PHYTOREMEDIATION
OF
HEAVY METALS

Submitted in partial fulfillment of the
requirements of the degree of

MASTER OF TECHNOLOGY
In
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By

CHRISTABELMORN BTHUH
(11200152)

Supervisor
Ms. ALKA YADAV

School of Civil Engineering

LOVELY PROFESSIONAL UNIVERSITY, PHAGWARA
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DECLARATION

I, CHRISTABELMORN BTHUH (11200152), hereby declare that this thesis report entitled "**PHYTOREMEDIATION OF HEAVY METALS"** submitted in the partial fulfillment for the requirements in the award of degree of Master of Civil Engineering, in the School of Civil Engineering, Lovely Professional University, Phagwara, is my own work. The material included in this report has not been submitted to any other university or institute either in part or in full for the award of any degree.

Date: CHRISTABELMORN BTHUH

Place:

CERTIFICATE

Certified that this project report entitled **"Phytoremediation of heavy metals"** submitted individually by student of School of Civil Engineering, Lovely Professional University, Phagwara, under my Supervision has been carried out the work in order to obtain the Award of Degree. This Report has not been Submitted to any other university or institution for the award of any degree.

Supervisor
Ms. Alka Yadav
Assistant Professor
School of Civil Engineering

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ABSTRACT

The issue of heavy metal contamination especially lead is growing up from year to year as more measures of lead are extracted and discharged into the natural environment such as water, air and soil and they are basically non-biodegradable and can accumulate into the environment, therefore, discover their way into the food chain which in turn can create problem or risk to human's health and all living organism as well. Phytoremediation is the new technology in which plants can be used as a tool to remediate heavy metals. In this study, a pot experiment was set up in which the Aloe Vera is cultivated with lead at different concentrations at 8ppm, 12 ppm and 16 ppm respectively and with exposure periods of 28 and 56 days respectively. The accumulation of lead was determined with the help of ICP-AES and result showed that the plant can accumulate lead in their organs and with the changed in pH the accumulation levels also affected. Aloe vera plant can be use as good phytoremediation process for lead and it is also less expensive and eco-friendly methods.

Keywords: *Heavy metal, Phytoremediation, Aloe Vera, ICP-AES.*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1.Background

Heavy metals are natural constituents of the world's covering. They have an atomic density greater than 5 g·cm−3 and a atomic number greater than 20. The most widely recognized heavy metal pollutants or contaminants are cadmium, chromium, copper, mercury, lead and zinc **(Laghlimi et al et.al., 2015).** The presence of heavy metals is one of the major sources of polluting the environment **(Ebrahim Panahpour et.al., 2013).** The contamination of soil and water with heavy metals causes many problems to the environment and also to the human health. An effective technological solution is required to look after these problems. Moreover, heavy metals are very unique class of toxicants and they cannot be broken down into non-toxic forms easily **(Hossein Ferraji et.al., 2014).** Due to fastest growing of industries and transportations, the continuous used of pesticides in agricultural field give rise to hazardous effects to the landscape and also the ground water as well as the surface water are getting polluted and the soil fertility has been detoriated **(Himanshi Sharma, 2016).** Lead is one of the heavy metals which are major environmental pollutants and causes a serious threat to our surrounding or environment, human health and animal health as well **(Sesan Abipdun Aransiola et.al., 2013).** Lead is that heavy metals which is not only toxic but also accumulate in individuals as well as the ability to affect the food chain as a whole and disturbed the health system of human beings, phytoplanktons and animals. Therefore, for lead proper treatment from soil and industrial waste water are require.

1.1.2. Source of lead:

Lead can reach the water system n soil through discharges or urban runoff such as industrial plants and sewage treatment plants. Many industrial processes use lead frequently which leads to the main cause of lead contamination in the

environment. The use of lead involves in varieties of industrial processes which includes smelting, mining, dumping of municipal sewage, manufacture of pesticides and fertilizers and burning of fossil fuels which contain lead in them. Also, varieties of commercial products and materials contain lead which include ceramics glazes, paints, television glass, batteries, ammunition, medical equipments like fetal monitors, x-ray shields and electrical equipments as well. Sources of lead are also from the use for roofing and from lead battery recycling sites. **(Lead chemical backgrounder, 2000)**

1.1.3. Effect of lead on human health:

Lead does no essential function in the human body and it is harmful after uptake from food, air or water. It is directly absorbed into the blood stream in human and is stored in bones and teeth (95% in bones and teeth) and soft tissues. On children, the major effect of lead is brain damage; and it can cause long-term effects rather than acute toxicity. Also, no symptom is shown to know that it is lead poisoning. For children, the critical age is from 6 months to 6 years. In adults, lead can damage or harm the heart, can cause a rise in blood pressure, kidneys damage, brain damage, miscarriages and subtle abortions, declined fertility of men, disruption of nervous system and blood (hematological) system. Lead poisoning in adults normally occurs from exposure to lead which is used in the place where they are working or outdoors such as inhalation of lead dust and fumes by the workers or swallowing lead dust while drinking, smoking or eating on the job is harmful for their health. Also, it can reach them by exposing during activities or any hobbies where lead is used. The one who got exposed of lead may carry with them home (i.e. dust) on their clothes and bodies which in turn can spread through the family members. Therefore, lead can cause diseases such as anemia, hepatitis, encephalopathy and nephritic syndrome and can accumulate to over a lifetime. In WHO (2004) allowable standard of lead is 0.15 mg/L and if it exceeds this limit it will affect the physical or mental development in infants and children while adults may have heart problem, kidney problem as mention above. **(S.D. Angima, 2008, Aransiola et.al., 2013)**

1.1.4. Effects of lead on the environment:

Lead can enter both water and soils through corrosions of lead paints and through lead pipelines which is corroded in the water transporting systems. Lead have the ability to accumulates in the bodies of soil organisms as well as water organisms and due to this, these organisms may suffer from lead poisoning. Shellfish can be totally get affected even if small concentrations of lead are present. Even phytoplanktons body functions can get disturbed when lead is present around them. Moreover, in seas phytoplanktons acts as a very important source of oxygen productions and many big sea animals eat them. In soil, lead intervention disturbed the soil functions in the farmlands or near highways and the soil organisms too when extreme or very high concentrations of lead present. Lead not only accumulate in individuals' organisms but can enter through the entire food chain and lead can be called a dangerous chemical.

Remediation techniques for heavy metals which polluted the soil can be solve using physico-chemicals processes such as precipitation, ion-exchange, evaporation, reverse osmosis and chemical reduction; but all these methods require external resources which are man-made and are also expensive. Phytoremediation technique is a reasonable, generally minimal effort way to deal with expelling or removing the metals from groundwater and the soil.

1.2. Phytoremediation

Phytoremediation means utilization of plants to treat contaminates sites and which it can be defined as the utilization of green plants to expel toxins from the nature or to safe them. Green technology is another name given to phytoremediation it connected to both organic and inorganic toxins that are present in soil, water or the air **(Selvaraj et al, 2013)**. Phytoremediation has been progressively received considerations over the late decades, as a rising and eco-friendly methodology that uses the characteristic properties of plants to remediate contaminated soils. By developing plants in the polluted sites, contaminants in soils will be evacuated, immobilized, or degraded, and the cost is a great deal less costly than other conventional techniques. Phytoremediation does not require costly equipment or profoundly specific work force, along these lines, it is generally simple to execute. It can accomplish for record-breaking treating an extensive variety of contaminants in an extensive variety of circumstances. **(Akhtar et al, 2013)**

1.3. Hyperaccumulator

Hyperaccumulators are those types of plant which can accumulate more concentration of heavy metals than other plants. Hyperaccumulators used for phytoextraction are said to have highest rates of accumulation and highest in translocation, it is also quick development and it has high generation of biomass **(Gunduz et al, 2012).** Plants that can clean soils does at least one of these: 1) plant which take up contaminant to their roots from soil; 2) the one who tie the contaminant physically or chemically to their roots; and 3) last the one which transfer the contaminant to shoot from the roots who can keep the contaminant away from leaching into the soil. To accumulate, degrade or volatilize the contaminants is not only the plants can do, but it can grow rapidly in various conditions and can be harvest easily. Aloe Vera plants is one that acts as and can be used as a hypeaccumulator.

1.3.1. Aloe Vera

Aloe Vera is one of the oldest plant on earth and it is a member of the Liliaceae family. It is a subtropical plant whose scientific name is *Aloe barbadensis*. The word aloe comes from Arabic word "aloeh" means "bitter and crystalline seva" and the word vera comes from Latin word "true" (Hidayati Karamina, 2014, www.aloe-vera-remedies.com). Aloe Vera can grow in different climate conditions. This plant can survive for a long periods without water. It can grow in dry areas with warm humid or in low rainfall regions but this plant cannot tolerate extremely cold temperature. It grows beautifully in hot climate and because of it double photosynthetic properties it needs more sunlight as compared to other plants. Also, this plant can be cultivated in many varieties of soil from loamy to sandy soils. The Aloe Vera plants grow best in light soil and it can tolerate upto 8.5 pH and even can tolerate soils which are salty in nature (www.agrifarming.in/aloe-vera-farming). Aloe Vera plant is also referred to as "miracle plant" as it has antiinflammatory, antiseptic and it helps to relieve the symptoms of severe illnesses like cancer and diabetes. It is also considered as an excellent treatment for nausea and stomach irritations. Aloe Vera is widely known to be used in beauty purposes like for skin care, hair care and health (http://www.gits4u.com/agri/agri5f.htm). It has been proved by many researcher to be use as hyperaccumulator and can be used as a good phytoremediation agents (**Panahpour et.al., 2013, Hidayati Karamina et.al., 2014, Swapnil Rai et.al.,2011, Fatemeh Shokri et.al.,2016**).

CHAPTER 2

TERMINOLOGY

Pb – Lead

Cd – Cadmium

Ni – Nickel

Fe – Iron

Cr – Chromium

Zn – Zinc

K – Potassium

Na – Sodium

Mg – Magnesium

Ca – Calcium

P – Phosphorus

ppm – Parts per million

EDTA – Ethylenediaminetetraacetic acid

AB-DTPA - Ammonium bicarbonate diethylenetriamine-pentaacetic acid

ICP-AES - Inductively Coupled Plasma-Atomic Emission Spectrophotometer

AAS - Atomic Absorption Spectroscopy

BCF – Bioconcentration factor

TF – Translocation factor

CHAPTER 3
REVIEW OF LITERATURE

Panahpour et.al.,2013 studied on the uptake of heavy metals by the Aloe vera plants on three regions. The aloe vera from these three regions were taken and the soil from where the plants grown, the gel and the leaves were taken for testing of heavy metals including Zn, Pb and Cd. One way variance analysis was conducted for statiscal analysis. It is found that in one region lead is more and in other cadmium and so on. Also, both lead and cadmium have the tendency to remain in the gel of aloe vera as compare to zinc. Hence, aloe vera can be a good hyperaccumulator plant.

Hamid Iqbal et.al.,2013 studied on pollution of heavy metals in two medicinal plants that is Aloe Vera and *Tamarix aphylla*. Both the plants samples were collected from three different regions one which are polluted, less polluted and non-polluted areas. Heavy metals including Pb, Cd, Ni, Cu, Fe, Cr and Zn were analysed for both the plants. From the results, it is found that the heavy metals tested were found more in concentrations from both the plants from the region which is polluted in different concentrations respectively. From the investigations, it is clear that the plant samples which were collected from polluted area are more contaminated with heavy metals as compared to less polluted and non-polluted areas. So, he concluded that each medicinal plant should be thoroughly analyzed for heavy metals before they used in the preparation of herbal products and also for pharmaceutical uses.

Hidayati Karamina et.al.,2014 studied on the decreasing or diminish the uptake of heavy metals by Aloe Vera by planting another hyper-accumulator plant along with Aloe Vera which act as a phytoremediation technique. Heavy metals studied were aluminium and lead from two species of Crotalaria that is Crotalaria sp. and Crotalaria junea L. Both these plants along with Aloe Vera were analysed. After analysis, it is found that high adsorption of lead and aluminium in the roots of Crotalaria junea L. and it has reduced the adsorptive ability of Aloe Vera's roots. Also, the author concluded that more research is needed to find out about these two plants species that is Crotalaria sp. and Crotalaria junea L their abilities to accumulate heavy metals in them.

Swapnil Rai et.al.,2011 studied on the different concentrations of heavy metals in Aloe Vera plants which were collected from different locations in India. Mature, healthy and fresh leaves of Aloe Vera were collected from six different sites in India to check the different concentrations of heavy metals including K, Na, Mg, Ca, Cd, Fe, Pb, Cu, Zn and P from the plants. After testing and analysis, the results showed that high metal concentration were recorded in Aloe Vera leaves. Also, the concentrations of different heavy metals depend upon the physiographical conditions, type of waste generated from the area and development activities. He concluded that the plant Aloe Vera has the ability to be a good absorber of heavy metals and can be used for removal of heavy metals from soil and waste water in order word it can be used as a good phytoremediation agent.

Fatemeh Shokri et.al.,2016 studied on the removal or cleaning up heavy metals from waste water of pharmaceutical research and educational laboratoriesby the help of Aloe Vera plants. The Aloe Vera plants were transplant into the research studies and contaminated with selected heavy metals including chromium, copper, lead, nickel and cadmium. The samples were taken for analysis after each 10 days of treatment upto 60 days completely. After testing and analyzing, it is found that Aloe Vera has high potential of taking up these toxics heavy metals within them. Among these heavy metals, it is showed that cadmium and lead accumulate is more in concentrations than other heavy metals in the plants. From the results, it can be concluded that Aloe Vera plants can be consider as a suitable and good hyperaccumulator plant which is effective and inexpensive adsorbent for the selected heavy metals.

Sesan Abiodum Aransiola et.al.,2013 studied on the plant Glycine Max L. to check its potential to remediate lead from the soil. The plant seeds were grown along with different concentrations of lead and the experiment were carried out up to a period of 12 weeks. The plants parts including leaves, stems, seeds and roots were taken for analysis after 12 weeks of exposure to lead. After analyzing it is found that more uptake of lead by the plants parts were found in the last stages of the growing periods especially by the seeds of the plants. The author lastly concluded that Glycine Max L. has the potential to remediate lead contaminations from soil and also, he recommended that if this plant is to be used for phytoremediation process then it should be harvested after 12 weeks of exposure and for their seeds it should be replanted for another cycle of clean-up process.

Hamanshi Sharma, 2016 reviewed on the phytoremediation of lead by two plants Vetiveria Zizanioides and Brassica Juncea. In this paper, the author reviewed about phytoremediation technique to be as the most promising approach to remove the contamination as this process is environmental friendly, socially accepted, cost effective, it is easy to maintain and also it has long term applicability. It is also mentioned for phytoremediation to be successful it needs a hyperaccumulator plant to remove and detoxify the surrounding contamination by physical, chemical and biological process of plants. Both these plants Vetiveria Zizanioides and Brassica Juncea has been studied in this paper and it is found that both have the potential to accumulate heavy metals within them but specifically both plants have more potential in accumulating lead than other heavy metals and it can be used for other heavy metals as well. After reviewing and studying various worked the author concluded that both these plants can be consider as a good hyperaccumulator for removal of lead from the contaminated soil.

Seema J Patel et al., (2013), worked on three plants that is sunflower, Indian mustard and water hyacinth to remediate two heavy metals (i.e. copper and lead) from soil and water respectively. The plants grown in presence of these heavy metals with different concentrations which were tested by Atomic Absorption Spectroscopy (AAS). From this experiment, she concluded that copper absorption by plants is more compared to lead. Among the three plants she found that sunflower is showing high absorption and much higher with the use of enhancer like EDTA. Also, the water hyacinth was analyzed on different parts like leaves, petioles and roots but roots showed high absorption of heavy metals compared to leaves and petioles. She finally concluded that phytoremediation can be considered as suitable for remediation of heavy metals that pollute the environment.

Amjab Husnain et al., (2013), studied on phytoremediation heavy metals from industrial waste by the plant Euphorbia prostrate. The objective of his worked were to find a low-cost phytoremediation and biosorption method. Different heavy metals like cadmium, chromium and lead were studied. Pot experiment were conducted in which the plants were grown with different concentrations of wastewater as well as with underground controlled tap water for 45 days before analysis. After investigation, he found that following germination of 45 days, plants flooded with wastewater in field demonstrated more accumulation of cadmium, in any case, the plants irrigated with tube good water did not accumulate cadmium. He presumed that Euphorbia prostrate tends to collect heavy metals in field furthermore it can be a suitable plant for both phytoremediation as well as biosorption of heavy metals.

Rafi Ullah et al., (2011), studied the phytoaccumulation of heavy metals by sunflower plants. He grows two cultivars of sunflower on the soil which are contaminated with different types of heavy metals like lead, chromium and cadmium in a pot using completely randomized design. Also, the test was performed with and without adding EDTA in which the leaves, shoot and roots of the plant were taken for testing. Statistical analysis was done for recorded data of eight weeks after sowing. After analysis, he concluded that heavy metals accumulation in plants treated with EDTA is more and also with increasing levels of heavy metals application, accumulation was also increased.

Waoo et al., (2014) studied on heavy metals extraction from industrial area and analysis on tendency of plants to accumulate heavy metals at high concentrations. This study is mainly focused on pollution of hazardous concentration of cadmium, nickel, chromium and lead. Plants were grown on contaminated site and the plant shoot were taken for testing. After analysis, he concluded that phytoremediation is a minimal cost effective, environmental-friendly most appropriate for reasonable for developing nations and urban areas. He included that in spite of its potential phytoremediation is yet to become distinctly a commercial accessible innovation in India.

Ziarati et al., (2014), worked on phytoremediation process for removing contaminated soil by the plant *Amaranthus* sp. In this study, it involved extraction of lead, cadmium, copper, nickel and zinc from plant roots and shoots. Distinctive soil pH was likewise keep up amid the examination and phytoextraction rate depended on various development growth of the plant. Impact of including dark and green tea leaves in the soil have likewise been examined. After examination, the results of this exploration he reasoned that *Amaranthus* sp in the soil which comprised of green tea and dried black leaves had appropriate capacity by phytoextraction technique furthermore it is prescribed to study more on species with different plants that can be able to accumulate heavy metals particularly the non-eatable plants.

Ochonogor et al., (2014), studied on phytoremediation of heavy metals with chromium and iron which were cultivated with *Psorolea pinnata* under greenhouse condition. Four weeks old plants were taken from the nursery and transplanted into the polluted soil and permitted to develop for 3 months in the green house. Plants were then harvested and taken for analysis. After analysis, he concluded that *Psorolea pinnata* showed it can bear high levels of concentration of metals with growth process at minimal inhibition. Therefore, it is useful plant for phytoremediation of toxic heavy metals.

Abhilash et al., (2016), studied on phytoremediation of heavy metals on industrial polluted soil by two plants *Spiracia oleracea* L and *Zeamays* L. Industrial waste water contaminated soil and control crops were collected for finding tangible phytoremediation standards end to identify efficient local waste water irrigated crop species for phytoremediation technique. Plants leaves, roots and stems were taken for testing. Bio-concentration factor (BCF) and Translocation factor (TF) were used for calculating or assessing the actual remediation. After analysis, it is found that *Spiracia oleracea* L and *Zeamays* L plant were more efficient in accumulating heavy metals as compared to other plants. Also, it shows that species grown at the industrial site are mostly enriched with heavy metals as compare to those at the other site, which tells that may be sludge cannot be use as organic fertilizer for obtaining better crops. *Zeamays* L is considered as better hyper accumulator and can be also the most suitable plants for phytoremediation.

Coupe et al., (2013), studied on remediating heavy metals from contaminated soil by using three different plants *Eucalyptus camaldeulensis*, *Medicago sativum* and *Brassica juncea*. A pot investigation was led by developing the plants on the contaminated soil and in addition on uncontaminated soil or control soil. Shoots and roots of the plants were tested after eight weeks. The analysis has been done by translocation factor (TF) and bio-concentration factor (BCF). After analysis and comparing the three plants it is found that camaldeulensis was suitable for phytoextraction of lead and zinc, while *Medicago sativum* and *Brassica juncea* were suitable for Phytostabilization of copper and chromium.

Akhtar et al., (2013) studied about bioremediation technique of lead and arsenic by plants and microbes from the polluted soil. In this review, they survey that different bioremediation forms like phytoremediation which incorporates expulsion of contaminants with the assistance of green plants and bioremediation which incorporates evacuation or removal of heavy metals by microorganisms are very successful. They studied the different components of bioremediation by both plants and organisms and how it helps in expelling lead and arsenic. They inferred that bioremediation procedure gives a powerful inventive measure to treatment of wide variety of contaminants and could be effective strategies to lessen the arsenic and lead contaminants of soil.

Mojiri et al., (2013) studied on phytoremediation of heavy metals from urban waste leachate by Southern cattail (*Thypha domingensis*). The point of this study was to check the phytoremediation of heavy metals from waste leachate in urban by the plant and improvement of studied parameters utilizing the central composite design strategies and response surface. Pots investigations were directed and both laboratory and statistical analysis were performed. The outcomes demonstrate that Thypha domingensis is a successful accumulator plant for nickel, lead and cadmium.

Jadia and Fulekar., (2008), studied on recent techniques for phytoremediation of heavy metals. In their paper, they learned about the technology includes in effective utilization of plants to detoxify or immobilize, remove ecological contaminants in a growth matrix through the organic substance or physical activities or process for plants. After reviewing and analysis various study areas they inferred that phytoremediation is another cleanup idea and research identified with this generally new innovation needs be advanced, emphasized and extended in developing countries since it is minimal cost. Likewise, they inferred that this technique is safe as it does not pose any destruction effect on soil fertility and structure compared to some other conventional innovation, for example, soil washing or corrosive extraction. It has been seen to be a more natural eco-friendly, low-cost and green technology.

Salaskar et al., (2011) worked on the bioremediation potential of Spinach for decontamination of cadmium in soil. The studies were conducted on greenhouse pot culture in order to check the efficiency of bioremediation technique for removing cadmium from the soil. Test were conducted in two stages that is uptake of cadmium by spinach and cadmium effect of spinach on dry matter yield. The plants were analyzed on different number of weeks. After the analysis, the results show the shoot of the spinach were able to accumulate cadmium which shows that spinach can be a useful plant for phytoremediation of cadmium from contaminated soils.

Ghosh and Singh., (2005) studied on phytoremediation of heavy metals and usage of its by-products. The review depends on the present status of phytoremediation innovations with specific emphasis on phytoextraction of soil heavy metals defilement or contamination. This paper explored about the versatility, bioavailability and plant reaction to presence of soil heavy metals. Different systems to improve phytoextraction and use of side effects have likewise been considered. It is report that a large portion of the reviews have been done in developed countries and information of reasonable plants is especially restricted in India.

Ali et al., (2012) worked on phytoremediation of heavy metals by *Trifolium alexandrinum.* The work experiments were conducted in pots in greenhouse. Heavy metals such as cadmium, lead, copper, and zinc were studied and it has been added to the pots were the plants are grown at different concentrations. After 98 days, the mature plants were separated into stem, roots and leaves for analysis its uptake of heavy metals from the soil. Bioconcentration and translocation factors were used for calculating and analyzing the study. After the following analysis, it is concluded that *Trifolium alexandrinum* is an effective plant for phytoremediation of cadmium, lead, copper and zinc. Also, they concluded that the plant has many advantages produces biomass considerably, and has short life cycle. It is also resistance to climatic conditions and can grow faster in a single development period.

Gunduz et al., (2012) worked on heavy metal phytoremediation potentials of *Lactuca sativa* L., *Spinacia oleracea* L., *Raphanus sativus* L and *Lepidium sativum* L. These plants were taken near the mining area and shifted to the plastic container which is filled with sandy soil along with some treatments provided. After 60 days of development the plants were uprooted from the pots and then it is taken for analysis. Remediation factor of the plants and ANOVA methods were used for analysis the result of the study. After analysis, it is found that plant species most suitable for remediation of arsenic and lead was found to be L *sativa*. For cadmium and iron none of the studied plants were suitable for the remediation of these two heavy metals. They additionally concluded that the suitable plants is the one which is called hyperaccumulator and which produced high biomass, but they concluded that it is hard to find almost same characteristics in similar plants.

Ferraji et al., (2014) studied on the of phytoremediation of lead-contaminated soil by Spinach (*Spinacia oleracea* L.). A pot experiment was conducted in which the plant species were grown with lead added at different concentrations. The plants were taken for testing after 7, 14 and 21 days respectively after growing in soil which is lead-contaminated. Focal composite outline and reaction surface procedure were utilized keeping in mind the end goal to clear up the way of the reaction surface in the investigative plant and for clarifying the independent variables of the ideal states. After analysis, the results showed that spinach has good potential to act as a phytoremediation tools in the studied soil and it is found that lead accumulate more in shoots than in roots. Also, after 21 days at best possible number of spinach planted in soil the efficiency of removal of lead was found to be 60.05%.

Taiwo et al., (2015) dealt with phytoremediation of heavy metals, for example, copper, zinc and lead from contaminated water by Water Hyacinth (Eichornia crassipes). The analysis has been led in the plastic compartments in which it included the planning of heavy metals arrangement in various concentration with variety in pH. The test was observed and examined for 8 weeks and were taken for testing utilizing Atomic Absorption Spectrophotometer (AAS). Further the results obtained were interpreted utilizing SPSS software to decide the mean concentration acquired from first week to eight weeks of the analysis. After the analyses, it is found that toward the end of 8 weeks of remediation, the water was beneath detection limit however it was accumulated in the plants. Additionally, the consequences of Bioconcentration factor demonstrated that the plant hyperaccumulated zinc more than other heavy metals, in this manner, they inferred that heavy metal take-up by Eichornia crassipes utilizing phytoremediation innovation is by all accounts a prosperous approach to remediate heavy metals from polluted site.

Laghlimi et al., (2015) studied on phytoremediation mechanism of heavy metals contaminated soils. Remediation of heavy metals from contaminated soil by different process were considered and it is found that many variables impact the decision of the reasonable phytoremediation technique for soil decontamination. It has also been found that it relies on upon the properties of soil, its level of heavy metals and qualities, types of plant species and temperature conditions can improve the remediation process. This paper discusses about factors affecting accumulation of heavy by different types of plant, the diverse methodologies of phytoremediation heavy metals from contaminated soils and favorable circumstances needed for phytoremediation process. He concluded that more research should be done on developing agricultural field in order to improve the phytoremediation technique at minimal time and low cost in removing the heavy metals from soil.

Selaraj et al., (2013) studied on phytoremediation of arsenic chloride by *Brassica juncea*.

He studied on two plants, *Abelmoschus esculentus* which was chosen as experimental plant and *Brassica juncea* was chosen as hyperaccumulator plant. The heavy metal arsenic was added with different concentrations to both the plants. The effect of various concentration of arsenic chloride on accumulation factor, translocation factor, morphometric characters and mobility index were analyzed on both the plants. Both the plants were co-developed together and it is found that when Abelmoschus esculentus was developed alone it diminish in development and poor in accumulation factor, translocation factor mobility of metals, yet when co-developed with Brassica juncea it began enhancing its development which tells that Brassica juncea demonstrated higher accumulation of arsenic, more translocation of as from root to shoot and great versatility of arsenic chloride. He concluded that *Brassica juncea* is best suited for remediating arsenic contaminated soil.

Nooshin Karimi (2013) studied on phytoremediation of chromium-contaminated soil by Alfalfa (Medicago sativa) and Sorghum bicolor (L) Moench. The plants were developed in pots in nursery with various concentration of chromium. The test has been taken following 50 days after these plants were developed and plants root and shoots were taken for testing the collection of chromium. Information were additionally investigated utilizing SPSS software. After analysis, it is found that both the plants Sorghum and Alfalfa could be suitable or useful plants for remediating chromium-polluted soil and it is found that chromium accumulate more in roots as compared in shoots, however Alfalfa indicated more capability of accumulating chromium than Sorghum.

Otaru et al., (2013) studied on the effectiveness of phytoremediation in the removal of heavy metals from soil using corn. Pots experiment were conducted in which the plants were grown with different concentrations of zinc, iron and lead. The samples were tested after 2 weeks' interval in a period of 8 weeks and plant leaves and stems were tested for checking the accumulation. After analysis, it is found that the highest removal of zinc concentration in the contaminated soil was in the sixth week and the highest removal of iron and lead were in the fourth week. The results showed that corn is a hyperaccumulator and it is effective in removal of heavy metal contaminated soil.

CHAPTER 4

RATIONALE SCOPE OF STUDY

The expansion in industrial activities has led to many contamination issues in the environment along with disintegration of a few biological systems in which the toxic or poisonous metals get accumulated. Also, other sources of operation led to the contamination of the surroundings especially the topsoil due to which leaching can occur and it reach to the groundwater and make it contaminated. Present scenario consideration shown the presence of heavy metals (lead) in the environment leads to the accumulation in the living tissues and also throughout the food chain causes a major health problem. Removal of lead which are harmful to our environment and treatment of such problems should be done as soon as possible. There are many different techniques in which lead can be treated or remove which are very expensive, so to remove or evacuate the dangerous metal through plants is one of the least expensive method and environmental friendly.

CHAPTER 5

OBJECTIVE OF STUDY

- To check the ability of the Aloe vera plant to remediate lead.
- To check the ability of Aloe vera to remediate at different concentrations of lead.
- To check the potential of Aloe vera plant to remediate at different growth perod.

CHAPTER 6

MATERIALS AND RESEARCH METHODOLOGY

The methodology for this dissertation can be explain by flow diagram shown in fig 6.1

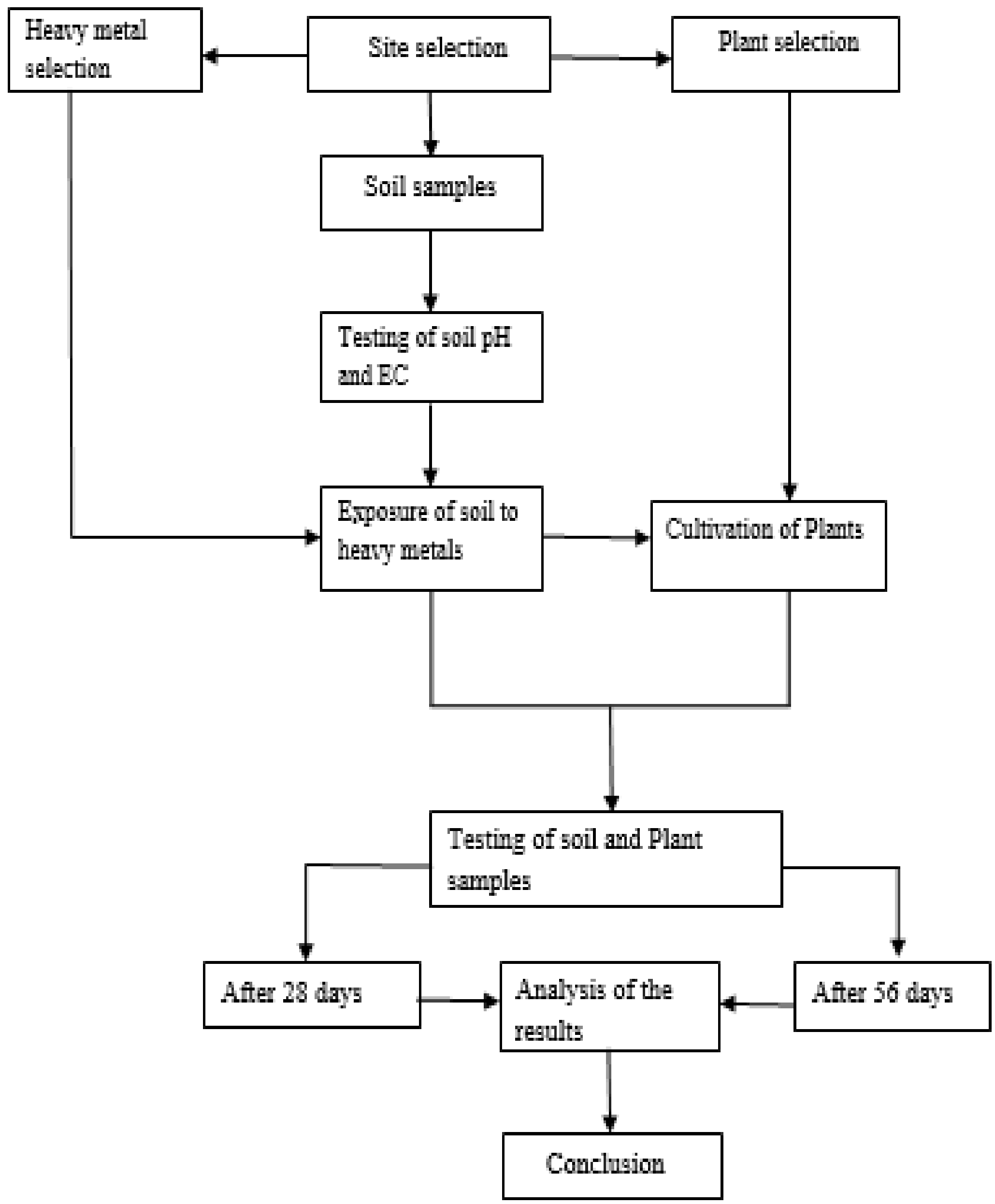

Fig 6.1 Flow diagram of methodology of Phytoremediation of heavy metals

6.1. Materials

The materials used in this project are 12 flower pots along with 12 trays, soil, Aloe Vera plants and lead acetate ($(CH_3COO)_2Pb.3H_2O$).

6.2. Selection of site

Before conducting the experiment, site selection is important in order to grow the plants for further study. An experimental setup was made for conducting the project to study the phytoremediation of lead by Aloe Vera plants at Lovely Professional University, Punjab. The experiment was taking place with the help of pots for planting the Aloe Vera plants to investigate the heavy metal (lead) accumulations in them. The experiment was conducted for the duration of 56 days and testing were done twice, one for 28 days and other for 56 days of exposure with lead. The experiment was conducted from the month of February to April. Each treatment had three replications of Aloe Vera.

6.3. Collection of soil and plant samples

The soils were collected from the field of School of Agriculture Exprimental Farm of Lovely Professional University. The soil was taken about 15 cm from the ground level for using as shown in fig 6.2. After the collection of soil, it was taken for sieving to remove unwanted materials and 5 kg were transfer to each pot. Initial pH and electrical conductivity were performed before planting.Twelve aloe vera plants were bought from the nursery and transfer to the pots were 5 kg soil are already present in each pots as shown in fig 3

Fig 6.2. Soil sample collected

Fig 6.3. Plants were planted in pots

6.4. Preparation of lead contaminants

The lead was added into the soil as lead acetate ($(CH_3COO)_2Pb.3H_2O$) and 1.83g of lead acetate was dissolved in a 1000 ml beaker with distilled water to make a stock solutions as shown in fig 6.4 of 8, 12 and 16 mililitres respectively. The different concentrations of the stock solutions were transfer to 100 ml capacity measuring cyclinder and make up to the mark to get the desires concentrations of 8ppm, 12ppm and 16 ppm respectively. After preparing it was then transfer to the soil. (Aransiola et.al., 2013).

Fig 6.4. Preparation of lead contaminant

6.5. Experimental design

The study was conducted in pots inside the campus of Lovely Professional University, Punjab as shown in fig 6.5. The treatments given were:

5 kg soil + plant (control)

- 5 kg soil + 8 ppm of lead
- 5 kg soil + 16 ppm of lead
- 5 kg soil + plant + 8 ppm of lead (with three replicates)
- 5 kg soil + plant + 12 ppm of lead (with three replicates)
- 5 kg soil + plant + 16 ppm of lead (with three replicates)

The plants were watering everyday with normal tap water and sampling of plants to check lead uptake and the soil residual lead content was done 28 days and 56 days after planting.

Fig 6.5. Experimental set up

. 6.6. Testing of soil and plant samples

Soil samples were analyzed for different soil parameters such as pH and electrical conductivity before plantations and after 28 days and 56 days. The detail procedure can be described as follows: -

6.6.1. Test for pH

10 gm of soil was taken into a beaker of 50 ml and 20 ml water was added with a continuous stirring for 30 mins. Then the sample was taken to the digital pH meter as shown in fig 6.6 and it was calibrated with 7.0 and 4.0 pH buffer standard solution. The sample was then tested.

Fig 6.6. pH meter

6.6.2. Test for Electrical Conductivity (EC)

10 gm of soil was taken into a beaker of 50 ml and 20 ml water was added with a continuous stirring for 30 mins. The EC meter as shown in fig 6.7 was inserted into the beaker and was swirled gently around in the soil-water extract. After approximately 30-60 seconds or after the EC reading has stabilized, the digital display on meter was noted.

Fig 6.7. Electrical conductivity

6.6.3. Soil digestion

Soil sampling of 10-12 cm depth were taken from each pot. The soil was dried in oven and then passed through a sieve of 2mm size. 10 gm of soil were taken and 20 ml of ammonium bicarbonate diethylenetriamine-pentaacetic acid (AB-DTPA) solution were added to the soil and shaked for 15 minutes and then it was filtered with the help of filter paper. The solutions were refrigerated until it was analyzed by Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES, iCAP 6000 series).

6.6.4. Plant digestion

Plant samples were harvested from each pot and were cleaned and washed with tap water twice and oven dried for 48 hours at 60 ^{o}C as shown in fig 6.8. After drying the dried plants were crushed with the help of mortar and pestle as shown in fig 6.9 into powered form and passed through 2 mm sieve size. Now 1gm weight of plant sample were taken in a volumetric flask of 50 ml and in the ratio 3:1 of HNO3 and Perchloric acid total of 10 ml was added and heated over a hot plate as shown in fig

6.10 until white fumes occurred. The samples were then transfer into a 25ml volumetric flask and make up to the mark. The sample were then taken for analyzed by using Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES, iCAP 6000 series) as shown in fig 6.11.

Fig 6.8. Dried plant sample

Fig 6.9. Plants were crushed

Fig 6.10. Digestion in hot plate

Fig 6.11. ICP-AES

CHAPTER 7

RESULTS AND DISCUSSION

7.1. The soil pH and electrical conductivity

Initial soil pH = 7.6 and EC = 0.337 Mhos/cm

7.1.1. After 28 days, the soil pH and EC are as shown in table 7.1

Table 7.1. Soil pH and EC after 28 days

Soil samples	pH	EC (Mhos/cm)
0 ppm	7.45	0.535
8 ppm + control	7.33	0.542
16 ppm + control	7.10	0.565
8 ppm (1,2,3)	7.28	0.542
12 ppm (1,2,3)	7.21	0.551
16 ppm (1,2,3)	7.08	0.585

7.1.2. After 56 days, the soil pH and EC are as shown in table 7.2

Table 7.2. Soil pH and EC after 56 days

Soil samples	pH	EC (Mhos/cm)
0 ppm	7.21	0.447
8 ppm + control	7.12	0.526
16 ppm + control	7.06	0.512

8 ppm (1,2,3)	6.89	0.523
12 ppm (1,2,3)	7.03	0.510
16 ppm (1,2,3)	7.01	0.520

7.2. Lead in soil and plants

Initial lead in soil = 0 ppm and in plant = 0 ppm

7.2.1. After 28 days, lead on soil and plants are as shown in table 7.3

Table 7.3. Lead in soil and plants after 28 days

Treatments	Soil (ppm)	Plants (ppm)	BCF (%)
0 ppm (control)	0	0	-
8 ppm (control)	2.05	-	-
16 ppm (control)	2.85	-	-
8 ppm (1,2,3)	1.44	0.03	0.375
12 ppm (1,2,3)	1.96	0.05	0.416
16 ppm (12,3)	2.12	0.09	0.562

7.2.2. After 56 days, lead on soil and plants are as shown in table 7.3

Table 7.4. Lead in soil and plants after 56 days

Treatments	Soil (ppm)	Plants (ppm)	BCF (%)
0 ppm (control)	0	0	-
8 ppm (control)	1.62	-	-
16 ppm (control)	2.02	-	-
8 ppm (1,2,3)	1.05	0.07	0.875
12 ppm (1,2,3)	1.25	0.08	0.667
16 ppm (1,2,3)	1.75	0.102	0.637

After analyzing the samples using ICP-AES, it is found that the pH of the soil decreases with concentrations added i.e. for 7.28, 7.21 and 7.08 (table 7.1), as metal availability is more in soil the pH decrease (Abhilash et al,2016). After 56 days, the pH keep on decreasing i.e. 6.89, 7.03 and 7.01. However, with electrical conductivity it was first increasing then decreasing with time as shown in table 7.1 and 7.2. Lead accumulation in plants and concentration in soil are shown in table 7.3 and 7.4., the results shown that after 28 days of exposure of Aloe Vera with lead treatment of 8ppm, 12ppm and 16ppm respectively the accumulation in plants are 0.03ppm, 0.05ppm and 0.09 respectively. And after 56 days the accumulation in plants are increasing with 0.07ppm, 0.08ppm and 0.102ppm respectively.

Comparison between the two readings the accumulation in 8ppm treatment has drastically increased by 0.04ppm wheras in 12ppm treatment it has increased by 0.02ppm and that of 16ppm treatment has increased by 0.012ppm. The lead uptake of the plants is affected with the change in pH of the soil (Ziarati and Alaedini, 2014) as it was determined in treatment of 8ppm soil after 28 days the pH was 7.28

and lead uptake was 0.03ppm, after 56 days the pH was 6.89 and lead uptake was 0.07ppm. Similarly, for treatment of 12ppm and 16ppm respectively after 28 days' pH was 7.21 and 7.08 was lead uptake was 0.05ppm and 0.09ppm and after 56 days' pH was 7.03 and 7.01 and lead uptake was 0.08ppm and 0.102ppm respectively. For 8ppm (control) and 16ppm (control) for both 28 and 56 days it has been found that the pH decrease i.e. from 7.33 and 7.10 to 7.12 to 7.06 respectively and the available lead determined were 2.05ppm and 2.85ppm after 28 days and 1.62ppm and 2.02ppm after 56 days. This shows the indication of mobility of lead ion with lower concentration of pH (Abhilash et at, 2016). As shown in fig 7.1 and fig 7.2 it explains the graphical representation the different levels of uptake of lead from Aloe vera plants.

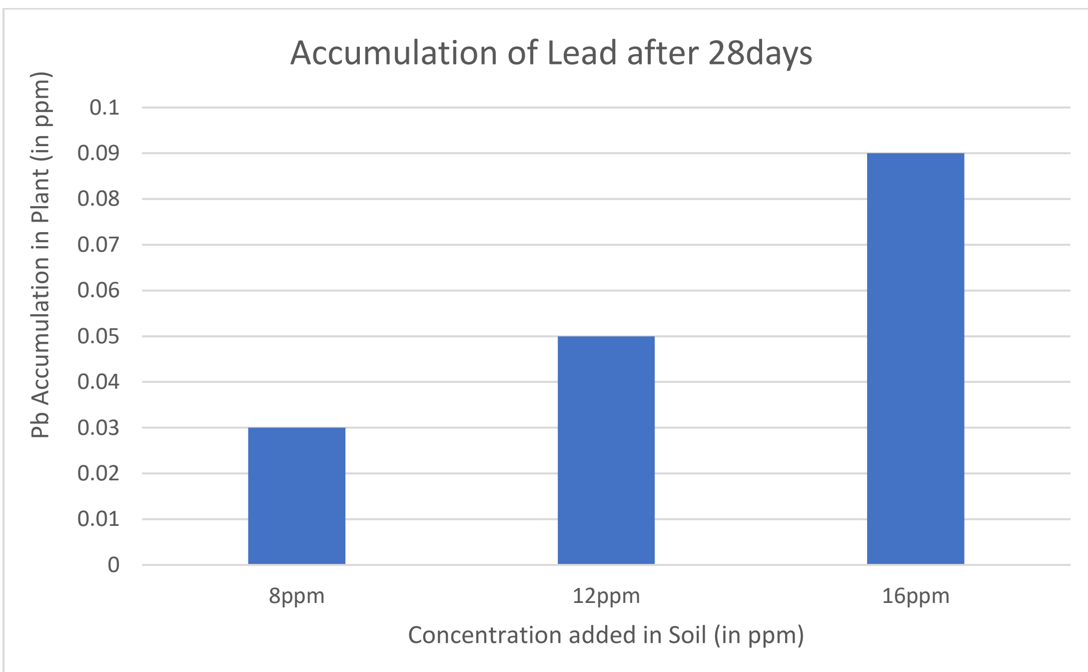

Fig 7.1. Uptake of lead by Aloe Vera after 28 days

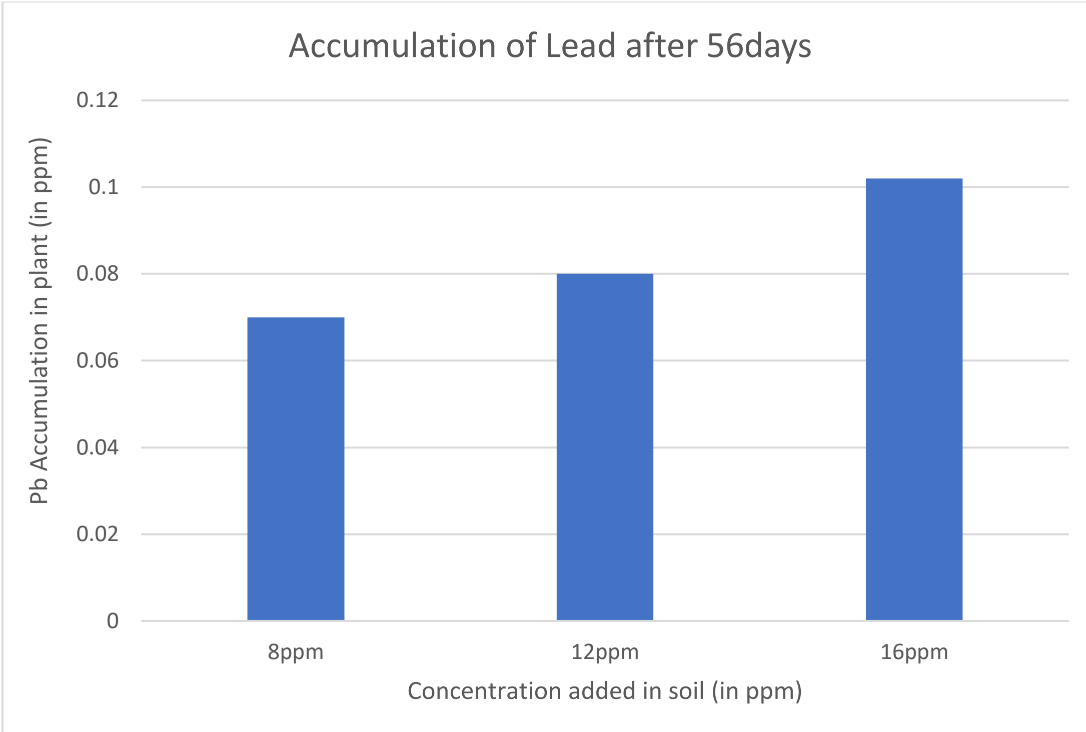

Fig 7.2. Uptake of lead by Aloe Vera after 56 days

BCF or bioconcentration factor is the efficiency of the plant to uptake heavy metals from soil and accumulate them in their tissues (Hazrat Ali et al, 2014) and it is given by the formula –

BCF = Metal concentration in plant (ppm) / Metal concentration in soil (ppm)

Also, it can be expressed in percent as -

BCF = (Metal conc. in plant(ppm) / Metal conc. in soil(ppm)) * 100

The BCF found in this work were more in the 8ppm treatment (after 56 days) i.e. 0.875% and lesser in 8ppm treatment (after 28 days). It is seen increasing in 12ppm treatment from 0.416% to 0.667% and that of 16ppm treatment from 0.562 to 0.637.

CHAPTER 8

CONCLUSION AND FUTURE SCOPE

From the investigation of checking the ability of Aloe vera plant to accumulate lead can be concluded that the plant has the potential and ability to accumulate lead in them from the soil as the BCF calculated are high as shown the above results. Plants can take up metals in them depending upon specific metal and plant species and also along with the environmental conditions. Aloe vera plant has proved to be a hyperaccumulator plant by accumulating metals like lead at different concentrations in their organs. At different time periods the accumulation factor of Aloe vera increases hence, this plant can be use for long time experiment. Other factors must also consider to get a good remediation result as in this study the change in pH affected the uptake of plants and many more environmental conditions which may affect the accumulation of any heavy metals. Therefore, phytoremediation can be considered as one of the suitable technique to clean up lead contaminant from soil with the help of Aloe Vera plant.

Future Scope

Aloe vera is used in many purposes so it is necessary to check the metals content in it as it acts as a hyeraccumulator plant. More research need to be done on the ability of the plants to accumulate heavy metals at different growth periods and its ability to withstand or grow on different concentration of heavy metals present with different environmental conditions.

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