PHYTOREMEDIATION OF LEAD AND ZINC IN POLLUTED YAMUNA SOIL USING *Helianthus annuus*- A NEW GREEN TECHNOLOGY

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ABSTRACT

*The heavy metals being discharged into the environment by various anthropogenic activities pose a great risk to ecological health and human wellbeing. The current conventional remediation techniques are expensive, time consuming, environmentally destructive and disturb soil native microflora. Phytoremediation is an indispensable tool box for wider application in the realm of environmental protection. It is a cost-effective alternative to high-energy, high-cost conventional methods , has aesthetic advantages and long term applicability and considered to be a "**Green Revolution**" in the field of innovative clean up technologies. Helianthus annuus was investigated for its potential to remove heavy metals, particularly Lead (Pb) and Zinc (Zn) present in contaminated/polluted Yamuna soil using phytoextraction. Yamuna soil was collected and studies were made at the regular intervals of one month. The distribution of lead in different parts of plant was studied. Results indicate that Lead and zinc concentrations in aerial parts and underground parts of plant in polluted soil sample was higher than the garden soil sample. As plant biomass increases, the amount of Lead and Zinc decreases in soil while increases relatively in different plant parts. The plant study was done considering and measuring their root length and shoot length and analysing them for their dry and fresh weight. The microwave digestion of soil and plant using EPA Digestion method-3051a was done and were analysed for heavy metal content by Flame Atomic Absorption Spectrometer Analyst 400 (Perkin Elmer, Shelton, CT, USA.).*

Keywords: Flame Atomic Absorption Spectrometer, Helianthus Annuus, Phytoremediation, Yamuna soil

I INTRODUCTION

Environmental pollution by heavy metals has become a serious problem in the world. The mobilization of heavy metals through extraction from ores and subsequent processing for different applications has led to the release of these elements into the environment. Modern day industries and activities are known to generate various dangers and risks that endanger biological communities worldwide [1]. Industrial operations and their unscientific waste transfer strategies are thought to be the primary source of heavy metal pollution in soil, water (ground & surface water) and deforestation [2]. About 90 % of the wastes generated from the extraction of metals are in the form of sulfides and these squanders contains high amount of heavy metal toxicity particularly

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Cu, Zn, Cd and Pb [3]. Although metals are also useful resources for the healthy environment, but at high concentration it leads to environmental degradation [4]. It is established that the harmful effects of metals are responsible for changed physiology of plants including restrained root development, photosynthesis [5], cell division and genotoxicity in plants [6].

1.1. Heavy metal impact on crops/plants and animals

Higher plants are known to induce reactions to heavy metals in their surroundings which meddle with the hereditary constitution of plants [7]. Adaptive response is one of the essential procedures where plants invariably react keeping in mind the goal to withstand adverse conditions presenting genomic security. Among the higher plants, horticultural yields have noteworthy capacity to move metals in their tissues, known as great bio-indicators and assume a key part in detecting metal harmfulness and resistance.

According to Environment Protection Agency (EPA), the eight most common heavy metal pollutants are As, Cd, Cr, Cu, Hg, Ni, Pb and Zn. Though some heavy metals are necessary for human growth and development, in higher concentration they cause antagonistic consequences for human wellbeing. The ecological problem of water and soil heavy metal contamination is of great importance in today's global scenario. The contamination of soil with heavy metals from seepage can potentially result in phytotoxicity [8]. In general, heavy metals attack the active sites of enzymes repressing essential enzyme function. Heavy metal ions particularly Pb^{+2} act as viable enzyme inhibitors. They can break the sulphur hydrogen bonds of enzymes. Geochemically, an element introduced into the soil may end up in one or more of the following forms: i) dissolved in soil solution; ii) held onto exchange sites of organic solids or inorganic constituents; iii) occluded or fixed into soil minerals; iv) precipitated with other compounds in soils and v) incorporated into biological material.

1.2. Impact of Lead and zinc toxicity

Metals are a natural part of terrestrial systems occurring in soil, rock, air, water and organisms. It is only when metals are present in bioavailable forms at extreme levels that they have the potential to become toxic to plants. Plant responses to metals are dose dependent. For essential metals, these reactions cover the stages from deficiency to tolerance to lethality. For non-essential metals, just the tolerance and lethality stages occur. Pb is a non-essential toxic element to plant and animals, extensively studied due to its probable adverse effect on nervous system and other parts of our body [9]. After the accumulation, it is released very slowly from our body; hence poisoning effect can occur without exposure to major doses. In soil, Pb exists mainly in +2 oxidation state. It is the least mobile heavy metal in soil, especially under reducing or non-acid condition. It generally enters the soil through the automobile exhaust and mining activities. The major biochemical effect of Pb is its interference with heme synthesis, leading to hematological damage. It inhibits several of the key enzymes involved in the overall process of heme synthesis. Zn is an essential micro-nutrient and enters soil through environmental pollution following industrial and agricultural activities, such as smelter and incinerator emissions, dispersal from mine wastes, excessive applications of Zn-containing fertilizers or pesticides and use of Zn-contaminated sewage sludge, manures or industrial wastes as fertilizers. Zinc toxicity symptoms include chlorosis and reddening of younger leaves with necrotic lesions on leaves in extreme cases. Hence, efficient planning and environmental management ensures minimized effects of heavy metal toxicity on the earth and hence help safeguard the ecosystem.

1.3. Remediation Strategies

The remediation of the soils contaminated with heavy metal poses number of difficulties. At international level various modern-day technologies are being used to clean the metal contaminated soils [10] but either they offer a temporary solution, are time consuming, environmentally destructive and disturbs soil native microflora or immobilize the metals or are not economically feasible when applied in expansive scale. The modern day bio-technological clean-up methods are found to be a successful hotspot for heavy metal remediation from soil [11].

Phytoremediation—a green solution to the problem of heavy metal pollution

Phytoremediation is a biological technique to reduce, degrade or immobilize the environmental pollutants (inorganic compounds) using crops/plants. The procedure is a financially savvy, non-intrusive and socially satisfactory approach to address the evacuation of natural contaminants [12] and hence thought to be a "Green Revolution" in the field of innovative remediation technologies. It delivers intact, biologically active soil. It can be used for removal of heavy metals and radionuclides as well as for organic pollutants (such as, polynuclear aromatic hydrocarbons, polychlorinated biphenyls, and pesticides). The idea of using plants to extract heavy metals from contaminated soils was reintroduced and developed by Chaney [13]. Plants generally handle the contaminants without affecting topsoil, thus conserving its utility and fertility. They may improve soil fertility with inputs of organic matter [14]. Green plants have an enormous ability to uptake pollutants from the environment and accomplish their detoxification by various mechanisms.

Contaminant uptake by plants and its mechanisms have been being explored by several researchers. It could be used to optimize the factors to improve the performance of plant uptake. According to Sinha et al.[15], the plants act both as "accumulators" and "excluders". Accumulators withstand the contaminants in their aerial tissues. They biodegrade or bio transform the contaminants into non-active forms in their tissues. The excluders restrict contaminant uptake into their biomass. Plants perform important secondary role in physically stabilizing the soil with their root system, preventing erosion, protecting the soil surface and reducing the impact of rain. At the same time, plant roots release nutrients that sustain a rich microbial community in the rhizosphere. In the present investigation an effort has been made to study with the use of *Helianthus annuus* for phytoremediation of lead from polluted Yamuna river soil.

1.4. Site characterization and selection

Today Yamuna is one of the most polluted rivers in the world due to high density population growth, rapid industrialization especially around New Delhi, where 15 drains discharge waste water into the river. Even the ground water has been affected by leachates that pass down from the dumping sites[16]. According to the

Central Pollution Control Board (CPCB), 70% of the pollution in river is from untreated sewage and the remaining 30% is from industrial sources, agricultural run-off, garbage etc. The water quality of Yamuna River falls under the category "E" which makes it fit only for recreation and industrial cooling, completely ruling out the possibility for underwater life. Almost every year mass death of fishes is reported. Biological Oxygen Demand (BOD) load increased by 2.5 times between 1980 and 2005: from 117 tonnes per day in 1980 to 276 in 2005. Thus, the aim of the present work is to develop an effective and eco-friendly approach to control soil pollution near Yamuna river using phytoremediation.

II EXPERIMENTATION

2.1. Instrumentation

A PerkinElmer AAnalyst 400 flame atomic absorption spectrometer equipped with deuterium lamp background correction and an air–acetylene burner for lead and zinc determination was used throughout the study. Microwave unit, Anton Paar, Model Multiwave 3000 was used to digest soil samples. To adjust the pH of solutions, ELICO pH meter model LI-614 was used. For making all solutions and washing, double distilled water was used from Double Distillation unit.

2.2. Reagents and materials

All chemicals used were of analytical reagent grade. All working solutions were prepared using double distilled water. A stock solution of 1000 μg mL^{-1} of Pb (II) and Zn (II) were prepared Pb $(NO_3)_2$ and $Zn(NO_3)_2$ in a 100 mL standard volumetric flask and was used to prepare fresh Pb(II) solutions daily by dilution. To adjust the pH of metal ion solutions, 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} NaOH solution were used. All glass apparatus were cleaned with 10% (v/v) nitric acid and rinsed with double distilled water before use. .

Seeds of Sun flower cultivated variety (*Helianthus annuus*) were used for the experiment purpose. This plant belongs to family Asteraceae, known for its ornamental flower, grows during the winter season. It is also important for its oil production property. In the present experiment we have used only ornamental variety so that lead absorbed by the roots of the plant cannot be the part of food chain. Surface sterilization of the seeds was done by using 0.1% mercuric chloride. Then they were soaked for two days in sterile distilled water before sowing.

2.3 Site Characterization and Sampling

Soil was collected from the bank of Yamuna River as shown in Fig.1 at the depth of 0-20 cm. The controlled soil was collected from the garden and pH of the soils was studied using pH meter. 5 g of soil sample and 25 mL of water was taken in a 50 mL beaker. The beaker was kept for agitation for one hour. After one hour pH of an aliquot of the supernatant solution was taken. The pH meter was standardized by pH 4 and pH 7 buffer solution. Ten seeds of *Helianthus annuus* per pot were used for sowing.

2.4. Green House Experiment

Green house pot culture experiment were conducted to study the effect of heavy metal i.e., lead and zinc on seed germination, root growth, shoot growth and phytoremediation by *Helianthus annuus*. Earthen pots are used for this purpose. 350 gm of soil is taken in each pot and seeds were sown in each pot.

2.5. Digestion of Soil and Plant

2.5.1 Hot Plate Method

0.5g sample is dissolved in 4.5mL HCl and 1.5 mL HNO_3 and placed in round bottom flask. It is sealed with condenser and placed on magnetic stirrer for 3-4 h. After cooling the solution, it is filtered and placed in 100 ml volumetric flask and makeup till the mark.

2.5.2 Microwave digestion of soil and plant

EPA Digestion method-3051a (microwave, HNO_3-HCl, total-recoverable). This technique provides rapid, safe and efficient digestion and is not susceptible to losses of volatile metals. This technique is time saving and energy efficient and no acid fumes are generated. For all these reasons the above technique can be considered as a green technique. 0.5g of soil sample was dissolved in 4.5 mL conc. nitric acid and 1.5mL conc. hydrochloric acid and placed in quartz microwave vessel. The vessel was sealed and heated in the microwave unit, Anton Paar, Model Multiwave 3000. After cooling the solution was placed in 100 mL standard flask and makeup till the mark.

III FIGURES AND TABLE

Table 1: Heavy Metal content in soil before and after Phytoremediation

Fig. 1 - Site of Soil Collection

Fig. 2 - Green House Experiment

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Sample	pH	Heavy Metal content (mg/kg)					
		Lead			Zinc		
		Before	After	%Remediation	Before	After	%Remediation
Yamuna	6.6	394	330	16.24	240	90	62.5
Garden	6.9	240	206	14.16	120	64	46.6

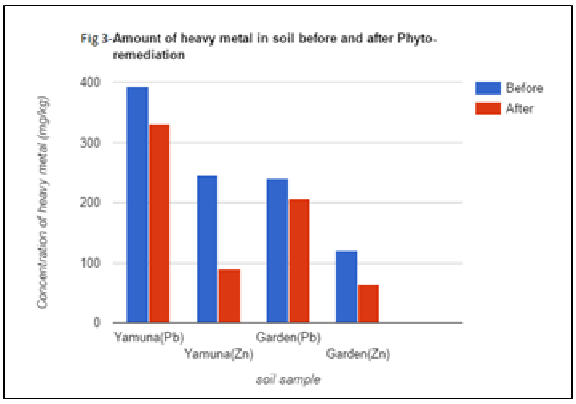


Fig 3-Amount of heavy metal in soil before and after Phyto-remediation

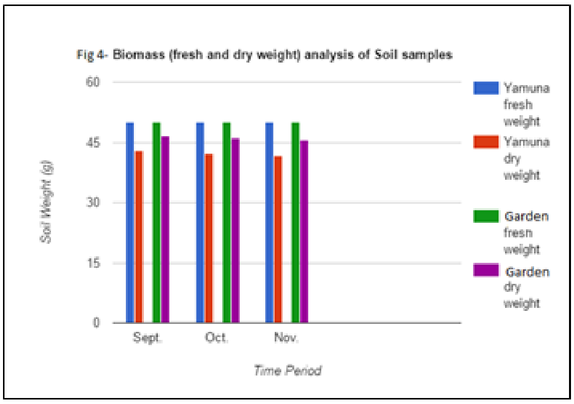


Fig 4- Biomass (fresh and dry weight) analysis of Soil samples

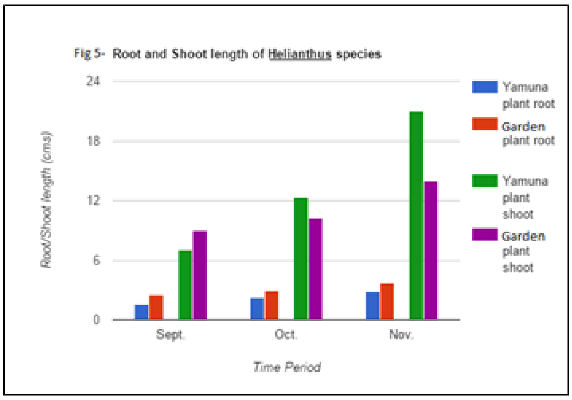


Fig 5- Root and Shoot length of Helianthus species

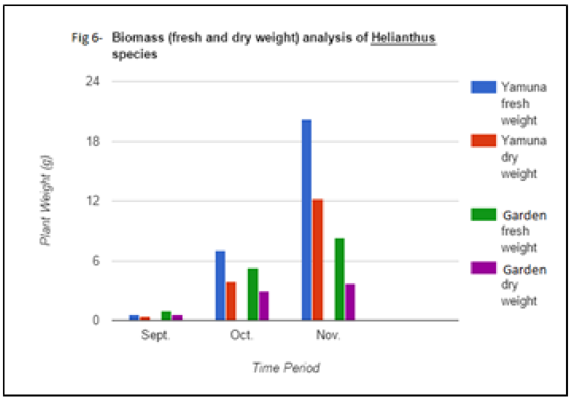


Fig 6- Biomass (fresh and dry weight) analysis of Helianthus species

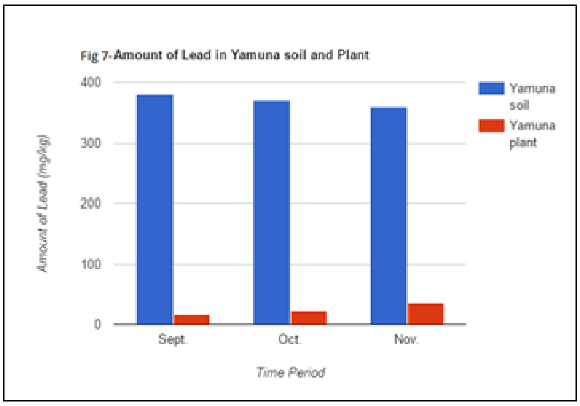
Table 2: Plant and Soil Biomass Analysis

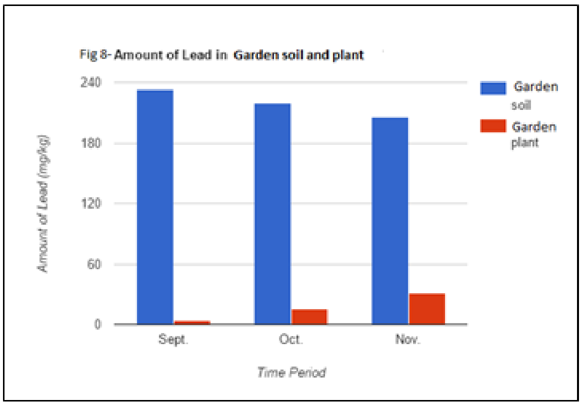
Sample	Shoot length (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Plant % moisture content	Soil fresh weight (g)	Soil dry weight (g)	Soil % moisture content

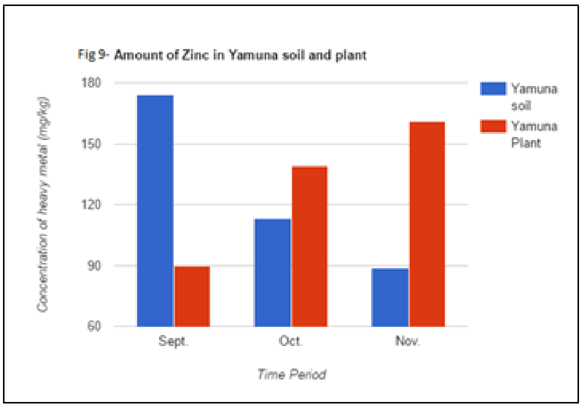
Yamuna	Sept.	7.0	1.6	0.544	0.418	30.14	50	43.0	16.27
	Oct.	12.3	2.2	7.014	3.94	72.91	50	42.3	18.20
	Nov.	21.0	2.8	20.22	12.2	65.73	50	41.7	19.90
Garden	Sept.	9.0	2.5	0.967	0.574	68.46	50	46.5	7.53
	Oct.	10.2	2.9	5.29	2.96	78.71	50	46.1	8.45
	Nov.	14.0	3.7	8.00	4.12	94.17	50	45.6	9.64

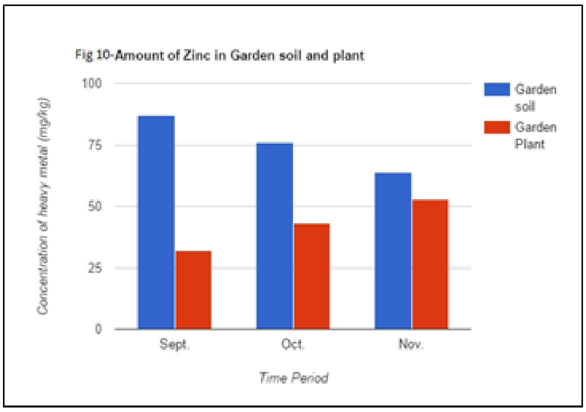
Table 3: Concentration of lead and Zinc in soil and Plants

Sample	Heavy Metal	Lead (mg/kg)		Zinc (mg/kg)	
		Soil	Plant	Soil	Plant
Yamuna	September	380	17	174	90
	October	371	23	113	139
	November	330	35	90	161
Garden	September	233	4	87	32
	October	220	16	76	43
	November	206	30	64	53









IV RESULT AND DISCUSSION

The fresh weight and dry weight of the plants was studied to compare the biomass amount and was found to be high in garden soil plants as compared to Yamuna soil plants. Observation regarding healthy growth rate of garden soil plants, considering they are of same age, is much better than that of Yamuna soil plants due to the presence of various contaminants (heavy metals) in Yamuna soil. The root length of Yamuna soil plants is considerably less than that of garden soil plant (Table 2) which is attributed to root as being the primary zone for accumulation of lead and zinc thus preserving metabolically and functionally active parts of plant (leaves and shoot) from deleterious effect of heavy metal. As growth of plant increases, the amount of lead and zinc in soil decreased while it increased in plant due to phytoextraction potentiality of *Helianthus* species. The heavy metal accumulation capacity for zinc was observed to be higher than that of lead in terms of heavy metal content in soil samples before and after remediation (Table 1)

The amount of Zinc taken up by plants in both soil samples is more than that of amount of Lead up taken because zinc being an essential micro-nutrient is required for proper growth and development in optimum concentration and hence is taken up by the plants more readily. Lead being toxic even at very low concentration affects the physiological and metabolic state of the plants thus limit uptake of lead. The amount of lead and zinc relatively present in soil and plant at different growth phases is summarized in Table 3. Hence the % remediation shows efficiency of *Helianthus annuus* as hyper accumulator of Lead and zinc employing phyto-extraction.

V CONCLUSION AND FURTHER RECOMMENDATIONS

Phytoremediation has the potential to treat sites polluted with more than one type of pollutant and is a promising technology for ecological restoration and biodiversity conservation. It is still a relatively new technology and is mostly in its testing stages and as such has not been used in many places as a full scale application. However it has been tested successfully in many places around the world for many different contaminants. Since lot of biomass is generated during this process raising a need for proper disposal and management. After burning of such plants, they can either be disposed securely in specific dumps or if monetarily attainable, prepared for bio-recovery of coveted metals (a practice known as phytomining) [17]. 'Mining with plants', or phytomining, is being considered as a safe and viable method for waste disposal. Phytomining is the production of a `crop' of a metal by developing high-biomass plants that gather metal in high amounts. This biomass containing heavy metals can be combusted to get crucial energy and the remaining slag is considered as "bio-mineral". This bio-mineral can be arranged for the recovery or extraction of the heavy metals.

In conclusion, Phytoremediation is more economically viable using the same tools and supplies as agriculture and is less disruptive to the environment and does not involve waiting for new plant communities to decolonize the site. It avoids excavation and transport of polluted media thus reducing the risk of spreading the contamination. And hence, is more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods.

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