The Effects of EDTA (ethylenediaminetetraacetic acid) and Earthworms (*Eisenia fetida*) on the growth of Mung Beans (*Vigna radiata*) grown in Lead contaminated soil.

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by

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APPROVAL SHEET

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"The Effects of EDTA (ethylenediaminetetraacetic acid) and Earthworms (*Eisenia fetida)* on the growth of Mung Beans (*Vigna radiata*) grown in Lead contaminated soil"

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Abstract

Soil lead contamination is a major environmental problem. Increasing attention has been given to the development of plant-based technology (phytoremediation) to remediate heavy metal-contaminated soils.

This study was supposed to determine the effects of EDTA (ethylenediaminetetraacetic acid) and earthworms (*Eisenia fetida*) on the lead uptake of mung beans (*Vigna radiata*). However, the Atomic Absorption Spectrometer would not be able to detect the extremely small amount of lead in the plant samples. Instead the researchers determined the significant differences in the root length, shoot length, number of leaves and weight after 5 hours of drying of mung bean grown in lead contaminated soil, treated with EDTA only, earthworms only, both EDTA and earthworms and no EDTA and earthworms (control).

Results showed that means of the plants treated with EDTA and earthworms had longer shoots, longer roots, greater number of leaves and weighed more after five hours of drying. One-way ANOVA and Fisher's LSD showed that only the shoot length and the weight after five hours of drying exhibited a significant difference.

This experiment is therefore inconclusive because the lead content of the plants was not measured and thus it is indeterminate whether or not the mung beans absorbed a significant amount of lead.

It is recommended that that the number of mung beans per replicate be increased and that AAS be used to measure the amount of lead absorbed by the plants.

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CHAPTER 1

INTRODUCTION

I. Background of the Study

Soil lead contamination is a major environmental problem facing the modern world (Body et al., 1991; Shen et al., 2002). Lead, a heavy metal, is the most abundant, globally distributed, best-recognized dangerous element that has been used since ancient times.

Despite the fact that its toxic effects has been recognized for several centuries, lead has been used as an anti-knocking gasoline additive since 1920 and remains an important component in the manufacturing of several commercial items such as storage batteries, cable coverings, casting, sheet lead, pipes and ammunition (Gambale et al., 2001). Evidence indicates that high concentrations of several metals have deleterious effects on human health. Lead has been tested and found to be carcinogenic, mutagenic, and teratogenic (Gambale et al., 2001). It affects the reproductive, nervous, gastrointestinal, immune, renal, cardiovascular, skeletal, muscular, and haemopoietic systems (Johnson et al., 1998; Gambale et al., 2001). there have been studies that show how lead affects plant. High concentrations of lead decreased the area of leaves, the content of green pigments in them, and the rate of photosynthesis(Kaznina et al., 2004). Plant growth was affected by the lead. plants grown in high lead treatment were significantly smaller compared to those grown in medium and low lead treatment(Uveges et al., 2002).

Because of lead's detrimental effects as a soil contaminant, there have been studies on how to reduce its concentration in contaminated soil. In spite of the evergrowing number of toxic metal contaminated sites, the most commonly used methods dealing with heavy metal pollution are either the extremely costly process of excavation and burial, or simply isolation of the contaminated sites. Such cleanup is practical only for small areas, often a half hectare or less, and cleaning one hectare to a depth of one meter costs between 600 thousand and 3 million dollars depending on the type and intensity of pollution (Moffat, 1995; Begonia, nd). As conventional cleanup technology is costly, and often harmful to desirable soil properties (texture, organic matter, pH), increasing attention has been given to the development of a plant-based technology (phytoremediation) to remediate heavy metal-contaminated soils. In the phytoremediation process, sequential crops of selected plant species can be cultivated to reduce the concentrations of heavy metals in contaminated soils to environmentally acceptable levels (Raskin et al., 1994; Shen et al., 2002). The plant species used in phytoremediation are known as hyperaccumulators. Hyperaccumulator plants possess an ability to take up abnormally high amounts of heavy metals in their shoots (Chaney et al., 1997; Shen et al., 1997; Shen et al., 2002). Certain plants may uptake as much as 5% of metals on a dry weight basis from the soil (Gambale et al., 2001).

High biomass species such as Maize (*Zea mays)*, Pea (*Pisum sativum)*, Oat (*Avena sativa)*, Indian Mustard (*Brassica juncea)*, and Mung bean (*Vigna radiata)*, possess an ability to uptake abnormally high amount of heavy metals in their shoots. A recent study showed that plants such as: Cabbage (*Brassica rapa)*, Mung bean (*Vigna radiata)*, and Wheat (*Triticum aestivum)*, accumulated lead from lead contaminated soil. (Shen et al., 2002)

Chelating agents enhance the transport of metals (e.g. Zn, Cd, Ni, Cr, Cu, Pb, and Fe) in soil to plants. Synthetic chelates, such as EDTA, have been shown to aid in the accumulation of Pb in the plant tissue. EDTA and other chelates have been used in soils and nutrients solutions to increase the solubility of metal cations and the translocation of Pb into shoots (Wallace et al., 1977;Checkai et al., 1987; Sadiq and Hussain, 1993). Application of EDTA to Pb-contaminated soils has been shown to induce the uptake of Pb by plants, causing Pb to accumulate to more than 1% (w/w) of shoot dry biomass (Vassil et al., 1998). However,in a recent study, chelator toxicity reduced the plant's biomass, thereby decreasing the amount of metal accumulation(Chen et al., 2001).

Some studies used earthworms in enhancing the lead uptake of plants. A recent study about the earthworm, *Lumbricus rubellus* and *Dendrobaena rubida*, the Chloragocytes and intestinal tissue showed significantly higher lead levels in contaminated earthworms than in control material (Ireland et al., 1976). A research study about *Lumbricus terrestris* was used in lead bioaccumulation and exposure to lead compounds of differing solubility. The study examined the relative effects of soluble and less soluble lead compounds on lead accumulation by *Lumbricus terrestris.* Results indicate that *Lumbricus terrestris*feeding on detritus in the upper soil levels can mobilize and accumulate lead from the principal transformation products of metallic lead, as at shooting ranges and lead smelter sites (Darling et al., 2001). Recent studies have shown that the presence of *Eisenia fetida* in the soil can aid in the phytoremediation process. Present results demonstrated that earthworm activity increases the mobility and bioavailability of heavy metals in soils (Wen et al.,2007).

This study used the ability of EDTA and earthworms, as lead uptake enhancer, in the remediation of mung bean on soil contaminated with lead acetate.

II. Statement of the Problem

What is the lead uptake of mung bean treated with EDTA and/or earthworms?

III. Objectives

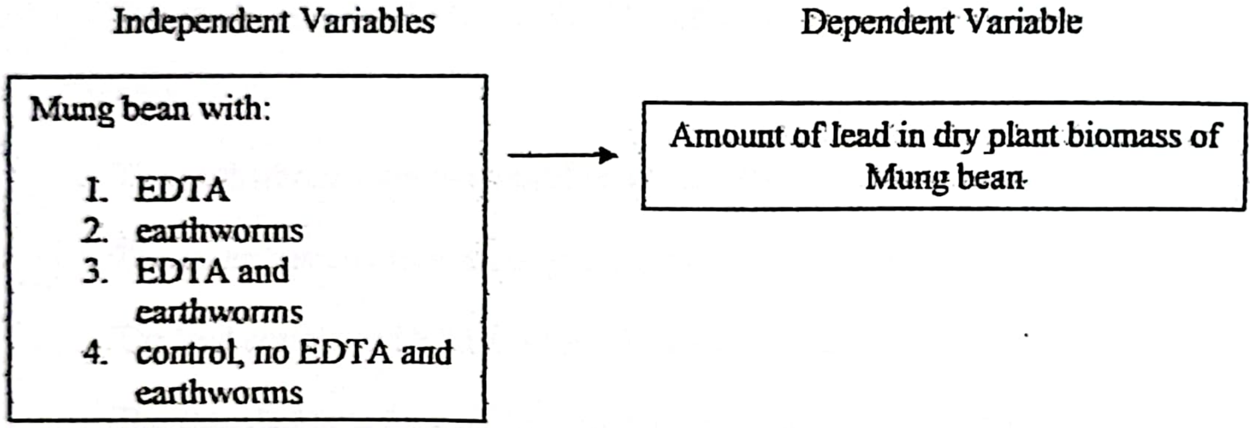
The objectives of this study were:

- To measure the amount of lead in the whole mung bean grown on lead-contaminated soil treated with EDTA
- To measure the amount of lead in the whole mung bean grown on lead-contaminated soil treated with earthworms
- To measure the amount of lead in the whole mung bean grown on lead-contaminated soil treated with earthworms and EDTA
- To compare the amount of lead accumulated by the mung bean with 1) EDTA, 2) earthworms, 3) EDTA and earthworms from 4) control, without EDTA and earthworms

IV. Hypothesis

There will be no significant difference on the lead uptake of the mung bean with and without earthworms and EDTA. There will also be no significant difference on the lead uptake of mung bean treated with 1) EDTA, 2) earthworms, 3) EDTA and earthworms, and 4)without EDTA nor earthworms.

V. Research Paradigm



VI. Significance of the Study

The information from the data gathered will be beneficial in the phytoremediation of lead-contaminated soil. The enhancement of the phytoremediation process will benefit the government agencies, companies and concerned NGOs such as the Department of Agriculture and Philippine Institute of Development Studies, which are responsible for cleaning up the contaminated soil. With the enhancement of the phytoremediation process, it will speed up the accumulation of heavy metals in the plants, thus spending less time than the usual. In the long run, this study will benefit the communities living on or near contaminated soil (dumpsites, junkyards and mines) by giving them information on how to enhance the cost effective process in remediating contaminated soil.

VII. Scope and Delimitations

The general purpose of this study was to determine the amount of lead accumulated by the plant when used with EDTA or with earthworm or with both the EDTA and earthworm.

The plant used in this study was the mung bean (*Vigna radiata*) which has been used in several studies on phytoremediation.

The study was conducted at the Philippine Science High School Western Visayas Campus.

The earthworms were purchased from the UPV.

The mung bean seeds were acquired from a local supermart.

The lead acetate and EDTA were purchased from the Far Eastern Drug.

The expected time for data gathering was one month and two weeks.

The samples were analyzed in the Analytical Chemistry Laboratory of Silliman University. However, the plant samples submitted were found to be very insufficient in amount for detecting lead. Because of that, the researchers decided to measure physical differences between plants instead to determine the effects of the different treatments on the growth of the plants: root length, shoot length, number of leaves and weight after five hours of drying.

The study was limited by the genetic capability of the mung bean to absorb lead and tolerate its presence, and by life span of the earthworms and their lead tolerance. The study was also limited by the competition of the earthworms for the availability of space in the pots and the nutrients in the soil which are needed for their growth. The study was limited by the number of mung bean plants per pot which is essential to the lead analysis of the plant using AAS.

VIII. Definitions

Atomic spectroscopy - the determination of elemental composition of a substance by means of its electromagnetic or mass spectrum. Atomic spectroscopy is closely related to other forms of spectroscopy.

- in this study, atomic spectrometry refers to the method on how determine the Pb element found in the dry plant biomass of mung bean.

Chelating Agent – a substance used to reduce the concentration of free metal ion in solution by compounding it. A compound that combines with metal ions to form stable ring structures

- in this study, chelating agent refers to EDTA which will be used to combine with lead acetate to form

Earthworm any of a number of oligochaetous worms that burrow in the soil.

- in this study, earthworms refer to the *Eisenia fetida*

EDTA - a popular acronym for the chemical compound ethylenediaminetetraacetic acid. It refers to the chelating agent that is widely used to sequester di- and trivalent metal ions.

Hyperaccumulators - plants that possess an ability to take up abnormally high amounts of heavy metals in their shoots.

- in this study, hyperaccumulators refer to the mung bean.

Lead - is a chemical element in the periodic table that has the symbol Pb and atomic number of 82. It is a soft, heavy, toxic and malleable poor metal which is bluish white when freshly cut but tarnishes to dull gray when exposed to air.

- in this study, lead refers to the Lead Acetate

Lead Acetate – a toxic soluble chemical compound, a white crystalline substance with a sweetish taste

Mobilizers – one that is responsible for making something movable or fluid.

- in this study, mobilizers refer to the earthworms.

Mung bean (*Vigna radiata*)- the dry plant biomass used as a hyperaccumulator in this study.

Phytoremediation - the treatment of environmental problems (bioremediation) through the use of plants

Soil – the top layer of the earth's surface, consisting of rock and mineral particles mixed with organic matter

- in this study, soil refers to the loam.

Solubilizers - makes something soluble or dispersible, especially by adding a detergent.

- in this study, solubilzers refer to EDTA that acts as a solubilzer of lead in the soil

CHAPTER 2

REVIEW OF RELATED LITERATURE

I. Phytoremediation

Phytoremediation is a process of decontamination of the soil by the use of plants to absorb heavy metals or other pollutants. Phytoremediation is aimed at providing an innovative, economical and environmental-friendly approach to removing toxic chemicals from hazardous waste sites. (Henry et al., 2000)

Contaminated soils and waters pose a major environmental and human health problem, which may be partially solved by the emerging phytoremediation technology. This cost-effective plant-based approach to remediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. Toxic heavy metals and organic pollutants are the major targets for phytoremediation. In recent years, knowledge of the physiological and molecular mechanisms of phytoremediation began to emerge together with biological and engineering strategies designed to optimize and improve phytoremediation. A study indicated that *Raphanus sativus*, a hyperaccumulator, was used to investigate the distribution of applied lead in the seed and seedling stage. Lead contamination of the embryo was inhibited by the testa until it ruptured during inhibition. In seedlings, lead moved into root cortex predominantly along the cell walls as far as the endodermis. Beyond the endodermis lead was localized extensively in the vascular tissues. There was little movement of lead from the vascular tissue into the surrounding tissue of the stem and cotyledons (Lane et al., 1977). Different processes of phytoremediation include: Phytoaccumulation, Phytodegradation, Phytostabilization, Phytoextraction and enhanced rhizosphere biodegradation.

II. Plants

A. Mung Bean

The mung bean (*Vigna radiata)* is a seed native in India. The beans are small, ovoid in shape and green in color. The mung bean is one of many species recently moved from the genus *Phaseolus* to *Vigna* and is still often seen cited as *Phaseolus aureus* or *Phaseolus radiatus*. These are all the same plant. Mung beans are tropical (or sub-tropical) crops, and require warm temperatures (optimal at 30-35°C). Loamy soil is best for mung bean cultivation. The germinated seeds are bean sprouts. Seeds are germinated at 65 to 70°F for 4-5 days. Mung beans are used in the phytoremediation of lead contaminated soil. It is a hyperaccumulator that has an ability to take up normally abnormally high amounts of heavy metals in their shoots (Yong et al., 1998). In a recent study during the first seven days, lead uptake by the mung bean was greater when EDTA was added than the control with no EDTA. After another seven days, lead uptake increased but the one treated with EDTA still showed the most hyperaccumulated lead (Shen et al., 2002). In other studies, the mung bean had a higher sensitivity to the EDTA treatment in soils. In the 2.5 and 5.0 mmol kg^{-1} EDTA treatments, the Pb concentrations in the shoots of the six dicotyledon species ranged from 1000 to 3000 mg kg^{-1} of dry matter (Chen et al., 2004).

III. Lead

Lead is a chemical element in the periodic table that has the symbol Pb and atomic number 82. A soft, heavy, toxic and malleable poor metal, lead is bluish white when freshly cut but tarnishes to dull gray when exposed to air. Lead is used in building construction, lead-acid batteries, bullets and shot, and is part of solder, pewter, and fusible alloys. Lead has the highest atomic number of all stable elements. Lead has a dull luster and is a dense, ductile, very soft, highly malleable, bluish-white metal that has poor electrical conductivity. Lead is also poisonous that may give adverse effects to plants such as soil ingestion, change of color in plant's leaves and change in the length of the plant (Gambale et al., 2001).

Despite the fact that its toxic effects has been recognized for several centuries, lead has been used as an anti-knocking gasoline additive since 1920 and remains an important component in the manufacturing of several commercial items such as storage batteries, cable coverings, casting, sheet lead, pipes and ammunition (Gambale et al., 2001). Soluble compounds of lead include: lead nitrate and lead acetate while lead coupled with chloride, bromide and iodide are said to be insoluble. The concentration of lead ranges from 200 to 1800mg/kg and a majority of the soil lead is considered available for plant uptake. 28 percent of the total lead occurred in the non-available form. The use of synthetic chelates will enhance the ability of the plant to take up lead by making the lead soluble and available to the plant (Gorman , 2002).

Lead may also be harmful to humans and may also give effects to the body. Such effects may be difficulty in breathing, affection of the oral and renal areas. (http://en.wikipedia.org/wiki/lead). Lead has also been tested and found to be carcinogenic, mutagenic, and teratogenic (Gambale et al., 2001).

Lead usually ends up as a soil contaminant by percolation of contaminated surface water to subsurface strata, leaching of wastes from landfills or direct discharge of industrial wastes to the soil.

The mobilization of metal contaminants, both in the soil and the plant, is another important factor influencing the success of phytoremediation. The amount of soluble Pb^{2+} in the soil appears to be a key factor to the enhancement of Pb^{2+} uptake by plants (Wu et al., 1999). There are two main amendment techniques that have been used to increase the bioavailability of lead in soils and the mobility of lead within plant tissues: lowering soil pH and adding synthetic chelates.

Lead (II) acetate is a chemical compound, a white crystalline substance with a sweetish taste. It is made by treating litharge (lead (II) oxide, PbO) with acetic acid. Like other lead compounds, it is very toxic. Lead acetate is soluble in water and glycerin. With water it forms the trihydrate, $Pb(CH_3COO)_2{\cdot}3H_2O$, a colorless or white efflorescent monoclinic crystalline. It is used in dyeing cotton and in making enamels and varnishes. Lead (II) acetate, among other lead salts, has been reported to cross the placenta and to the embryo leading to fetal mortality. Lead salts also have teratogenic effect in some animal species. Lead can be absorbed through the respiratory system. Local irritation of bronchia and lungs can occur and, in cases of acute exposure, symptoms such as metallic taste, chest and abdominal pain, and increased lead blood levels may follow. Lead and lead compounds may be absorbed through the skin on prolonged exposure; the symptoms of lead poisoning described for ingestion exposure may occur. Contact over short periods may cause local irritation, redness and pain. Before dealing with this kind of compound, protective gloves, Safety goggles, or eye protection in combination with breathing protection should be worn to prevent skin and eye contact and prevent inhalation problems.

IV. Earthworms

Earthworm activity aerates and mixes the soil, and is constructive to mineralization and nutrient uptake by vegetation. Certain species of earthworm come to the surface and graze on the higher concentrations of organic matter present there, mixing it with the mineral soil. The earthworm is essential to composting; the process of converting dead organic matter into rich humus, a medium vital to the growth of healthy plants, and thus ensuring the continuance of the cycle of fertility. The earthworm is of great value in keeping the soil structure open, creating a multitude of channels which allow the processes of both aeration and drainage to occur. Various species of worms are used in vermiculture, the practice of feeding organic waste to earthworms to decompose (digest) it, a form of composting by the use of worms. These are usually *Eisenia fetida* or the Brandling worm, also known as the Tiger worm or Red Wriggler, and are distinct from soil-dwelling earthworms (http://en.wikipedia.org/wiki/Earthworm). Earthworms have the potential to accumulate significant levels of lead, and thus earthworm ingestion may result in lead transfer to higher trophic levels (Darling et al., 2001). In a recent study about the earthworm, *Lumbricus rubellus* and *Dendrobaena rubida*, the Chloragocytes and intestinal tissue showed significantly higher lead levels in contaminated earthworms than in control material (Ireland et al., 1976). A research study about *Lumbricus terrestris* was used in lead bioaccumulation and exposure to lead compounds of differing solubility. The study examined the relative effects of soluble and less soluble lead compounds on lead accumulation by *Lumbricus terrestris*. Results indicate that *Lumbricus* *terrestris*feeding on detritus in the upper soil levels can mobilize and accumulate lead from the principal transformation products of metallic lead, as at shooting ranges and lead smelter sites (Darling et al., 2001). Not only *Lumbricus terrestris* was used in lead bioaccumulation but in iron phosphate and metaldehyde slug pellet formulations too wherein exposure to iron phophate increased earthworm mortality but surviving earthworms gained less mass than those exposed to metaldehyde (Langan et al., 2005). A recent study about the Cadmium, Zinc, and Lead uptake by earthwórms wherein Cd, Zn, and Pb appeared to be more strongly accumulated by L. rubellus when present in soil with a low pH value. Cu was the only exception in this regard; its uptake by L. rubellus was not significantly influenced by soil pH. The organic matter content of the soil, mainly, garden soil, played a significant role only in the worm uptake of Pb. Soil Pb content, soil pH, and soil organic matter content together accounted for almost 70 percent of the variance in worm Pb content. The results indicate that L. rubellus accumulates Pb more strongly in soil with a low pH and low organic matter content than in soil with higher values of these parameters. The demonstrated influence of pH and organic matter content on element concentration in earthworms emphasizes the importance of soil factors in governing the entrance of toxic metal elements into the food web. Studies have estimated that the potential life span of earthworms of the family Lumbricids specifically *Eisenia fetida* under field conditions is 4 to 8 years while earthworms under laboratory conditions 4 and a half years (Korschelt, 1914). Other studies have also cited that metal mobility increases with earthworm density (Ashton et al., 2006).

A. *Eisenia fetida*

Eisenia fetida, known under various common names, including redworms, brandling worms and red wiggler worms, is a species of earthworm adapted to the environment of decaying organic material. Eisenia fetida are able to tolerate a temperature range of 40 to 90 degrees Fahrenheit, with an ideal range of 70-75 degrees. This moderate-sized earthworm has a very distinctive striped appearance. The pink to purplish red pigmentation occurs in bands separated by unpigmented areas which have a yellowish hue. The red pigmentation is largely restricted to the upper surface of the body. The common names of brandling and tiger worm refer to the stripy appearance, the red streaks supposedly resembling brand marks. The brandling is sometimes found in deep woodland leafmould. It can be extremely abundant in manure and compost heaps. When the worm is irritated it exudes a musty (foetid) yellow fluid onto the body through small pores on the upper surface. This seems to be a defence against predators, the characteristic red and yellow stripes being a warning to predators to leave it well alone. This species grows and reproduces extremely quickly and is much used in worm farming. Although far from being a typical earthworm in its way of life this species is frequently used to test the toxicity of soil pollutants. It thrives in rotting vegetation, compost, and manure. It is rarely found in soil, and prefers conditions where other worms cannot survive. They are encountered most often as fishing bait. Also, because they can speed the process of converting compost into soil, they are used in areas where they occur naturally. In addition, they are commonly used to process kitchen waste into worm castings. This process is known as vermiculture, with an end result of *vermicompost*.

Recent studies have shown that the presence of *Eisenia fetida* in the soil can aid in the phytoremediation process. A recent study demonstrated that earthworm activity increases the mobility and bioavailability of heavy metals in soils (Wen et al., 2004).

V. EDTA

Because hyper accumulators cannot completely extract large amounts of metal from the soil and into their bodies, EDTA was created to further enhance the solubility of metals in the soil. EDTA is a popular acronym for the chemical compound ethylenediaminetetraacetic acid. EDTA refers to the chelating agent that is widely used to sequester di- and trivalent metal ions. Recalcitrant chelating agents such as EDTA are an environmental concern predominantly because of their persistence and strong metal chelating properties. Chelating agents enhance the transport of metals (e.g. Zn, Cd, Ni, Cr, Cu, Pb, and Fe) in soils to plants. Synthetic chelates ,such as EDTA, have been shown to aid in the accumulation of Pb^{2+} in the plant tissue. EDTA and other chelates have been used in soils and nutrient solutions to increase the solubility of metal cations and the translocation of Pb into shoots (Wallace et al., 1977; Checkai et al., 1987; Sadiq and Hussain, 1993).

Despite an overall increase of Pb in the shoots, there are differences, however, in the extent of accumulation at equivalent chelate levels among various plant species (Huang et al., 1997). In recent research, EDTA has been shown to induce heavy metal uptake in selected high bio-mass hyper accumulating plants such as: corn (*Zea mays*), indian mustard (*Brassica juncea*) and sunflower (*Helianthus annus)* and have been used to extract metals from soils and have been widely studied and used in the field of agriculture. Although the presence of EDTA may not affect lead uptake, but it was also proven to improve translocation of metals from roots to shoots (Lee et al., 2003). A study demonstrated that chelants increased lead uptake into the shoots but reduced Copper and Zinc uptake from solution. Uptake of essential metals that are normally taken up via the symplastic pathway, is reduced by the addition of chelates by rendering the metal unavailable to the plant's metal transporters into the symplast. Nevertheless, high concentrations of chelants and soluble metal in the substrate can induce plant uptake. Uptake of metals in the presence of chelates requires hat the metal pass directly into the root xylem via the apoplastic pathway, in part helped by the disruption of the root endodermis caused by high concentrations of chelants in solution. It is also said that solubilisation does not necessarily induce bioaccumulation. There are environmental concerns about the use of induced bioaccumulation due to metal leaching through the soil profile, possibly entering groundwater. While EDTA is the most often studied compound for chelant-enhanced phytoextraction, its use in this role is unacceptable due to the severe risk of leaching high concentrations of mobile metal-complexes to groundwater (Robinson et al., 2006). Other studies have also cited that EDTA amendment did not significantly reduce earthworm survival rates, EDTA treatment of field-derived soils significantly increase earthworm concentrations in nickel and lead but reproduction rates were very low in lead and zinc contaminated soils (Jones et al., 2006).

VI. Effects of Lead and EDTA on the growth of Mung Bean (*Vigna radiata*)

Some studies showed that EDTA at a rate of 0.5 g/kg significantly increased the shoot concentrations of Cd and Ni from 34 and 15 to 115 and 117 mg/kg, respectively. The total removal efficiency for EDTA was 59 µg/plant (Chen et al., 2001). Other studies also showed that The effect of lead (in the form of $Pb(CH_3COO)_2$ $3H_2O$ at concentrations of 200, 400, and 800 mg/kg substrate) was studied. Exposure to low concentrations of lead (200 mg/kg) slightly increased the content of chlorophyll in leaves and the rate of photosynthesis. Medium concentrations of lead (400 mg/kg) had no marked effect on the parameters analyzed, whereas high concentrations (800 mg/kg) decreased the area of leaves, the content of green pigments in them, and the rate of photosynthesis (Kaznina et al., 2004). Plants grown in lead-free conditions were larger than plants exposed to lead. Plants in the low (500 mg/l) and medium (1000 mg/l) lead treatments did not differ from each other, while plants in the high (2000 mg/l) lead treatment were significantly smaller. However, the biomass allocation to roots was not significantly different among treatments (Uveges et al., 2002). Some studies also showed that the increase in root and shoot growth was 20% and 19.5%, respectively, in the presence of $(CH_3COO)_2Pb$ (660 M). Moreover, concentration of accumulated lead in root and shoot was also reduced in the presence of this isolate ranging from 37.5 to 93.19% however; the 19.58% decrease in chlorophyll content in the case of lead acetate remained unchanged (Tripathi et al., 2005). About 3.5% of soils Pb were leached from the soil columns after the application of 5.0 mmol kg^{-1} of EDTA. The growth of sunflowers in the soil columns had little effect on the amount of metals that were leached out. This was probably due to the shallowness of the layer of soil, the short time-span of the uptake of metals by the plant and the plant's simple root systems.

CHAPTER 3

METHODOLOGY

Research Design:

Experimental Organism:

Mung Bean (*Vigna radiata*)

Variables:

Earthworm (*Eisenia fetida*)

EDTA (ethylenediaminetetraacetic acid)

Set-ups (three replications each):

Mung beans grown in Pb-contaminated soil treated with:

1. EDTA (pots labeled: B,D,J,S)

2. *Eisenia fetida* (pots labeled: G,I,P,Q)

3. EDTA and *Eisenia fetida* (pots labeled: R,T,C)

Control:

No EDTA and *Eisenia fetida* but mung beans only (pots labeled: A,E,F)

Measurements:

The amount of lead in the mung beans was to be measured in milligrams of lead (Pb) per kilogram of dry plant biomass (mgPb/kg). However, since it was not possible to measure this, the following parameters were measured instead:

Root Length (cm)

Shoot Length (cm)

Number of Leaves

Weight after 5 hours of drying

I. Contaminating the Soil

A. Materials

80g lead acetate $Pb(CH_3COO)_2 \cdot 3H_2O$

4 500mL beakers

4 spoons

1 analytical balance

1 weighing scale

4 1.5L bottles of distilled water

1 fume hood

1 pair of gloves, mask, lab gown and pair of goggles per person

1 pH meter

1 lead(Pb) probe

36L loam

12 3L plastic pots with bottom perforations (labeled A- L)

12 plastic saucers

B. Gathering/ Construction of Materials

250g of lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$) were purchased at a local chemistry supply store. The excess lead acetate were turned in to the Chemistry Department of PSHS-WV for proper disposal/storage. Lab equipment was borrowed from the PSHS-WV Laboratory. Other materials are readily available.

C.1 Procedure

The loam was put into the plastic pots and was weighed. The lead probe will then be use to determine the concentration (if any) of lead in the soil prior to contamination. Once the soil is weighed, the amount of lead nitrate to be added was computed so that every kilogram of soil will have 1000mg of lead acetate (1000mg $Pb(CH_3COO)_2 \cdot 3H_2O$/ kg soil).

Under a fume hood so as to prevent inhalation of the fumes, the lead acetate was weighed to the proper amount for each corresponding pot and will mixed with 400mL water. The lead acetate dissolved in the water will then be poured evenly into its corresponding pot to contaminate the soil. The lead probe was used to measure the amount of lead in the soil on the day of contamination and on the succeeding days.

C.2 Safety

When handling lead acetate the researchers will abide to the highest possible safety practices. The lead will always be handled under a fume hood and will not be allowed to come in contact with the skin. Lab gowns were always worn. Chemical safety goggles were used to protect the eyes. Food and drinks will not be allowed into the lab and experiment area.

II. Planting the Plants and Introduction of Worms and EDTA into the soil

A. Materials

1 230cm x 25cm x 15cm seedbed filled with loam

20 mung bean (*Vigna radiata*) seeds

1 pH meter

1 lead(Pb) probe

2 sets gardening tools

40 earthworms (*Eisenia fetida*)

EDTA (in a 50mM solution)

B. Gathering/ Construction of Materials

The seedbed was made of materials and soil available at the PSHS-WV campus. All seeds were purchased at an agricultural supply store. The weighing scale was borrowed from PSHS-WV Laboratories. The earthworms were purchased at the University of the Philippines in the Visayas. The EDTA was purchased at the Far Eastern Drug Store in Cebu City.

C. Procedure

The plants were grown outdoors under natural light conditions at Philippine Science High School Western Visayas Campus. Twenty mung bean seeds were sown 6'' apart in one seed bed. After seven days, the emerged seedlings were thinned to 12 seedlings and transferred to individual plastic pots filled with the soil contaminated with lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$). The plants were allowed to acclimatize to the soil for 3 days. After the acclimatization period, 5 earthworms from the species *Eisenia fetida* were introduced to the pots labeled GIPQRTC. After 1 more day, the pots labeled BDJSRTC were treated with 30mL of 50mM EDTA by application to soil surface. The pots labeled A, E and F will serve as control set-ups and will not have EDTA and worms.

III. Maintenance of Plants for the Duration of the Experiment

A. Materials

1 notebook for notes and observations

1 watering can with graduations

distilled water

sticks

polyethylene film

chicken wire

12 wooden/ plastic signs to aid in pot identification

4 large warning signs to deter people

B. Gathering/ Construction of Materials

The materials can be purchased from a local hardware or garden supplies store or can be improvised with available materials.

C. Procedure

The plants were harvested 10 days after the transplanting of seedlings to the pots containing the contaminated soil. Any metal toxicity symptoms (e.g. discoloration, pigmentation, yellowing, stunting, necrosis) that were exhibited by the plants were visually noted during the experimentation period.

To ensure the safety of everyone, the area within 5m radius of the study site were off-limits to everyone except the researchers and their advisers. All people entering the study site were required to adhere to standard safety procedures. (See I.C.2 Safety)

Excess soil moisture draining from perforations at the bottom of each pot were trapped in a plastic saucer beneath each pot to prevent leaching into the soil and cross contamination among plants. The drainage that accumulates in the saucers were watered back to the plant to prevent possible loss of lead in the growing medium.

When rain was imminent, plants were covered with a clear polyethylene film that were secured on chicken wire and placed above the plants.

IV. Collecting the Plants and Measuring the amount and concentration of lead (Pb) in the Plant Biomass

A. Materials

Gardening tools

Analytical Balance

Distilled water

3 Hot plates

mortar and pestle

Ziploc bag (labeled and numbered)

12 round bottom flasks

Concentrated acid solution

(4 parts:12 mol HCl 1 part: 18 mol HNO_3)

graduated cylinder

reagent bottles

fume hood

12 clamps

12 iron stands

B. Gathering/ Construction of Materials

The materials were borrowed from the PSHS-WV Laboratories. The samples were sent to the Silliman University Chemistry Department for Atomic Absorption Spectrometry. However, the plant samples submitted were found to be very insufficient in amount and were sent back to the researchers because the chances of detecting lead would be extremely small.

V. Collecting the Plants and Measuring the Root Length, Shoot Length, Number of Leaves and Weight after Five Hours of Drying

A. Materials

Gardening tools

Gloves

Analytical Balance

Distilled water

Ziploc bag (labeled and numbered)

Oven

Paper

Thread and Scissors

Vernier Caliper

B. Gathering/ Construction of Materials

The equipment was borrowed from the PSHS-WV Laboratories. The other materials were readily available.

C. Procedure

After 10 days, the plants were harvested. The whole plants were washed with distilled water to remove adhering debris. The shoot and root lengths were measured by cutting the thread to the same length as that of the plants root and shoot and then measuring the length of the thread using the vernier caliper. The number of leaves was visually noted. The plants were then oven dried at 90°C for 5 hours to remove the moisture.

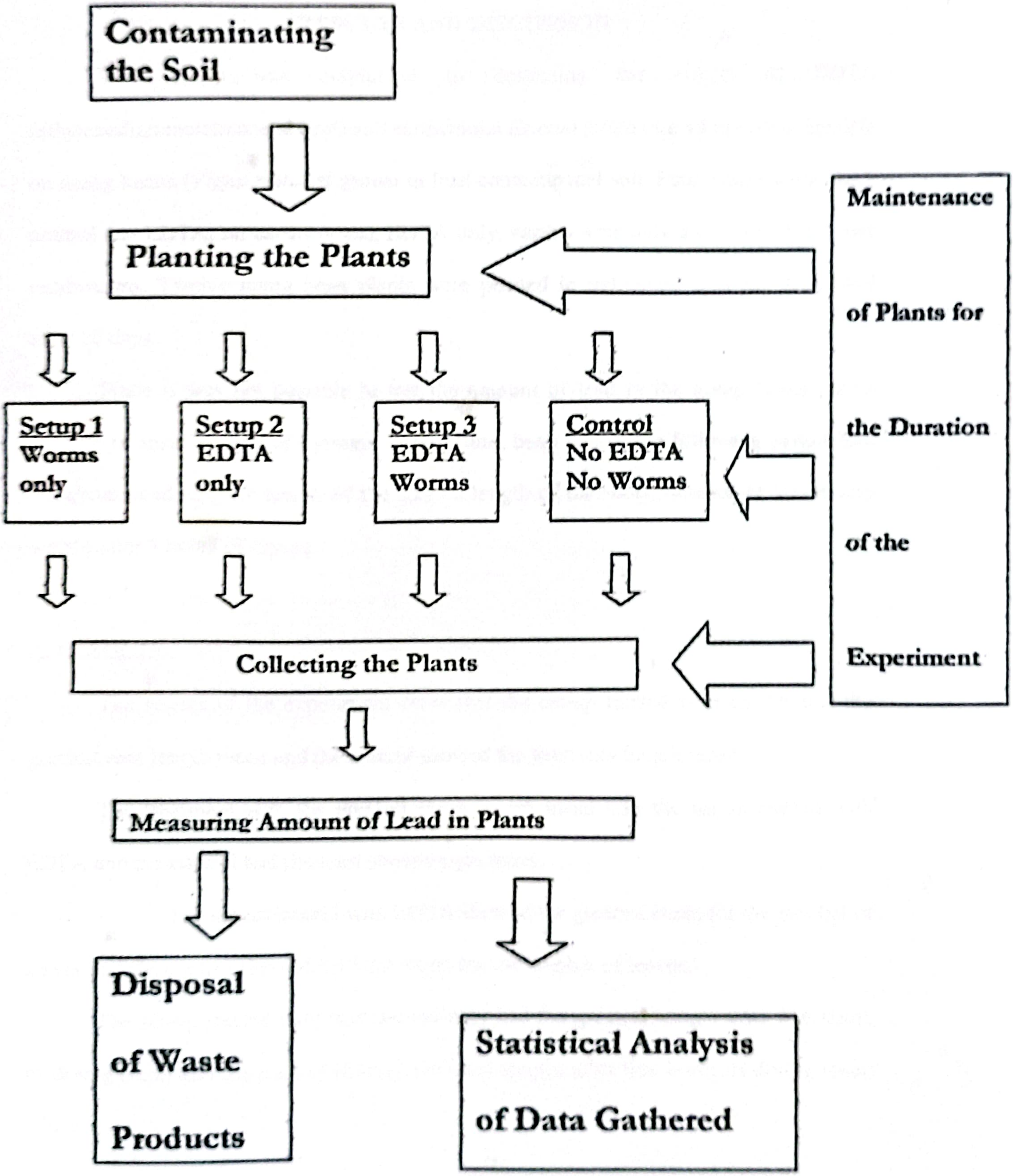
VI. Disposal of Waste Products

The waste products were disposed of accordingly. The remaining contaminated soil was isolated, along with the remains of the unanalyzed plant samples and the (dead) earthworms.

VII. Statistical Analysis of Data Gathered

The results of the study were analyzed by the One-Way Analysis of Variance (ANOVA) of the data gathered from the experiment. If the results of the One Way ANOVA were significant, the data was subjected to the Fisher's Least Significant Difference Post-hoc Test.

Flowchart of Procedure:



CHAPTER 4

RESULTS AND DISCUSSION

This study was conducted to determine the effects of EDTA (ethylenediaminetetraacetic acid) and earthworms *Eisenia fetida* as lead uptake enhancers on mung beans (*Vigna radiata)* grown in lead contaminated soil. Four setups were used: control (no EDTA, no earthworms), EDTA only, earthworms only and both EDTA and earthworms. Twelve mung bean plants were planted in individual pots and harvested after 10 days.

Since it was not possible to test the amount of lead in the mung beans plants because of insufficiency of biomass of the mung bean plants, the following parameters were measured instead: length of the shoots, length of the roots, number of leaves and weight after 5 hours of drying.

A. RESULTS

The results of the experiment show that the set-up treated with EDTA had the greatest root length mean and the control showed the least root length mean.

The treatment with the greatest shoot length mean was the set-up treated with EDTA and the control had the least shoot length mean.

The set-up treated with EDTA showed the greatest mean for the number of leaves and the control showed the least mean for the number of leaves.

The set-up treated with earthworms only had the greatest weight after five hours of drying mean and the control showed the least weight after five hours of drying mean (Table 1).

Table.1 Means of root lengths, shoot lengths, number of leaves, wet weight, weight after 5 hours of drying of mung beans

Treatments	Root Length (cm)	Shoot Length (cm)	No. of Leaves	Weight after 5 hours of drying (mg)
1.Control	3.16	14.46	4.67	0.0214
2.EDTA	5.51	24.52	6.75	0.0413
3.Worms	4.48	21.09	5.25	0.1048
4.EDTA &worms	4.36	24.23	6.67	0.0418

One-way ANOVA of the root lengths and number of leaves showed that the means of the root lengths have no significant difference. The results showed that the lengths of the roots of the mung beans in different setups were not affected by the presence or absence of EDTA and earthworms.

However, One-way Analysis of Variance (Table 2) showed that the means of the shoot lengths in four different setups have a significant difference. The same result was shown in Table 2 of the weight after 5 hours of drying of mung bean (*Vigna radiata*) in the four different setups.

Table.2 One-Way ANOVA (Analysis of Variance) of root length, shoot length, number of leaves and dry weight after five hours of drying

		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
ROOT LENGTH	Between Groups	9.515	3	3.172	1.280	.334	Not Significant
	Within Groups	24.781	10	2.478			
	Total	34.296	13				
		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
SHOOT LENGTH	Between Groups	207.617	3	69.206	38.537	.000	Significant
	Within Groups	17.958	10	1.796			
	Total	225.576	13				
		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
NUMBER OF LEAVES	Between Groups	17.845	3	5.948	1.798	.211	Not Significant
	Within Groups	33.083	10	3.308			
	Total	50.929	13				
WEIGHT AFTER 5 HOURS OF DRYING		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
	Between Groups	.001	3	.000	9.249	.003	Significant
	Within Groups	.000	10	.000			
	Total	.002	13				

Fisher's Least Significant Difference Post-Hoc Test (Table 3) was used to determine the significant difference in the shoot length and weight. The test showed that the mean shoot lengths of the set up treated with both EDTA and earthworm and EDTA only are statistically equal and greater than the shoot length in the setup treated with earthworm and the control. The mean shoot length of the control setup is less than that of the setup treated with earthworm. In summary, the relative ordering of the mean shoot lengths is as follows:

Both=EDTA > earthworms >control.

Table.3 Fisher's Least Significant Difference Post-Hoc Test to compare the mean shoot lengths of the mung bean plants in the control, with EDTA, with earthworms and both EDTA and earthworms experimental setups

Set-up	Set-up	Mean Difference (I-J)	Std. Error	Sig.	Interpretation
Control	EDTA	-10.05667(*)	1.02351	.000	**Significant**
	Earthworms	-6.62667(*)	1.02351	.000	**Significant**
	EDTA & earthworms	-9.76667(*)	1.09418	.000	**Significant**
EDTA	Control	10.05667(*)	1.02351	.000	**Significant**
	Earthworms	3.43000(*)	.94759	.005	**Significant**
	EDTA & earthworms	.29000	1.02351	.783	Not Significant
earthworms	Control	6.62667(*)	1.02351	.000	**Significant**
	EDTA	-3.43000(*)	.94759	.005	**Significant**
	EDTA & earthworms	-3.14000(*)	1.02351	.012	**Significant**
EDTA & earthworms	Control	9.76667(*)	1.09418	.000	**Significant**
	EDTA	-.29000	1.02351	.783	Not Significant
	Earthworms	3.14000(*)	1.02351	.012	**Significant**

* The mean difference is significant at the .05 level.

The results of Fisher's Least Significant Difference Post-Hoc Test (Table 4) showed that the mean weight after 5 hours of drying of the both EDTA and earthworms and EDTA only setups are statistically equal, which are both greater than the mean weight after 5 hours of drying of the earthworms only and control setups. The mean weight after 5 hours of drying of the control setup is statistically equal to that of the earthworms only setup. In summary, the relative ordering of the mean shoot lengths is as follows:

Both=EDTA > earthworms = control.

Table.4 Fisher's Least Significant Difference Post-Hoc Test to compare the mean dry weights of the mung bean plants in the control, with EDTA, with earthworms and both EDTA and earthworms experimental setups

Set-up	Set-up	Mean Difference (I-J)	Std. Error	Sig.	Interpretation
Control	EDTA	-.01934(*)	.00488	.003	Significant
	Earthworms	-.00347	.00488	.494	Not Significant
	EDTA & earthworms	-.02040(*)	.00522	.003	Significant
EDTA	Control	.01934(*)	.00488	.003	Significant
	Earthworms	.01588(*)	.00452	.006	Significant
	EDTA & earthworms	-.00106	.00488	.833	Not Significant
earthworms	Control	.00347	.00488	.494	Not Significant
	EDTA	-.01588(*)	.00452	.006	Significant
	EDTA & earthworms	-.01693(*)	.00488	.006	Significant
EDTA & earthworms	Control	.02040(*)	.00522	.003	Significant
	EDTA	.00106	.00488	.833	Not Significant
	Earthworms	.01693(*)	.00488	.006	Significant

* The mean difference is significant at the .05 level.

B. Discussion

The plant samples were sent to the Analytical Chemistry Laboratory of Silliman University for lead analysis. However the plant samples submitted were found to be very insufficient in amount and were sent back to the researchers because the chances of detecting lead would be extremely small.

For the reason that the lead absorbed by the mung beans would not be possible to detect, the researchers decided to measure the physical differences between plants instead to determine the effects of the different treatments on the growth of the plants: root length, shoot length, number of leaves and weight after five hours of drying.

Physical differences can indicate whether plants can absorb more Pb. Generally, plants that absorb more Pb tend to develop slower than plants that absorb less Pb and exhibit signs of phytotoxicity. High concentrations of Pb decrease the area of leaves, the content of green pigments in them, and the rate of photosynthesis (Kaznina et al., 2004). Plants in the high lead treatment were significantly smaller (Uveges et al., 2002).

Thus, plants that have absorbed more Pb are expected to be smaller than plants that absorb less Pb. This is because the abnormal amount of Pb in the plant. Heavy metals, being phytotoxic, cause growth inhibition and even plant death. (Tripathi et al., 2005). A recent study also showed that chelator toxicity reduced the plant's biomass, thereby decreasing the amount of metal accumulation (Chen et al., 2001).

This generalization cannot be verified in this study because Pb uptake has not been determined. The results only showed that the EDTA which is suppose to be a metal chelator and the earthworm which is suppose to be a metal mobilizer have actually increased the size of the plant in terms of shoot length and weight after 5 hours of drying. If EDTA and earthworm are suppose to increase the Pb uptake of the plant, the result of this study is actually in contrast to the statements of Chen et al. and Kaznina et al. that Pb absorbing plant are usually slow developing.

If the statement of Chen et al. and Kaznina et al. is true, then the results of this study must be the result of extraneous variables. The results from the experimental setups showed greater development (longer roots and shoots, more leaves) as opposed to the control setup. The result of extraneous variables, such as unequal exposure to wind and rain, could have possibly led to the decrease of the concentration on lead in the soil wherein the plants were grown, thus allowing the plants grown with EDTA and earthworms to grow and develop faster than the setup without EDTA and earthworms (control). Another extraneous variable would be the variations in the genetic composition of each mongo plant since only one plant was used in each replicate.

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study aimed to determine the effects of EDTA (ethlylenediaminetetraacetic acid) and earthworms *Eisenia fetida* the physiological effects of EDTA (ethylenediaminetetraacetic acid) and earthworms *Eisenia fetida* as lead uptake enhancers on mung beans (*Vigna radiata)* grown in lead contaminated soil.

It specifically aimed to:

1. Compare the root lengths of mung beans grown in four different setups:

Control (no EDTA, no earthworms), EDTA only, earthworms only, and Both EDTA and earthworm

2. Compare the shoot lengths of mung beans grown in four different setups:

Control (no EDTA, no earthworms), EDTA only, earthworms only, and Both EDTA and earthworms

3. Compare the number of leaves of mung beans grown in four different setups:

Control (no EDTA, no earthworms), EDTA only, earthworms only, and Both EDTA and earthworms

4. Compare the weight after 5 hours of drying of mung beans grown in four different setups:

Control (no EDTA, no earthworms), EDTA only, earthworms only, and Both EDTA and earthworms

It was hypothesized that there would be no significant difference in the:

(A) Root lengths, (B) shoot lengths, (C) number of leaves, and (D) weight after 5 hours of drying of the mung beans grown in lead contaminated soil in four different setups.

A. Summary

1. The amount of lead in each plant was not measured. The plants were found to be very insufficient in amount and were sent back to the researchers because the chances of detecting lead would be extremely small
2. Physical differences have shown that plants treated with EDTA and earthworms had longer shoots, longer roots, a greater number of leaves and weighed more after five hours of drying. However, only the shoot length and the weight after five hours of drying showed a significant difference.

B. Conclusions

The presence of earthworms and EDTA in lead contaminated soil has an effect on the mung beans grown in the contaminated soil. When grown with EDTA and earthworms in lead contaminated soil, the shoot length of the mung beans and weight after 5 hours of drying increases but has no effect on the root length and number of leaves. However, it is indeterminate whether or not the mung beans absorbed a significant amount of lead without the use Atomic Absorption Spectrometry (AAS). The results of this experiment are inconclusive because the amount of lead in the plants was not measured and only one plant per replicate was used.

C. Recommendations

The researchers recommend that AAS or atomic absorption spectrometry be use to determine the amount of lead on the plants.

To minimize extraneous variables, the researchers also recommend that the plants be grown indoors under laboratory conditions.

The researchers recommend that the test organisms that will be used should be of uniform height at the beginning of the experiment to further reduce extraneous variables.

To increase the reliability of the AAS, the researchers strongly recommend that forty to fifty mung bean plants be grown in each pot instead of just one. Also, the researchers would like to recommend that future researches related to this study be conducted using other hyperaccumulator plants with higher biomass than that of mung beans.

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APPENDIX A

RAW DATA

	LENGTHS		NUMBER OF LEAVES			
Plant Samples	Root Length	Shoot Length	Big	Small	Total	Weight after 5 hours of drying
A	2.85	11.79	2	1	3	0.0171
B	5.65	25.61	5	2	7	0.0509
C	6.19	22.90	5	1	6	0.0460
D	6.47	24.11	5	5	10	0.0379
E	3.91	15.27	2	5	7	0.0266
F	2.71	16.33	2	2	4	0.0206
G	5.79	21.65	5	1	6	0.0171
I	2.81	21.79	2	3	5	0.0267
J	6.30	23.66	2	3	5	0.0374
K	5.51	15.70	3	2	5	0.0268
P	6.79	20.72	2	1	3	0.0355
Q	2.54	20.20	5	2	7	0.0203
R	3.71	25.18	6	2	8	0.0389
S	3.61	24.70	4	1	5	0.0369
T	3.19	24.61	4	2	6	0.0406

Control -AEF
EDTA -BDJS
Worms -GIPQ
Both EDTA and earthworms -RTC

APPENDIX B

STATISTICAL ANALYSIS

Means of root lengths, shoot lengths, number of leaves, wet weight, weight after 5 hours of drying of mung beans

Treatments	Root Length (cm)	Shoot Length (cm)	No. of Leaves	Weight after 5 hours of drying (mg)
1.Control	3.16	14.46	4.67	0.0214
2.EDTA	5.51	24.52	6.75	0.0413
3.Worms	4.48	21.09	5.25	0.1048
4.EDTA &worms	4.36	24.23	6.67	0.0418

One-Way ANOVA (Analysis of Variance) of root length, shoot length, number of leaves and dry weight after five hours of drying

number of leaves and dry weight after five hours of drying

		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
ROOT LENGTH	Between Groups	9.515	3	3.172	1.280	.334	Not Significant
	Within Groups	24.781	10	2.478			
	Total	34.296	13				
		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
SHOOT LENGTH	Between Groups	207.617	3	69.206	38.537	.000	Significant
	Within Groups	17.958	10	1.796			
	Total	225.576	13				
		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
NUMBER OF LEAVES	Between Groups	17.845	3	5.948	1.798	.211	Not Significant
	Within Groups	33.083	10	3.308			
	Total	50.929	13				
		Sum of Squares	Df	Mean Square	F	Sig.	Interpretation
WEIGHT AFTER 5 HOURS OF DRYING	Between Groups	.001	3	.000	9.249	.003	Significant
	Within Groups	.000	10	.000			
	Total	.002	13				

Fisher's Least Significant Difference Post-Hoc Test to compare the mean shoot lengths of the mung bean plants in the control, with EDTA, with earthworms and both EDTA and earthworms experimental setups

Set-up	Set-up	Mean Difference (I-J)	Std. Error	Sig.	Interpretation
Control	EDTA	-10.05667(*)	1.02351	.000	Significant
	Earthworms	-6.62667(*)	1.02351	.000	Significant
	EDTA & earthworms	-9.76667(*)	1.09418	.000	Significant
EDTA	Control	10.05667(*)	1.02351	.000	Significant
	Earthworms	3.43000(*)	.94759	.005	Significant
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earthworms	Control	6.62667(*)	1.02351	.000	Significant
	EDTA	-3.43000(*)	.94759	.005	Significant
	EDTA & earthworms	-3.14000(*)	1.02351	.012	Significant
EDTA & earthworms	Control	9.76667(*)	1.09418	.000	Significant
	EDTA	-.29000	1.02351	.783	Not Significant
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Fisher's Least Significant Difference Post-Hoc Test to compare the mean dry weights of the mung bean plants in the control, with EDTA, with earthworms and both EDTA and earthworms experimental setups

Set-up	Set-up	Mean Difference (I-J)	Std. Error	Sig.	Interpretation
Control	EDTA	-.01934(*)	.00488	.003	Significant
	Earthworms	-.00347	.00488	.494	Not Significant
	EDTA & earthworms	-.02040(*)	.00522	.003	Significant
EDTA	Control	.01934(*)	.00488	.003	Significant
	Earthworms	.01588(*)	.00452	.006	Significant
	EDTA & earthworms	-.00106	.00488	.833	Not Significant
earthworms	Control	.00347	.00488	.494	Not Significant
	EDTA	-.01588(*)	.00452	.006	Significant
	EDTA & earthworms	-.01693(*)	.00488	.006	Significant
EDTA & earthworms	Control	.02040(*)	.00522	.003	Significant
	EDTA	.00106	.00488	.833	Not Significant
	Earthworms	.01693(*)	.00488	.006	Significant

APPENDIX C

PLATES

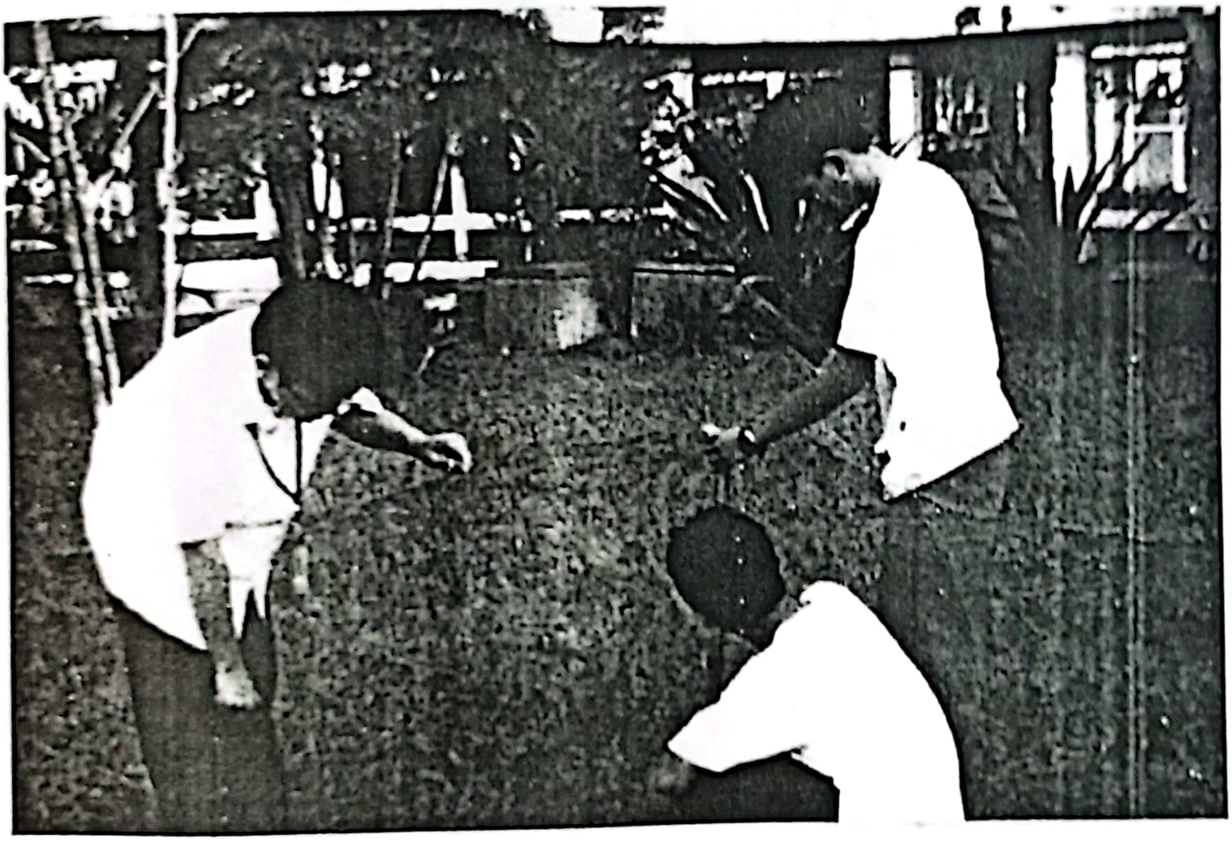

Plate 1. Setting up of research site

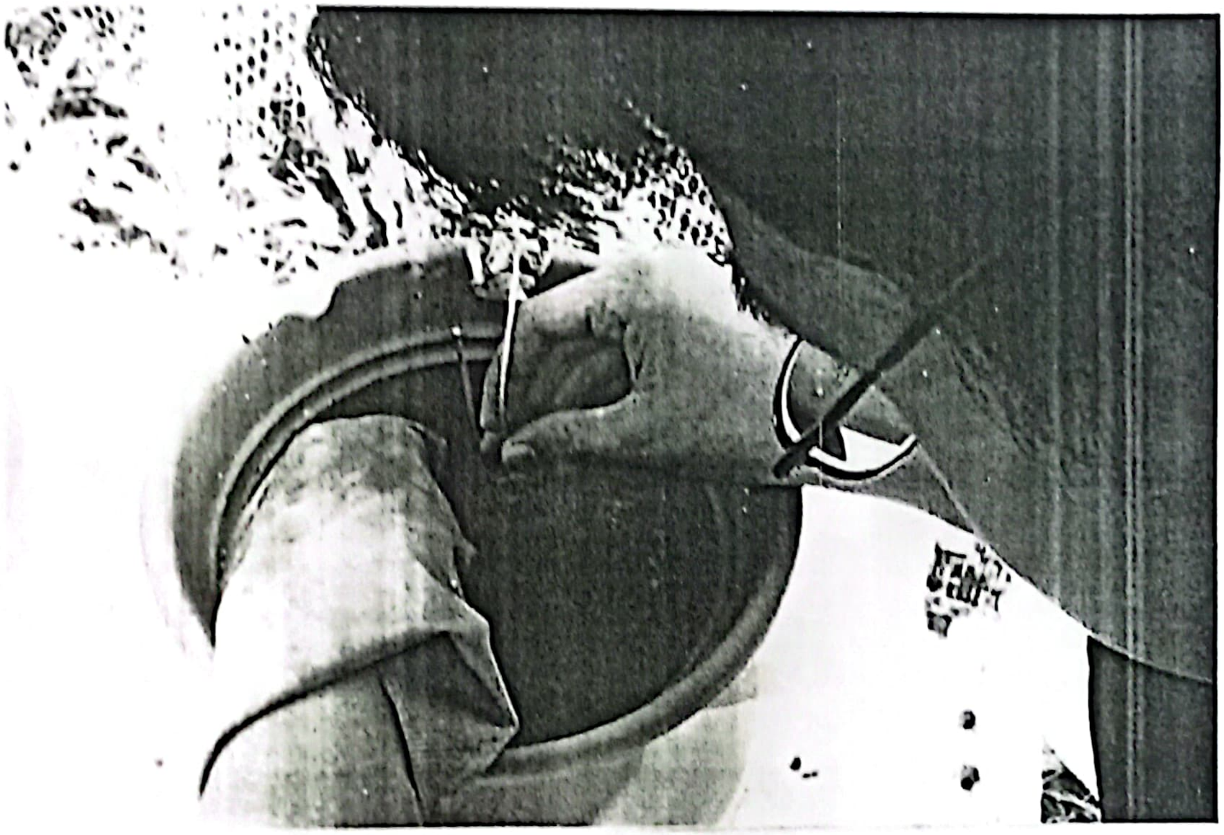

Plate 2. Planting of mung bean plants in individual plastic pots with lead contaminated soil

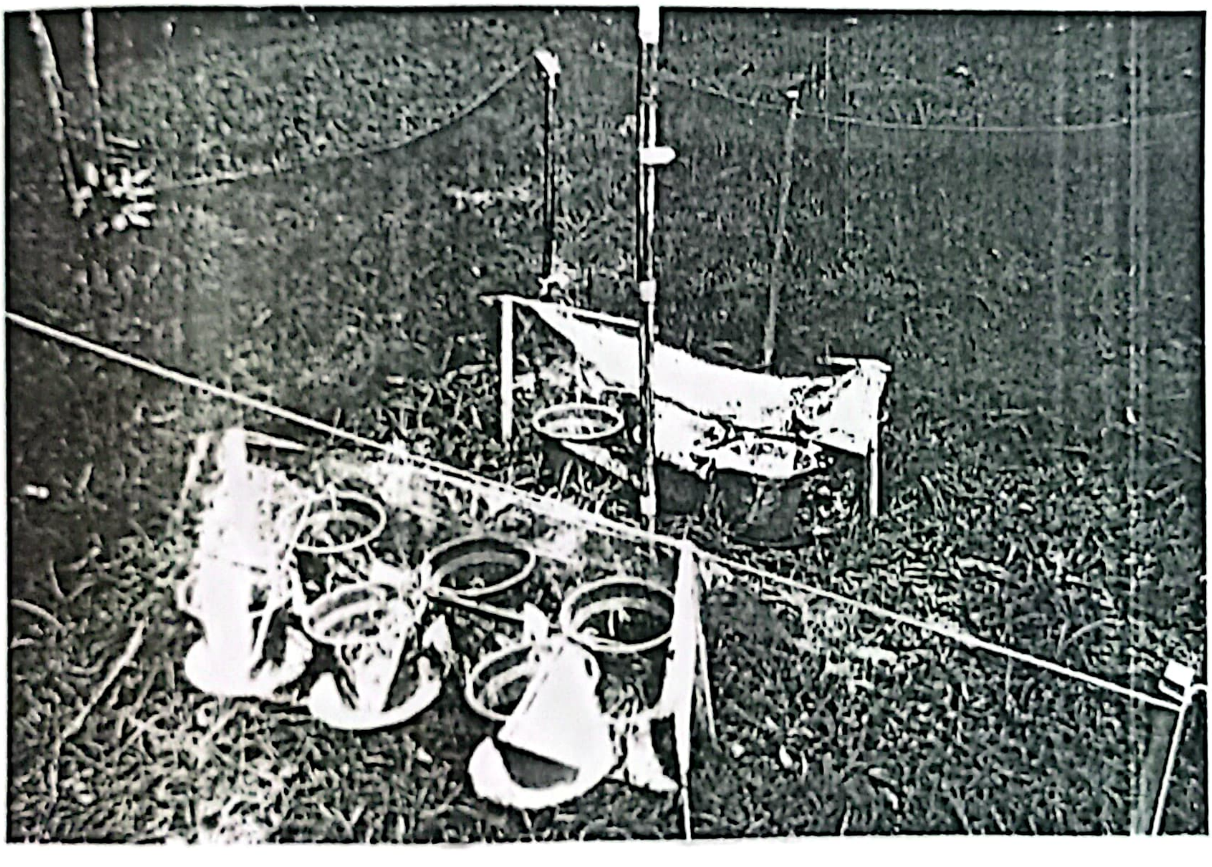

Plate 3. Research site containing pots with lead contaminated soil and individual mung bean plants

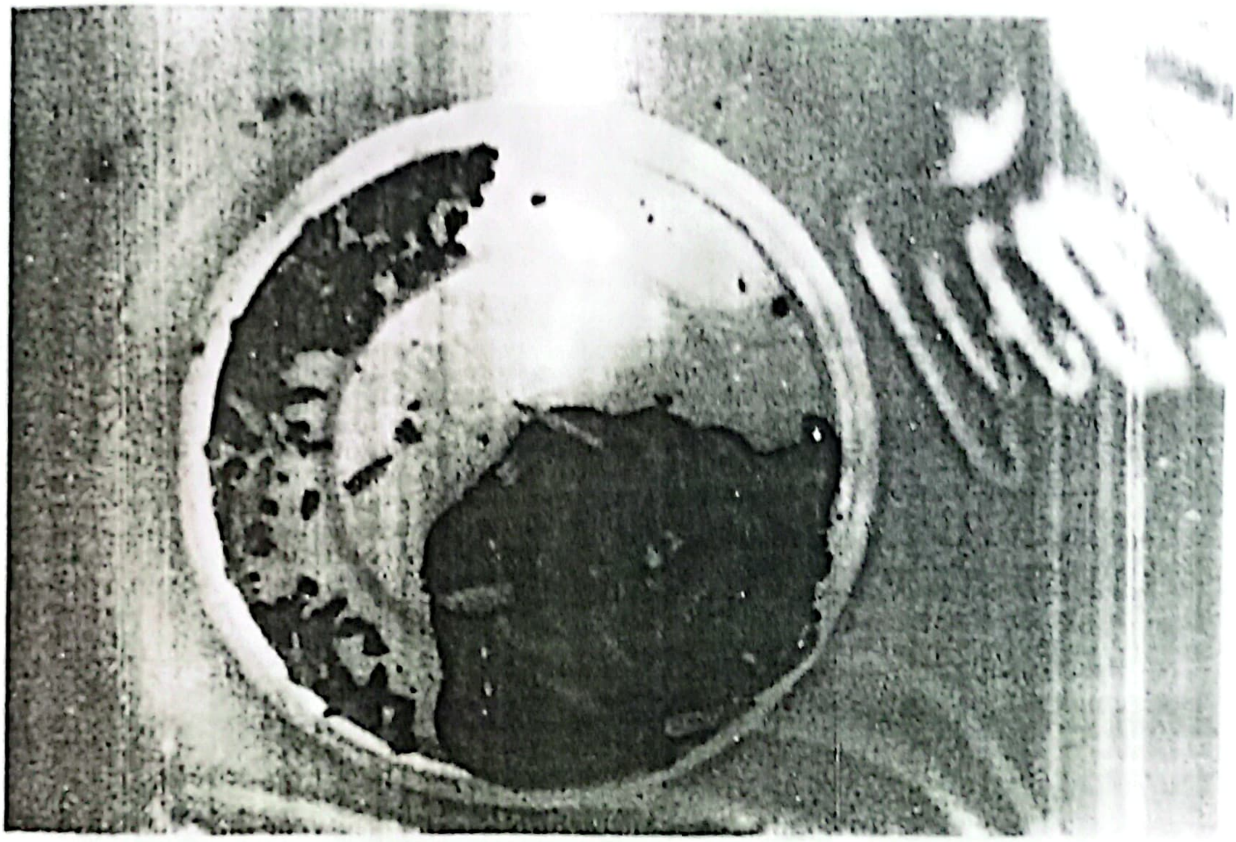

Plate 4. Earthworms *Eisenia fetida* in plastic cups

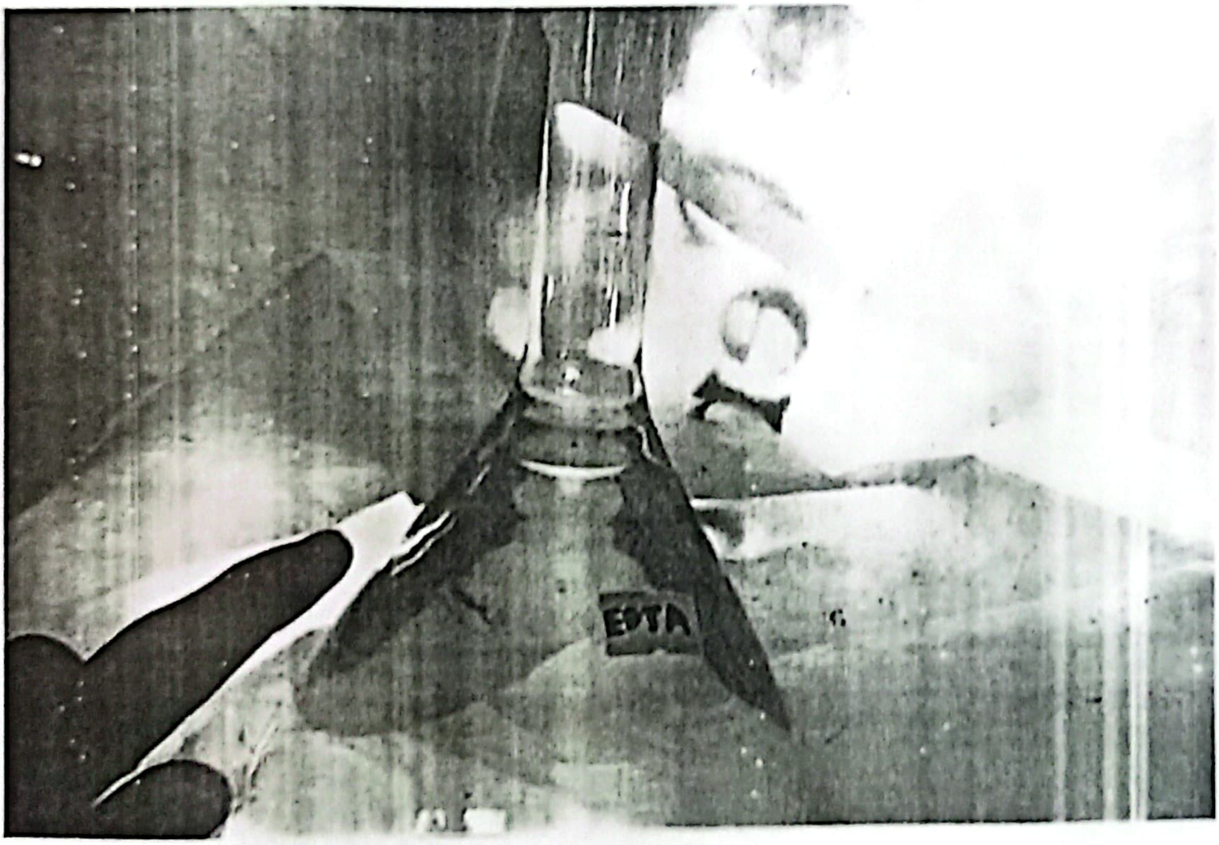

Plate 5. EDTA (ethylenediaminetetraacetic acid) dissolved in water in a 1L round bottom flask

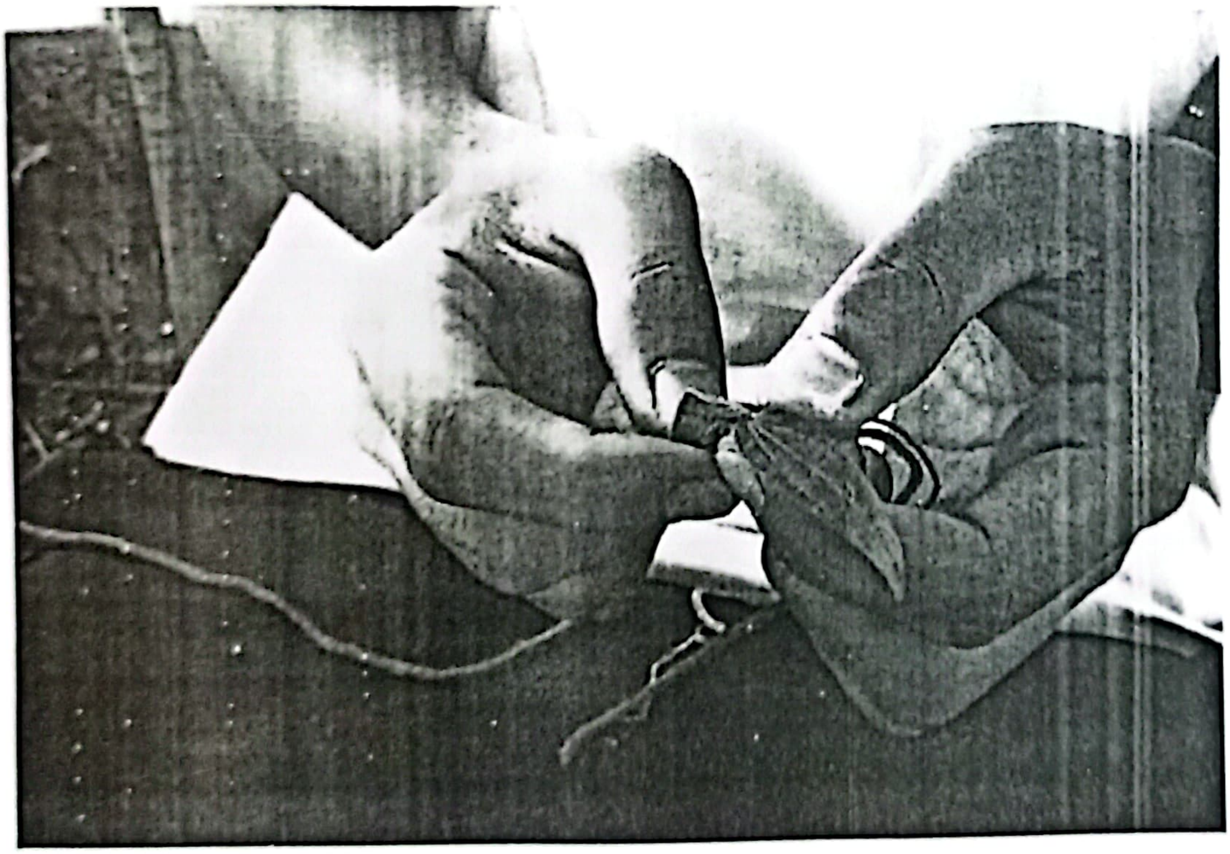

Plate 6. Measuring of the root length, shoot length and number of leaves using a yarn and Vernier caliper/ruler