EFFECT OF DIPA AND EDTA ON LEAD PHYTO-AVAILABILITY AND UPTAKE

By

NEELAM NAQVI

NUST201463474MSCEE65214F

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

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بسم الله الرحمن الرحيم

CERTIFICATE

It is certified that the contents and forms of the thesis entitled **"Effect of DIPA and EDTA on lead phytoavailability and uptake"** submitted by Ms. Neelam Naqvi has been found satisfactory for the requirements of the degree of Master of Science in Environmental Science.

Supervisor: _____
Dr. Muhammad Arshad
Associate Professor
IESE, SCEE, NUST

Member: _____
Dr. M. Qasim Hayat
Assistant Professor
ASAB, NUST

Member: _____
Dr. Zeshan
Assistant Professor
IESE, SCEE, NUST

Traveler there is no path

The path forms itself as you walk it.

Antonio Machado

Dedicated to my beloved mother

To the memory of my father: whom I missthe most.

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Neelam Naqvi

Contents

LIST OF ABBREVIATIONS

EDTA	Ethylene diamine tetra acetic acid
DIPA	Diiso propanol amine
Pb	Lead
AAS	Atomic Absorption Spectroscopy
FDA	Food and Drug Administration
$MgCl_2$	Magnesium Chloride
BCF	Bioconcentration Factor
TF	Translocation Factor
mmol kg^{-1}	Millimole per Kilogram
mg kg^{-1}	Milligram per Kilogram
Conc.	Concentration
NTA	Nitrilotriacetic acid
HEIDA	Hydroxy ethyl imino di acetate acid
P	Probability of null
T	Student's t-value
Coef	Coefficient
SE Coef	Standard error coefficient

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ABSTRACT

Lead (Pb) is challenging to remediate due to its persistent toxicity, non-biodegradability, low mobilization and bioaccumulation in food chain. Chelating agents may enhance Pb phytoavailability and phytoextraction. The aim of this study was to investigate effect of EDTA and DIPA to enhance Pb phytoavailability and uptake. For this purpose soil was spiked with Pb concentration (0, 500, 750, 1000 and 1500 mg kg^{-1}) and amended with EDTA and DIPA at dosage level (0, 1.5, 3, 5, 7.5, 10 mmol kg^{-1}) for plantation of *Pelargonium hortorum*. Soil samples were extracted with $MgCl_2$, plant samples were digested with HNO_3:HCl in (3:1) and analyzed through Atomic Absorption Spectroscopy (AAS). The behavior of both EDTA and DIPA was monitored in aspect of vegetative traits, Pb phytoavailability and plant uptake. Biomass of *Pelargonium hortorum* was decreased with increase in concentration of Pb and chelating agents. Phytoavailability of Pb at 1500 mg kg^{-1} with EDTA 10 mmol kg^{-1} was 1.4-folds in comparison to DIPA at same dosage. *Pelargonium hortorum* found to accumulate Pb in following order EDTA> DIPA> Control. EDTA and DIPA at 10 mmol $kg^{-1}$with Pb 1000 mg kg^{-1} were found to uptake Pb 6-fold and 3-foldsin comparison to Pb 1000 mg kg^{-1} alone. Pb uptake decreased at 1500 mg kg^{-1} with both chelating agents. Translocation factor of *Pelargonium hortorum* was <1. On the whole, 10 mmol kg^{-1} of EDTA and DIPA performed better among all dosage. Increasing concentration of cheating agents enhanced phytoavailability and uptake of Pb. Data from present research provide a new insight to use DIPA in phytoremediation to reclaim contaminated soil.

Chapter 1

INTRODUCTION

1.1 Background

World has been facing a dilemma of heavy metal contamination in soil. Heavy metals are basically ubiquitous in environment and badly affect soil and water ecosystem. They enter naturally through weathering of parent material, erosion and volcanic activity while their anthropogenic sources include mining, smelting, electroplating and many other industrial processes (Alaribe and Agamuthu 2015; Chen *et al*., 2000). Emissions from burning of waste containing these heavy metals during combustion let them to enter in our environment (Li *et al*., 2015; Chen *et al.,* 2015).

Due to their persistent toxicity, non-biodegradability, wide distribution and bioaccumulation in food chain, they are considered as the most troublesome type of pollutants (Li*et al*., 2015). Because of their long time persistency and residence in soil, heavy metals imply technology challenge in order to utilize the soil again (Salazar and Pignata 2014).

Heavy metals such as, copper, cadmium, Lead, mercury, zinc and nickel are considered to have densities greater than $5g/cm^3$ (Chen *et al*., 2015). Severe persistence of these heavy metals makes them most toxic in the environment whereas soil contamination depends on level of contamination (Zhou *et al.,* 2014). Toxic metals like Pb and Cd exposures are connected with a lot of conditions. On top of it their sources to enter soil may also vary (Norton *et al*., 2015).

Trace amount of few heavy metals such as Zn, Cu and Ni is significant because they are considered as micronutrients essential for human body. While other

heavy metals such as Cr, As, Pb and Cd are carcinogenic even in trace amounts (Li *et al*., 2015).

Mobilization of Pb is monitored to be exceptionally low, less than 1%. This factor hinders phytoremediation of Pb (Sarkar *et al*., 2008). Plant biomass and growth may also observe to get reduced due to the toxicity caused by the insolubility and immobilization of Pb (Mani *et al*., 2016; Chen *et al*., 2015).

Past studies show that to increase the mobility of heavy metals different natural and synthetic chelating agents are used. The efficiency of these chelating agents may vary with type of soil and plant used (Chirakkara *et al*., 2015). The Phytoavailability of heavy metals also depend on type of chelant, metal specification, plant metabolism and soil (Mahar *et al*., 2016; Levresse *et al*., 2012).

EDTA an amendment in past was used as fertilizer but now used as a supplement in soil washing and found to make metal-EDTA complexes to increase solubility and Phytoavailability of metal in soil. The application of EDTA increases Pb solubility by making Pb-EDTA complex and increasing the availability for phytoextraction (Wu *et al*., 2003). While the solubility of metal with EDTA is influenced by different factors such as; soil pH, metal concentration and metal specie (Saifullah *et al*., 2009).

Appeal of EDTA is enhanced due to its high efficiency of extraction, metal-EDTA complexes, low biodegradability and advances in recovery and recycling (Zhang *et al*., 2013). EDTA is regarded as biologically stable and most commonly used chelants among all (Hu *et al*., 2014). It enhances bioavailability as well as is an effective synthetic chelating agent (Najeeb *et al*., 2009). In mobilization of metals from solid phase to soil solution EDTA turns to be the consistent amendment (Park *et al*., 2011).

Another amendment DIPA is basically an aliphatic amine of isopropyl alcohol mostly used in industrial application such as in different variety of cosmetics and skin care products. This is often used to check the dermal toxicity in animals (Saghir *et al*., 2007; Stott *et al*., 2008; Johnson *et al*., 2007).

Heavy metal if present in soil are not easy to reclaim and also the recovery of soil to its original state is a difficult task. Plants growing in such contaminated soil also get stressed. Plants remediating soil not only uptake those heavy metals but also recover the soil. Soil basically provides a platform to heavy metals so they can store, exchange and enter food chain. Soil contaminated with heavy metals when provide an interacting environment to air, water and rock may cause harmful impacts on human and animal (Obiora *et al*., 2016).

An advantageous and environmental friendly process for the removal of contamination from soil and water is of immense importance. Using plant for this purpose is known as phytoremediation (Chen *et al*., 2015). It is considered as a suitable alternative to approach soil decontamination even at large scale (Bauddh *et al*., 2015; Chen *et al*., 2007). This technique not only degrades, stabilize and eliminate pollutants from soil but also avert and remediate it. Plants used for the function of phytoremediation improve its performance in combination with enhancing agents (Romeh, 2015; Vigliotta *et al*., 2016). These enhancing agents help increasing the metal mobility in soil solution and involve the absorption of metals by root and further translocated to aerial parts of plant in the process known as phytoextraction (Paulo *et al*., 2014).

The method of phytoremediation has many advantages over other techniques which include social and esthetic values, cost effectiveness, sustainable and environment responsive (Chen *et al*., 2014). Its value may rise even more when the

plants used for the treatment of contaminated soil are native. For a successful attempt to execute this technique is to understand the condition of plant, its biomass production, toxicity intensity with metal, ability to grow, plant organ in which metal will be collected and growth time period (Salazar and Pignata 2014).

Plants used in the process of phytoremediation often use two approaches; one is to use a natural hyperaccumulator plant and second is to use a plant with high biomass whose efficiency increases with the use of chelates (Chibuike *et al*., 2014). Hyperaccumulator plants are those with low biomass, slow growth, and high tolerance to contaminant and have a potential to extract pollutant from soil (Chen *et al*., 2004; Farid *et al*., 2013; Chaney *et al*., 1997). On other side high biomass plant species could also accumulate a variety of heavy metals and possess characteristics rapid growth, high biomass, extensive root system and are capable to tolerate high amount of heavy metal (Sheoran *et al*., 2016; Chibuike *et al*., 2014).

Hyperaccumulator specie allows phytoextraction in which plants uptake contaminants from soil in its root and then transfer to the above ground parts of plants (Mahar *et al*., 2016). The efficiency of removing a targeting heavy metal from soil is based on availability of that metal in soil solution with amendments and extraction in roots and further translocation to shoots (Saifullah *et al*., 2009; Chen *et al*., 2006).

Plant selection for the process of phytoremediation should have these two characteristics; one is to have high biomass along with fast growth and other is to accumulate more metal (Patel and Patra 2015).

Main limitation in process of phytoextraction occurs when plants poses low bioavailability and have a limited translocation factor (Dede and Ozdemir 2016). When the ability of plant to transfer heavy metal in above ground part of plant is low, chelate induced phytoextraction could help out in increasing the removal of

contamination from soil (Najeeb *et al*., 2009). All such problems can be avoided with a vast knowledge.

Scented geraniums (*Pelargonium*) are known for its commercial application as in cosmetic industry and as flavoring in foods. The geranium oil and its major components have gained acceptance in food industry as by the approval from American Food and Drug Administration (FDA) in food use (Nakbanpote *et al.,* 2016). A research on six scented pelargonium cultivars had already been conducted to know their potential for phytoremediation. Among which three were found to be Pb hyperaccumulator (Attar, Atomic snowflake and Colorinda) (Arshad *et al.,* 2008).

1.2 Objectives

Keeping in view all the insight gained from the latest research, it could be hypothesized that DIPA and EDTA may enhance Pb accumulation in plant. The specific objectives were;

a) To compare EDTA (ethylene diamine tetra acetic acid) and DIPA (Di iso propanol amine) for desorbing Pb from soil,
b) To evaluate the potential of scented geranium *(Pelargonium hortorum)* for phytoremediation of Pb contaminated soil.

1.3 Scope of Study

Use of amendments for enhancing the process of phytoremediation is of great attention worldwide. Using ornamental plants for phytoremediation is more preferable. Various results however are showing the influence of amendments in phytoremediation. The scope of the study was to provide a substantial assessment of EDTA and DIPA on phytoavailability and uptake of Pb in growth response of scented geranium.

Chapter 2

LITERATURE REVIEW

2.1 Heavy Metal and its Removal

Non-biodegradable nature of heavy metals allows them to persist in the environment. Their persistence in soil for such a long period of time raised issue of environment and health. Presence of such metals in environment led them to accumulate and enter in food chain. This contamination requires great attention in order to remediate (Ali *et al*., 2013).

Presence of these heavy metals in soil not only cause accumulation in food chain as well as disturb soil ecology and water quality. Excessive presence of heavy metals in soil cause ecological imbalance. The adverse harmful effects of heavy metals presence in soil demands to remediate such issue (Alaribe and Agamuthu 2015).

2.2 Lead as Heavy Metal

Heavy metals such as Pb is considered to have limited bioavailability in soil, as Pb is mostly bound to organic and inorganic constituents or are present as insoluble precipitates (Sallami *et al*., 2013).

2.2.1 Sources of Pb

Aerial emission from combustion of leaded petrol, battery manufacture, herbicides, insecticides, mining, smelting, anti-spark linings and leaded paints are some anthropogenic sources of Pb in environment (Mahar *et al*., 2015; Zaier *et al*., 2010).

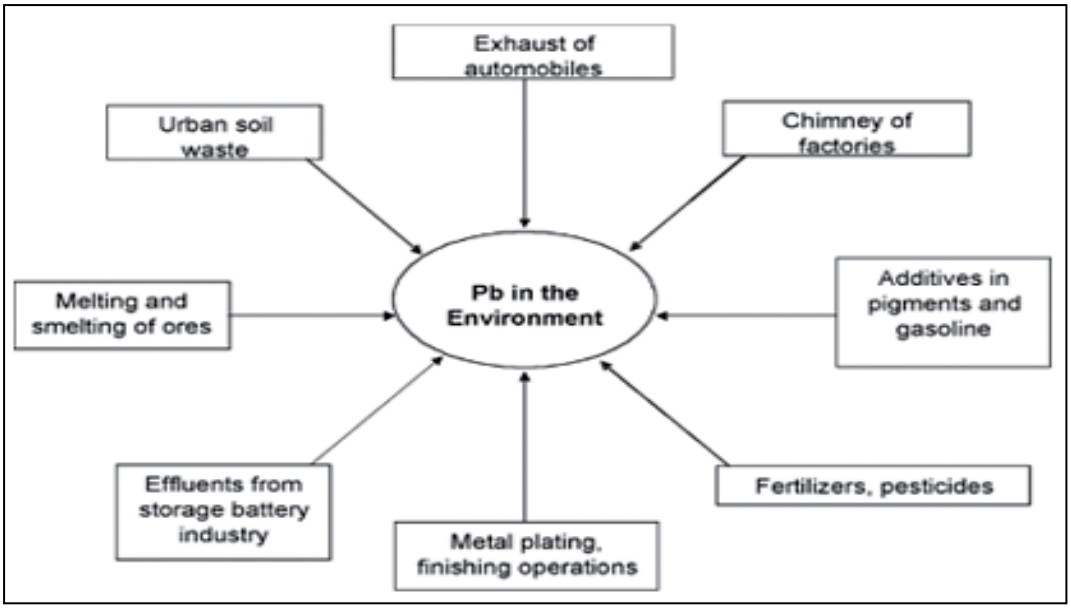

Figure 2.1: Sources of Pb pollution in environment (Sharma and Dubey 2005)

2.2.2 Exposure Routes

There can be different exposure routes to Pb which include; dermal, inhalative and oral exposure. Different heavy metals cause many undesirable effects on human when exposed to a metal through any of this route (Ali *et al.*, 2013).

2.2.3 Pb Toxicity

Pb poisoning in children may impact their brain development, decreased RBC, slower reflexes and slow learning while adults may suffer through miscarriages, shortened life, increase blood pressure and neurological damage etc (Ali *et al.*, 2013 ; (Tchounwou *et al*., 2014). Pb is carcinogenic to humans even at low concentration of 400–500 mg Pb kg soil US-EPA (2001).

2.3 Phytoremediation of Heavy Metals

Among different methods to remediate soil contaminated with heavy metals, phytoremediation got consideration because of its ease and benefits. This technique was preferred due to its efficiency, cost effectiveness and environmental restoration technology (Greipsson, 2011). This technique involve the process of phytoextraction

or phytoaccumulation in which the metals present in soil were first taken by roots of the plants and additionally translocated to the above ground parts of the plant (Aranisola *et al*., 2013). Through phytoremediation not only soil was recovered to its original condition but also different hyperaccumulator species were discovered which eventually used further for this purpose. This makes phytoremediation an important method to be used in active modern researches (Ali *et al*., 2013).

Kumar *et al*. (2013) reviewed minimization of heavy metals with technique of phytoremediation in which it was concluded that use of plant makes it acceptable to reclaim the environment by reducing toxicity caused by heavy metal pollution. Efficiency of phytoremediation was observed by studying potential of 15 different plants through experimentation to find out the hyperaccumulator specie. The results notably demonstrate that most of the plants have great potential for high concentration of heavy metals. Among which *Salvia spinosa* was considered as hyperaccumulator and are suggested for phytoremediation of contaminated soil (Kazemeini *et al*., 2013).

Potential of plant for phytoremediation of Pb contaminated soil was confirmed in another study by Cheng *et al*. (2005)in which it was observed that corn could be used as a bioenergy source and can remediate soil as well. *Magnolia grandiflora*, *Ligustrum vulgare* and *Phoenix dactylifera* showed its potential for heavy metal accumulation and answer that different part of these plants can be used as biomonitors when it comes to the determination of heavy metal concentration (Demirayak *et al*., 2011).

For removal of heavy metals from soil 16 different plant species were studied to accumulate toxic metals (Pb, Cu, Zn, Co, Ni, and Cr). On the basis of bioconcentration factor, translocation factor and bioaccumulation it was evaluated that concentration in roots of these plants were in pattern of Cu>Cr>Zn>Ni>Pb>Co and

shoot concentration follow Cu> Zn> Cr> Pb>Co Ni> whereas no plant species is observed to be hyperaccumulator but was suggested for phytostabilization of some heavy metals (Malik *et al.*, 2010).

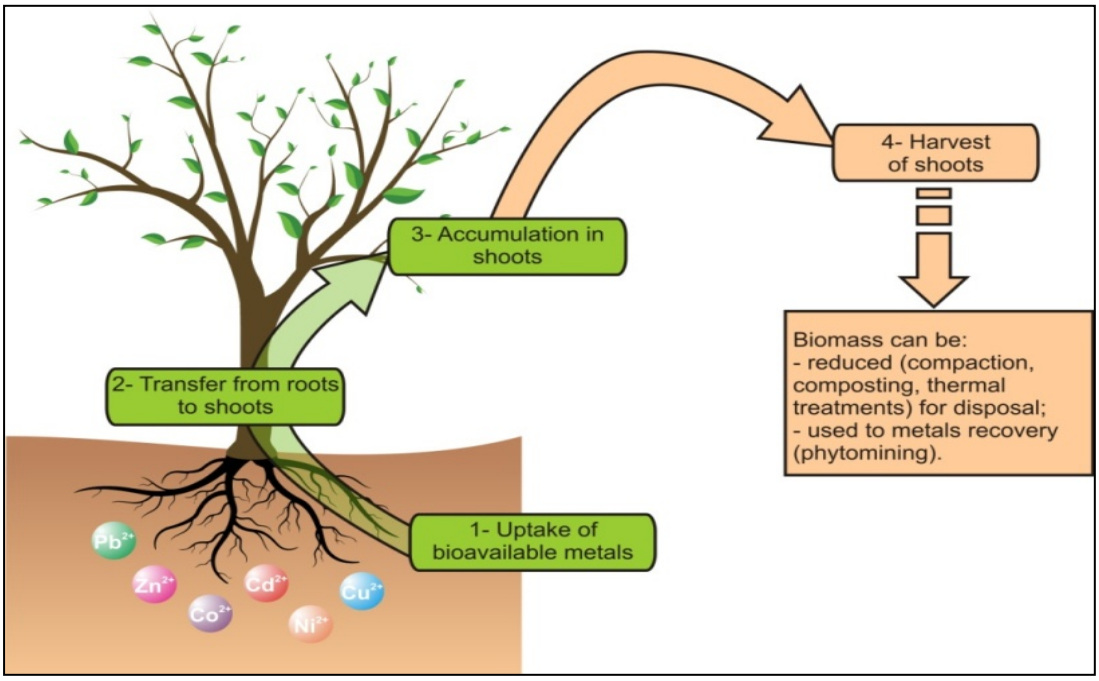

Figure 2.2: Schematic representation of phytoextraction of heavy metals from soil (Favas *et al.*, 2014)

Fire Phoenix and *Medicago sativa* L.were evaluated for removal efficiency of PAHs. The results advised that *Fire Phoenix* and *Medicago sativa* L.should be practiced in PAHs contaminated soil for remediation (Xiao *et al.*, 2015).Potential of plants to remediate heavy metals contaminated soil and its advantages such as social and esthetic values, cost effectiveness, sustainable and environment responsive made this technique preferable on top of others (Chen *et al.*, 2014; Chen *et al.*, 2015).

2.4 Potential of Amendment in Chemically Induced Phytoremediation

Bioavailability of heavy metals in soil get influenced by different factors such as, soil moisture, soil texture, cations exchange capacity, soil pH, biochemical

processes and redox potential (John and Leventhal, 1995). Whereas plant related features which control metal bioavailability involve; root absorption factor, root depth and density, acidification of rhizosphere, growth of plant, and biomass of plant species (Sheoran *et al*., 2016).

Chemically induced phytoremediation basically means introduction of chelants to help phytoextraction (Luo *et al*., 2006). To enhance the metal extraction process chelation technology is known to be used frequently due to its performance. However while using this chelation technology a balance between metal extraction stability and biodegradability of these chelants is significant (Chauhan *et al*., 2015)

Chelants speed up the process of heavy metal availability which was bound to solid phase of soil by breaking the state of equilibrium between soil liquid and solid phase of heavy metal. A new state of equilibrium is achieved when soil intensity adsorbed to metal-chelant is reduced and metals are released in soil solution (Sheoran *et al*., 2016).

Chelating agents are known for their potential to free heavy metal present on cation anion exchange site by forming metal chelate complexes. By making these complexes, chelating agents enhance bioavailability of metals to uptake by plant (Dipu *et al*., 2012). Alaribe and Agamuthu (2015) studied potential of another plant *Lantana camara* with organic additives (EFB and SMC) as amendments in soil spiked with different doses of Pb. Results reported that *Lantana camara* has potential for phytoremediation with organic additives as amendments. As with EFB and SMC metal reduction of 52.06 to 88.03% and 45.10 to 82.73% was observed respectively.

Pedron *et al*., (2014) evaluated the potential of chelating agents on Pb contaminated site. Oxalic acid (OSS) and EDDS were used along with EDTA in Pb assisted phytoextraction. They hypothesized that EDSS was more successful in

mobilizing Pb from soil particles and is considered as an appropriate alternative to EDTA for remediation of Pb contaminated soil. Same procedure was done with plant *Brassica juncea* and results showed an increased concentration of Pb with chelators as compared to control. Ruley *et al*. (2006) monitored capability of amendments (EDTA, HEDTA, DTPA, NTA and citric acid) for Pb accumulation in *Sesbania drummondii* and reported that accumulation of Pb in roots increases 4-5folds while in shoots 40-folds increased were observed in the presence of chelators. Kim *et al*. (2016) observed in his study potential of EDTA with reducing agents for heavy metals (Pb, As, Cu and Zn) extraction. The results depicted that overall efficiency of almost 90% was observed when combination of reducing agents and EDTA were applied.

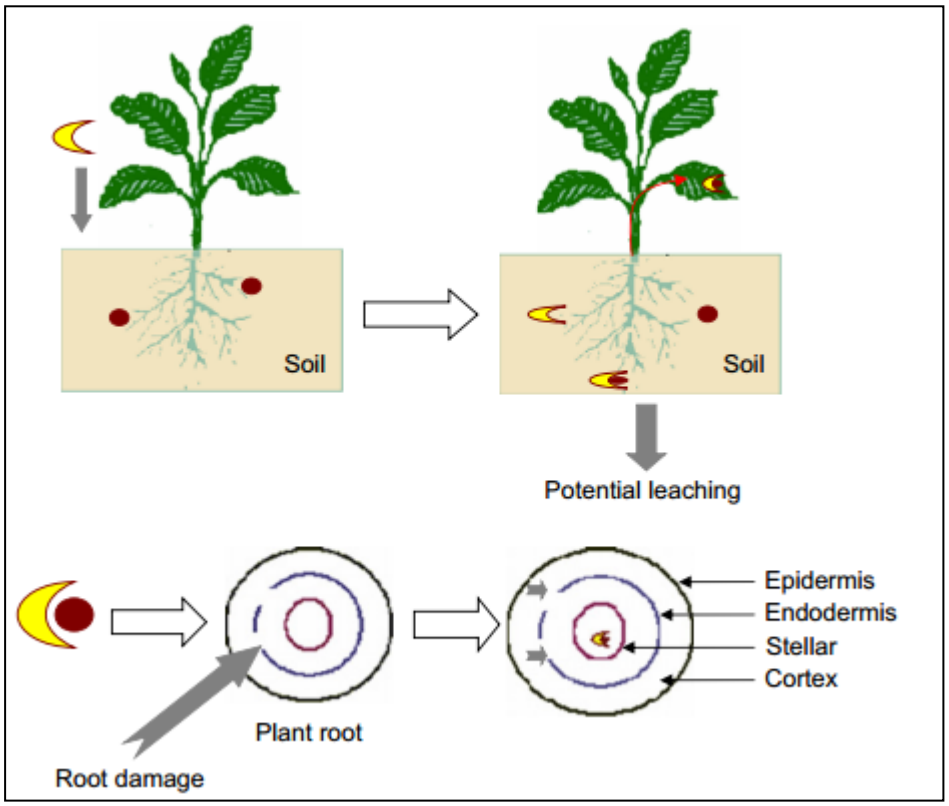

Figure 2.3: The schematic representation of uptake of metal-chelant complexes by plant roots, their translocation upward, and the potential leaching of metals into the surrounding environment in the process of chelant-enhanced phytoextraction (circle

and crescent is representing metals and the applied chelant in soil respectively.

(Lestan *et al*., 2008)

2.5 Role of EDTA

Application of chelating agents in contaminated soil with plant based technique show positive changes in soil condition. Chelating agents like EDTA, EDDS and NTA were found to be exceptionally useful in enhancing the process of phytoextraction. Different experiments revealed that outcome of application of chelating agents depend upon type of soil, plant used, its toxicity to plant etc. It was also observed that effectiveness of these chelating agents vary according to the type of heavy metal they are exposed to (Evangelou *et al*., 2007).

Different reports (Farid *et al*. 2013; Shahid *et al*. 2014) concluded that EDTA assisted Phytoextraction increases plant growth parameters as well as accumulation of different heavy metals (Cd, Cr, Pb, and Zn). EDTA not only available the metals by making complexes but also increase the transportation of metal from root to shoot. EDTA was considered successful to accumulate Pb in shoots to more than 1% of dry biomass of shoot. The ability of chelants such as EDTA, EDDS and NTA were observed upon metal extraction. Results showed that stability of metal-chelant complex follow the trend as EDTA > EDDS > NTA in metal extraction (Chauhan *et al*., 2015). Chiu *et al*.(2005) tested different chelating agents such as NTA, HEIDA and EDTA on enhancement of metal uptake in two different plants such as *Vitiveria zizaniodes* (vetiver) and *Zea mays*. With high biomass potential, Vetiver and *Zea mays* both showed potential to increase concentration of heavy metals in their shoots with the application of these chelators.

2.6 Role of EDTA in Metal Accumulation

Chelating power of chelants gets affected due to pH. It may happen as remobilization of other chelant metal complex if present and due to dissolution of minerals such as Ca^{2+} as presence of cations like Ca^{2+} effect metal-chelant complex and its mobility. Extraction of metals with the application of EDTA gets influenced due to a struggle between Ca^{2+} and heavy metal present in soil. (Luo *et al*., 2006).

Among different amendments like citric acid, sulphur etc EDTA was known for its best results with Pb by increasing its solubility. Different studies suggested that EDTA has capacity to make metals like Pb available to plant which has low solubility. So the evidence on ability of EDTA by detaching metals from soil and making complexes to increase their availability strengthens this point to use it for phytoremediation (Shahid *et al*., 2015).

Suthar *et al*. (2014) investigated different dosage of EDTA (0, 1.25, 2.5 and 5mMkg^{-1}) in soil contaminated with Pb and Cd with maize and sesbania. They reported that in comparison to control 13.1 and 3.1 fold increase in soluble fraction was observed in Pb and Cd when treated with 5mMkg^{-1} of EDTA. On plant efficiency also 5mMkg^{-1} of concentration turns out to be best.

The role of citric acid and EDTA was observed on improving metal accumulation, plant growth and Mn toxicity stress alleviation. Three-week-old plantlets of *J.effusus* were subjected to various treatments in the hydroponics as: Mn (50, 100 and 500M) alone, Mn (500 M) + citric acid (5 mM), and Mn (500 M) + EDTA (5 mM). It was noticed that Mn accumulation and translocation was increased by both EDTA and Citric acid (Najeeb *et al*., 2009).

2.7 EDTA Application

Neugschwandtner *et al*.(2008) believed time of application and the way to add these amendments play an important role to carry out a successful phytoextraction process. In study carried out by these authors, they found that application of EDTA in single dose form increases the efficiency of phytoremediation by uptake of Pb and Cd as compare to split dose.

Amendments such as EDTA should be applied before 2 weeks of harvesting plant for an effectual phytoextraction (Wang *et al*., 2009). Chiu *et al*. (2005) tested application of different chelating agents such as NTA, HEIDA and EDTA on 16-20 days before harvesting plant. They reported that surge time played an important role in application of amendments. 16-20 days surge time maximize accumulation of heavy metals in both *Vitiveria zizaniodes* and *Zea mays*.

2.8 Uptake of Pb with EDTA

Saifullah *et al*. (2009) examined bioavailability of heavy metal like Pb and effect of chelating agents like EDTA to enhance their availability and accumulation. The strong chelating abilities of EDTA allow it to assist Pb based phytoremediation. Through EDTA metal concentration is increased in above ground parts of plant as the mobility and the translocation from root to shoot is raised.

Jez *et al*. (2015) investigated the effect of EDTA to reduce Pb concentration in soil. The concentration of Pb was determined both before and after soil washing with 60mmol kg^{-1} of EDTA. Results confirmed that treating Pb contaminated soil with EDTA could help improving soil condition by removing Pb from soil. Zhang *et al*. (2013) in his study evaluates the potential of EDTA and three of its derivatives; CDTA, BDTA and PDTA respectively for the washing of soil and extracting metals

such as Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Ca^{2+}, and Fe^{3+}. The results show that EDTA and its derivates were capable for soil washing.

Considering phytoremediation as an effective method to remediate contaminated soil, Cho *et al*. (2008) studied *Allium fistulosum* (green onions) for phytoremediation of lead spiked soil in both presence and absence of EDTA and PDTA growth and accumulation of Pb. It was clearly noticed that in the presence of EDTA accumulation of Pb in stem of green onions was enhanced and had stronger effect. Pb accumulation was enhanced to about 225 mg kg^{-1} in the presence of EDTA while with no EDTA it only goes up to 25 mg kg^{-1} which shows poor bioavailability of Pb without chelants.

Multiple ornamental grasses and plants were exposed to soil contaminated with Pb, Cd and Zn with high dosage of EDTA (120 mmol kg^{-1}). Plants grown for remediation on of soil exhibit high biomass production and is considered suitable for greening (Jelusic and Lestan 2015). The effect at different doses of EDTA and Citric acid was studied to increase the potential accumulation in *Marigold* on soil contaminated with Cu, Zn, Pb and Cd. It was recommended to use *Marigold* for the phytoextraction of these heavy metals from soil using EDTA and Citric acid as amendments (Sinhal *et al.,* 2009). To increase the availability of Pb to *Eucalyptus camaldeulensis* different dosage of EDTA such as (0, 5, 10 and 15mmol kg^{-1}) was studied. Soil amended with 15 mmol kg^{-1} of EDTA. So it was suggested that these dosage could be used to enhance availability of Pb to soil and uptake by plant such as *E. camaldeulensis* (Sallami *et al*., 2013).

Effects of chelate induced phytoextraction of Pb were observed on *Cynara cardunculus.* Analysis of both Pb-EDTA and Pb-EDDS complex after giving exposure reveal that EDTA was more capable of up-taking Pb to root and further

translocation towards shoot. Thus Pb spiked EDTA treated soil proof that it has more capacity to enhance Pb extraction than EDDS (Epelde *et al*., 2008).

The effect on the growth of an ornamental plant *Arundo donax* L. grown in soil contaminated with As, Pb and Cd with citric acid, EDTA and acetic acid as amendments was studied. At concentration of 2.5 mmol kg^{-1} of citric acid and acetic acid and 5 mmol kg^{-1} of EDTA the As Pb and Cd concentration in shoots were amazingly increased. So it was considered that these amendments can be used for remediation of these heavy metals with the help of ornamental plants (Miao *et al*., 2011).

Bidens maximowicziana another hyperaccumulator plant was reported with an extraordinary capacity for Pb accumulation. To the above ground parts of plant the translocation of Pb was promoted with increasing dosage of EDTA application and gave a Pb distribution order like this; leaf > stem > root. The results suggested that *Bidens maximowicziana* can be used for remediation of Pb contaminated soil with EDTA (Qi *et al*., 2007).

EDTA helped to increase the phytoavailability and accumulation of Pb in contaminated soil. With increase in concentration of EDTA, effect on phytoavailability and uptake enhances were proved (Zaier *et al*., 2014; Cho *et al*., 2008; Meer *et al*., 2005; Saifulah *et al*., 2009; Sallami *et al*., 2013).

2.9 Uptake in Pelargonium

An important oil bearing crop *Pelargonium graveolens* L. Hér was selected with different ratio of tannery sludge (TS) and soil rich in heavy metals. The results of this study best justify that this plant is a good phytostabilizer of heavy metals present in TS and soil and can be used to accumulate heavy metal from TS and soil as well as is an alternative for oil yield (Patel and Patra 2015).

Availability and uptake of different heavy metals (Cd, Cu, Zn, Pb, Ni, Cr) were observed in *Pelargonium hortorum*. It was reported that availability of metal depends directly on level of metals applied whereas heavy metals concentration was maximum in roots as compare to aerial parts (Orrono and Lavado 2011; Orrono *et al*., 2012).

Effect on growth and accumulation of heavy metals were studied in heavy metal contaminated soil with (Cd, Cr, Cu, Pb, Ni and Zn) on *Pelargonium hortorum.* Effects such as reduction in biomass production and heavy metal accumulation pattern followed roots > shoots were reported by Orrono and Lavado (2009).

Chapter 3

MATERIALS AND METHODOLOGY

3.1 Soil source

Soil sample was taken from the premises of National University of Sciences and Technology (NUST) Islamabad.

3.2 Soil Characterization

Physiochemical characteristics of soil determined were: pH, EC, and Organic matter, texture and soil composition.

3.2.1 Soil pH

Soil pH was checked to ensure its strength for plant growth. 10g of air dried soil (< 2 mm) was taken in 100 mL glass beaker. 50 mL of distilled water was added using a measuring cylinder. It was left on a shaker at 100 rpm for 30 min. In suspension combine electrode (HANNA 8520) was placed and reading was taken after 30 seconds (Tang *et al*., 2014).

3.2.2 Soil Electrical conductivity

Soil EC was measured to make sure about the ability of dissolved material to conduct electricity through it. 10 g of soil (< 2 mm) was taken in 100 mL glass beaker. 50 mL of distilled water was added using a measuring cylinder. It was left on a shaker at 100 rpm for 30 min. Soil EC was then measured by putting EC meter electrode into suspension made and reading was taken as get stable (Meers *et al*., 2005).

3.2.3 Organic Matter Content

Organic matter content was determined by dry combustion method. Pre-weighing 10 g of soil in china dish and let to heat in a muffle furnace (NEY M-525) at

350 ºC for 3 hours. After heating for 3 hours china dish was placed in desiccator for about 30 min to cool down. It was then weighted again to find out the percentage organic matter content of soil (Cheng *et al*., 2015).

3.2.4 Moisture Content

Ten gram air dried soil (< 2 mm) was taken in a Petri dish. It was dried in an oven, with lid unfitted, at 105 ˚C overnight. Afterward it was removed from oven; cooled in a desiccator for about 30 min and re-weighed. Moisture content was calculated using following relation:

$$\% \text{ moisture in soil} = \frac{\text{wet soil} - \text{dry soil}}{\text{dry soil}} \times 100$$

3.2.5 Soil Texture

Soil texture was determined to find out its suitability for plant growth. For this soil and sand was taken in 1:1. 25 g of soil and sand was measured separately and mixed in a 100 mL beaker by adding distilled water to make a paste. The paste was continuously mixed well with the help of glass spatula. Time to time spatula was removed from the beaker to check if the soil mixture drop down from it to find out the right proportion (Salvich and Petterson 1993).

3.2.6 Elemental Analysis

Soil composition was found out through x-ray fluorescence (XRF) (JSX-3202M) elemental analyzer. Soil sample was set in a ring and pressure was applied to make a pellet. This pellet was further placed in the equipment and took few seconds to find out the elemental composition of sample (Cheng *et al*., 2015).

3.3 Soil Preparation

Soil sample was taken and air dried for 3 days. A Ball Mill (0001/SCME/particulate technology lab/Eqpt/0092) was used for grinding soil in fine powder by adding balls in ball mill. Balls and soil were added to ball mill in form of

1:4 soil and balls. The rotation for ball mill was then set to 40-50 rpm for 20 min. After the completion of grinding soil for 20min it was further sieved through 2mm sieve manually.

3.3.1 Soil Spiking

The prepared soil was then weighed and spiked by adding Pb according to treatments selected. Pb was added to soil in powder form. The level of soil metal contamination were control, T1 (500), T2 (750), T3 (1000), T4 (1500) mg kg^{-1}. The spiked soil after mixing well was packed in sampling bags for 7 days and labeled according to treatments.

3.3.2 Amendments

Two different amendments such as, Ethylene diamine tetra acetic acid (EDTA) and Diisopropanol amine (DIPA) were taken to enhance the availability of Pb in soil by forming Pb-EDTA and Pb-DIPA complexes for uptake by plant. The dosages were selected as 0, 1.5, 3, 5, 7.5 and 10 mmol kg^{-1} respectively.

3.4 Soil Experiment

3.4.1 EDTA Amended Soil

Soil experiment was conducted by application of EDTA in previously spiked soil to enhance the mobility of Pb. Firstly 10 g of soil was weighed in each Petri plate with 5 replicates for each set. Set was labeled as T0, T1, T2 T3 and T4 with each dosage of EDTA. Each of the selected dosage (0, 1.5, 3, 5, 7.5 and 10 mmol kg^{-1}) were added in spiked soil in solution form. Each one of the dosage was dissolved in 3mL of de-ionized water and supplemented in petri plate through pipette.

3.4.2 DIPA Amended Soil

To enhance the availability of Pb, another amendment DIPA was introduced. Initially 10g of soil was weighed in each Petri plate with 5 replicates for each set. Set

was labeled as T0, T1, T2 T3 and T4 with each dosage of DIPA. Each one of the chosen dosage (0, 1.5, 3, 5, 7.5 and 10 mmol kg^{-1}) were added in spiked soil in solution form. Each one of the dosage was dissolved in 3mL of de-ionized water on an Orbital Shaker for perfect mixing and supplemented in petri plate with the help of pipette.

3.4.3 Incubation

After that these Petri plates with amended soil were covered carefully and enclosed with Para films to maintain moisture level. Then allowed to place in incubator (IN-110 Memmert) at 25˚C for a contact time of 7days.

3.4.4 Oven Dry

After completing an exposure period of 7 days petri plates were removed from incubator and put in oven for a drying time period of 1 day at 70 ˚C (Neugschwandtner *et al*., 2008). After being oven dried for 1day these soil petri plates were taken out from oven and cool at room temperature for some time. Soil from these petri plates were then separated with the help of spatula and sealed in sampling bags. These sampling bags were named according to the samples and set for analysis.

3.5 Metal Availability in Soil

Pb contaminated soil sample for both EDTA and DIPA were analyzed by taking 5 g of soil in a volumetric flask, labeled and extracted with 50 mL of 1M $MgCl_2$ on an Orbital shaker (LABCON, SPO-MP8) for 2 hours (Sarkar *et al*., 2008). After shaking samples for 2hours, samples were removed from the shaker. Then samples were allowed to filter with the help of Whatman No. 52 filter paper. The bottles with filtered samples were stored at 4˚C prior to analysis.

3.6 Heavy Metal Analysis through Atomic Absorption Spectroscopy

Mobile Pb in the form of EDTA-Pb complex and DIPA-Pb complex in experimental soil were determined by using Flame Atomic Absorption Spectroscopy (FAAS).

3.7 Soil Preparation for Pot Experiment

Soil collected from NUST was air dried for 3 days. After drying soil for 3days it was crushed into fine powder by using a Ball Mill (0001/SCME/particulate technology lab/Eqpt/0092). Following the process of grinding the soil was then screen through a sieve (< 2 mm) manually.

3.8 Soil Spiking for Pot Experiment

Sieved soil was then weighed according to each set of treatment (control, 500, 750, 1000, and 1500 mg kg^{-1}). Each treatment includes 30 pots. The soil was weighed according to each treatment and spiked with Pb in powder form. Pb was mixed in soil manually. Contact time of 7 days was given.

3.9 Greenhouse Experiment

The seeds of *Pelargonium hortorum* were collected locally and germinated in contamination free soil for 6 weeks. After a period of 6 weeks seedlings were removed from uncontaminated soil and transplanted in spiked soil. Each pot contains 700 g of spiked soil and allows the exposure time of 90 days in a greenhouse constructed in National University of Science and Technology (NUST) Islamabad. The room was maintained to avoid unnecessary temperature and light.

3.9.1 EDTA Supplement

When plants were exposed to Pb contaminated soil for a time period of 90 days in Greenhouse, EDTA amendment was supplemented 3 weeks before harvesting. EDTA dosage (0, 1.5, 3, 5, 7.5 and 10 mmol kg^{-1}) was applied to each pot in form of

solution. A session of 3weeks was given after application of 40mL of EDTA to each pot to enhance Pb uptake in plant. Each set of plant included 5replicates.

3.9.2 DIPA Supplement

Another set of *Pelargonium hortorum* was allowed to grow in Greenhouse with an exposure period of 90 days and 5replicates each. In this set second amendment DIPA was applied to plants growing in spiked soil. DIPA was introduced in the form of solution. Each dosage of DIPA (0, 1.5, 3, 5, 7.5 and 10 mmol kg^{-1}) was supplemented by dissolving in de-ionized water on an Orbital Shaker for almost 20 min. 40 mL solution was given to each pot for selected dosage of DIPA. Exposures time period of 3 weeks were specified before harvesting the set. Plants were monitored throughout the exposure period. Nutrient solution was given after 2 weeks and plants were watered daily to keep the soil moist.

3.10 Harvesting

Plants were removed from pots carefully. After taking out plant from pot it was washed thoroughly with distilled water.

3.11 Measurement of Physiological Parameters

a) pH

After removing plant from pots they were washed with distilled water to remove soil attach on the roots of plant. The pH was measure at that moment for each plant by putting a glass electrode in a 100 mL beaker. The pH meter was calibrated before measurements were taken.

b) Root and Shoot Length

Plant was separated into root and shoot with the help of scissor. Root and shoot length were measured one by one by using a scale (cm).

c) Number of leaves and fresh weight

Number of leaves present on the shoot of plant were counted carefully and noticed. Roots and shoots weight was taken individually on weigh balance in grams (Biotechnology lab/NUST). After weighing, root and shoot samples were packed in sampling bags and labeled accordingly.

e) Dry Weight

Samples of both root and shoot were placed in oven for removing moisture at 70 ˚C for 48 hours (Tauqeer *et al*., 2016). After drying the biomass of root and shoot were weighed again. Plant sample were then stored in labeled sampling bags.

3.12 Analysis of Pb in Plant

3.12.1 Wet Digestion

After harvesting plant and drying in an oven a process of wet digestion take place for analysis of Pb content in root and shoots of plant. 0.1 g of crushed plants sample (roots, shoots) were weighed. With 0.1 g of plant sample in 25 mL of volumetric flask 15 mL of concentrated HNO_3 and concentrated HCL (3:1) was added in flask through pipette each time when acid digestion is done. Flask was placed on hot plate in a fume hood and heated. Temperature was increased slowly from 50 ˚C up to 150 ˚C with time (Saifullah *et al*., 2010).

Heating process was continued on plate until the sample color changes to transparent and all the traces of plant material were digested completely. After the solution become colorless, sample were removed from the hot plate and filtered through Whatman No. 52 filter paper. The filtered solution were diluted to 50 mL with distil water in volumetric flask and stored at 4 ºC for Pb analysis.

3.12.2 Atomic Absorption Spectrophotometer

For the analysis of Pb in prepared sample through (AAS), standard solution with the same medium as samples was prepared for Pb at right concentration (Martin *et al.*, 2007). Analysis through AAS (HITACHI (Japan), Model 180-80 Polarizedzeemanatomic absorption spectrophotometer) was done by PINSTECH Islamabad. During analysis condition of AAS was as given in the table below:

Table 3.1: Analytical Conditions for Lead (Pb)

Sample	Standard solutions
Lamp current	7.5 Ma
Resonance absorption line	283.3 nm
Slit width	1.3 nm
Burner type	Standard
Burner height	7.5 mm
Oxidant	Air (1.6kg/cm^2) (9.4 L/min)
Fuel	Acetylene (C_2H_2) (0.30 kg/cm^2) (2.3 L/min)
Measurement mode	Direct
Equation type	Linear fit
Background/ ZAA	On
Recorder output	Direct (5sec)
Scale expansion	1.0 X

Table 3.2: Measurement Conditions

Analytical mode	AAS (concentration)
Measurement mode	Direct
Concentration units	(mg L^{-1})
Standards	5.0 (mg L^{-1})
	10.0 (mg L^{-1})
	20.0 (mg L^{-1})
Three more supplement standards for higher and lower concentrations range cover	1.0, 30.0 and 50.0 (mg L^{-1})
Correlation Coefficient of standard curve	0.9998

Standard Solution Preparation

Stock solution; Pb standard solution 1000 mg/L from Merck (cat. no. 19776.0500)

- Concentration 1001 ± 2 mg/L $Pb(NO_3)_2$ in HNO_3 (0.5 mol/l)
- Working standard; 100mg/L dilute from stock solution
- Standards prepared from working standard solution

3.15.3 Measurements

The Translocation factor (TF) and Bioconcentration factor (BCF) of Pb per plant was calculated from the following relation given by (Aransiola *et al*., 2013).

$$\textbf{BCF} = \frac{\text{metal conc. in roots (mg kg}^{-1})}{\text{Metal conc. in soil (mg kg}^{-1})}$$

$$\textbf{TF} = \frac{\text{metal conc. in } C_{aerial} \text{ of plant}}{\text{metal conc. in roots of plant}}$$

C_{aerial} = Metal conc. in stem, leaf, seed

Pb uptake was measured using the following formula given by (Frietas *et al*., 2009).

Pb uptake = [(shoot dry weight × shoot Pb conc.) + (root dry weight × root Pb conc.)]

3.16 Statistical Analysis

Data collected during experimentation was subjected to Regression and Analysis of Variance (ANOVA). Results were analyzed using two way ANOVA, with $\partial = 0.05$ using Minitab 16. Comparative graphs were made using Tableau. Statistical significance was established when $p<0.05$.

Chapter 4

RESULTS AND DISCUSSION

4.1 Soil Characterization

Soil physiochemical characteristics determined were pH, EC, organic matter content, texture and elemental composition. The results are presented in Table 4.1: it shows all the parameters and their respected values.

Table 4.1: Physiochemical characteristics results of Soil

Parameters	Observed Values	Method
pH	7.11	pH meter
EC	56.7mS/cm	EC meter
Texture	Loamy	Paste method
Organic matter content	0.17%	Dry combustion
Pb	-	XRF

The elemental composition was basically determined to find out the already present Pb in soil. The results of elemental composition of soil through XRF are presented in Figure 4.1. The results showed that no amount of Pb is present in selected soil.

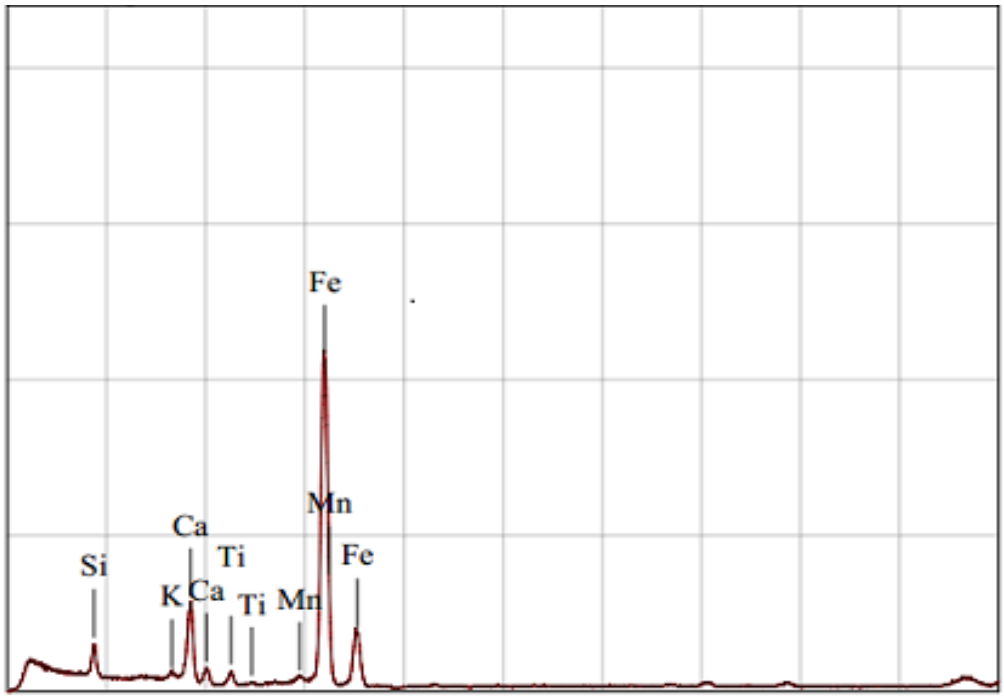

Figure 4.1: Elemental composition of soil through XRF showing absence of Pb

4.2 Phytoavailability of Pb in Soil

4.2.1 Phytoavailability of Pb with EDTA

The results representing the relationship between Pb availability and added EDTA are presented in Figure 4.2. In non spiked soil (control) Pb was not detected through AAS, which verifies the result of XRF where Pb was not detected in control. In non-amended soil with increase in concentration of Pb from 500 mg kg^{-1} to 1500 mg kg^{-1} phytoavailability of Pb was observed to be increased from a minimum of 7.16 mg kg^{-1} to 39.5 mg kg^{-1} respectively. This increase in phytoavailability due to increase in concentration of Pb represented normal availability of Pb in soil.

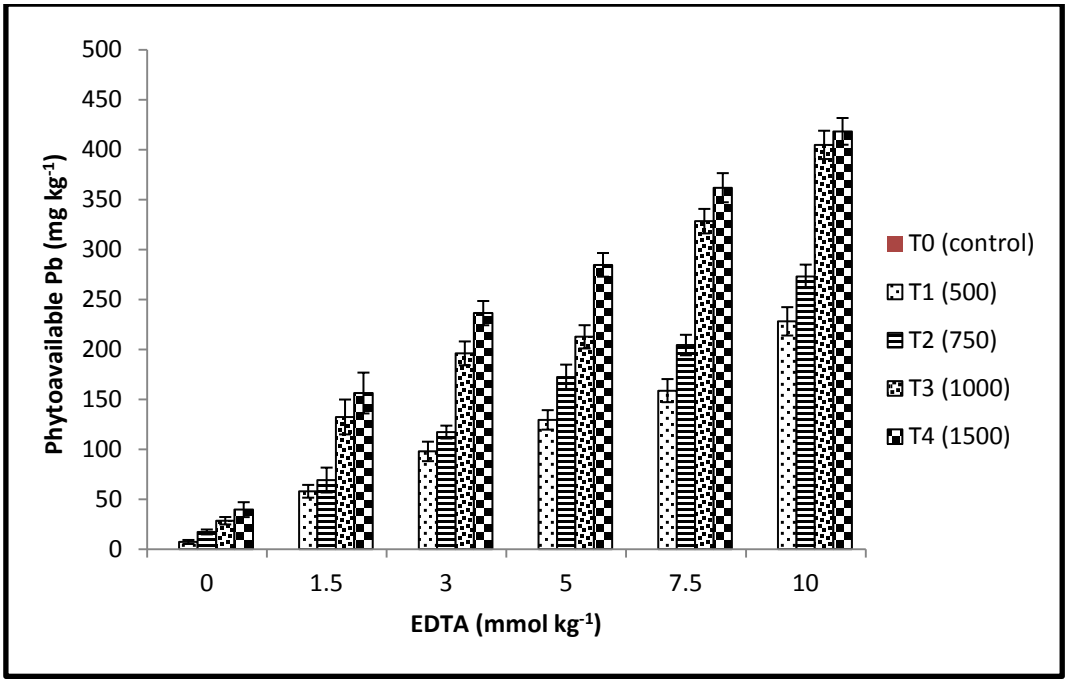

Figure 4.2: Lead phytoavailability in response to EDTA application

In above figure, in control EDTA application is not showing any effect because of absence of Pb. The concentration of phytoavailable Pb is noticed to range between 7.26 to 418.24 mg kg^{-1} in soil spiked with Pb 500 mg kg^{-1} with no EDTA to Pb 1500 mg kg^{-1} with 10 mmol kg-1 of EDTA. It was observed that with increase in concentration of EDTA, the availability of Pb is increased. While with Pb 1500 mg kg^{-1} and a dose of 10 mmol kg^{-1} of EDTA set out a trend of 9.5-fold increase in comparison of 1500 mg kg^{-1} of Pb with no EDTA. Study done by Epelde *et al*. (2008) also reveals that EDTA could help in enhancing the accessibility of Pb by making Pb-EDTA bonds. Lai *et al.* (2005) also proved in his study that soil solution concentration of metal treated soil with Pb, Cd, Zn and Fe is increased with application of EDTA. Overall experimental results of Pb movability concluded that availability of Pb is enhanced with increase in concentration of Pb as well as with increase in application of EDTA.

EDTA is considered as the most frequently suggested chelant to boost phytoavailability of metals. Mobility of Pb was observed to increase with EDTA

application. EDTA was effective in mobilizing metals from soil by forming metal-EDTA complexes and provided them in available form. Results of phytoavailability of Pb in soil with application of EDTA also represent that Pb-EDTA complexes were formed to increase the movability of Pb in soil. Experimental results of Pb movability with EDTA also concluded that availability of Pb was enhanced with application of EDTA. This observation was in agreement with study done on EDTA-enhanced phytoextraction in which EDTA form soluble complexes with Cd and Zn to increase their mobility (Lambrechts *et al*., 2011).

EDTA was found to be an organic amendment to form bonds with the metal present and increase their solubility to further taken up by the plant in phytoremediation. Another study shows a similar trend where with increase in concentration of EDTA increased the phytoavailability of Pb to 80% for its accumulation by plant in phytoextraction (Cui *et al*., 2007).

4.2.2 Phytoavailability of Pb with DIPA

No literature is available on DIPA as an amendment in soil. To increase the phytoavailability of Pb DIPA was applied as an amendment in Pb spiked soil. Figure 4.3 represents the mean value for Pb at applied concentration of DIPA with ± SD. The graphical representation of soil experiment with DIPA exhibit that availability of Pb was increased with increase in DIPA concentration.

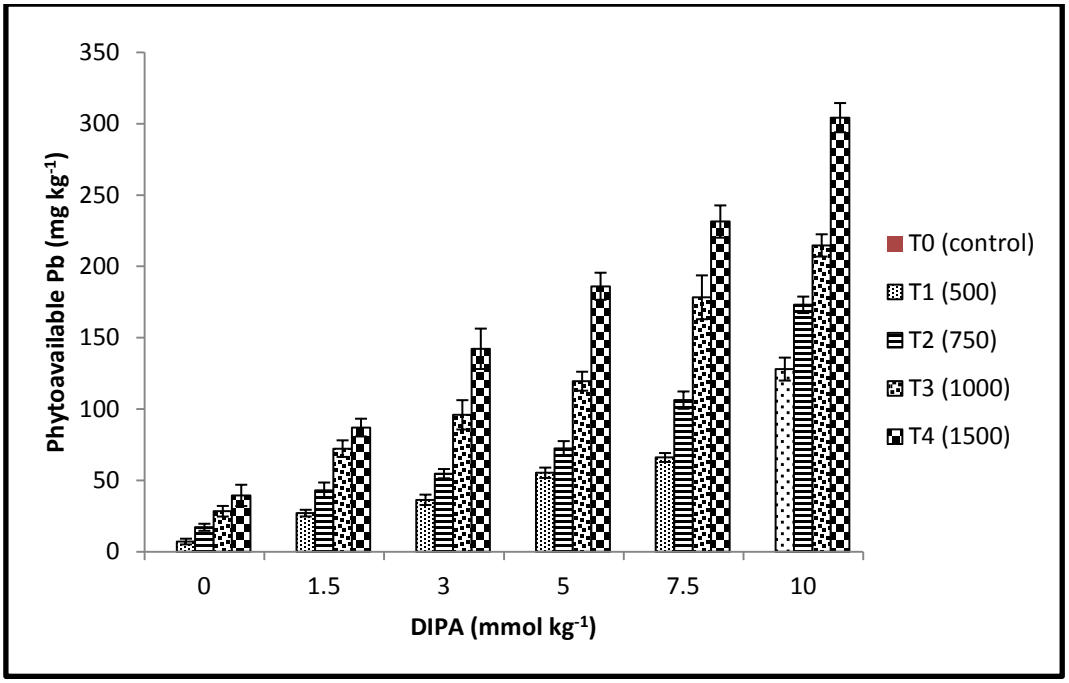

Figure 4.3: Lead phytoavailability in response to DIPA application

At 7.5 and 10 mmol kg^{-1} of DIPA availability of Pb in 1500 mg kg^{-1} was increased exponentially in comparison with DIPA 5 mmol kg^{-1}. The concentration of Pb (available Pb) is ranged from a minimum of 7.16 to a maximum 304 mg kg^{-1}in a soil spiked with Pb 500 mg kg^{-1} to Pb 1500 mg kg^{-1} with DIPA 10 mmol kg^{-1} (fig 4.3).

According to Cui *et al*. (2007), the proportion of amendment and type of chelant is important factors which influence phytoavailability. DIPA and EDTA with increased dosage tend to increase the availability of Pb in soil contaminated with up to 1500 mg $kg^{-1}$of Pb. Both amendments showed different results that could be due to potential capacity for phytoavailability of this particular metal to the type and amount of chelant supplemented. Meer *et al*., (2005) also observed that for effective results proportion of amendment depend upon chelant-metal interaction. Same increased dosage of EDDS confirmed highest mobilization with it for Cu but slightly persuade its activity for Cd and Pb.

4.2.3 Comparison of DIPA and EDTA for Pb Phytoavailability

Comparison of DIPA and EDTA for phytoavailability of Pb in soil is given in Figure 4.4.

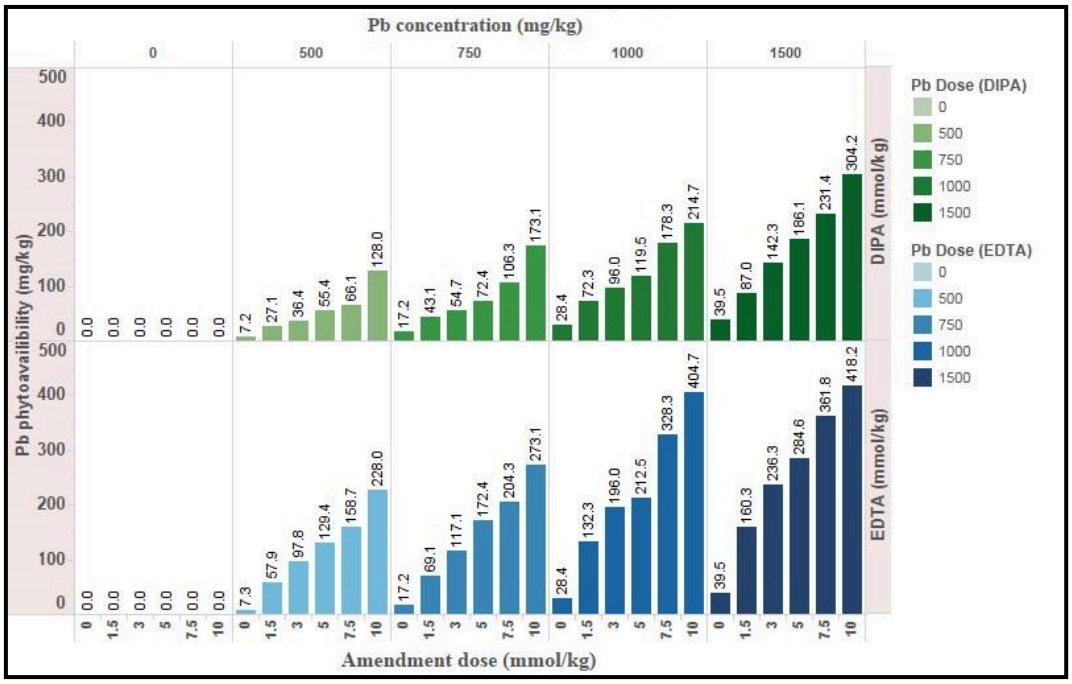

Figure 4.4: Comparison of DIPA and EDTA for Pb phytoavailability in Soil

For the phytoavailability of Pb the same dose of DIPA and EDTA exhibit different trends. At 10 mmol $kg^{-1}$of EDTA and DIPA illustrated availability of 418 mg kg^{-1} and 304 mg kg^{-1} in soil spiked with 1500 mg $kg^{-1}$of Pb. According to the past research Chiu *et al*. (2005) at the same concentration of 20 mmol kg^{-1} of HEIDA, CDTA, HEDTA, EDTA, DTPA and EGTA for Cu mobility in soil only HEIDA was capable to increase phytoavailability of Cu in soil. Meers *et al*. (2005) also observed that for mobilization of Pb EDTA was more effective than EDDS.

Concentration of metal-chelate complex was considered to be an important factor when such an experiment was carried out. Presence of a chelate is not specific to a heavy metal neither to the dosage. The interference due to presence of cations in soil played an important role for Pb phytoavailability. The difference in mobility of Pb by DIPA and EDTA also exhibited this trend. Cations in soil with EDTA and

DIPA also show different interaction for phytoavailability of Pb. Formation of metal-chelate complex followed mechanism where EDTA detached metal from minerals with increased in its solubility (Shahid *et al*., 2009; Zhang *et al*., 2010). Evangelou *et al*. (2007) also confirmed this tendency of Pb movability by EDTA and DIPA where EDTA and HEDTA enhanced Pb availability while CDTA and DTPA hindered it.

The availability of metal is due to pH of soil. A decrease in soil pH of soil causes mobility of metal to increase in soil for accumulation by plant. The interaction of HEIDA and Cu decreased the pH of soil and increased its availability (Chiu *et al*., 2005). Presence of Pb in soil changed the chemistry of soil and confirmed its toxicity. Combinations of different chemical, biological and environmental factors changed the chemistry of soil for bioavailability of heavy metals (Aransiola *et al*., 2013). In present study the contact of Pb with EDTA was more helpful in reducing the pH of soil to increase its phytoavailability then its relationship with DIPA. In regression analysis both EDTA and DIPA were found to significantly increase the phytoavailability of Pb in soil but EDTA efficiency was more than DIPA (fig 4.4 a, b, c, d).

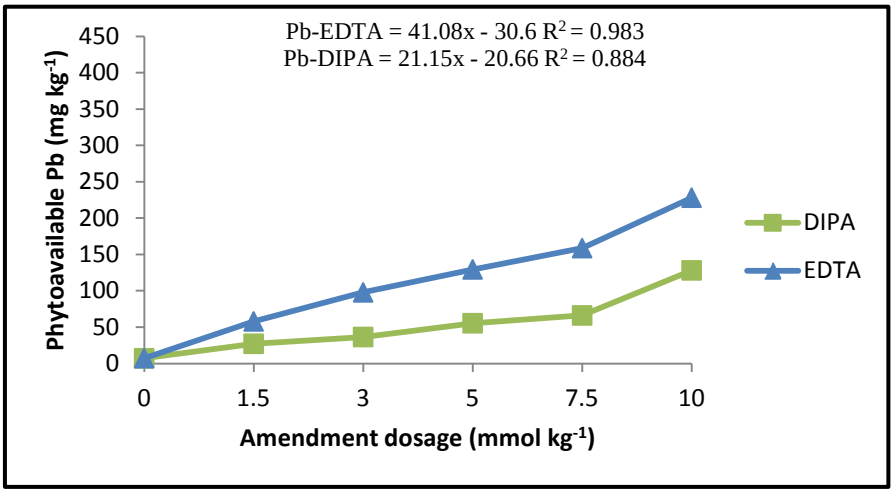

Figure 4.4 (a): Regression between 500 mg kg $^{-1}$ of Pb and different dosage of EDTA and DIPA for Pb phytoavailability in soil

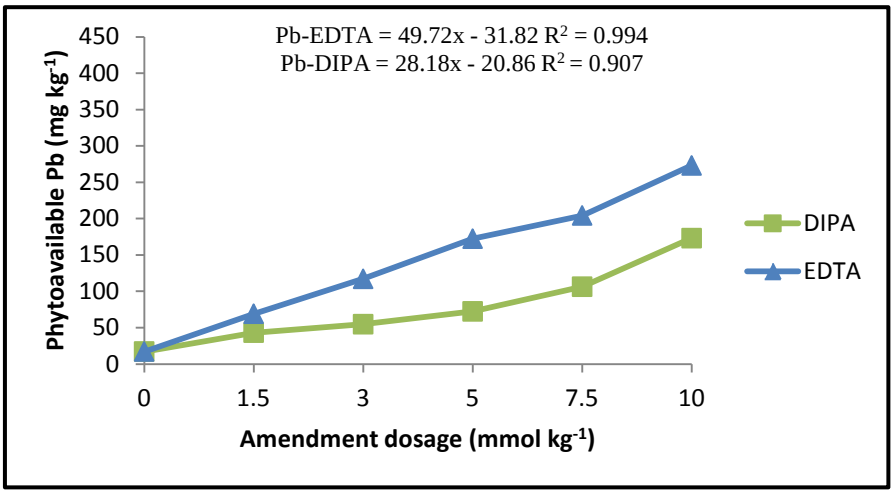

Fig. 4.4 (b): Regression between 750 mg kg $^{-1}$of Pb and different dosage of EDTA and DIPA for Pb phytoavailability in soil

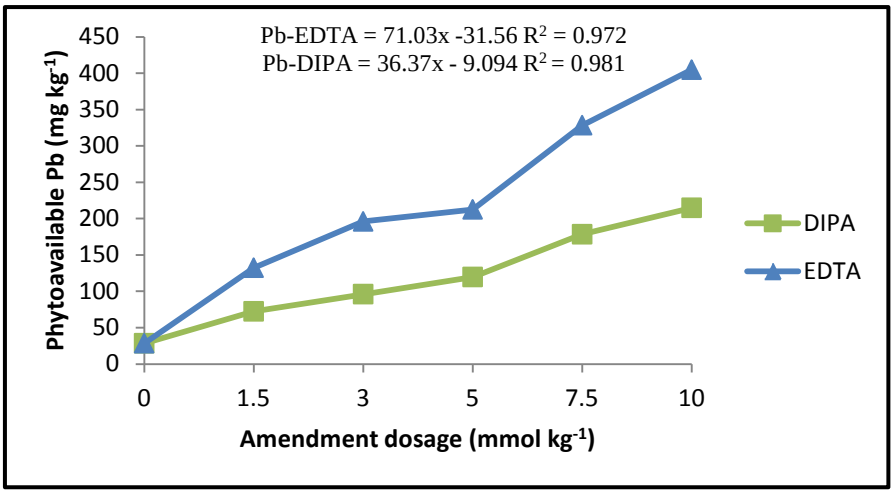

Figure 4.4 (c): Regression between 1000 mg kg $^{-1}$ of Pb and different dosage of EDTA and DIPA for Pb phytoavailability in soil

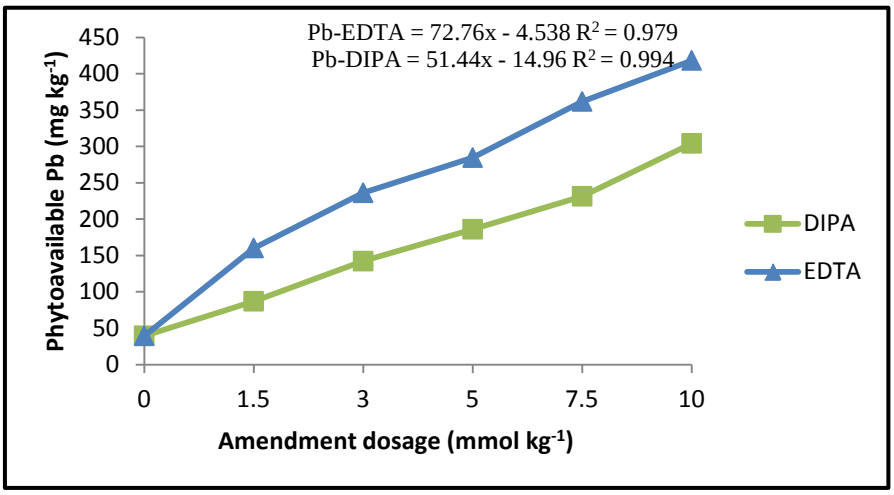

Figure 4.4 (d): Regression between 1500 mg kg $^{-1}$ of Pb and different dosage of EDTA and DIPA for Pb phytoavailability in soil

Positive coefficient value indicated a direct relationship, where increased in amendment dose with increased in Pb dose increased Pb phytoavailability (Table 4.2). For example increase in one unit of DIPA and EDTA in 1500 mg kg^{-1} of Pb caused Pb phytoavailability to increased 25 and 35 units respectively. Increased in amendment dose and Pb dose showed statistically significant result ($P < 0.05$) as smaller 'P' value specify the rejection of null hypothesis.

Table 4.2: Results of Regression and Analysis of Variance on Pb phytoavailability

Pb (mg kg^{-1})	Chelating agent	Coef	SE Coef	T	P
500	EDTA	20.31	0.78	25.98	3.85E-21
	DIPA	10.73	0.61	17.31	1.73E-16
750	EDTA	24.51	0.80	30.3	5.98E-23
	DIPA	14.34	0.65	22.04	3.1E-19
1000	EDTA	35.18	1.35	25.8	4.21E-21
	DIPA	18.12	0.57	31.39	2.29E-23
1500	EDTA	35.59	1.77	20.05	3.76E-18
	DIPA	25.46	0.71	35.49	7.95E-25

4.3 Plant Experiment with EDTA and DIPA

To evaluate the effect of EDTA and DIPA on scented geranium *(Pelargonium hortorum)*, a pot experiment was conducted under greenhouse condition. Five replicates for each treatment of Pb with each dose of EDTA and DIPA separately were prepared to study Pb uptake and growth performance in scented geranium.

4.4 Effect of EDTA and DIPA on Growth of *Pelargonium hortorum*

4.4.1 Root Length

Figure 4.5 and 4.6 illustrate the influence of EDTA and DIPA on root length of *Pelargonium hortorum*. In (fig 4.5), root length showed a decrease in trend with respect to the increase in dosage of EDTA and Pb in Pb spiked soil. Root length illustrated a maximum value of 19.1cm in control and a lowest value of 10.2 cm in *Pelargonium hortorum* grown in soil spiked with 1500 mg kg^{-1} of Pb and amended with (10 mmol kg^{-1}) of EDTA. Root length decreased by 26% in Pb (1500 mg kg^{-1}) and amended with (10 mmol kg^{-1}) of EDTA in comparison to Pb 1500 mg kg^{-1} Pb alone. Increased in dosage of EDTA from control to 10 mmol kg^{-1} in absence of Pb was observed to indicate no effect of amendment alone (fig. 4.5).

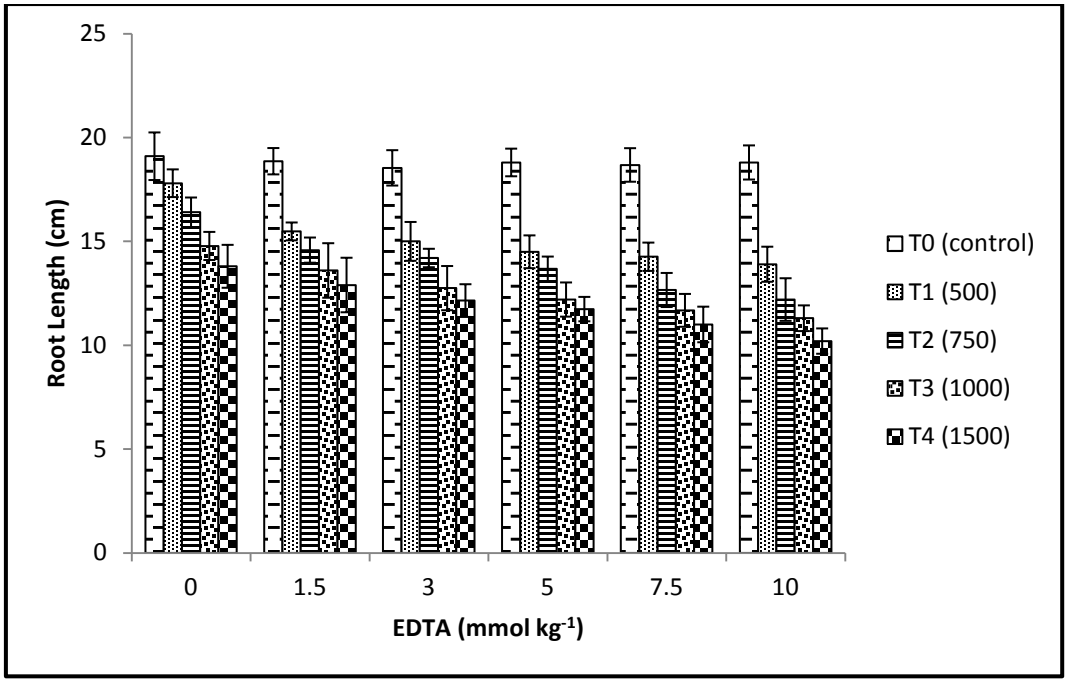

Figure 4.5: Root length of *Pelargonium hortorum* in response to EDTA application

Root length responds different with or without Pb as well as with EDTA. With increase in concentration of Pb from control to 1500 mg kg^{-1} root length decreased by 19.1 cm to 13.8 cm. This observation was in accordance with the study done by Cui *et al*. (2007) where root length of *zinnia* get inhibited with increased concentration of Pb with respect to control. Also Pb when treated with EDTA showed a decreasing trend in root length of plant. Whereas Cui *et al.* (2007) study was unlikely to the results of root length with application of EDTA in Pb polluted soil where in his study Pb toxicity was reduced and root length improved in presence of Pb and EDTA and our study reported that Pb in soil when treated with EDTA confirm that Pb toxicity to root was increased and root length decreased.

Pelargonium hortorum when exposed to Pb in combination with increased in EDTA dosage when observed revealed that plant height and root length was reduced. Same negative effect for Pb and EDTA on root length of maize was also reported by (Hadi *et al*., 2010).

In Figure 4.6, root length was observed to decrease in presence of Pb and DIPA. But this decrease in root length throughout the increase in concentration of DIPA is lesser then as it was noticed in presence of EDTA, as in presence of Pb 1500 mg kg^{-1} with EDTA and DIPA 10 mmol kg^{-1} root length dropped down to 10.2 cm and 11 cm respectively in comparison to root length of 13.8 cm in Pb 1500 mg kg^{-1} alone. Whereas in comparison to control 10 mmol kg^{-1} of EDTA and DIPA in 1500 mg kg^{-1} of Pb exhibited a decline of 46% and 42%. Same dose of EDTA and DIPA showed different results on the root length of *Pelargonium hortorum* this indicate signs of different capacity and reaction. Root length of plant in presence of EDTA was found to be more sensitive than in presence of DIPA.

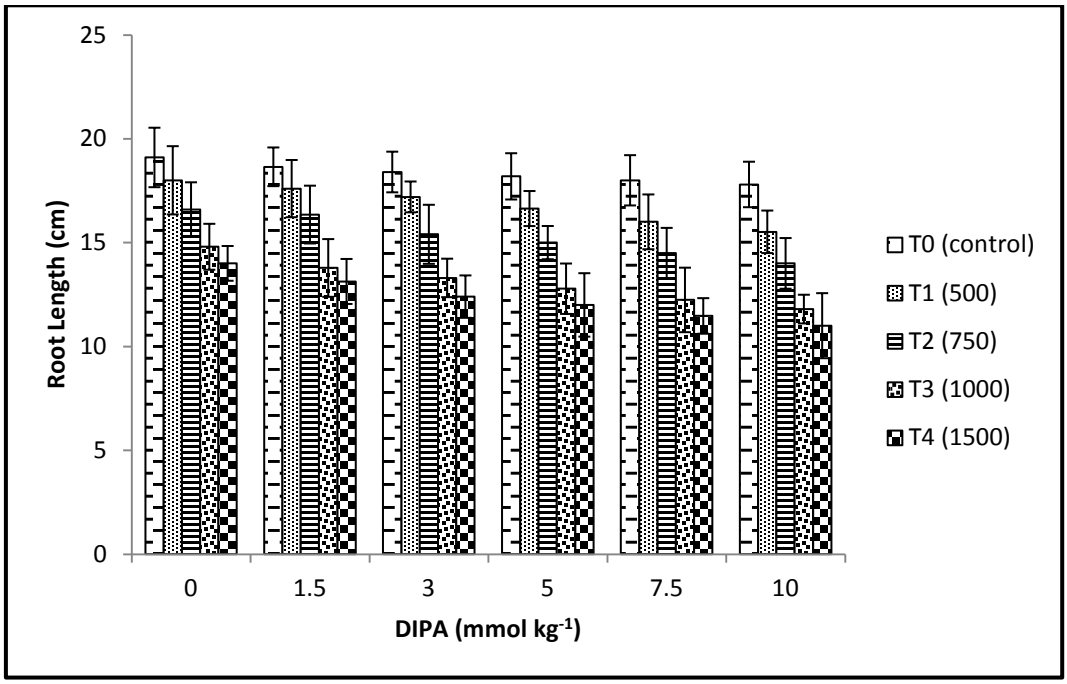

Figure 4.6: Root length of *Pelargonium hortorum* in response to DIPA application

With the application of DIPA alone (without Pb) root length decreased from 19 cm in control to 17.8 cm in treatment of 10 mmol kg^{-1} of DIPA (fig. 4.6). In comparison with control root length was observed to reduce by another study in presence of Pb + NTA and Pb + Citric acid as well as the presence of chelators alone

root length show different trends among which DTPA was found to decrease the root shoot length at dose of (1.5, 2.5, 5 and 10 mmol kg^{-1}) (Ruley *et al*., 2006).

Root growth basically indicates the health condition of a plant. Two process, cell division and root elongation complete root growth. (Singh *et al*., 2016) reported that the activity of this combination (root elongation + cell division) get disturbed due to the toxicity caused by presence of heavy metals in several plants. Our study present that increased in concentration of Pb without amendment reduced root length was due to the stress caused by increase in concentration of Pb to plant metabolism.

4.4.2 Shoot Length

The dose and response graph of EDTA on shoot of *Pelargonium hortorum* is shown in Figure 4.7. It was observed that with increase in concentration of EDTA with and without Pb shoots length decreased. The shoot lengths dropped down from maximum of 15.6 cm to minimum value of 9.3 cm in soil without Pb to 1500 mg kg^{-1} of Pb with 10 mmol kg^{-1} of EDTA while in presence of Pb 1500 mg kg^{-1}alone shoot length was dropped to 12.6 cm. Almost 26% decreased in shoot length of *Pelargonium hortorum* was measured in combination of EDTA 10 mmolkg^{-1} and Pb 1500mgkg^{-1} as compared to Pb 1500 mg kg^{-1} alone.

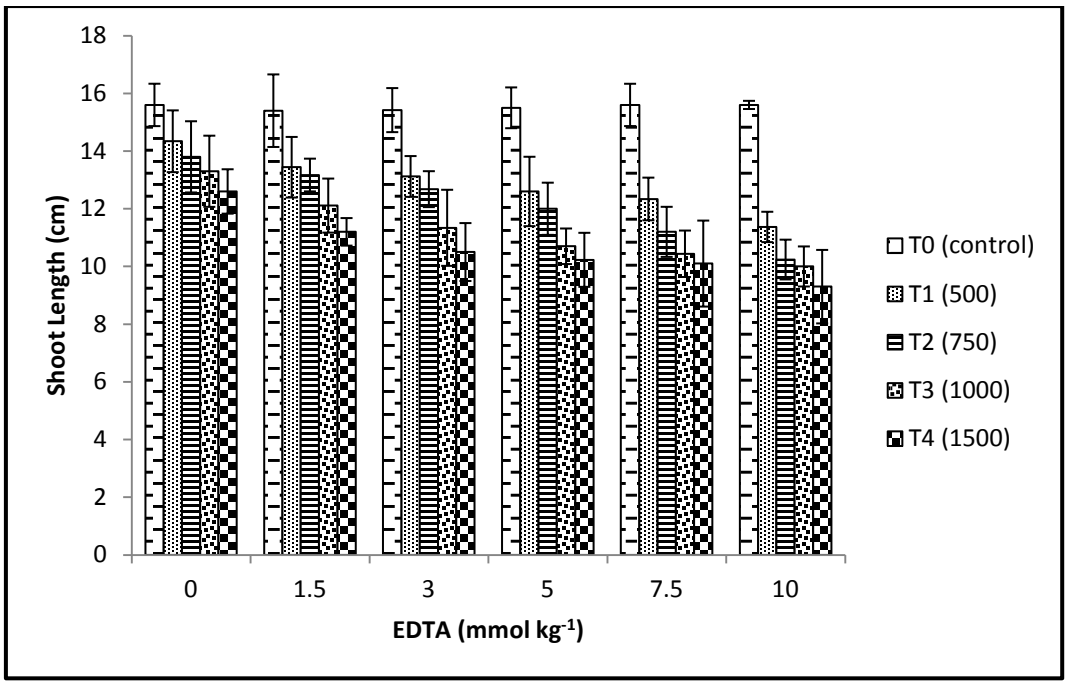

Figure 4.7: Shoot length of *Pelargonium hortorum*in response to EDTA **a**pplication

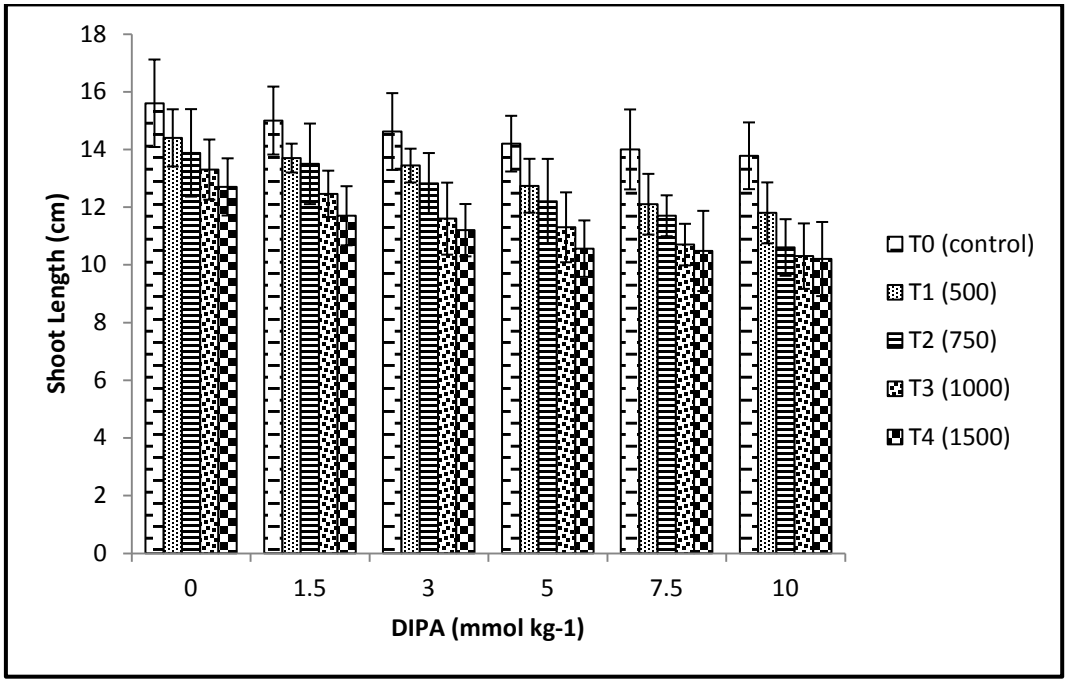

Figure 4.8: Shoot length of *Pelargonium hortorum*in response to DIPA **a**pplication

In Figure 4.8 shoot length of *Pelargonium hortorum* in addition of DIPA display the same decreasing trend as in EDTA. Shoot length of *Pelargonium hortorum* reveal to be stressed under the increased concentration of Pb. Control

without Pb to Pb 1500 mg kg^{-1} shoot length decreased from 15.6 cm to 12.6 cm which an almost 19% decrease. This is mainly due to the reason of increased stress caused by Pb. Cui *et al*. (2007) also revealed in his study that shoot length of *Zinnia* get inhibited with increasing concentration of Pb due to its toxicity.

Shoot length in Pb 1500 mg kg^{-1} alone to Pb 1500 mg kg^{-1} with DIPA 10 mmol kg^{-1} represented almost 23% drop down. This observed decrease in length of shoot with application of DIPA was less than as with EDTA. The reduction in shoot length at EDTA agreed with the study done by Ruley *et al.* (2006), in his study he observed that significant reduction occur in shoots of *Sesbania* when treated Pb contaminated soil with EDTA. Lambrechts *et al*. (2011) also reported decrease in shoot elongation of *L. perenne* when amended heavy metals polluted soil with EDTA.

With the application of both DIPA and EDTA for Pb accumulation, shoot length of *Pelargonium hortorum* was found to reduce with increased concentration of both Pb and amendment. This proved that they are basically dose dependent. DIPA as chelator alone was found to be sensitive to the shoot length of *Pelargonium hortorum* as with increase in dosage of DIPA shoot length get reduced. The toxic effect of chelator was also verified by (Oviedo and Rodriguez 2003). In their study they explained that chelators had their toxic effects on interaction with plants. As chelators alone inhibit cellular division and biomass production as well as interfere with chlorophyll synthesis.

4.4.3 Plant biomass

Application of DIPA and EDTA for Pb uptake lower the dry biomass of root and shoot of *Pelargonium hortorum* as compared to control without Pb. Whereas dry biomass of root and shoot with use of DIPA and EDTA decreased in comparison to treatment of Pb without chelators. Figure 4.9 and 4.10 illustrated that root dry biomass

decreased due to EDTA and DIPA. Figure 4.11 and 4.12 gives the graphical representation of shoot dry biomass with application of EDTA and DIPA separately. Root and shoot dry biomass exhibited a trend in which dry biomass of root and shoot decreased with increase in concentration of Pb + amendment. This result was unlikely to the study done by (Kanwal *et al*., 2014) where application of EDTA help to mitigate the negative effect caused by Pb on *Brassica napus.* Andrade *et al*. (2014) in his study indicate that addition of EDTA in Cd contaminated soil decrease dry biomass of both root and shoot in *Brachiaria decumbens.*

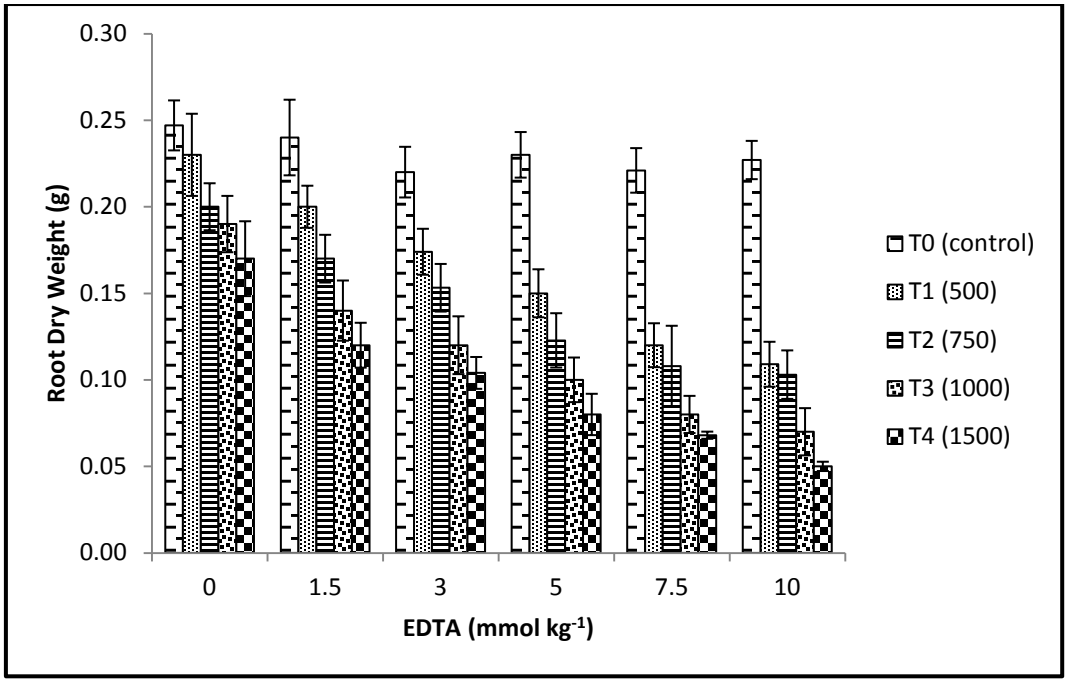

Figure 4.9: Root dry weight of *Pelargonium hortorum* in response to EDTA application

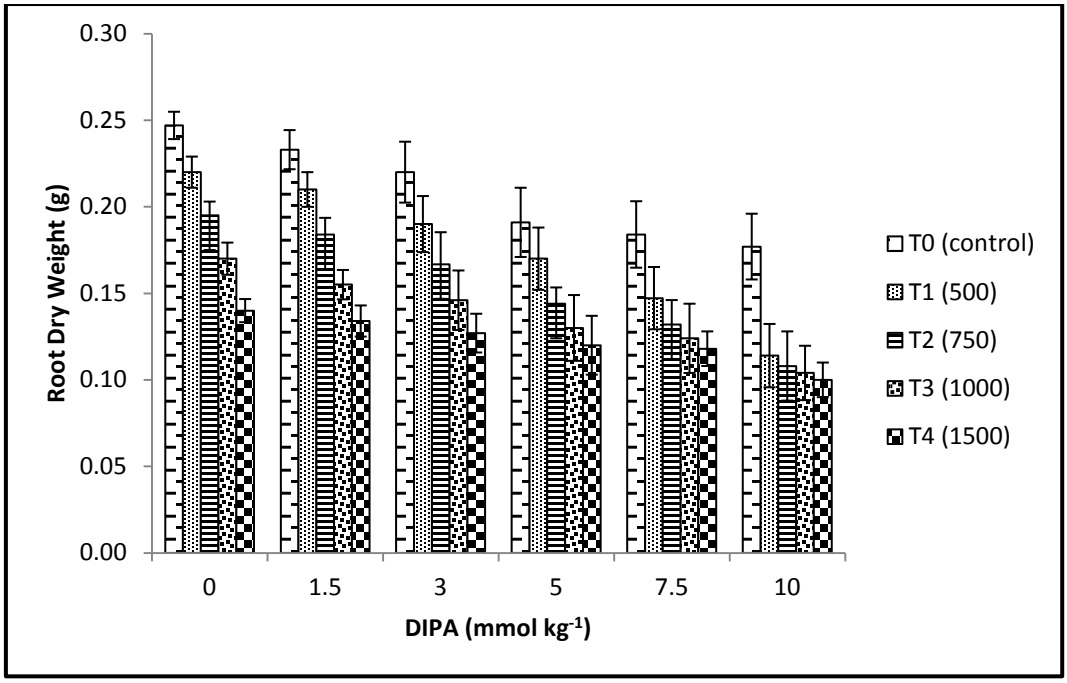

Figure 4.10: Root dry weight of *Pelargonium hortorum* in response to DIPA application

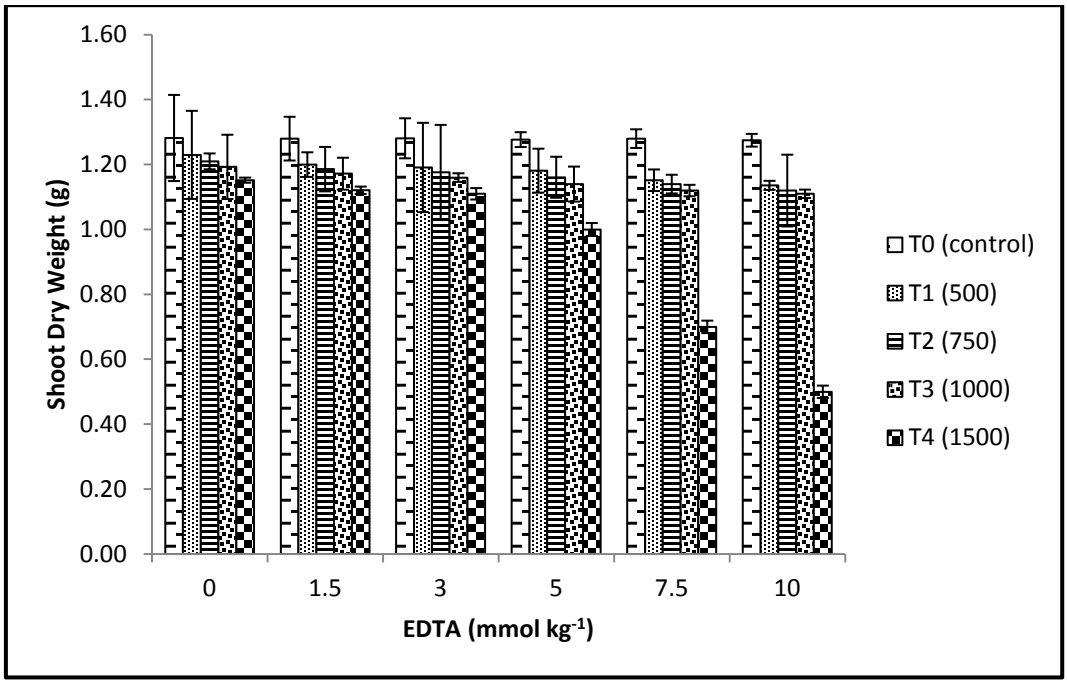

Figure 4.11: Shoot dry weight of *Pelargonium hortorum* in response to EDTA application

In treatment of Pb 1500 mg kg^{-1} + EDTA 10 mmol kg^{-1}3.4-fold decrease in dry biomass of root and 2.3-fold in dry biomass of shoot was observed as compare to Pb 1500 mg kg^{-1} alone (fig 4.9 and 4.11). Whereas in Pb 1500 mg kg^{-1} + DIPA 10

mmol kg^{-1} treatment a decrease of 1.4-fold in both dry biomass of root and shoot were detected comparatively to Pb 1500 mg kg^{-1} alone (fig 4.10 and 4.12). According to Luo *et al*. (2006) dry biomass of *Z. mays* observed to decreased with increase in dosage of chelators due to accumulation of Pb which suppresses its biomass. EDTA with increase in its dosage from 3 to 10 mmol kg^{-1} declared to decrease plant dry biomass (Epstein *et al*., 1999).

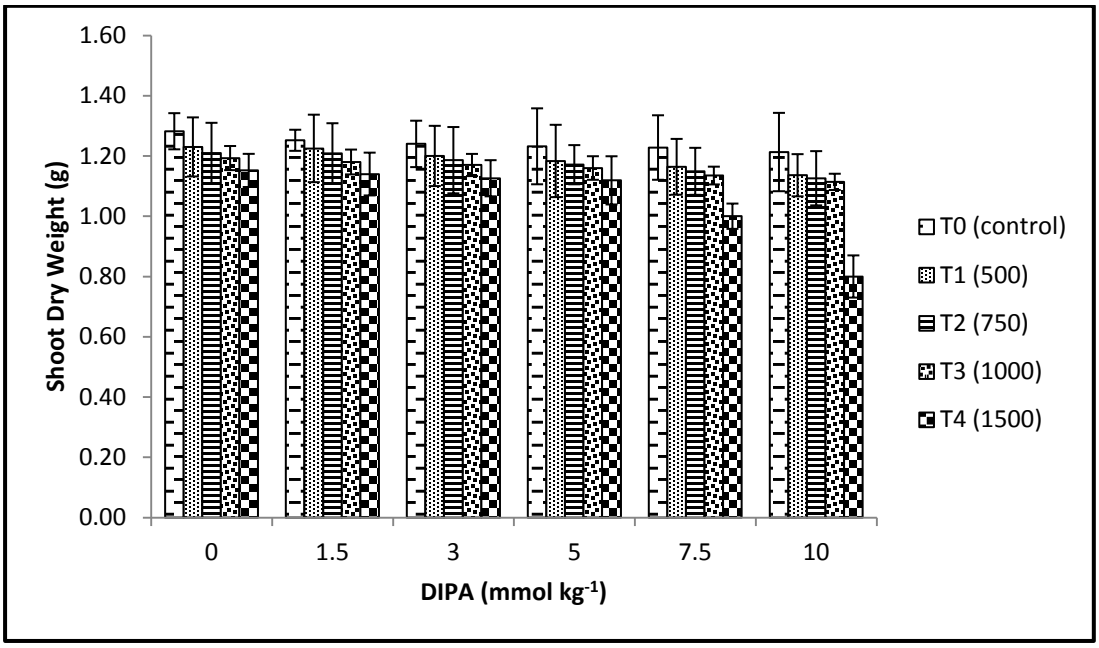

Figure 4.12: Shoot dry weight of *Pelargonium hortorum* in response to DIPA application

Reduction of 64% in dry biomass of root and 56% in dry biomass of shoot were inspected in treatment with Pb 1500 mg kg^{-1} + EDTA 10 mmol kg^{-1} as compared to Pb 1500 mg kg^{-1} alone (fig 4.9 and 4.11). Freitas *et al*. (2009) also observed a decrease in dry biomass production with application of EDTA and NTA as compared to control (with Pb and without chelators). In the presence of EDTA and DIPA only, dry biomass of both root and shoot get influenced by DIPA and decreased with increase in its dosage but remain unaffected by the use of EDTA. Our results are unlikely to Vassil *et al*. (1998) who observed that presence of EDTA alone also affect

plant physiological behavior. Whereas instead of EDTA, DIPA was monitored to cause reduction in dry biomass of plant and affect plant physiology.

Dry biomass of shoot with EDTA show no significant affect whereas both root and shoot dry biomass influenced by the use of DIPA. The root dry biomass decreased to 0.17 from 0.24 g and shoot biomass dropped down to 1.21 from 1.28 g. These results were in accordance with study where EDTA after its application had observed to cause no clear symptoms of toxicity to *B. maximowicziana* and also measured a decrease in plant biomass with increased in concentration of Pb and EDTA (Wang *et al*., 2007). The decline in dry biomass of root and shoot both with DIPA and EDTA was mainly due to the toxicity caused by the combination of Pb + DIPA and Pb + EDTA for Pb accumulation in plant. EDTA when applied to Pb contaminated soil reduced the dry biomass yield of both corn and bean (Luo *et al*., 2005). Figure 4.13 and 4.14 display the trend of number of leaves in presence of EDTA and DIPA, respectively.

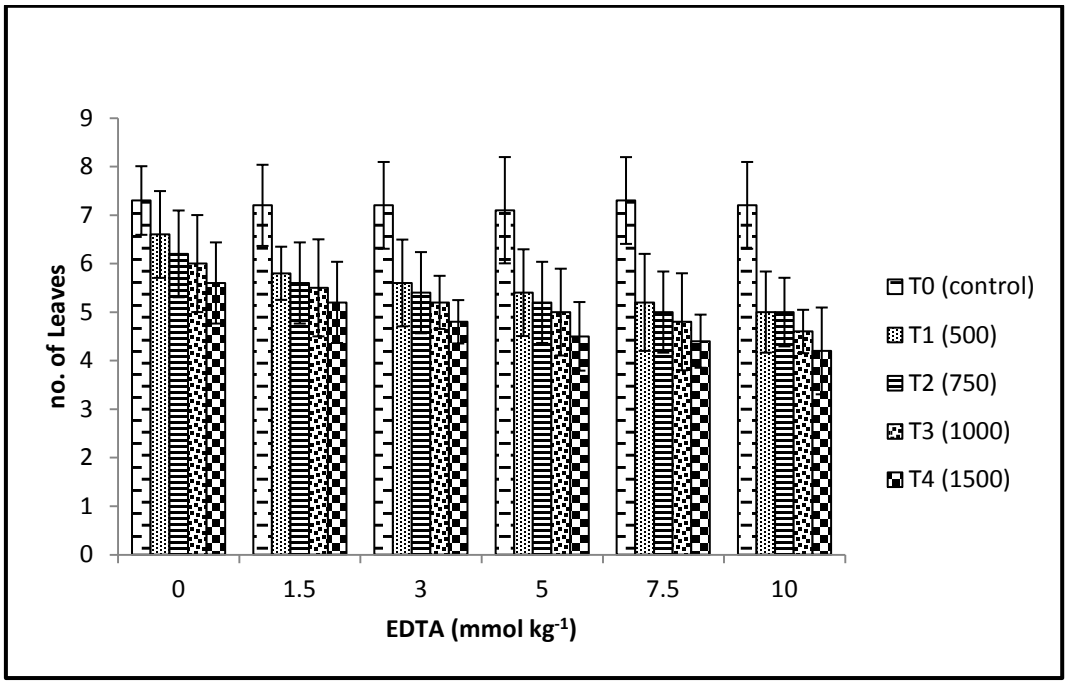

Figure 4.13: No. of leaves in *Pelargonium hortorum* in response to EDTA application

Loss of leaves and biomass is observed in Pelargonium when treated with 10 mmol kg^{-1} of EDTA (fig 4.13). Epelde *et al*. (2008) study also agrees with the results of EDTA 10 mmol kg^{-1} on plant condition observed in *Pelargonium* for the reason that this same concentration of EDTA influenced condition of *Cynara cardunculus.* It was also verified that increase in concentration of EDTA affect condition of leaves. With increased concentration of Pb along with EDTA and DIPA caused number of leaves to decrease in *Pelargonium hortorum*. With passage of time new leaves were noticed to be grown.

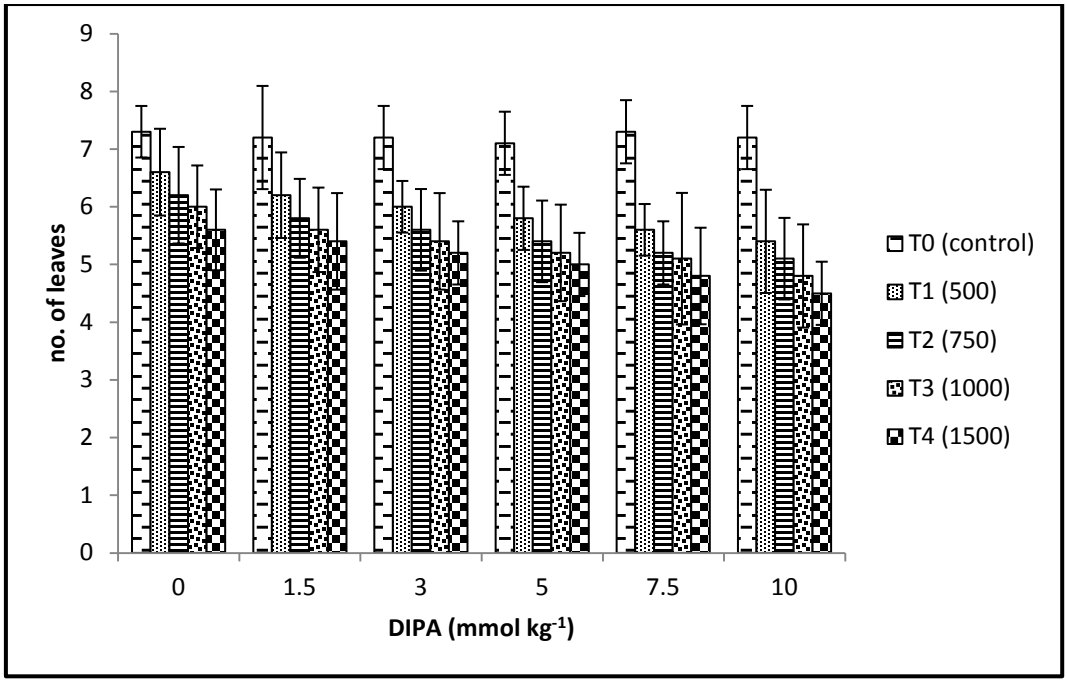

Figure 4.14: No. of leaves in *Pelargonium hortorum* in response to DIPA application

Due to EDTA and Pb toxicity shoot biomass observed to be decrease as well leaves show clear symptoms of toxicity and cause drop down in number of leaves with increased dosage of EDTA and Pb. This observation was in complete agreement with study done by Chen *et al*. (2004) where dry biomass decrease in maize, sunflower and cabbage as well as their leaves show symptoms of chlorosis and necrosis. The same reduction in number of leaves was noticed in case of treatment with Pb + DIPA (fig 4.14). This indicate that DIPA was also toxic to plant in

combination with Pb. Harmful effect of EDTA and DIPA in combination with Pb was in agreement with the study of Hadi *et al*. (2010) that EDTA found capable to increased metal mobility in soil solution and influence plant growth.

Surge time of amendment was also an important factor in plant condition as well in metal extraction. DIPA and EDTA were added in solution form 3weeks before harvesting. On harvest the dry biomass of plant with both DIPA and EDTA examined to be reduced. Study done by Chiu *et al*. (2005) noticed different surge time suitable for chelators according to metals they are interacting with and type of plant used. According to him yet there is lack of information about time of application of amendment. In his study for Cu extraction by HEIDA 5-16 days were sufficient whereas for Zn and As through NTA 20 days were even not sufficient for metal uptake. This indicates the importance of harvest after application of amendment for maximum uptake by plant and plant physiology.

4.5 Rhizosphere pH

Figures 4.15 and 4.16 show the effect of EDTA and DIPA on rhizosphere pH.

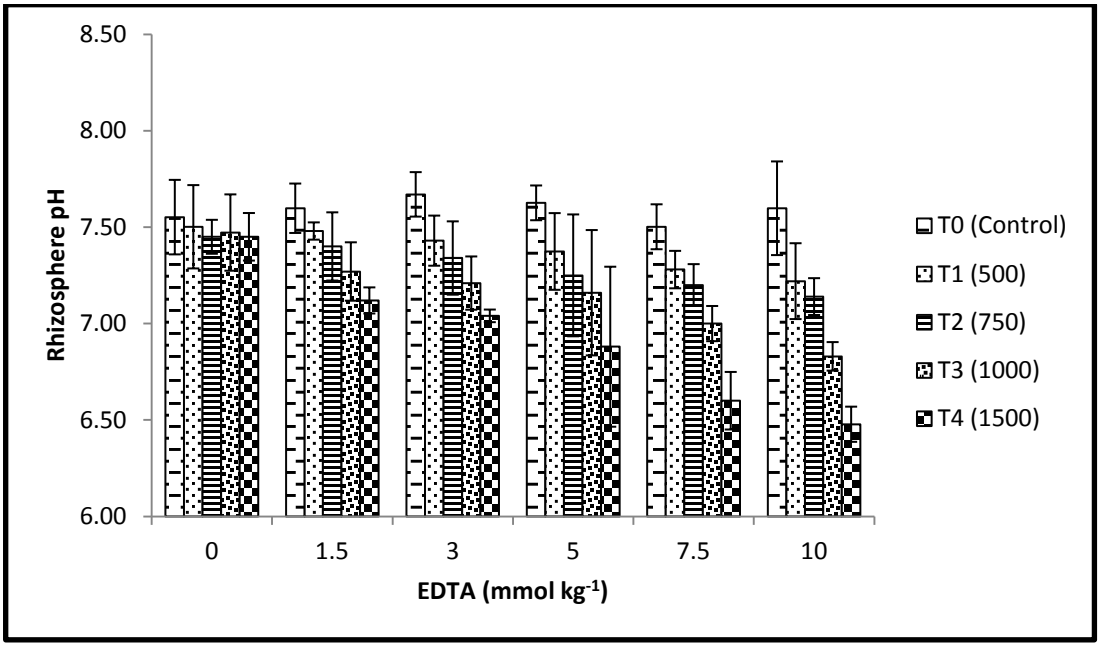

Figure 4.15: Rhizosphere pH in response to EDTA application

In comparison of amended soil with 10 mmol kg^{-1} of EDTA and Pb 1500 mg kg^{-1} with 1500 mg kg^{-1} of Pb only, a decreased of 0.9 units in rhizosphere pH was observed (fig 4.15). The phenomena behind decrease in pH is due to amendment application which relates to study done by Park *et al.* (2011) where *Lolium perenne* rhizosphere decreased due to presence of biosolid. In comparison of amended soil with 10 mmol kg^{-1} of DIPA and Pb 1500 mg kg^{-1} with 10 mmol kg^{-1} DIPA alone, an increase of 0.3 units in rhizosphere pH was observed (fig 4.16). An increase of 0.5 units in rhizosphere pH was calculated in soil contaminated with 1500 mg kg^{-1} of Pb in contrast to 1500 mg kg^{-1} of Pb and DIPA 10 mmol kg^{-1}. Rhizosphere pH in control (without amendment) with increase in dosage of Pb was not affected. From control up to Pb 1500 mg kg^{-1} rhizosphere pH remained almost same within range of 7.5 whereas in control (without Pb) increased in dosage of DIPA up to 10 mmol kg^{-1} slightly increased rhizosphere pH (fig 4.16).

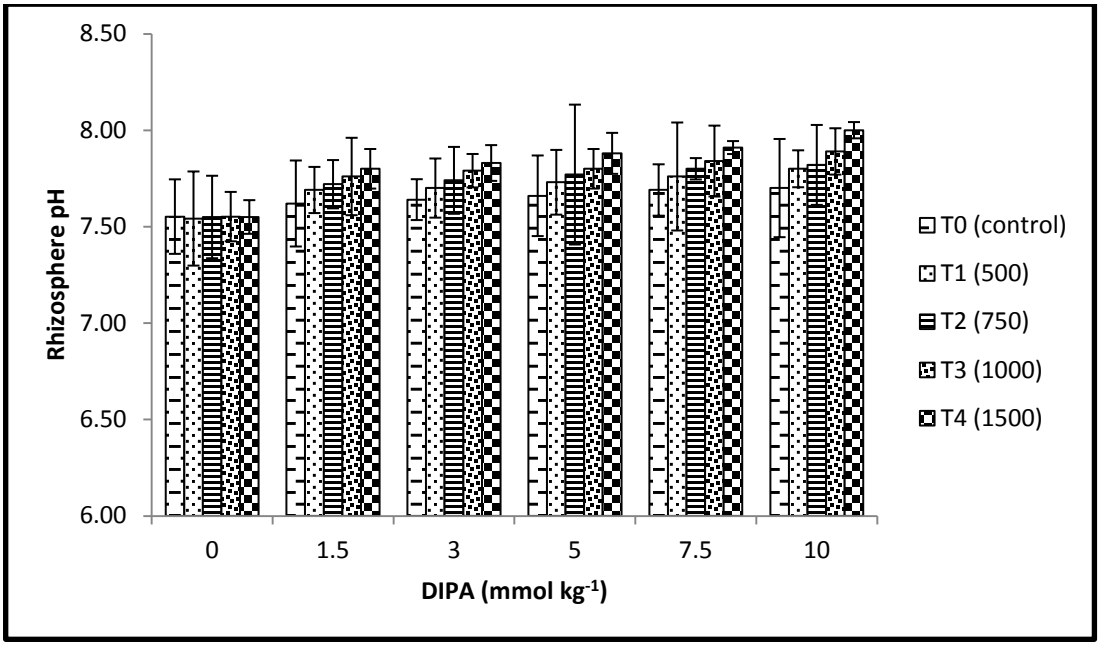

Figure 4.16: Rhizosphere pH in response to DIPA application

In the presence of biochar in soil contaminated with Cd and Zn, pH of rhizosphere reduced and move toward acidification. Rhizosphere acidification is

possible due to uptake of N and P by plant which influences the pH. As the uptake of N and P releases cations from the roots of plant and acidifies rhizosphere pH (Houben *et al.,* 2015). According to (Neumann *et al*., 1999) rhizosphere acidification depends upon environmental constraint, nutrients limitation and response of plant to them.

Rhizosphere pH is mainly the pH of soil around the roots induced by root activities. The release of H^+ and OH^- ion to balance cations anion uptake at crossing point of soil and root is a reason behind pH changes in rhizosphere. According to Arshad *et al*. (2016) Attar cultivars in Pb contaminated soil acidifies pH probably due to release of H^+ ion while Concolor alkalinize rhizosphere pH as a result of OH^- ion discharged. Same in (fig. 4.15 and 4.16) *Pelargonium hortorum* in presence of Pb with EDTA acidifies rhizosphere pH possibly by release of H^+ ion whereas *Pelargonium hortorum* in occurrence of Pb with DIPA alkalinize rhizosphere pH due to release on OH^- ion.

4.6 Pb Concentration in Roots and Shoots of *Pelargonium hortorum*

Figure 4.17 and 4.18 presents the Pb concentration in both roots and shoots of *Pelargonium hortorum* with use of both EDTA and DIPA. In graph we can visualize that concentration of Pb in roots and shoots of *Pelargonium hortorum* increased with increase in concentration of Pb in soil. In the absence of both EDTA and DIPA the increased in Pb concentration of root and shoots of *Pelargonium hortorum* exhibit the normal availability of Pb to plant. Lai *et al*. (2005) studied the same effect of Pb accumulation in rainbow pink.

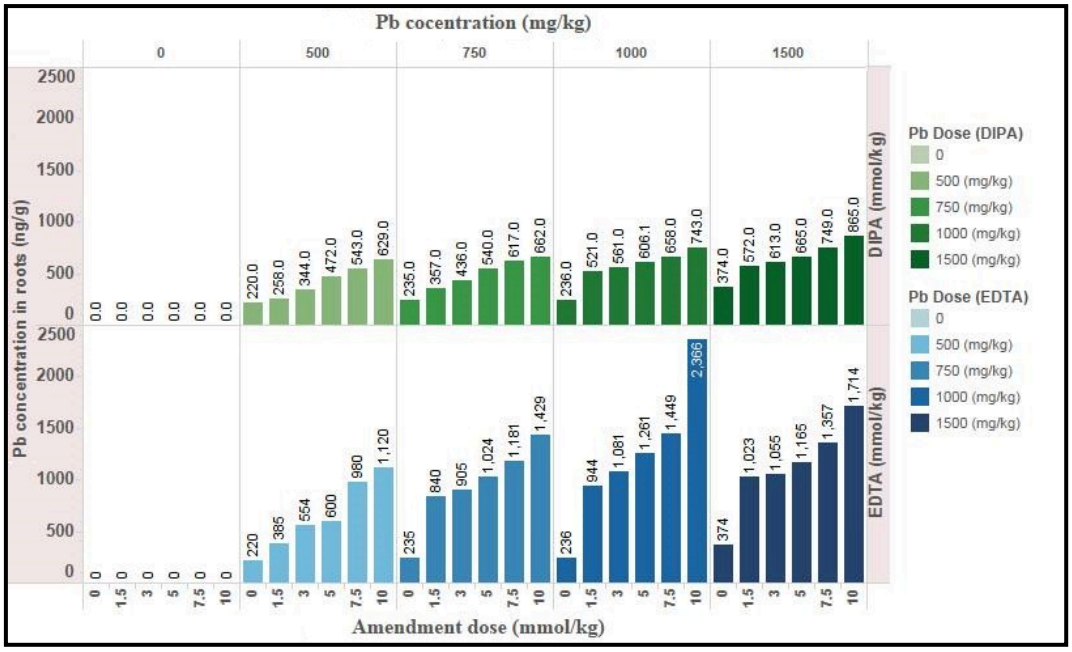

Figure 4.17: Pb concentration in roots of *Pelargonium hortorum* in response to EDTA and DIPA application

The increase of 78% and 56% in Pb concentration in roots of *Pelargonium hortorum* were observed within treatment of Pb1500 mg kg $^{-1}$ + EDTA 10 mmol kg^{-1} and Pb 1500 mg kg $^{-1}$ + DIPA 10 mmol kg^{-1} as compare to control Pb 1500 mg kg $^{-1}$ without EDTA and DIPA (fig 4.17). The maximum concentration of Pb in roots was noticed in combination of Pb 1000 mg kg^{-1} and EDTA 10 mmol kg^{-1}. Concentration of Pb in roots at Pb 1000 mg kg^{-1} and EDTA 10 mmol kg^{-1} is 10-fold additional then concentration of Pb in roots at Pb 1000 mg kg^{-1} without EDTA.

Both EDTA and DIPA were found to be successful in increasing the concentration of Pb in roots of *Pelargonium hortorum*. But comparatively DIPA was detected to be less effective than EDTA in increasing Pb concentration in roots. Wang *et al*. (2007) studied the effect of EDTA and Citric acid on Pb phytoremediation by *B. maximowicziana*. His investigation also reveals that EDTA was more capable to increase concentration of Pb in roots of plant than citric acid. This is mainly due to the reason that EDTA + Pb form stronger complexes than any other amendment with Pb.

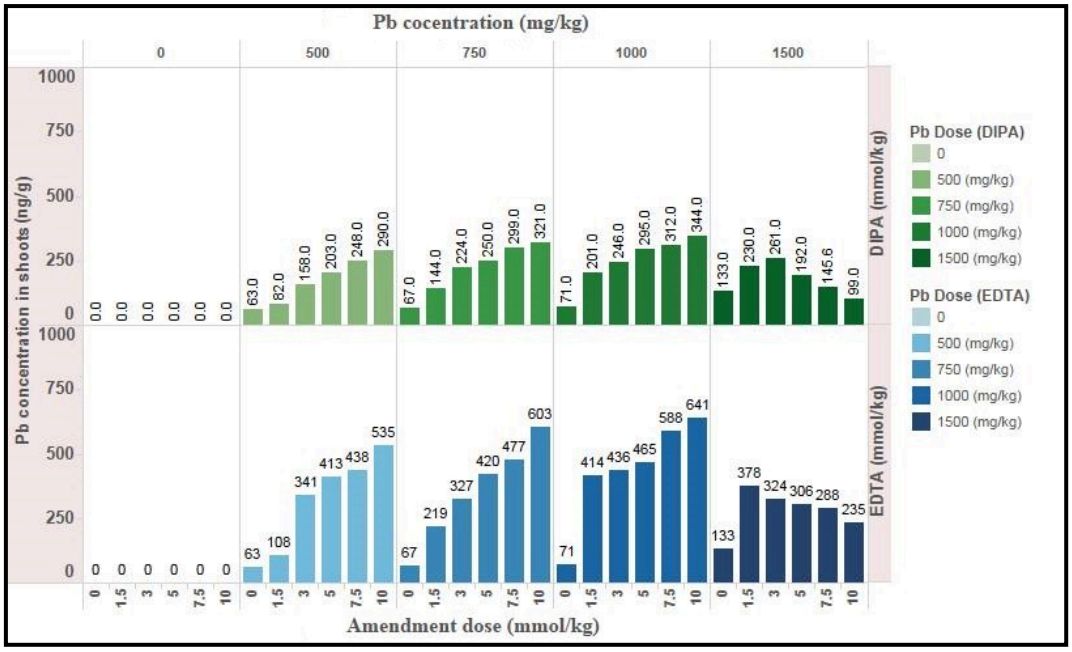

Figure 4.18: Pb concentration in shoots of *Pelargonium hortorum* in response to EDTA and DIPA application

In shoots of *Pelargonium hortorum* an increase of 88% and 73% in Pb concentration were observed within treatment of Pb 1000 mg kg $^{-1}$ + EDTA 10 mmol kg^{-1} and Pb 1000 mg kg $^{-1}$ + DIPA 10 mmol kg^{-1} as compare to control Pb 1000 mg kg $^{-1}$ without EDTA and DIPA (fig 4.18). The maximum concentration of Pb in shoots was noticed in combination of Pb 1000 mg kg^{-1} and EDTA 10 mmol kg^{-1} whereas as slight decrease in concentration of Pb in shoots were observed after EDTA 1.5 mmol kg^{-1} in Pb 1500 mg kg^{-1}. Concentration of Pb in shoots of *Pelargonium hortorum* at Pb 1500 mg kg^{-1} after treated with DIPA 3 mmol kg^{-1}showed a decline (Fig 4.18).

In comparison to DIPA, EDTA was more capable to increase Pb concentration in shoots of *Pelargonium hortorum*. Another study verifies the capability of EDTA for increased concentration of Pb in roots and shoots of *B. maximowicziana* than Citric acid (Wang *et al*., 2007). Corn and bean when investigated in Pb contaminated soil under the effect of both EDDS and EDTA also gives the same result that EDTA was better than EDDS to increase the concentration of Pb in shoots of plant (Luo *et*

al., 2005). To reclaim the contaminated soil to its original state, Pb concentration in shoots of more than 1% of its dry biomass was essential to reduce Pb concentration in soil by 500 mg kg^{-1} over a period of 20-25 years using plants with a high biomass yield 20,000 kg ha^{-1} of dry matter.

Increased concentration of EDTA up to 20 mmol kg^{-1} increase Pb concentration in maize and was more competent than NTA to remediate soil contaminated with Pb (Freitas *et al*., 2009). Pb accumulation in roots and shoots of plant vary due to genetic differences in plant for metal uptake and translocation. Application of EDTA enhance Pb uptake by plant which stressed out the plant even more and cause reduction in its dry biomass. The level of accumulation and translocation than further depend on plant sensitivity to cellular activity and level of Pb contamination (Chen *et al*., 2004). Both EDTA and DIPA increased Pb concentration in roots and shoots of *Pelargonium hortorum*.

R^2 value depicted a positive correlation among increased level of Pb with increased dosage of EDTA and DIPA in concentration of Pb in roots of *Pelargonium hortorum* (fig 4.18 a, b, c, d).

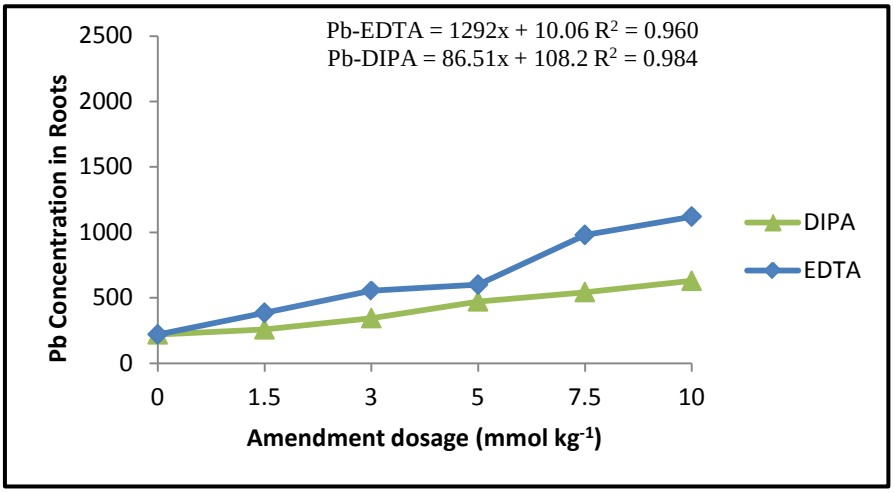

Figure 4.18 (a): Regression between 500 mg kg^{-1} of Pb in root concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

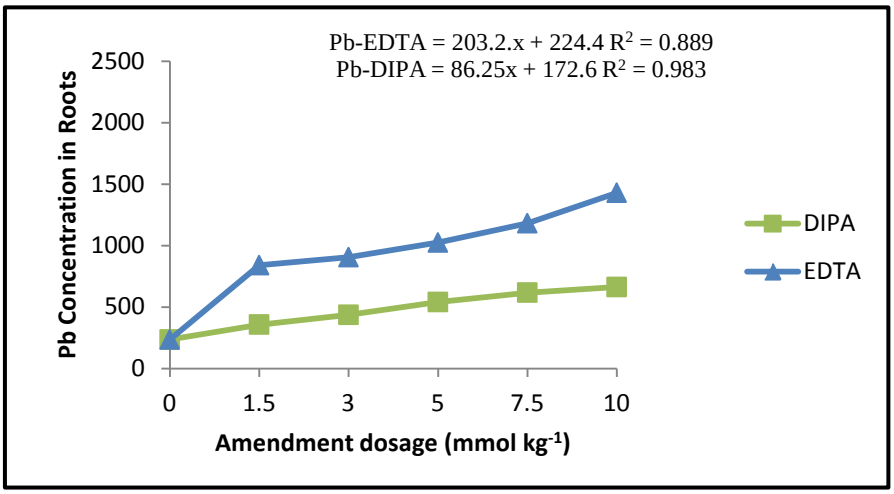

Figure 4.18 (b): Regression between 750 mg kg^{-1} of Pb in root concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

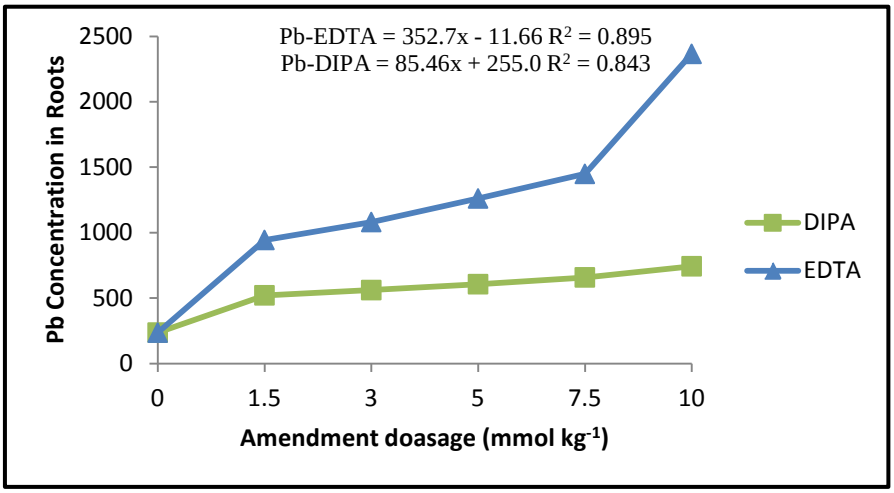

Figure 4.18 (c): Regression between 1000 mg kg^{-1} of Pb in root concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

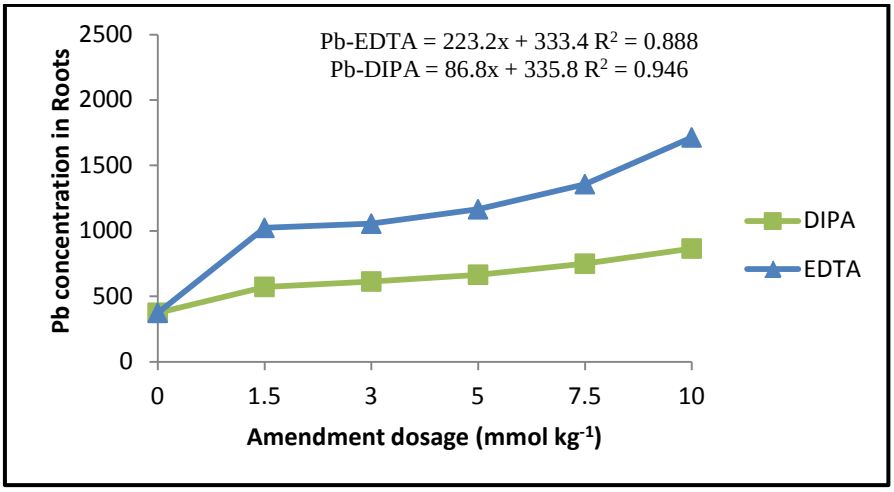

Figure 4.18 (d): Regression between 1500 mg kg^{-1} of Pb in root concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

In (fig 4.18 e, f, g, h) R^2 value indicated the positive relation and an upward trend except at Pb 1500 mg kg^{-1}. Low R^2 value at level of Pb 1500 mg kg^{-1} in relationship with EDTA and DIPA point out the weak correlation and showed a downward trend.

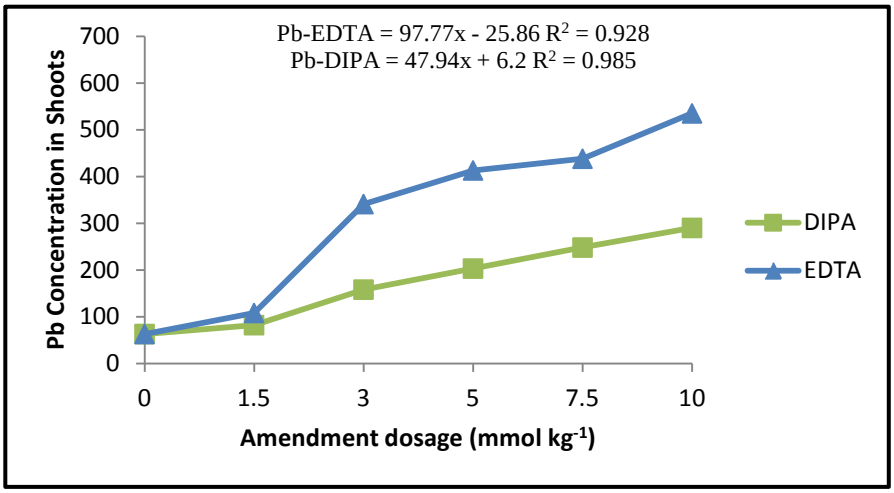

Figure 4.18 (e): Regression between 500 mg kg^{-1} of Pb in shoot concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

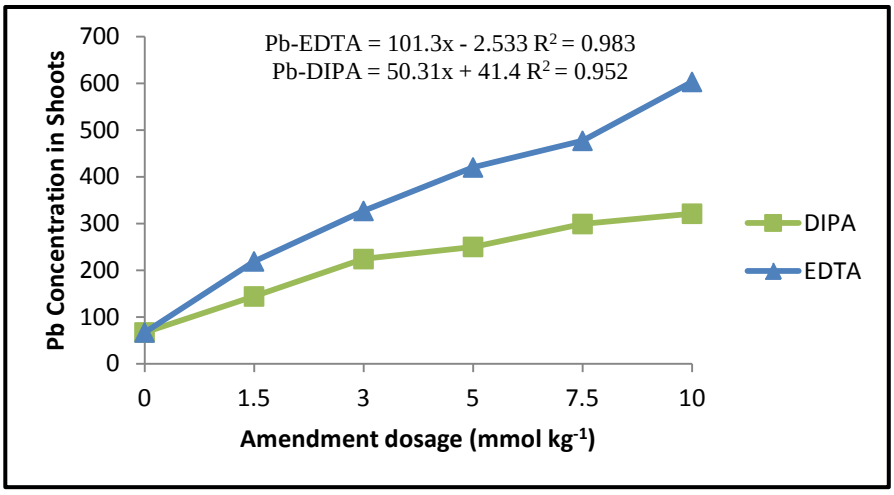

Figure 4.18 (f): Regression between 750 mg kg^{-1} of Pb in shoot concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

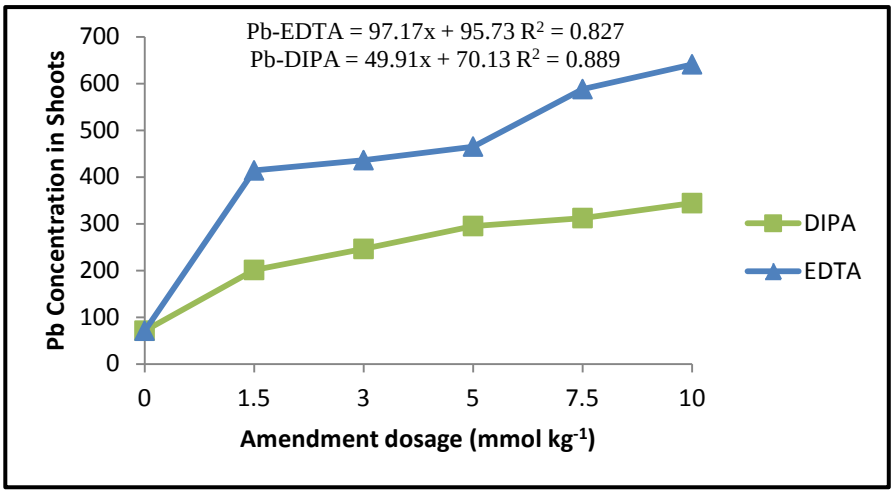

Figure 4.18 (g): Regression between 1000 mg kg^{-1} of Pb in shoot concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

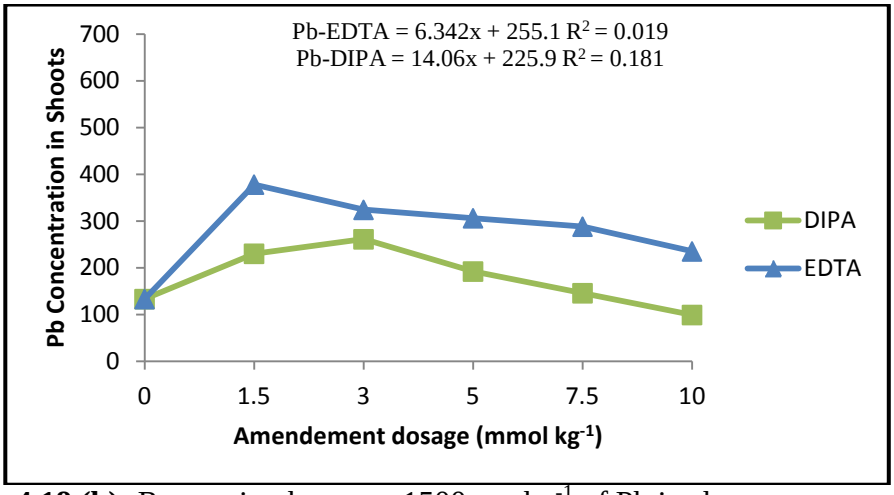

Figure 4.18 (h): Regression between 1500 mg kg^{-1} of Pb in shoot concentration of *Pelargonium hortorum* with different dosage of EDTA and DIPA.

In Table 4.3 & 4.4positive coefficient value indicates a direct relationship, where increased in amendment dose and Pb dose increased Pb concentration in roots and shoots of *Pelargonium hortorum*. Except for DIPA with 1500 mg kg^{-1} of Pb, where one unit increase in DIPA dosage cause 8 unit decrease in Pb concentration in shoots (Table 4.4). Increase in amendment and Pb dose show statistically significant result ($P < 0.05$) with 95% confidence interval. Excluding EDTA and DIPA with Pb 1500 mg kg^{-1} for Pb concentration shoots, where ($P > 0.05$).

Table 4.3: Results of Regression and Analysis of Variance on Pb concentration in roots of *Pelargonium hortorum*

Pb (mg kg^{-1})	Chelating agents	Coef	SE Coef	T	P
500	EDTA	90.47	9.47	9.54	2.65E-10
	DIPA	42.88	3.09	13.8	4.68E-14
750	EDTA	98.30	23.04	4.26	0.000205
	DIPA	41.9	3.40	12.3	7.84E-13
1000	EDTA	175.7	16.44	10.6	2.2E-11
	DIPA	40.80	4.54	8.97	9.83E-10
1500	EDTA	109.09	17.7	6.15	1.19E-06
	DIPA	42.51	3.85	11.01	1.08E-11

Table 4.4: Results of Regression and Analysis of Variance on Pb concentration in shoots of *Pelargonium hortorum*

Pb (mg kg^{-1})	Chelating agent	Coef	SE Coef	T	P
500	EDTA	47.09	5.14	9.14	6.62E-10
	DIPA	23.57	1.56	15.07	5.8E-15
750	EDTA	45.80	4.16	10.9	1.15E-11
	DIPA	24.19	2.23	10.8	1.65E-11
1000	EDTA	46.57	7.06	6.59	3.75E-07
	DIPA	23.71	2.46	9.63	2.17E-10
1500	EDTA	1.28	6.42	0.19	0.84
	DIPA	-8.48	3.21	-2.63	0.013

4.7 Pb Uptake in *Pelargonium hortorum* as Effect of EDTA and DIPA

In particular throughout the study main focus was to enhance the uptake of Pb through EDTA and DIPA. Figure 4.19 presents the effect of EDTA and DIPA on Pb uptake in *Pelargonium hortorum*. The trend in graph shows that increasing concentration of Pb in soil causes uptake in plant to increase without any amendment.

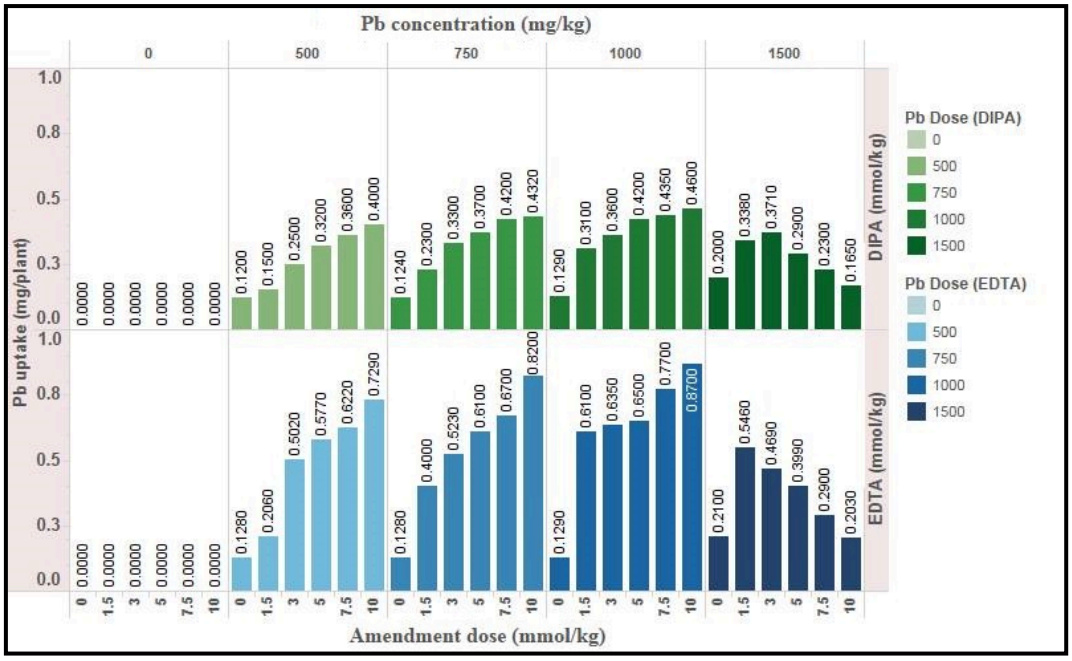

Figure 4.19: Pb uptake by *Pelargonium hortorum* in response to EDTA and DIPA application

The increase in uptake from 0.13 mg/plant in presence of Pb 1000 mg kg^{-1} to 0.87 mg/plant in treatment with Pb 1000 mg kg^{-1} + EDTA 10 mmol kg^{-1} showed 6-fold increase in the uptake of Pb (fig 4.19). In treatment with Pb 1000 mg kg^{-1} + DIPA 10 mmol kg^{-1} the uptake of Pb was measured to be 0.46 mg/plant which are 3-fold increase than 0.13 mg/plant in presence of Pb 1000 mg kg^{-1}. Similar to the studies on effect of EDTA as chelator, in another study addition of EDTA in Pb contaminated soil enhanced uptake of Pb in *Sesbania* more than any other amendment such as DTPA, HEDTA, NTA and citric acid (Ruley *et al*., 2006).

Pb accumulation in *Pelargonium hortorum* under the effect of EDTA and DIPA exhibited that EDTA showed significant increase in Pb accumulation as compared to DIPA (fig 4.19). According to a study done by Hadi *et al*. (2010) EDTA was stronger to form combination with Pb as compared to GA3 and IAA and their strong complex allowed more accumulation of Pb with EDTA than any other chelant.

According to another study, EDTA as a chelating agent enhanced heavy metals uptake in maize with its increased dose of application (Chiu *et al*., 2005). In uptake of heavy metals few factors are involved which change the chemistry. Different plants, type and level of heavy metal contamination, and dose + type of chelant they are exposed to enhance heavy metal accumulation make the process of bioavailability and accumulation (Cui *et al*., 2007).

Keeping in view the trend in fig 4.19, increased dosage of both DIPA and EDTA with increase in concentration of Pb enhanced the uptake of Pb in *Pelargonium hortorum*. EDTA application of 10 mmol kg^{-1} performed best in uptake of Pb with its increased concentration. This is similar to another study done by (Epstein *et al*., 1999) where 10 mmol kg^{-1} of EDTA performed best for uptake of Pb in *Brassica juncea* than the lower doses of EDTA. Continuous decrease in uptake at Pb 1500 mg kg^{-1} with all increased dosage of EDTA after 1.5 mmol kg^{-1} and for DIPA 3 mmol kg^{-1} (fig 4.19) represented that survival of *Pelargonium hortorum* under the stress to uptake large amount of Pb was not possible. Decrease in uptake of Pb at higher concentration of Pb and with increased dosage of EDTA and DIPA was might be due to stress cause by the increase in phytoavailability of Pb to plant. Meer *et al*. (2005) in his study confirm that increase phytoavailability of metal may cause metal toxicity to plant and depressed its potential growth. Both EDTA and DIPA increase Pb uptake in *Pelargonium hortorum*. EDTA was noticed to be more effectual than DIPA in increased Pb uptake by plant (4.19 a, b, c, d).

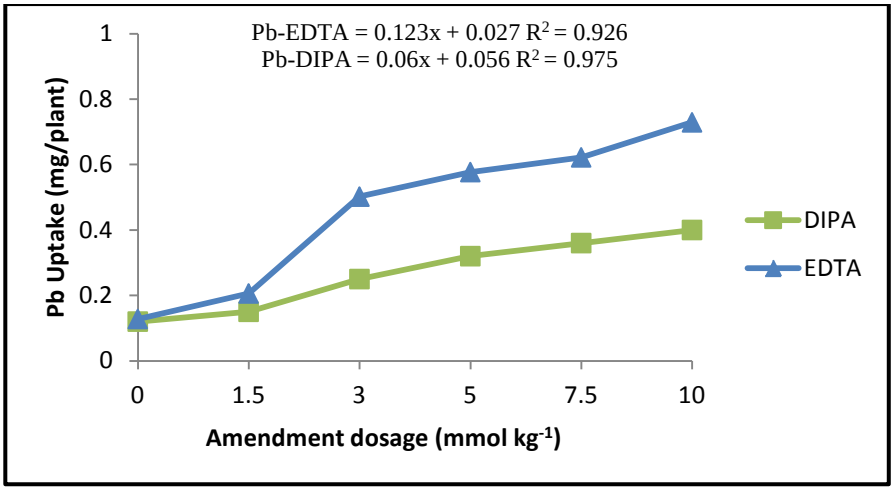

Figure 4.19 (a): Regression between 500 mg kg^{-1} of uptake in *Pelargonium hortorum* with different dosage of EDTA and DIPA.

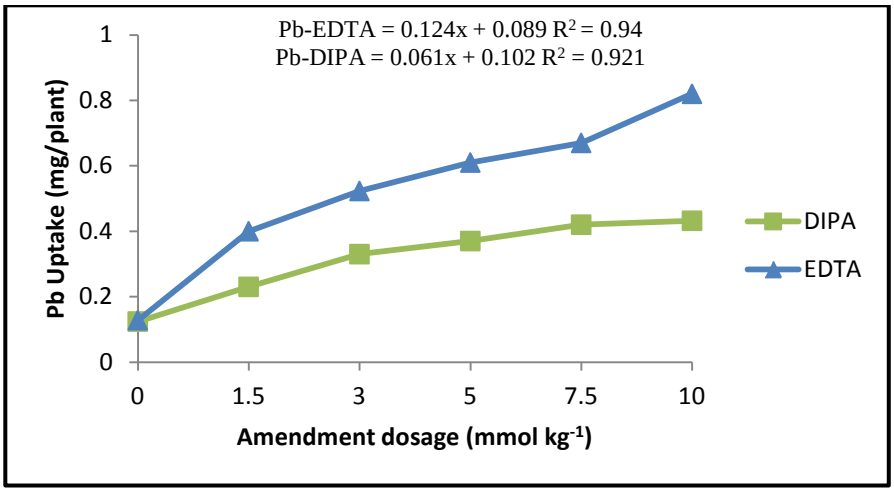

Figure 4.19 (b): Regression between 750 mg kg^{-1} of uptake in *Pelargonium hortorum* with different dosage of EDTA and DIPA.

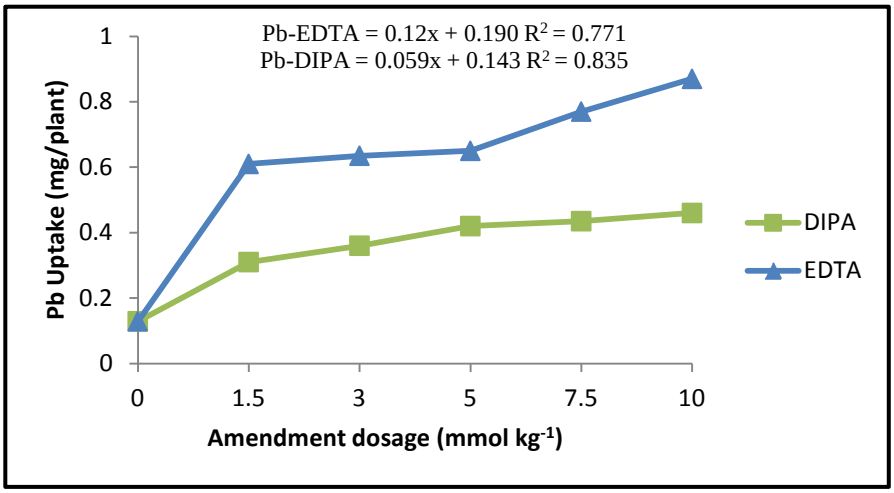

Figure 4.19 (c): Regression between 1000 mg kg^{-1} of uptake in *Pelargonium hortorum* with different dosage of EDTA and DIPA.

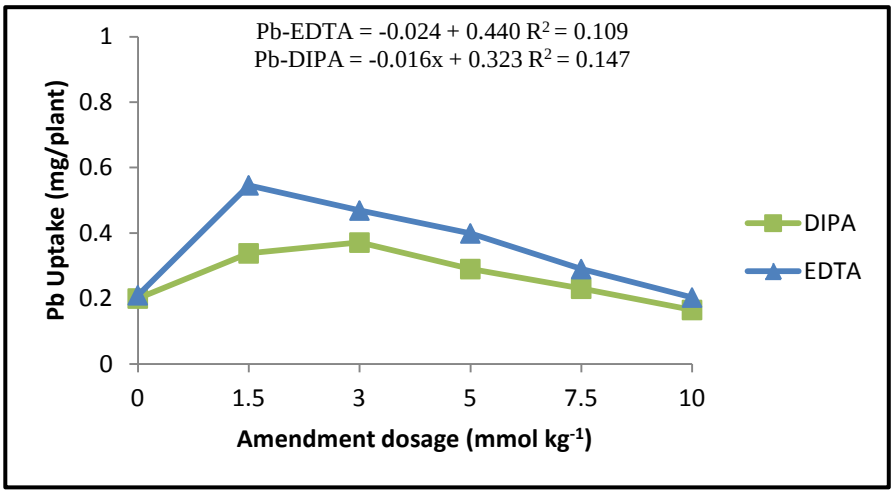

Figure 4.19 (d): Regression between 1500 mg kg^{-1} of uptake in *Pelargonium hortorum* with different dosage of EDTA and DIPA.

Table 4.5: Results of Regression and Analysis of Variance on Pb uptake in *Pelargonium hortorum*

Pb (mg kg^{-1})	Chelating agent	Coef	SE Coef	T	P
500	EDTA	0.059	0.011	5.11	0.006
	DIPA	0.029	0.003	7.70	0.001
750	EDTA	0.06	0.01	5.62	0.004
	DIPA	0.02	0.006	4.74	0.009
1000	EDTA	0.057	0.018	3.12	0.03
	DIPA	0.028	0.008	3.46	0.025
1500	EDTA	-0.015	0.017	-0.9	0.41
	DIPA	-0.01	0.009	-1.08	0.33

Pb uptake by *Pelargonium hortorum* with both EDTA and DIPA show significance ($P \leq 0.05$) with confidence interval 95% except for EDTA and DIPA with Pb 1500 mg kg^{-1} (Table 4.5).

4.7.1 Translocation factor

The success of phytoremediation depends on translocation factor. TF is the ratio of amount of metal to shoot than in its roots. Figure 4.20 exhibit TF of Pb to shoots from that in roots of *Pelargonium hortorum*. The highest TF of 0.68 was calculated in Pb 500 mg kg^{-1} with EDTA 5 mmol kg^{-1}. TF of 0.49 was determined in treatment of Pb 750 mg kg^{-1} and DIPA 3 mmol kg^{-1}. The translocation factor in presence of both EDTA and DIPA is less than 1 which reported that at these concentrations of Pb with different dosage of EDTA and DIPA was not capable to translocate higher amount of Pb in its shoot of *Pelargonium hortorum* than in its roots. According to Aranisola *et al*. (2013) TF > 1 exhibithigh potential of metal extraction from contaminated soil and its translocation from roots to shoots. In another similar study it was expressed that plant with TF value > 1 is a good option

for phytoremediation due to its capacity to accumulate sufficient amount of metal from soil to roots and then into shoots (Wei and Chen 2006).

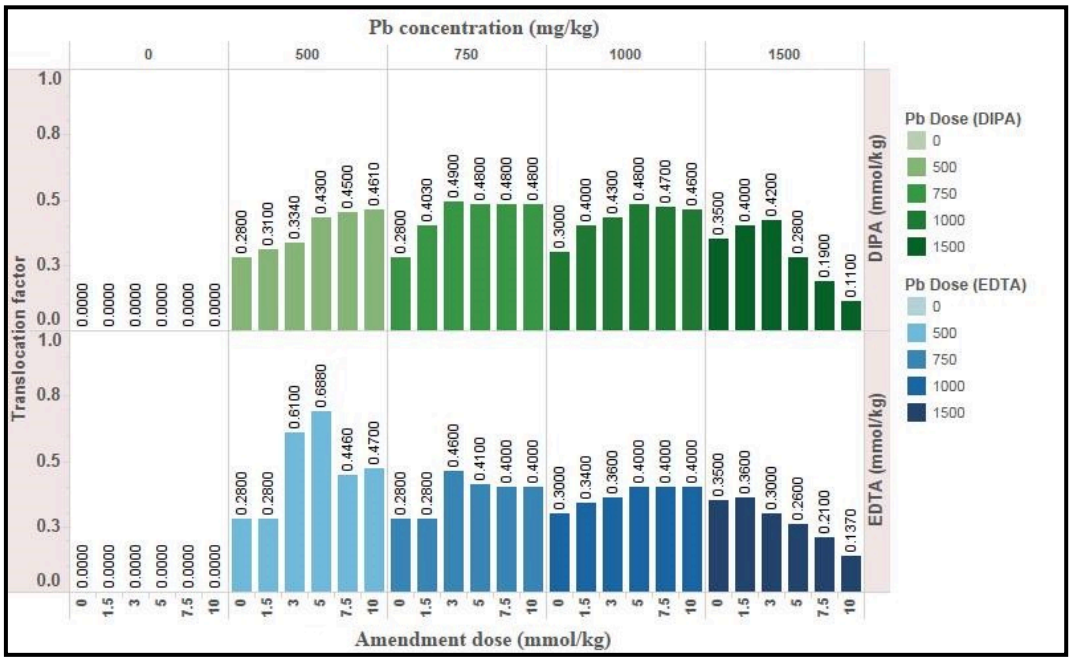

Figure 4.20: Translocation factor of Pb by *Pelargonium hortorum* in response to EDTA and DIPA application

4.7.2 Bioconcentration Factor

Bioconcentration factor basically considered as the potential to accumulate amount of metal in plant than in soil. Figure 4.21 represent value of BCF of *Pelargonium hortorum* in addition of both EDTA and DIPA to Pb contaminated soil. Overall *Pelargonium hortorum* exhibit a trend of > 1 BCF up to Pb 1000 mg kg^{-1} in treatment with EDTA. Whereas in presence of DIPA only 7.5 and 10 mmol kg^{-1} at Pb 500 mg kg^{-1} have BCF > 1. Plants with BCF > 1 are considered to be accumulators and can be used in phytoremediation while plants with BCF value < 1 are not recommended for phytoremediation (Baker, 1981). BCF > 10 by plants are considered to be hyperaccumulator (Ma *et al*., 2001).

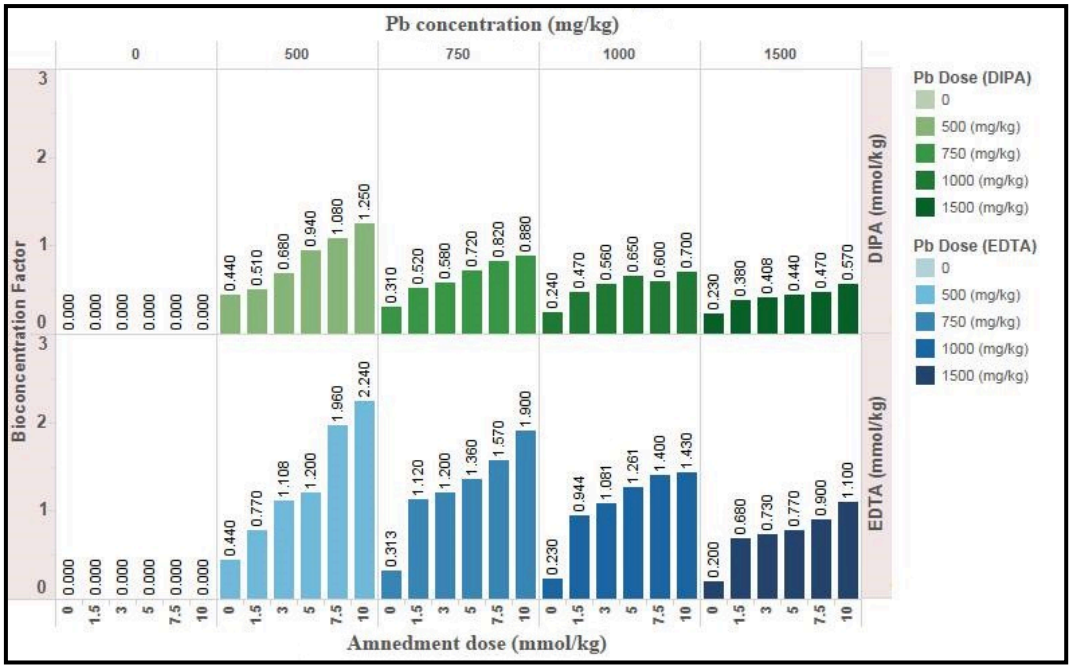

Figure 4.21: Bioconcentration factor of Pb by *Pelargonium hortorum* in response to EDTA and DIPA application

Pelargonium hortorum show BCF > 1 with use of EDTA and a particular dose of DIPA allow it be consider in the category of accumulators according to study done by (Baker, 1981) but not an hyperaccumulator according to criteria mentioned by (Ma *et al*., 2001). According to study done by (Aranisola *et al*., 2013) declared that plant with combination of high BCF and low TF can be utilized for phytostabilization of heavy metals in contaminated soil. Hence low TF value and high BCF value by *Pelargonium hortorum* indicate that they can be used for the purpose of phytostabilization in Pb contaminated soil.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Application of chelating agents in heavy metal contaminated soil affect plant growth in term of root shoot length and biomass. Phytoavailability of Pb spiked with 1500 mg kg^{-1} increased up to 304 mg kg^{-1} by DIPA and 418 mg kg^{-1} by EDTA. Dry biomass of shoots decreased to 1.4-fold with DIPA and 2.3-fold with EDTA. Pb was taken up by roots of *Pelargonium hortorum* more than its aerial parts were confirmed by translocation factor <1. Uptake was maximum at dosage of 10 mmol kg^{-1} of both DIPA and EDTA by 3-fold and 6-fold respectively in soil contaminated with 1000 mg kg^{-1} of Pb in comparison to Pb 1000 mg kg^{-1} alone. It was concluded that EDTA and DIPA both were helpful in phytoavailability of Pb and uptake. Between both chelating agents, EDTA was found stronger to make complexes with Pb and enhance phytoavailability and uptake.

5.2 Recommendations

In spite of the rapid progress in the field of phytoremediation of heavy metals around the globe, we need tremendous scientific and practical approaches for further investigation on this subject. Based on the results of the study, following recommendations can be made for further work, which could be done by investigating further prospects like:

a) The effects of DIPA and EDTA should be observed in combination on Pb and other heavy metals.

b) Application of EDTA and DIPA can be evaluated at different surge times with a variety of plants.

c) An interaction among heavy metal, chelants and rhizosphere communities could be studied.

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