highest amount of Cu from contaminated soil, due to further solubilization of metals from non-labile fraction of soil, EDTA treatment could not create reduction effects on Cu concentration in plants as much as done by humic substances and combined extractants (Table 4.7.21).

Therefore it can be concluded that among the extractants, the combined 0.4 % FA and 0.04 M EDTA resulted the lowest mobility index of Cu in calcareous soil and thereby yielded the minimum Cu content in both plants.

4.7.6 Experiment with soil of cable Industry, Chittagong

The contaminated soil is located near cable industry of Chittagong Coastal Plain. The detail properties of soil of cable industry were described in section 4.2.6. The soil is slightly alkaline in nature. Copper was the interested element of the contaminated area.

Experiment with *Amaranthus gangeticus*: The vegetative growth including plant height, root and leaf length, total plant biomass and yield of red amaranth plant grown in untreated and treated contaminated soil of cable industry of Chittagong (CC) and non-contaminated soil of same region are presented in Table 4.7.22. The plant height, leaf and root length of red amaranth were observed lowest in contaminated soil. Consequently the yields (fresh and dry weight) of plants were also found minimum with plants grown in contaminated soil. A visual observation of the treatment effects on the growth of red amaranth is presented in Figure 4.7.29.

Table 4.7.22. Vegetative growth and yield attributes of *Amaranthus gangeticus* grown in treated and untreated contaminated soils and non-contaminated soil of Chittagong (CC).

Treatment	Plant height (cm)	Leaf length (cm)	Root length (cm)	Fresh weight (g plant^{-1})	Yield (Dry weight) (g pot^{-1})
Contaminated	5.89c	3.43c	4.03c	5.39d	5.78e
0.4 % HA	21.45a	6.18a	6.23a	10.62b	13.22bc
0.4 % FA	20.97a	5.89ab	6.12a	10.59b	13.01c
0.4 %HA + 0.06 M EDTA	21.89a	6.09a	6.53a	11.68a	14.26a
0.4 % FA + 0.06 M EDTA	22.09a	6.14a	5.93a	11.59a	13.98ab
0.1 M EDTA	15.21b	5.31b	5.08b	9.04c	9.34d
Non-contaminated	21.11a	6.02a	5.88ab	10.42b	12.87c

The contaminated soils treated with humic substances, EDTA and combined HS + EDTA extractants created impact on the growth and yield of *Amarantus gangeticus* which were statistically higher than plant grown in 0.1 M EDTA treatment soil. Abolina and Tashkhadzhaev (1968) reported that humic substances activated the biochemical processes in plants (respiration, photosynthesis and chlorophyll content) and increased the quality and yield of vegetable plants.

Fig. 4.7.29. Photograph of *Amaranthus gangeticus* plant grown in treated and untreated contaminated soils of cable industry of Chittagong.

The maximum yield of red amaranth plant was recorded with plants grown in soil where combined 0.4 % HA + 0.06 M EDTA and 0.04 % FA + 0.06 M EDTA extractants were used for metal removal. These extractants created beneficial effects on the yield (dry weight) of plant and resulted 146 and 141 % over that of contaminated soil, respectively. These yield values were also statistically higher than plant grown in non-contaminated soil. The only HA and FA treated soils favored the increased yield (dry weight) of plants of 128 and 125 % over that of contaminated soils, respectively (Table. 4.7.22)

Experiment with rice: The vegetative growth and yield attribute data of *Oryza sativa* grown in different treated and untreated contaminated and non contaminated soils are given in Table 4.7.23. Like red amaranth contaminated soil caused the lowest vegetative growth and grain yield of rice plant. The biochemical processes of plants were inhibited due to metal toxicity in contaminated soil and thereby reduced growth and yield of rice plant. Visual differences of growth of rice are shown in Figure 4.7.30.

Table 4.7.23. Vegetative growth and yield attributes of *Oryza sativa* grown in treated and untreated contaminated soils and non-contaminated soil of Chittagong (CC).

Treatment	Plant dry weight (g pot^{-1})	Plant Height (cm)	Panicle Length (cm)	100 grain weight (g)	Total grain weight (g pot^{-1})
Contaminated	155.82d	74.80c	19.30b	1.6504c	16.02d
0.4 % HA	217.08ab	87.47ab	22.02a	1.9654b	19.14c
0.4 % FA	190.33c	77.22c	19.30b	1.9907b	19.46bc
0.4 %HA + 0.06 M EDTA	226.14a	91.69a	22.92a	2.357a	21.17a
0.4 % FA + 0.06 M EDTA	208.05abc	83.25abc	21.11ab	2.3938a	21.31a
0.1 M EDTA	203.54bc	81.14bc	20.66ab	1.8281b	18.45c
Uncontaminated	217.08ab	87.47ab	22.02a	2.2654a	20.64ab

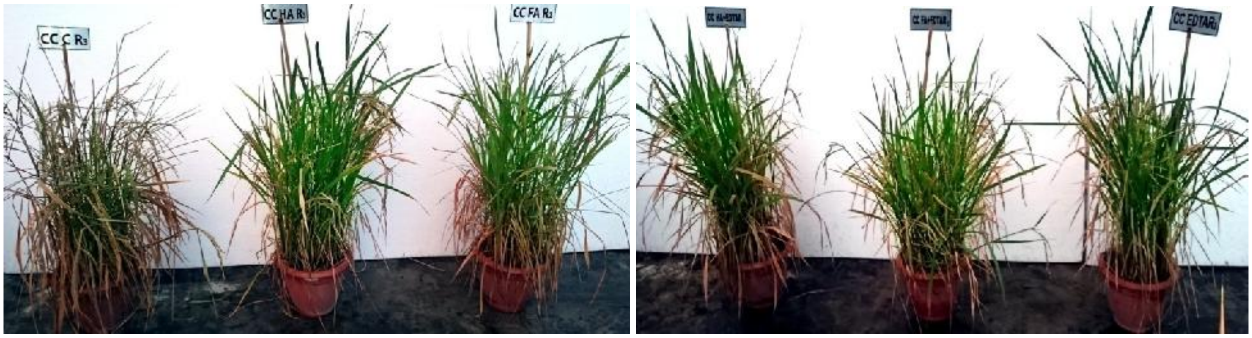


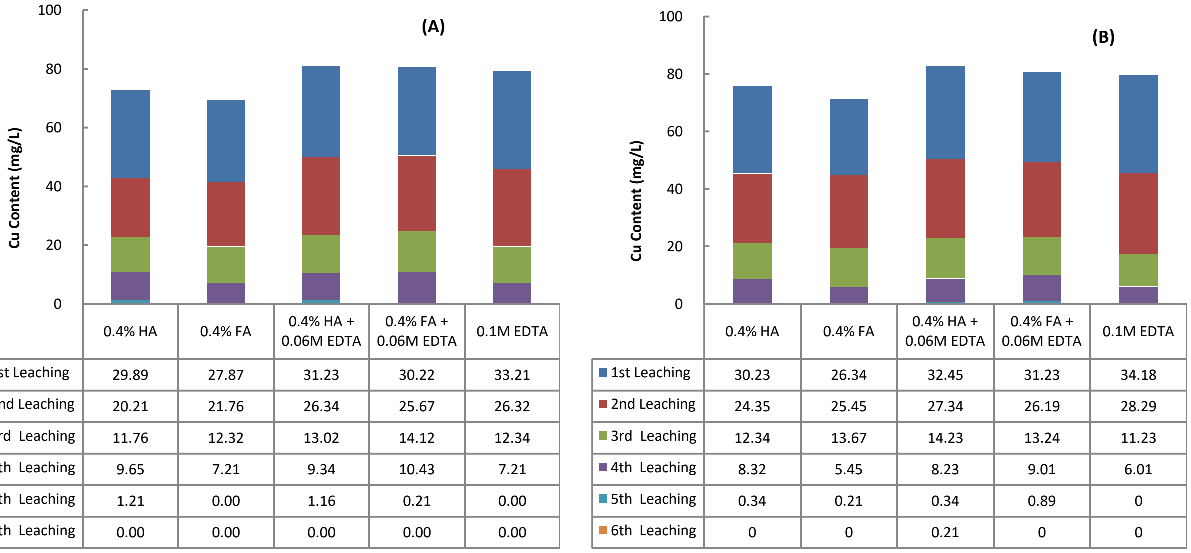
Fig. 4.7.30. Photograph of *Oryza sativa* (BRRI dhan 49) plant grown in treated and untreated contaminated soils of cable industry of Chittagong.

The soils washed with humic substances created increased effects on plant height, dry weight, panicle length and grain yields of rice plant. Soils treated with each of humic and fulvic acids at the concentration of 0.4 % created impact on increased grain yields of rice plant and the yields were 19.5 and 21.5 % over that of plant grown in contaminated soil, respectively (Table 4.7.23). In many studies, humic and fulvic acids were reported to increase the root length (Vaughan & Malcolm 1979), and to increase the fresh weights and yields of crop plants (Chen *et al.,* 2004a, b).

The maximum vegetative growth and grain yield of rice plant were observed in soils where combined 0.4 % HA + 0.06 M EDTA and 0.4 % FA + 0.06 M EDTA extractants were used for metal removal. These extractants favored the increased grain yield of 32.1 and 33 % over that of contaminated soil, respectively (Table 4.7.23).

The concentrations of Cu in leachate were observed higher in the first four extractions and then concentrations were sharply decreased at 5th and 6th extractions (Fig. 4.7.31).

It was found from the fractionation of soil after plant harvest that soil treated with humic substances retained less than 7 and 10% of Cu in water soluble and exchangeable fractions. A range of 63 to 67 % and 64 to 65 % of Cu were present in carbonate bound fraction during plant harvest where humic and fulvic acids were used for soil reclamation. Therefore the mobility indexes of Cu were recorded 13.3 to 14% and 13 to 14.3% in soil treated with humic and fulvic acids, respectively. However organic matter bound Cu increased 14 to 16% and 13 to 14 % in humic and fulvic acids treated soils over that of contaminated soil, respectively (Fig. 4.7.31).



(A)

	0.4% HA	0.4% FA	0.4% HA + 0.06M EDTA	0.4% FA + 0.06M EDTA	0.1M EDTA
🟦 1st Leaching	29.89	27.87	31.23	30.22	33.21
🟥 2nd Leaching	20.21	21.76	26.34	25.67	26.32
🟩 3rd Leaching	11.76	12.32	13.02	14.12	12.34
🟪 4th Leaching	9.65	7.21	9.34	10.43	7.21
🟦 5th Leaching	1.21	0.00	1.16	0.21	0.00
🟧 6th Leaching	0.00	0.00	0.00	0.00	0.00

(B)

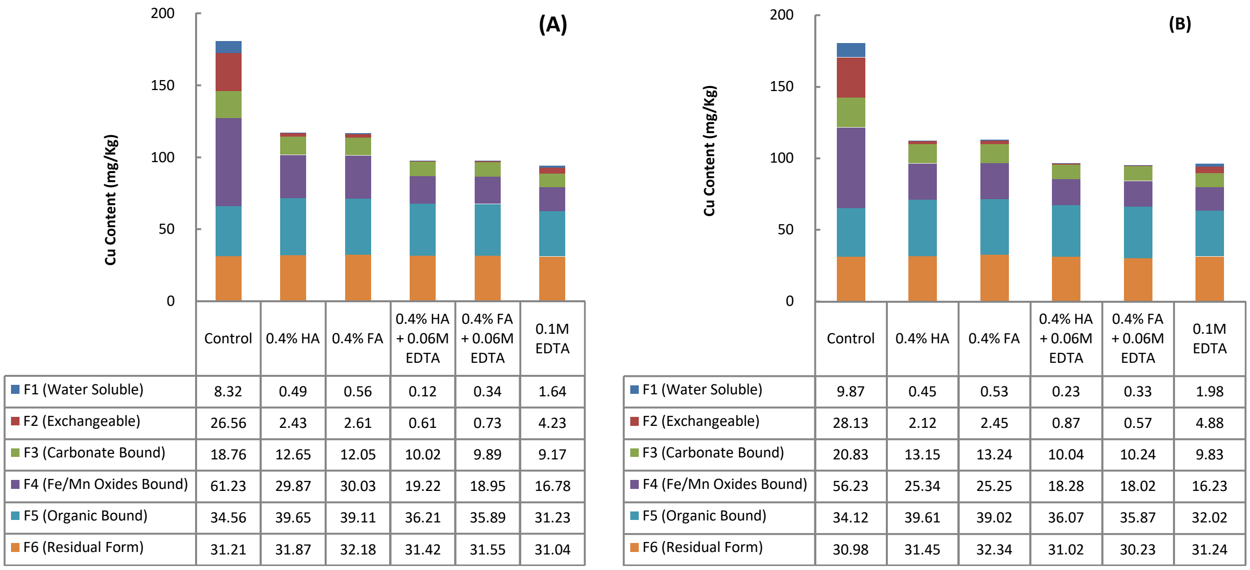
	0.4% HA	0.4% FA	0.4% HA + 0.06M EDTA	0.4% FA + 0.06M EDTA	0.1M EDTA
🟦 1st Leaching	30.23	26.34	32.45	31.23	34.18
🟥 2nd Leaching	24.35	25.45	27.34	26.19	28.29
🟩 3rd Leaching	12.34	13.67	14.23	13.24	11.23
🟪 4th Leaching	8.32	5.45	8.23	9.01	6.01
🟦 5th Leaching	0.34	0.21	0.34	0.89	0
🟧 6th Leaching	0	0	0.21	0	0

Fig. 4.7.31. Concentration of Cu (mg L^{-1}) in leachate after extraction of contaminated CC soil with different extractants where *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants were grown.

Concentration of Cu (mg L^{-1}) in leachate after extraction with different extractants of contaminated CC soil used to grow *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.

The Cu extraction capacity of 0.1 M EDTA was higher than that of humic substances (Fig. 4.7.31). Comparing EDTA and humic substances, Borggaard *et al.*, (2009) found that EDTA extracted 67 % and humic substances 41 % of the total Cu content, i.e. EDTA is a more efficient Cu extractant than HS. The superiority of EDTA is not surprising considering the different carboxylic acid densities with 400 mmol/mol C in EDTA and 100 mmol/mol C in HS and the high Cu-EDTA stability constant (Martell and Smith, 1974).

The fractionation study of soil after treating with only 0.1 M EDTA indicated that more than 20, 16 and 48 % of Cu were retained in water soluble, exchangeable and carbonate bound fractions during plant harvest (Fig 4.7.32). Therefore soil remediated with EDTA caused the mobility index of Cu of 16 to 17.4% which was comparatively higher than the soil treated with humic substances and combined extractants (Table 4.7.24).



(A)

	Control	0.4% HA	0.4% FA	0.4% HA + 0.06M EDTA	0.4% FA + 0.06M EDTA	0.1M EDTA
■ F1 (Water Soluble)	8.32	0.49	0.56	0.12	0.34	1.64
■ F2 (Exchangeable)	26.56	2.43	2.61	0.61	0.73	4.23
■ F3 (Carbonate Bound)	18.76	12.65	12.05	10.02	9.89	9.17
■ F4 (Fe/Mn Oxides Bound)	61.23	29.87	30.03	19.22	18.95	16.78
■ F5 (Organic Bound)	34.56	39.65	39.11	36.21	35.89	31.23
■ F6 (Residual Form)	31.21	31.87	32.18	31.42	31.55	31.04

(B)

	Control	0.4% HA	0.4% FA	0.4% HA + 0.06M EDTA	0.4% FA + 0.06M EDTA	0.1M EDTA
■ F1 (Water Soluble)	9.87	0.45	0.53	0.23	0.33	1.98
■ F2 (Exchangeable)	28.13	2.12	2.45	0.87	0.57	4.88
■ F3 (Carbonate Bound)	20.83	13.15	13.24	10.04	10.24	9.83
■ F4 (Fe/Mn Oxides Bound)	56.23	25.34	25.25	18.28	18.02	16.23
■ F5 (Organic Bound)	34.12	39.61	39.02	36.07	35.87	32.02
■ F6 (Residual Form)	30.98	31.45	32.34	31.02	30.23	31.24

Fig. 4.7.32. Fractionation of Cu in treated and untreated contaminated CC soils after harvest of *Amaranthus gangeticus* (A) and *Oryza sativa* (B) plants.

The combined 0.4 % HA + 0.06 M EDTA and 0.4 % FA + 0.06 M EDTA increased the extraction capacity of Cu from contaminated soils of Chittagong which were similar as extracted by only 0.1 M EDTA (Fig 4.7.31). Soils treated with these extractants retained less than 5 and 4% of Cu in water soluble and exchangeable fractions, respectively. The combined HA + EDTA and FA + EDTA remediated soils also retained 48 to 54 % and 49 to 53 % of Cu in carbonate bound fraction, respectively (Fig 4.7.32). Therefore the mobility indexes of Cu were recorded lowest of 11 to 11.5% and 11.3 to 11.7% in soils treated with the above respective combined extractants (Table 4.7.24). The excess humic substances in soil after extraction inhibited metal mobility by forming stable HS-Metal complex with free available metal ions. Consequently combined extractants increased 3 - 5 % of Cu in organic matter bound fraction over that of contaminated soil (Fig 4.7.32).

Table 4.7.24. Mobility indexes of Cu (%) in treated and untreated contaminated CC soils after plant harvest.

Treatment	Soil of Amaranthus plant	Soil of Rice plant
Contaminated (control)	29.69±1.45	32.65±1.61
0.4 % HA	13.31±0.64	14.02±0.71
0.4 % FA	13.06±0.63	14.38±0.73
0.4 %HA + 0.06 M EDTA	11.01±0.52	11.54±0.55
0.4 % FA + 0.06 M EDTA	11.26±0.55	11.69±0.54
0.1 M EDTA	15.98±0.82	17.39±0.83

Concentration of Cu in plants: The highest Cu concentrations in different parts of red amaranth plant when grown in contaminated soil were 106.3, 23.34 and 38.89 mg kg^{-1} for root, stem and leave, respectively. Root of rice plant also obtained the maximum amount of Cu of 129 mg kg^{-1} from contaminated CC soils. Followed by stem and grain of rice where the contents were 16.33 and 6.56 mg kg^{-1} of Cu, respectively (Table 4.7.25).

Table 4.7.25. Concentration of Cu (mg kg^{-1}) in different parts of *Amaranthus gangeticus* and *Oryza sativa* plants grown in treated and untreated contaminated and non-contaminated soils of Chittagong (CC).

Treatment	*Amaranthus gangeticus*			*Oryza sativa*		
	Root	Stem	Leave	Root	Stem	Grain
Contaminated	106.3a	23.34a	38.89a	129.2a	16.33a	6.56a
0.4 % HA	9.61c	6.76c	8.92b	11.34c	8.54b	1.12d
0.4 % FA	10.02c	7.11c	9.76b	12.12c	9.12b	1.82c
0.4 %HA + 0.06 M EDTA	8.02cd	5.78cd	4.21c	10.91c	5.12c	1.14d
0.4 % FA + 0.06 M EDTA	9.23c	6.02cd	4.12c	11.67c	4.69cd	1.01d
0.1 M EDTA	15.23b	9.22b	9.88b	20.55b	9.32b	3.23b
Non-contaminated	6.45d	3.21d	3.26d	7.23d	3.32d	0.45e

The soil treated with each 0.4 % HA and FA significantly reduced the concentration of Cu in both plants. The lowest Cu contents in both red amaranth and rice plant were recorded in plants grown in soils treated with the combined 0.4 % HA + 0.06 M EDTA and 0.4 % FA +

0.06 M EDTA extractants. These combined extractants increased the removal efficiencies of Cu from soils and consequently reduced the concentration of Cu in plants (Table 4.7.25).

In contrast, the treatment of soil with 0.1 M EDTA extractant considerably reduced the concentration of Cu in plants but the values were higher than in plant grown in HS and combined HS + EDTA treated soils. Free EDTA in soil enhanced further dissolution of Cu from non-labile fractions to plant available forms, thereby increased Cu content in plants (Table 4.7.25). But excess humic substances in soil decreased metal mobility from soil to plant. Wang *et al.* (2010) indicated that humic substances in soil reduced the availability of the soluble and exchangeable forms of metals, including copper and cadmium to plant uptake.

Therefore soils treated with only humic substances and combined HS + EDTA extractants equally effective to reduce Cu concentration in plants compared to that only EDTA treatment. So the use of only 0.4% HA or 0.4% FA is enough to reduce Cu content in plants and consider as suitable extractants for metal removal from contaminated soil of Chittagong.

The above pot experiments indicated that soils treated with only EDTA were harmful due to further dissolution of metals from non-labile fraction with time. Humic substances might be alternative to reduce metals toxicity in contaminated soils. But humic substances were not enough to reduced metals as soils were calcareous or highly polluted with metals. In these cases small amount of EDTA might be incorporated with humic substances for metal removal from soils.

Based on soil properties and metal concentration, and comparing the removal efficiencies with reducing metal mobility in soil the following extractants are recommend for particular metal contaminated soil (Table 4.7.26).

Table 4.7.26 The recommended suitable extractants for particular metal contaminated soils.

Sl No	**Contaminated Soil**	**Suitable extractants**
1	Soil of Madhupur Tract Battery Industry	0.4% Fulvic acid
2	Calcareous soil of Kushtia having cable industry	Combined 0.4% FA + 0.04 M EDTA
3	Tannery Industrial area, Hazaribagh, Dhaka	0.4% Humic acid, 0.4% Fulvic acid
4	Ship breaking Industry, Shitakunda	Combined 0.4% HA + 0.04 M EDTA Combined 0.4% FA + 0.04 M EDTA
5	Calcareous soil of Ishurdi having Battery industry	Combined 0.4% FA + 0.04 M EDTA
6	Cable Industry, Chittagong	0.4% Humic acid, 0.4% Fulvic acid

5. SUMMARY

Metals are required in minute quantities for normal growth and development of plants, but turn out to be toxic once their available concentration exceeds a particular level. Soils are contaminated with heavy metals through natural and anthropogenic activities. Soil acts as a natural sorbent of heavy metals and like other organic pollutants they do not undergo microbial or chemical degradation and their concentrations in soils persist for a long time after introduction. A range of technologies exist to remediate of metals-contaminated soils. These technologies are physical separation, chemical extraction, immobilization, toxicity reduction and phytoremediation. The present research work was carried out to evaluate different chemical technologies and of these chemical extraction and chemical Immobilization are mostly used for soil remediation.

A survey oriented work was carried out to indentify some metal contaminated soils in Bangladesh. Samples were collected mainly from different industrial areas. The contaminated areas were categorized according to the types and levels of metal pollution. Six representative sampling sites were selected: two battery industrial areas located in red soil of Madhupur tract and calcareous soils of Ishwardi, tannery industrial area of Hazaribagh, ship breaking yard of Shitakunda and two cable industries located in calcareous soil of Kushtia and coastal plain soil of Chittagong. Distribution of metals in contaminated areas was determined by collecting a number of samples in and around the industrial areas. The concentrations of metals were plotted in GIS maps and prepared cartographic contour line by using some special analyst tools of ArcGIS 9.3.1 which represented the distribution of metals in contaminated areas.

The concentrations of metals were found high in contaminated areas. Total lead concentrations were recorded 245 and 164 mg kg^{-1} in soils near battery industries of Madhupur tract and Ishwardi, respectively. High amount of chromium (256 mg kg^{-1}) was found in tannery industrial areas. All metal contents were recorded very high in soil of ship breaking yard and the values were 93, 25, 71, 69, 192, 845 mg kg^{-1} of Pb, Cd, Cr, Ni, Cu and Zn, respectively. Soils of cable industries of Kushtia and Chittagong contained high amount of Cu which were 151 and 196 mg kg^{-1}, respectively. The fractions of metals contaminated soils were determined following sequential extraction procedure to measure the relative proportions of metals retained by each soil fraction (water soluble, exchangeable, carbonate bound, oxides bound, organic matter bound and residual form). The mobility indexes of metals were also calculated from first three labile fractions of soil. In acidic red soil of Madhupur tract most of the Pb was bound to non-labile Fe/Mn oxide fractions, but in calcareous soil of Ishwardi high amount of Pb was retained in carbonate bound fraction. Therefore, the mobility index of Pb was recorded high in soil of Ishwardi (40.9 %) than that in Madhupur soil (17.8 %). A large amount of Cr was found in immobile organic matter bound forms and the mobility index of Cr was recorded lowest in soil of Hazaribagh tannery industrial area. In ship breaking yard most of the metals were associated with non-lablile fractions. Among the metals Cd and Cr concentrations were recorded highest and lowest in labile fractions, respectively. So the mobility index of Cd was 67 % and Cr was 18.6%. And the mobility indexes of other metals were in the range of 28.6 to 33.5 % in soil of ship breaking yard. The cable industry in calcareous soil of Kushtia contained high amount of Cu in carbonate bound forms and the mobility index of Cu was also found high (43.7 %) in soil. In soil of another cable industry of Chittagong, most of the Cu was presented in non-labile fraction and the mobility index was recorded 28.4 %.

The different chemical extractants were used for metal removal from soils. Initially six synthetic chemical extractants: distilled water, buffered salt solution (1 M NH_4OAc at pH 7.0), salt of divalent cation (0.1 M $CaCl_2$), 0.1 M HCl acid solution, chelating agents of 0.1 M EDTA and 0.005 M DTPA were used. To determine the extractability of these extractants spiked soils of non-contaminated point sources of same regions were used. The soils were artificially spiked with six metals: Pb, Cd, Cr, Ni, Cu and Zn and maintained approximately 100 mg kg^{-1} of each metal. A leaching experiment was set up using 25 cm tall leaching tube containing 20 g of spiked soils and each time 100 ml of extractants were used.

Among the metals, the highest amounts of Cd were removed by extractants from soils because large amounts of Cd (50 to 65%) were present in labile fractions of soils. In contrast the lowest amounts of Cr were leached by extractants due to the presence of Cr mostly in non-labile fractions (Fe/Mn oxides and organic matter bound forms). The removed portions of other fours metals (Ni, Cu, Pb and Zn) from soils were comparatively higher than Cr but lower than that of Cd.

In general the metal removal efficiencies of extractants were found higher in light textured soils than that of heavy textured and calcareous soils. The high clay and silt contents of soil bound metals tightly by their surface charge and inhibit their migration to other media. Consequently, the higher the proportion of the clay and silt in soil, the harder is the extraction of metals.

Among the extractants distilled water removed the lowest amounts of metals from soils because very few amount of metals were presented in water soluble form. Among the salt solutions, the metal removal efficiencies of NH_4OAc were comparatively higher than that of $CaCl_2$. It was observed from fractionation study that buffered NH_4OAc effectively extracted metals from different soil fractions of exchangeable, carbonate and oxide bound forms of soils compared to $CaCl_2$.

The extractant 0.1 M HCl removed significantly high amounts of metals from spiked soils of Madhupur tract, Hazaribaagh, Shitakunda and Chittagong. The extractability of heavy metals increased with decreasing pH of soil solution. The pH of soils controls the solubility and hydrolysis of metal hydroxides, carbonates, phosphates and also organic matter. The metal fractionation of spiked soils indicated that the extractant HCl not only leached metals from labile forms but also dissolved metals from non-labile forms of soils.

The extraction of heavy metals with 0.1 M HCl solution showed some difficulties. Acid solution markedly reduced the pH of soils. Acid solutions degrade soil crystalline structure and also leached a high amount of plant essential elements like Ca, Mg, Fe and Mn besides the removal of heavy metals. Furthermore, 0.1 M HCl could not extract sufficient amount of metals from calcareous soils of Ishwardi and Kushtia because HCl becomes neutralized by reacting with calcareous minerals. There HCl was not a suitable extractant to extract metals from soils.

Organic acids and chelating agents are suggested as alternatives to mineral acid for less damaging leaching effects. The chelators EDTA and DTPA removed considerable amounts of metals from all soils. Among them, the extractability of EDTA was comparatively higher than DTPA. The extractant EDTA removed significantly higher amounts of metals from all spiked soils including the calcareous soils. The fractionation study of EDTA treated soil indicated that most of the metals were removed from the water soluble, exchangeable, carbonate and oxide bound forms of soils after washing. Therefore the mobility indexes of metals were in general found lowest in soils after extraction with EDTA. So the highest degree of metal decontamination was achieved by washing soils with 0.1 M EDTA with less surface damage, which indicated that EDTA is the most effective synthetic chemical extractant for the reclamation of metals from different types of soils.

Extractant EDTA removed high amount of metals from soils, but the use of such chemical is also problematic due to its persistence in the environment. The addition of EDTA and other strong ligands increase heavy metal leaching from the topsoil. Despite its potentiality, use of EDTA as a soil washing solution has disadvantages including high costs, slow biodegradability, ecosystem toxicity and further dissolution of metals from non-labile fraction with time.

Soluble humic substances (HS) might be an alternative to use instead of synthetic chemicals such as mineral acid or EDTA. Humic substances act as a chelating agent for metal ion extraction. These natural extractants are less expensive, biodegradable, environment friendly, less destructive to soil structure and also improve soil properties.

Two peat soils were collected one from Khulna University campus and another from Gopalganj basin. Humic substances were extracted from peat soils. The characterization of humic substances was done by determining various parameters to evaluate their maturity. The quantifications of functional groups of humic substances were also performed. The complexing capacities of humic substances with metal ions depend on its maturity, molecular dimension and presence of functional groups. The optical density, E4:E6 ratio and coagulation thresholds of humic substances of Khulna region indicated more maturity due to intense humification process than that of HS in Gopalganj region. Consequently the functional groups including carboxylic (-COOH), phenolic (-OH) and aliphatic (-OH) and yields of humic substances of Khulna region were relatively higher (from FTIR spectroscopy and potentiometric titration) which increased its metal holding capacity over that of Gopanganj region. Therefore the humic substances of Khulna region were selected for further investigation.

Different concentrations of humic and fulvic acids were applied on spiked soils of six different regions. The concentrations of humic substances were selected in between 0.1 and 0.5 %, from which the most effective doses of humic substances were determined for removal of metals from particular soil.

The extraction capacities of humic substances largely varied with the pH of soil and extractant used. The complex reaction of HS and metal ions caused by the carboxyl and hydroxyl groups of HS which was started at pH 6. The association of metal-HS complex increased with increasing pH. The soils of Madhupur and Hazaribagh were slightly acidic in nature and the maximum extraction of metals was observed by humic substances at pH 6.0 to 7.0. In contrast calcareous soils were alkaline in nature and humic substances removed the highest amount of metals at pH 8.0. Humic substances also leached the highest amount of metals from soils of Shitakunda and Chittagong at pH 7.0. So the metal removal efficiencies of humic substances were highly depended on the pH of soil.

Like synthetic extractants humic substances removed the highest amount of Cd and lowest amount of Cr from all soils, because most of the Cd and Cr were distributed in labile and non-labile fractions of soils, respectively. Humic substances also extracted considerable amount of Cu and Ni from soils which were comparatively higher than that of Pb and Zn.

It was generally found that humic substances extracted considerably higher amount of metals from light textured soil compared to that of heavy textured soil. Heavy metals in clay/silt fraction are more difficult to be extracted than those in sand probably because heavy metals were strongly bound to clay particles, and the combination is too strong to make the metals transfer to other media.

Among the humic substances, humic acid removed comparatively lower amount of metals from heavy textured soil of Madhupur, Ishwardi and Kushtia than that of fulvic acid. This was probably due to that higher molecular structure of humic acid takes longer time to penetrate heavy textured soil and form immobile HS-metal complex at high concentration. In contrast the lower molecular structure and higher functional groups containing fulvic acid can easily penetrated heavy textured soils and extracted more metals compared to HA.

On the other hand humic acid leached somewhat higher amount of metals from light textured soil of Hazaribagh and Shitakunda compare to fulvic acid. This might be due to that fulvic acid with it's lower molecular structure, percolated the light textured soil quickly (2 - 3 hours) compared to humic acid (required more than 6 hours). Therefore humic acid got more contact time with soil particles and extracted higher amount of metals from spiked soil than that of FA.

The extraction capacities of natural and synthetic chelating materials indicated that synthetic chelator EDTA extracted 37 - 65 % Pb, 60 - 71 % Cd, 24 - 34 % Cr, 35 - 63 % Ni, 35 - 60 % Cu and 43 - 58 % Zn and, the natural chelator humic substances extracted maximum 16 - 45 % Pb, 44 - 70 % Cd, 16 - 33 % Cr, 22 - 51 % Ni, 24 - 52 % Cu and 21 - 45 % Zn of total amount from different spiked soils. Therefore EDTA in general extracted comparatively higher amount of metals from soils than that of humic substances.

Humic substances were less suited for removing metals from contaminated calcareous soil. The major cation Ca may interfere with chelating process of HS in calcareous soil. In addition humic acid could not extract sufficient amount of metals from heavy textured soil due to its large molecular structure.

To overcome these difficulties and to improve the extraction efficiency of metals, EDTA was combined with humic substances. Different concentrations of EDTA from 0.02 to 0.1 M were mixed with humic and fulvic acids. These combined HA + EDTA and FA + EDTA extractants were then used for metal removal from spiked soils of different regions.

It was found that the extraction capacities of humic substances were significantly increased when small amount of EDTA was combined with. In spiked soil of Madhupur tract, fulvic acid at the concentration of 0.4 % in combination with 0.04 M EDTA extracted similar amount of metals from soil as was done by only 0.1 M EDTA solution. Humic acid also positively influenced the extraction capacity of metals by mixing with EDTA solution, but HA required more EDTA (0.06 M EDTA) to removed similar amounts of metals as extracted with combined FA + EDTA extractant.

Humic substances were unable to extract much metals from calcareous soils, but in combination with EDTA, extracted considerable amount of metals. Humic and fulvic acids required 0.06 M and 0.04 M EDTA to removed statistically similar amount of metals from calcareous soils of Ishwardi and Kushtia as extracted with only 0.1 M EDTA solution. Therefore, fulvic acid required comparative lower amount of EDTA to remove similar amount of metal than that of combined HA + EDTA solution.

Humic substances extracted comparatively higher amounts of metals from light textured soils of Hazaribagh and Shitakunda. Therefore, both humic and fulvic acids in general required low amount of EDTA (0.04 M) to remove statistically similar amounts of metals as extracted with only 0.1 M EDTA solution. In spiked soil of Chittagong, the removal efficiencies of metals by humic substances were increased by mixing with both 0.04 and 0.06 M EDTA solutions and the amounts extracted by 0.1 M EDTA solution only was similar to those of the extractants.

In order to study the mobility and bioavailability of metals from soil to plant, pot experiments were conducted with red amaranth (*Amaranthus gangeticus*) and rice (*Oryza sativa*) BRRIdhan-49. Six pot experiments were conducted with six different contaminated soils. Seven treatments were applied for each contaminated soil. One treatment contained only contaminated soil to represent the control and one pot contained non-contaminated soil of same region. Five other treatments were to wash contaminated soils with humic acid, fulvic acid, 0.1 M EDTA, and combined HA + EDTA and FA + EDTA solutions. Contaminated soils were leached with these extractants several times, until very few or no metals were detected in leachate.

The vegetative growth and yields of red amaranth and also the growth and grain yields of rice plants were found lowest when grown in contaminated soils due to plant injury from metal toxicity. The growth and yield of plants increased in soils when humic substances, EDTA and combined extractants were used for metal removal. These extractants removed toxic metals from soil and thereby created favourable effects on increased growth and yields of plant.

Pot experiments with the Pb contaminated soil of Madhupur tract indicated that only 0.1 M EDTA and combined 0.4 % FA + 0.4 M EDTA extracted the higher amount of Pb from soil. Fulvic acid at the concentration of 0.4 % also extracted high amount of Pb from soil which was higher than that of only 0.3 % HA and combined 0.3 % HA + 0.06 M EDTA extractant. The fractionation of contaminated soil of Madhupur tract during plant harvest indicated that soils remediated with combined FA + EDTA and only FA extractants yielded the lowest amount of Pb present in labile fraction and the least mobility indexes of Pb in soil which thereby caused the minimum Pb concentration in plant materials. Though the combined FA + EDTA and only FA extractants created effects on the minimum content of Pb in plants, the use of only 0.4 % fulvic acid is recommended for metal removal from contaminated soil of Maduhupur tract without EDTA.

In calcareous soil of Ishwardi large amount of Pb was found to exist in carbonate bound fraction which created the high mobility indexes of Pb (47%) in soil. The combined 0.4 % FA + 0.4 M EDTA leached the highest amount of Pb from soil which was similar as extracted by 0.1 M EDTA alone. The extractants HA, FA and combined HA+ EDTA leached comparatively lower amount of Pb than that of only EDTA and combined FA + EDTA extractant. Although 0.1 M EDTA extracted the highest amount of Pb from contaminated soil of Ishwardi, due to the presence of higher amount of Pb in water soluble and exchangeable fractions, EDTA created the relatively higher mobility index of Pb than that of combined FA + EDTA treated soils. Among the extractants, combined FA + EDTA caused the lowest mobility index of Pb in soil and also caused the minimum concentrations of Pb in both plants after remediation.

Pot experiment with contaminated soil of Hazahibagh indicated that Cr was present at high concentration in soil and the most of the metal was retained in non-labile fraction and thus the mobility index of Cr was recorded only 11.6 to 12.5 %. All extractants leached considerable amounts of Cr from soil. The soils treated with 0.4 % HA, 0.4 % FA alone and combined 0.4 % HA + 0.04 M EDTA and 0.4% FA + 0.04 M EDTA extractants retained less than 10 % of Cr in labile fractions and mobility indexes were recorded below 2 %, whereas only EDTA treated soil retained 17% of Cr in soil during plant harvest with mobility index of 2.75 to 2.81 %. By comparing the effects of extractants on contaminated soil of Hazaribagh, it can be concluded that only HA, FA and combined HS + EDTA extractants reduced Cr mobility in soil and as a consequence in plants more competently compare to that of only EDTA treatment. Therefore, the use of only 0.4 % humic acid or 0.4 % fulvic acids is adequate for removal of Cr from soil of Hazaribagh without using EDTA.

The soil of Shitakunda was highly contaminated with multi metals. From pot experiment with contaminated soil of Shitakunda it was found that the extractants 0.1 M EDTA, combined 0.4 % HA + 0.04 M EDTA and 0.4 % FA + 0.04 M EDTA leached considerable amounts of metals from soil which were higher than that of only 0.4 % HA and 0.4 % FA extractants. As soil contained high amount of metals, only HA or FA was not enough to remove them all. The fractionation of soil after plant harvest indicated that soils treated with combined HA + EDTA and FA + EDTA extractants retained lowest amount of metals in water soluble and exchangeable forms and the mobility indexes of metals were also recorded minimum. The soils remediated with only 0.4 % HA, 0.4 % FA and 0.1 M EDTA also reduced the mobility indexes of metals in soil but the values were lower than that of soils treated with combined extractants. Therefore, combined HA + EDTA and FA + EDTA treated soils supplied the minimum metal contents in plants. So these combined extractants are considered as best suitable extractants for metal removal from contaminated saline soil of Shitakunda.

Similar to the calcareous soil of Ishwardi a large amount of Cu was present in carbonate adsorbed fraction of calcareous soil of Kushtia which was responsible for the high mobility indexes of Cu (41.61 and 40.88 %) in soil. The combined 0.4 % FA + 0.4 M EDTA and only

0.1 M EDTA leached the higher amounts of Cu from soil over that of 0.3 % HA, 0.4 % FA and combined 0.3 % HA + 0.06 M EDTA extractants. Solution of 0.1 M EDTA extracted the highest amount of Cu from contaminated soil of Kushtia. The presence of higher amount of Cu in water soluble and exchangeable forms of EDTA treated soil caused relatively higher mobility index of Cu than that of combined FA + EDTA treatment. Therefore among the extractants, the combined 0.4 % FA + 0.04 M EDTA created the lowest mobility index of Cu in soil and as a consequence the minimum concentration of Cu in both crops after remediation.

The saline soil of Chittagong was mainly contaminated with copper. From pot experiment with contaminated soil of Chittagong it was observed that the extractants 0.1 M EDTA, combined 0.4 % HA + 0.06 M EDTA and 0.4 % FA + 0.06 M EDTA leached considerable amounts of metals from soils which were higher than that of only with 0.4 % HA and 0.4 % FA extractants. But the fractionation study indicated that soils treated with only 0.4 % HA, 0.4 % FA and combined HA + EDTA and FA + EDTA extractants retained 5 to 10% metals in water soluble and exchangeable forms, which were lower than that obtained with only 0.1 M EDTA treatment. Consequently soils remediated with only humic substances and combined HS + EDTA extractant similarly reduced the Cu contents in plants. So the use of only 0.4 % HA or 0.4 % FA is enough to reduce Cu content in plants and consider as suitable extractants for metal removal from contaminated soil of Chittagong.

From all pot experiments it was observed that soils treated with only 0.1 M EDTA extractants retained comparatively higher amount of metals in water soluble and exchangeable forms because excess free EDTA in soil enhanced further dissolution of metals from non-labile to labile fractions.

On the other hand, residual humic substances in soil after extraction with humic substances and combined HS + EDTA extractions reduced the mobility of metals by forming immobile HS-metal complex which inhibited metal accumulation by plant. Although EDTA extracted the highest amount of metal from contaminated soil, due to further solubilization of metals from non-labile fraction, EDTA treatment could not reduce metal content in plants as much as done by humic substances and combined extractants.

6. CONCLUSION

Contamination of soils with heavy metals is a widespread and serious problem all over the world which affects human health and environmental quality. Heavy metals are associated with the components of soil and these associations indicate both their mobility in the soils and bioavailability to plant. The degree at which metals are associated with different soil fractions depends on soil properties as pH, organic matter content, redox potential, soil texture, cation exchange capacity, clay minerals, carbonate content and Fe/Mn oxides in soil.

Once soils are polluted with metals, they do not undergo biological and chemical degradation like organic pollutants and persists for a long period of time. A range of technologies exist to remediate the metals contaminated soils. The present research focused on different chemical technologies for extraction and immobilization of metals. Different synthetic and natural extractants were applied to remediate metals contaminated soils in the experiments.

The findings of results indicated that soil treated with only synthetic extractants (HCl, EDTA) are harmful due to the destruction of soil crystalline structure, loss of other soil essential elements and further dissolution of metals with time. Natural extracting agents (humic substances) might be alternative to remediate metals contaminated soil. But humic substances were not found enough to reduced metals if soils are calcareous or highly polluted with metals. In these cases synthetic chelatants should be incorporated with humic substances.

Soil texture is an important parameter to take into account to select the most appropriate analytical technique to remove and to determine soil contaminants. Results indicated that metals were more quantitatively removed by fulvic acid rather than humic acid when a soil is fine textured. Whereas both humic substances give equivalent results in coarse-textured soils. As the isolation and extraction of humic acid is cost effective rather than that of fulvic acid, the use of humic acid should be recommended as a standard procedure for coarse textured soil.

The next step to adopt a cleaning technique for the disposal of leachate obtained from the extraction. The collected leachate contains heavy metals complexed with humic substances, which may be treated by evaporating and burning of organic matter and turn the heavy metals into slag and regaining of the metals by electrolysis process. The leachate can also be precipitated by adding strong base. Therefore soluble humic substances are suggested as cleaning agent of polluted soils instead of the environmentally problematic synthetic chemical because of their biological toxicity. Humic substances can even improve biomass activity in washed soils and contribute to a further natural attenuation once the soils are disposed after an *ex situ* remediation process. Moreover, the fractions of humic substance remaining in soil may play a favourable action in plant growth and thus assist in the full recovery of the treated soils.

Therefore, humic substances alone and/or combined with small amount of EDTA effectively reduced metal mobility in soil and as a consequence of bioavailability in plant. The suitability of these extractants for particular contaminated soils might be selected by considering soil properties and level of metal concentrations.

7. REFERENCES

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Appendix 8.1

8.1 Extraction and quantification of humic substances

Extraction of Humic Acid and Fulvic Acid was done according to new standard method of International Humic Substances Society (IHSS) (Lamar *et al.,* 2014)

Step 1. Alkaline Extraction

Prepare analytical samples by crushing dried peat soil to a fine powder using a mortar and pestle so that 100% of the crushed analytical sample passes through a U.S. Standard Sieve mesh size No. 60 making sure that the powder is well mixed.

Determine the moisture content gravimetrically as follows:

Weigh an aluminum weigh boat and record mass (Wt1); transfer 2 ± 0.5 g test portion of the analytical sample into the weigh boat, weigh, and record mass (W1); place in drying oven for 24 ± 0.2 h at 90°C; after 24 h, remove from drying oven and place in desiccator to cool for 1 h; weigh and record mass of weigh boat and dry test portion (W2). Determine the moisture ratio using Equation 1 (Step 7).

Next, weigh a second test portion from the analytical sample estimated to contain approximately 2.5 g HA and record the test portion mass to four decimal places (W3) into a precalibrated/premarked 1 L Erlenmeyer flask. Add 0.1 M NaOH with stirring, and make to a final volume of 1 L. Determine the dry weight of the test portion using Equation 2 (Step 7).

For liquid materials, thoroughly mix the analytical sample by stirring with a glass rod for 1 min, ensuring that any residue that may have fallen to the bottom of the container is thoroughly mixed. Then add an aliquot of a test portion of the analytical sample, noting the volume (V1), into a precalibrated/premarked 1 L Erlenmeyer flask, and bring to a final volume of 1 L with 0.1 M NaOH. The test portion should result in a concentration of 200 to 600 mg/L HA plus FA after dilution with 0.1 M NaOH. The aliquot volume will be based on the HA and FA product concentrations that were claimed by the manufacturer. Determine the density (g/mL) of the liquid material by weighing 10 mL of well-mixed analytical sample in a pretared graduated cylinder (D1). Determine the weight of the liquid test portion using Equation 3 (Step 7).

Add a 1.2 cm long magnetic stir bar, replace the air in the headspace with N2, and cover with Parafilm. Mix vigorously on a stir plate (e.g., 200–300 rpm). Stir solid materials for 6 h to extract humic substances and the liquid materials for 1 h to ensure dissolution of all HA and FA.

From this point on, the method is the same for both solid and liquid materials.

After stirring, remove the flask from the stir plate, transfer the contents to centrifuge tubes, and centrifuge the entire volume to separate any insoluble material from the dissolved HA and FA. Centrifuge at 3900 × g for 10 min (50 mL centrifuge tubes used). Discard the insoluble precipitates and collect the alkaline supernatant containing the HA and FA in a clean 1 L Erlenmeyer flask.

Step 2. Separation of HA

While the extract solution is being mixed with a stir bar, carefully insert a pH electrode into the middle portion of the solution. To flocculate the HA, add concentrated HCl (1:1) drop wise to the alkaline extract until pH 1.0 ± 0.05 is reached.

Cover the flask with Parafilm and mix for 1 h. Check pH and readjust to pH 1.0 with additional concentrated HCl if necessary. If the pH should fall below 0.95, adjust pH back to 1.0 ± 0.05 with the 0.1 M NaOH solution. Continue to let the acidified extract mix and check pH occasionally until it is stable at pH = 1 ± 0.05 for 5 min.

Once the pH is stable, remove the flask from mixer and cover with Parafilm. Let the mixture sit unstirred until precipitated HA has fallen to the bottom of the flask. The time for HA to precipitate from solution varies greatly among products, but a typical time range is 1 to 6 h.

(a) Once the HA has completely precipitated, decant the FA-containing extract (fulvic fraction) into a clean 1 L Erlenmeyer flask, being careful not to include any of the HA precipitate. Typically >900 mL can be decanted while excluding any HA precipitate. With some products, the precipitated HA will remain in suspension as colloidal particles. If so, centrifuge the entire volume to separate the flocculated HA from the acidified fulvic fraction.

(b) Pour the remaining mixture into centrifuge tubes and centrifuge at 3900 × g for 0.5 h to separate the HA precipitate. If necessary a higher g force or longer centrifugation time can be used to obtain a clean separation of the fulvic extract supernatant from the HA precipitate. Add the supernatant to the FA-containing acidified extract.

Step 3. Determination of HA Concentration

(a) Place the centrifuge tubes containing the precipitated HA in a drying oven set at 90°C, and dry the HA to constant weight (typically 24 h). Constant weight is achieved when the tube and HA (or FA) weigh the same after an additional 2 h drying time.

(b) After drying, remove the tubes from the drying oven and place in a desiccator to cool to room temperature. After cooling, quantitatively transfer the residue from the tube by scraping it from the sides and bottom of the tube with a spatula, transfer to a tared weigh boat, and record the mass (W4HA). This residue is the “Extracted HA”.

Step 4. Determination of Ash Content

Transfer the extracted HA to a preweighed (Wt2) ceramic dish that had been previously dried in a drying oven set at 90°C and then cooled in a desiccator to room temperature. After recording the combined mass of the extracted HA and dish (W5), combust in a muffle oven for 4 h at 500°C. While still warm, remove the dish and contents from the muffle oven and place in a desiccators to cool. Once cool, weigh the dish with ash (W6) and calculate the ash ratio (Equation 4, Step 7). Determine the final mass of the extracted HA by correcting for ash content using Equation 5 (Step 7).

Step 5. Separation of Fulvic Acid

Separate FA from the other acid-soluble compounds in the fulvic fraction by using a 40 × 250 mm glass column prepared with a nonionic macroporous acrylic ester resin (i.e., Supelite DAX-8). Through selective adsorption, hydrophilic acid-soluble components do not bind to the resin and are removed. Pass the fulvic fraction through the column using a peristaltic pump, under low pressure, via the top of the column. It is critical that the top of the resin in the column remains covered with solution until all the extract has been added to prevent drying of the resin.

Once the fulvic fraction has been completely loaded onto the resin, wash the resin with deionized water by pumping it through the top of the column using the peristaltic pump under low pressure. Discard the effluent. Wash the column until the absorbance at 350 nm of the column effluent is equal (e.g., within 0.015 absorbance units) to that of the deionized water used to wash the column, using deionized water to zero (i.e., blank) the spectrophotometer. A wavelength of 350 nm gives strong absorbance by FA and allows the use of a spectrophotometer that only measures visual wavelengths.

Desorb the FA by back elution (i.e., influent introduced into the bottom of column) by pumping 0.1 M NaOH using the peristaltic pump. Most of the FA is adsorbed to the very top of the DAX-8 resin. Desorption from the column bottom uses a minimal amount of 0.1 M NaOH to fully desorb the FA. All the FA has been desorbed when the absorbance of the column effluent is equal to the absorbance of influent at 350 nm. Use 0.1 M NaOH as the spectrophotometric blank. Add the effluent taken to check absorbance of the desorbed FA solution.

Protonate and de-ash the FA by passing repeatedly (by gravity feed) through Amberlite IR120 hydrogen form ion exchange resin contained in a 5 × 50 cm column until the electrical conductivity of the effluent is <120 uS/m as measured with a conductivity meter. To ensure that all the FA is removed from the resin after the final pass, wash the column with deionized water until the absorbance of effluent at 350 nm is the same (e.g., within 0.015 absorbance units) as the deionized water used to wash the column. Use deionized H2O as the spectrophotometric blank. Add the wash and any effluent portions taken to check absorbance to the purified FA solution. To help with removal of all FA, the resin can be agitated (e.g., using a long glass or plastic rod) several times.

Concentrate the FA to a volume of approximately 15 ± 2 mL by using a rotary evaporator at 55°C. Completely transfer the 15 mL fulvic acid concentrate to a 50 mL plastic centrifuge tube and dry at 90°C to constant dryness in a drying oven. Freeze drying is an alternative to oven drying. After drying, as described for the HA above under Step 4, place the tube in a desiccator to cool. Remove FA from the tube by complete scraping of the tube sides and bottom with a spatula, and weigh it on pretared weigh paper (W8). This material is the "Extracted FA". Determine the residual ash content of extracted FA as described under Step 4 for HA and calculate the ash ratio (Equation 4, Step 7). Finally, determine the weight of the extracted FA without ash using Equation 6 in Step 7.

Step 6. Column Regeneration

Regenerate the DAX-8 resin by pumping 0.1 M HCl [8.33 mL concentrated HCl/1000 mL final volume deionized (DI) water] through the bottom of the column until the pH of the effluent is equal to the pH of the influent. Use the peristaltic pump to pump all reagents through the DAX-8 column during regeneration. Next rinse the column with DI water by pumping it into the top of the column until the pH of the effluent equals the pH of the influent (i.e., DI water).

Regenerate the H^+ form cation exchange resin in a batch process by pouring the resin into a large beaker (e.g., 4 L plastic beaker), pour off the water, and cover the resin with 1 M HCl (83.3 mL concentrated HCl/1000 mL final volume DI water). Let stand for a minimum of 30 min with occasional stirring (e.g., once every 5 min). Remove the excess acid from the resin by pouring off the acid and covering the resin with DI water. Stir vigorously with a stirring rod for 15 s, then let the resin and rinse water sit for 5 min. Repeat the process until the pH of the rinse water equals the pH of the DI water.

Load the regenerated resin back into the column. Once loaded, rinse the resin with DI water and check the pH of the effluent. If it is still lower than the pH of the DI water before it is passed through the column, continue to rinse the column with DI water until the pH of the effluent is within 0.1 pH units of the pH of the influent.

Step 7. Calculations

Moisture ratio = $[(W_1 - W_2)/(W_1 - W_{t1})]$………. (1)

Dry test portion dry weight = (W_3) (1 – moisture ratio) …………….(2)

Liquid test portion weight (g) = (V_1) (D_1) ………………..(3)

Ash ratio = $[(W_5 – W_6)/(W_5 – W_{t2})]$ …………………….(4)

Weight of extracted HA without ash (g) = $(W_{4HA})(1 – \text{Ash Ratio})$ ………………(5)

Weight of extracted FA without ash (g) = $(W_{4FA})(1\text{-Ash Ratio})$ …………(6)

where

W_1 = weight of test portion taken for moisture plus ceramic dish before drying, g;
W_2 = weight of test portion taken for moisture plus ceramic dish after drying, g;
W_{t1} = weight of ceramic dish, g;
W_3 = test portion weight, g;
V_1 = volume of liquid test portion, mL;
D_1 = weight/unit volume of liquid test portion, g/mL;
W_{4HA} = weight of extracted HA, g; W_{4FA} = weight of extracted FA, g;
W_{t2} = weight of ceramic dish used for ashing, g;
W_5 = weight of extracted HA or FA taken for ash plus ceramic dish before combusting, g; and
W6 = weight of ash plus ceramic dish after combusting, g.

(**a**) For solid materials, determine the percentages of FA and HA (dry weight basis) as follows:

FA, % = [Ashless FA (g)/test portion dry weight)] × 100

HA, % = [Ashless HA (g)/test portion dry weight)] × 100

(**b**) For liquid materials:

FA, % = [Ashless FA (g)/liquid test portion (g)] × 100

HA, % = [Ashless HA (g)/liquid test portion (g)] × 100

Appendix 8.2

8.2 Characterization of humic substances

Humic substances are heterogeneous, high-molecular-weight organic substances that are composed of humic acid, fulvic acid and humins, all of which have a different solubility in alkaline and acid solutions (Zhang *et al.*, 2013). Humic substances consists of a complex mixture of organic substances covering a very wide range in molecular size from small water soluble compounds to large insoluble molecules mainly formed during microbial degradation of dead organic materials (Stevenson and Cole, 1999). Due to surface carboxylic acid and phenolic groups, HS can form complexes with most heavy metals (Christensen and Christensen, 1999; Stevenson and Cole, 1999; Weng *et al.*, 2002; Hruˇska *et al.*, 2003). The properties of humic substances such as optical density, E_4:E_6 ratio, coagulation threshold and functional groups were analyzed. These properties were very important to determine the maturity, molecular condensation, hydrophobic nature as well as complex forming capacity of HS with metals.

8.2.1. Optical Density: Optical density of humic substances decreased with increasing wavelength. Among two different sources of humic and fulvic acids, optical density of humic substances showed considerable variation.

The optical densities of humic and fulvic acids extracted from peat from Gopalganj region was lower than that of Khulna region. During comparative studies of the chemical structure and the optical properties of humic and fulvic acids of different origins it was found that younger humic substances, in a chemical sense, have a lower optical density than more mature humic substances (Kononova, 1966). This indicated that humic substances extracted from peat of Gopalganj region were younger than that of Khulna region.

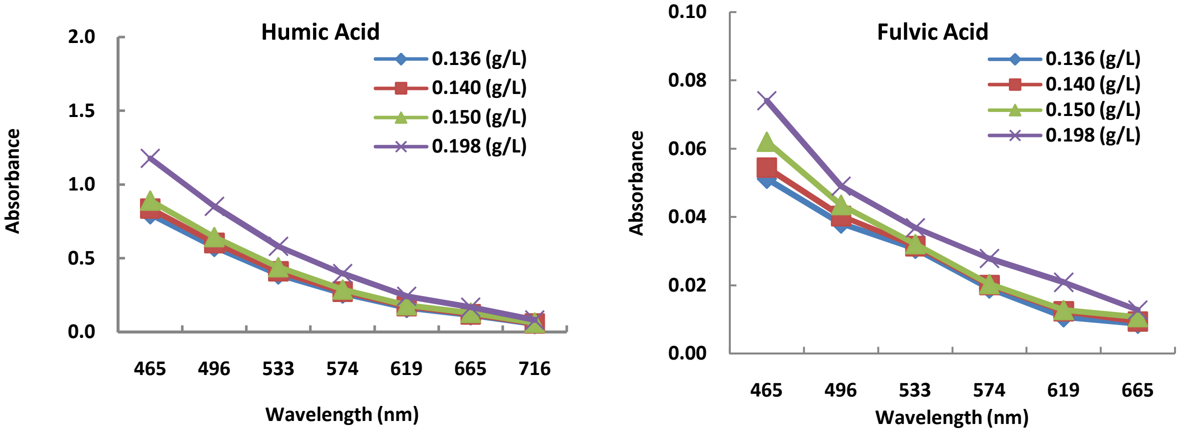

Fig. 8.2.1. Optical density of humic and fulvic acids extracted from peat collected from Gopalganj region at different carbon concentration.

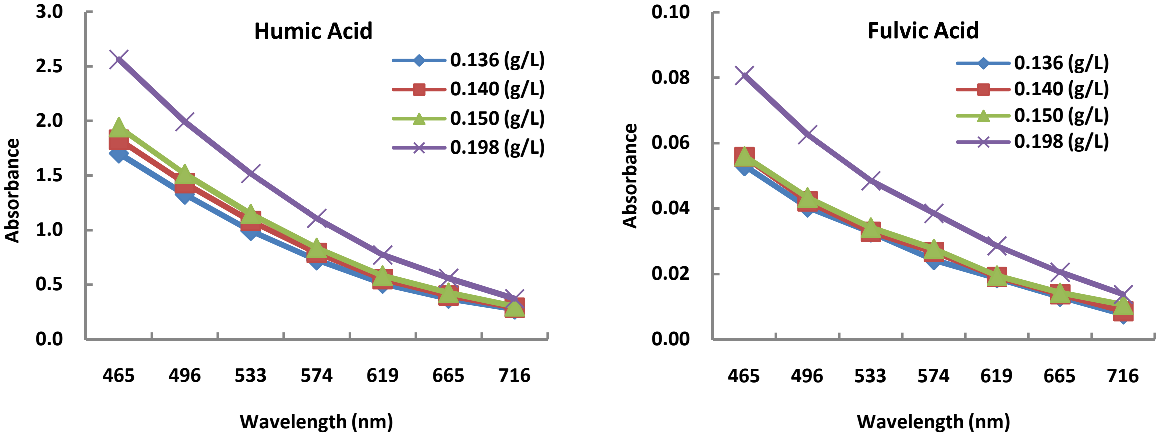

Fig. 8.2.2. Optical density of humic acid and fulvic acid extracted from peat collected from Khulna region at different carbon concentration

8.2.2 E_4/E_6 Ratio: The optical parameter E_4/E_6 ratio expresses molecular condensation. Scheffer (1954) showed that the ratio of the extinction E at the wavelengths 465 nm and 665 nm (the so-called E_4/E_6 ratio) is independent of the concentration of carbon in solution and by reflecting the steepness of the spectrophotometric curve, is a characteristic of humic substances.

Table 8.2.1 The E_4/E_6 ratio of Humic and fulvic acids of peat collected from different regions.

Carbon Concentration (g/L)	**Gopalganj**		**Khulna**	
	Humic Acid	**Fulvic Acid**	**Humic Acid**	**Fulvic Acid**
0.136	5.818	6.991	4.077	4.589
0.140	5.798	6.900	4.043	4.569
0.150	5.804	6.890	3.944	4.537
0.196	5.789	6.981	3.937	4.578

The mean E_4:E_6 ratio value was high (HA 6.94 and FA 5.8) in peat of Gopalganj region followed by Khulna region (HA 4.57 and FA 3.99). According to Chen *et al.* (1977), the E_4/E_6 ratio is a parameter inversely related to the molecular dimension. Chanyasak *et al.*, (1982) reported that E_4/E_6 ratio has been immensely useful as an assessment index, which usually decreases during humification. This index is widely used as an indicator for evaluating the maturity of humic substances. As shown in Table 8.2.1, the E_4/E_6 ratio of both HA and FA of Khulna region decreased with increasing humification process and higher molecular weight compound was formed by increased condensation and aromatization processes which produced more polycondensed humic substances during humification. So, in respect of E_4/E_6 ratio, it can be concluded that humic substances of Khulna region were more mature than that of Gopalganj region. Kononova (1966) found that the E_4/E_6 ratio ≥ 5.0 indicates a reduced hydrophobic structure while that ≤ 5.0 suggests an increased hydrophobic structure. So, according to E_4/E_6 ratio humic substances of Khulna region showed increased hydrophobic and HS of Gopalganj region indicated reduced hydrophobic structure.

8.2.3 Coagulation Threshold: Requirement of time as well as $CaCl_2$ concentrations for coagulation of humic and fulvic acids were investigated by Kononova (1966). Data present in Table 8.2.2 showed the coagulation threshold values obtained with humic and fulvic acids from different regions. These coagulation results indicated that peat of Khulna region has more condensed molecular structure of HA and FA than Gopalganj region.

Table 8.2.2 Coagulation threshold of humic and fulvic acids of peat collected from different regions.

Time required for coagulation	**Gopalganj**		**Khulna**	
	Humic Acid	**Fulvic Acid**	**Humic Acid**	**Fulvic Acid**
	Concentration of Calcium chloride (meq/L)			
1 hour	9.0	9.5	8.0	8.5
4 hours	8.0	8.0	6.5	7.0
6 hours	7.0	8.0	6.0	7.0
24 hours	6.0	7.5	5.5	6.0

8.2.4. Elemental compositions: Results of elemental compositions of HA and FA are presented in Table 8.2.3. Decreasing carbon content may be explained by more intense decomposition of organic and decreasing H content indicated substitution or fusion of aliphatic chains to form aromatic groups' carbon in HA of Khulna region. A relative increase of nitrogen in HA could be due to incorporation of N in HA through condensation of lignin with proteins (Stevenson, 1994) or due to complexation of N with lignin (Hsu and Lo, 1999). Peat of both regions showed the lower C/O ratio in FA than HA indicated the presence of more O-containing group in FA such as carboxylic-ketonic (C=O, COO-) and phenolic-alcoholic (OH). Moreover the HA and FA of Khulna region showed the lower C/O ratio in indicated more O-containing group (C=O, COO, OH) than that of Gopalganj region.

Table 8.2.3 Elemental composition (%) of humic and fulvic acids from peat of different regions.

Sources	Humic Substances	C	H	N	S	O	C/H	C/O
Gopalganj Peat Soil	**Humic acid**	48.4	5.6	3.73	0.51	41.76	8.64	1.16
	Fulvic acid	43.2	5.8	2.17	0.81	48.02	7.45	0.90
Khulna Peat Soil	**Humic acid**	47.3	5.3	4.08	0.31	43.04	8.92	1.10
	Fulvic acid	41.5	5.4	3.88	0.65	48.56	7.69	0.85

8.2.5. FTIR Spectroscopy: From FTIR spectra of humic acid of Gopalganj (GHA): one small peaks at 2963 cm^{-1} (aliphatic C–H), a sharp peak of unsaturated C=C at 1655 cm^{-1} (aromatic C=C, COO-, C=O), a sharp peak at 1561 cm^{-1} (amide II bonds), a peak at 1415 cm^{-1} (phenolic OH deformation and C–O stretch), a sharp peak at 1158 cm^{-1} (symmetric bonding of aliphatic CH_2, OH, or C–O stretch of various groups), and a peak at 801 cm^{-1} (aromatic CH out of plane bending) were observed (Fig 8.2.3 A).

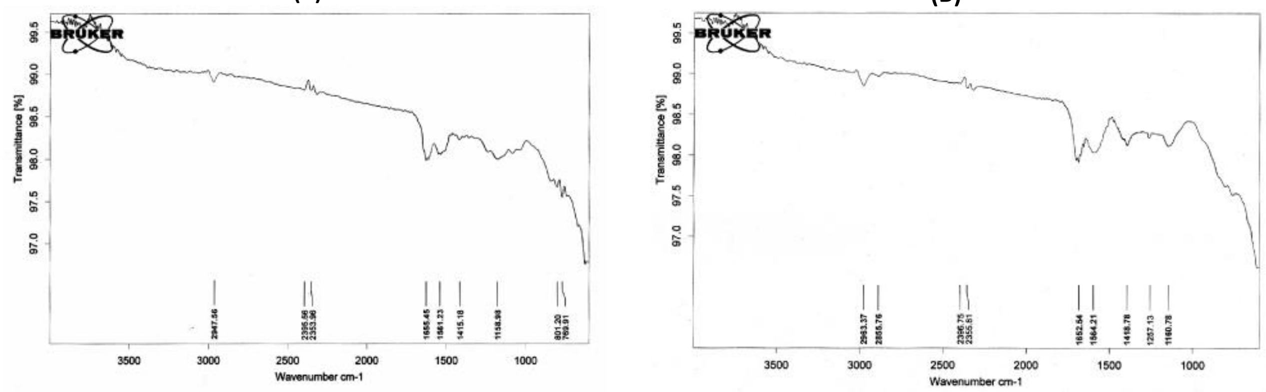
(A) **(B)**

Fig. 8.2.3. FTIR Spectrum of Humic acid (HA) of Gopalganj peat soil (A) and Khulna peat soil (B)

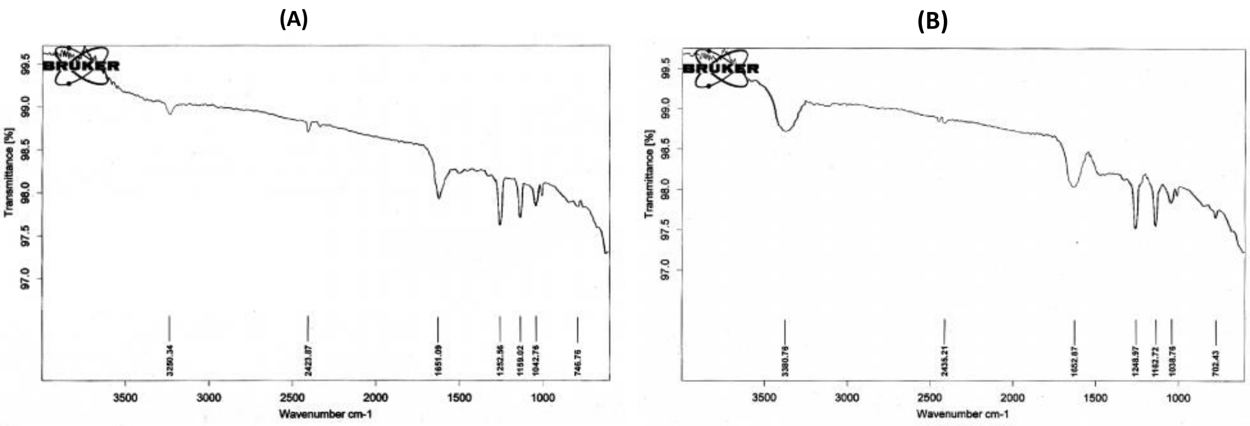

Fig. 8.2.4. FTIR Spectrum of Fulvic acid (FA) of Gopalganj peat soil (A) and Khulna peat soil (B)

The main absorbance bands in humic acid spectra of Khulna region were two small peaks at 2963 and 2855 cm^{-1} (aliphatic C–H),), a sharp peak of unsaturated C=C at 1652 cm^{-1} (aromatic C=C, COO–, C=O), a sharp peak at 1564 cm^{-1} (amide II bonds), a peak at 1418 cm^{-1} (phenolic OH deformation and C–O stretch), a shoulder at 1257 cm^{-1} (aromatic C, C–O stretch), a sharp peak at 1160 cm^{-1} (symmetric bonding of aliphatic CH_2, OH, or C–O stretch of various groups), a peak in the 1100– 1000 cm^{-1} range (C–O stretch of polysaccharide, Si–O stretch) observed (Fig 8.2.3 B). Inbar *et al.* (1990) found similar peak in FTIR spectra of humic acid.

The band assigned to aliphatic C–H stretch occurred at 2950 cm^{-1} was lower and the band 2850 cm^{-1} was absent in GHA. Peaks in the polysaccharide region at 1160 cm^{-1} decreased, while the 1420 cm^{-1} peak became sharper in KHA, as compared to a HA spectrum of Gopalganj peat, which indicated less –OCH_3 and –OH polysaccharide groups in HA of Gopalganj peat (Fig 8.2.3). The relative height of the aromatic region at 1650 cm^{-1} and 1250 cm^{-1} rose as the humification process proceeded from GHA to KHA which indicated the humic substances of Khulna region had become more uniform and stable with the attainment of maturity level (Hsu and Lo, 1999). The ratio between the aromatic carbon/ aliphatic carbon (1650/2850 cm^{-1}/cm^{-1}) increased from GHA to KHA as 0.79 to 1.54 and the aromatic carbon/amide II ratio increased from 0.94 to 1.52, respectively (Fig 8.2.3). These changes in FTIR spectra indicate that easily degradable organic matter constituents such as short aliphatic chains, polysaccharides and alcohols are chemically or biologically oxidized, leading to increased aromatic structures of high stability (Gerasimovwicz and Bayler, 1985).

The FA spectrum was similar to that of HA, as shown in Figure 4.5.4. A relatively weaker peak at 1650 cm^{-1} as compared to HA was found, indicating that fewer C=C bonds were present in FA and a lower degree of aromaticity. However, a sharp peak occurred at 1040 nm regions (C–O stretch of polysaccharide, Si–O stretch), which did not occur in the HA spectra (Fig. 8.2.4). This indicated that more O-containing group (C=O, COO-) than that of HA fraction.