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A POT EXPERIMENT FRAMEWORK ELUCIDATING THE BIOCHEMICAL BASIS FOR *HELIANTHUS ANNUS L.* PHYTOREMEDIATION CAPACITY AGAINST LEAD CHLORIDE ($PbCl_2$) IN THE SOIL

[1]S.Surendranath Babu, [2]M.Puneeth Varma, [3*]T.Damodharam

[1]Research Scholar, [2]Research Scholar, [3] Professor

[1,3]Department of Environmental Science,[2] Department of Virology

[1]Sri Venkateswara University,Tirupati,Andhra Pradesh, India

Abstract: Pb-contaminated soil acts as a phytotoxic element in environmental rehabilitation and cleaning with hyperaccumilator plants, which can accumulate these contaminants most efficiently and effectively, and serves as a significant and economically advantageous measure for long-term and environmental preservation. Crops such as sunflower (profitable edible oil crop) with high biomass and economically second only to soy bean among vegan oils with high protein content. The sole approach for high biomass and bioenergy for phytoremediation is a strategy for gaining additional benefits in addition to remedial efforts. This study used physiologically matured sunflower pots with concentrations of Pb (50,100,150,200,250 mg/kg-1) from the 30th, 60th and 90th harvesting days. Based on the bioaccumulation coefficient (BAC), bioconcentration factor (BCF), and translocation factor (TF), As a result Pb absorption is larger in the root ($\pm$0.58) than in the shoot ($\pm$ 0.10)than in the leaves ($\pm$0.07).

Index Terms: Lead chloride, Biochemistry, Helianthus annus L., Phytoremediation.

III.I INTRODUCTION:

To address this issue, phytoremediation is a novel, cost-effective, and environmentally friendly metal cleanup approach by using plants from contaminated soil and water with minimal environmental degradation. (Koźmińska et al., 2018; Afonso et al., 2019; Abou-Shanab, 2011; Sharma et al., 2015; Ogundola et al., 2022). There are various types of bioremediation, such as phytodegradation, phytoextraction, phytofiltration, phytostabilization, and phytovolatization. The mechanisms of remediation with heavy metals can differ from site to site based on the source of each particular pollutant, and the accumulation of metals was found to be highest in the plant's roots, followed by its shoots. (Fulekar.,2016; Adejube et al.,2017). Plants chosen for phytoremediation should have fast growth rates, significant biomass generation, heavy metal hyperaccumulation, wide distribution, translocation process between stem and shoot, leniency of hazardous heavy metal repercussions, resistance to pests and pathogens, adaptability to changing environmental conditions, ease of development and harvest time, and no enticement to herbivores (Shabani and Sayadi, 2012; Ali et al., 2013).

In the environment, lead is the most potential hazardous heavy metal. Its usage dates back to ancient times because of its significant physico-chemical characteristics. It is a widespread environmental chemical throughout the world (Mahaffay et al., 1990). Lead is used in the construction of boats, bearings, buildings, paints, lead batteries, vehicles, fuel, pipelines, ceramics, plastics, as well as smelting, mining operations, and the armaments industry. Lead poisoning affects in humans by, skin contact and inhalation contribute to indirect lead intake; ingestion of Pb-contaminated food and drink is a direct cause of accumulation. Acute Pb exposure causes renal, reproductive, and brain problems, whereas chronic Pb toxicity affects the CNS and PNS. (Ara and Usmani et al., 2015). Lead also impairs haemoglobin production. Pregnant women who have low calcium, iron, or zinc levels are especially vulnerable to the consequences of lead buildup.Changes in behavior, reduced IQ, sluggish learning in youngsters, diarrhea, anemia, skin allergies, chronic renal malfunctioning, and many other symptoms(Cerazy and Cottingham et al., 2010, Kwong et al., 2004, Barbier et al., 2005).

Lead reduces plant absorption of key minerals including magnesium and iron, which hinders chlorophyll production. It damages the photosynthetic machinery due to its affinity for protein N- and S-ligands. Increased chlorophyllase activity promotes chlorophyll breakdown in lead-treated plants.Lead treatment is said to have a greater impact on chlorophyll b than on chlorophyll a. Lead effects have been documented for both donor and acceptor sites of photosynthesis-2 (PS II), the cytochrome b/f complex, and photosynthesis-1 (PS I) (Stefanov K, Seizova K et al.,1995;Rebechini HM, Hanzely L. et al.,1974;Ahmed A, Tajmir–Riahi HA. et al.,1993). Research on the phytoextraction process's ability to remove lead (Pb) produced positive results in the past. (Hadi and Bano, 2009). In this studies the phytoextraction capability of sunflower (Helianthus annuus L.) during its first development in Pb-contaminated soil. The wet/dry weights of the plants and heavy-metal concentrations were determined (Xin Zhao et al.,2023).The high Pb conc in sunflower tend to avoid toxicity in the physiologically most active portions of the plants by reducing Pb translocation to the epigeous portion, and by promoting the re-translocation of toxic metals from shoots to the roots (Batista et al., 2017).Nevertheless, in this method, the harvested sunflower plants can be easily and safely processed by ash, composting or drying, or they can be recycled and removed from the secondary pollutant class (Lasat, 2002; Töre & Özkoç, 2022).

II. MATERIALS AND METHODS

2.1. Plant material and experimental conditions:

The soil was obtained within the botanical conservatory (1-30 cm depth) at Sri Venkateswara University in Tirupati. The finely crushed soil then sterilised by suteclave allowed to dry for seven days, then pulverised using a mortar and pestle before being put over 2 mm tubes of sieving. The maximal water retention ability of the soill (300 ml water kg soil (3) was determined (Keen, 1931). Considering the research of Ramesh and Damodharam, (2017) the fundamental characteristics about the soil was determined. Clay (54.2%), silt (10.6%), soil (33.9%) pH (6.6), electrical conductivity (0.39 Mbovcm), organic matter g/kg), available phosphorus (50.26 mg/kg), available potassium (97 45 mg/kg), total nitrogen (55.13 kg), total copper (11 mg/kg), and soil Pb (0.36 mg/kg) had been it's physiochemical attributes (6.2 g/kg) Throughout this completely randomised designed (CRD) experiment, plastic pots (9 -12 cm) have bech unilised, while each pot contained 1 kg of soil. Helianthus annus seeds have been collected from plants thriving in uncontaminatedlocations; there were a minimum of 6 seeds within each pot. Because of its adiptable tature, Prophorous cannot require an excessive amount of water for development, therefore tap water is supplied price per week. One seedling (Phesterophorus plantlet) was placed into every pot after being chosen for its size being uniforth. Thirty pots all together were put to use with five of them allocated to each treatment.

When the seeds were sown, the ambient temperature had been 32/20°C (day night) and 26/18°C when the crop had been harvested. For the experiment of the control, water has been provided every day to keep the soil moistened at a level between 75 - 85%. In contrast to the treatment condition, soil had been experimentally supplied with various $PbCl_2$, concentrations (5, 10, 15 and 20 mg/kg). Throughout the course of the study, the soil received no extra fertiliser applications.

2.2. Determination of plant grosseth parameters:

In order to compare the parameters of plant growth responses of being treated and untreated seedlings subjected to varying amounts of pbcl2, stress, seedlings of helianthus annus that were 30 - 90 days old were collected. Plants were harvested and then washed with tap water and each plant separated into three parts (roots, stems, and leaves)

The roots were further washed with a solution containing 5 mM Tris-HCl pH 6.0 and 5 mM EDTA, and they were then rinsed with distilled water in order to remove the surface-bound metal ions. Plant height and root length were measured with a centimetre ruler from the root and shoot joint to the apices. The fresh of each plant (roots, stems, and leaves) was measured using an analytical balance

2.3. Determination of phytoremediation indices:

2.3.1. Quantification of lead levels in plant and soil:

According to mentioned earlier in (Usman et al.,2019), lead quantification within the soil as well as the plant (helianthus annus seedlings of 30, 60, and 90 days old exposed to the concentrations of 50 - 250 mg/kg-1) shoot along with root has been carried out. In brief, employing an extensive volume digesting apparatus at alternate temperatures, samples of around 0.5 g (plants) and 0.25 g (soil) were subjected to digestion with a solution of nitric acid (HNO3) along with solution of hydrogen peroxide (H_2O) or hydrogen fluoride (HF) as long as solutions became transparent. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to analyse digested materials. Pb levels in each sample have been measured using five separate samples along with standard reference materials.

2.3.2. Computations of phytoremediation indices:

The bio-concentration factor (BCF), which measures the ratio of metal concentration in plant roots to soil concentration, the translocation factor (TF), which measures the ratio of metal concentration in the shoot to metal concentration in the root, and a bio-absorption coefficient (BAC) were all assessed as that of earlier reported in (Raj et al.,2020).

2.4. Statistical Analysis:

The results were expressed as the mean standard error of the five determinations.

III. Results

Physiological parameters

The lead levels applied and its accumulation in plants produced negative effects and produced significant ($p \geq 0.05$) effect on RWC response of the plants which decreased to 51% by increasing the level of Pb in the soil to 250 mg.kg-1 soil compared to 85% at Pb 0. Photosynthetic pigments were compared in sunflower plants treated with lead polluted soil. The levels of photosynthetic pigments i.e. chlorophyll a and b and carotenoids not affected significantly as the Pb concentrations increased, compared with control. (Table 1).

Effect of lead concentrations on some physiological traits of sunflower plot:

Leaves

Treatment	Dose Pb mg.Kg^{-1}						Mean	LSD 0.05
	0	50	100	150	200	250	-	-
% RWC	85	79	76	62	63	51	67	29.52
Chlorophyll – a Fresh weight (mg.g^{-1})	5.61	5.51	4.85	5.01	4.61	4.27	4.91	N.S
Chlorophyll – b Fresh weight (mg.g^{-1})	3.17	3.13	3.39	2.79	2.81	3.01	3.05	N.S
Carotenoids Fresh weight (mg.g^{-1})	2.57	2.91	2.31	2.32	1.98	2.13	2.34	N.S

Growth Traits

At 100 mg .kg-1 soil of Pb, there was a significant decrease in plant height to76 cm, which started to drop more sharply to 75.67 and 75, respectively at Pb concentrations of 200 and 250mg.kg-1soil compared with control which gave maximum height of 80.67 cm(Table 2). Generally, the data depicted in table 2 clearly shows that there were no significant differences between the plants for root dry weight, shoot dry weight and whole plant dry weight when they were treated with lead at concentrations of 0, 50,100,150, 200 or 250 mg.kg-1 soil after 30 days from sowing.

Effect on Plant height and Dry weight of organs and Whole plant

organs	Dose Pb mg.Kg^{-1}						Mean	LSD (0.05)
	0	50	100	150	200	250	-	-
	Plant height (cm)							
	60.61	78.57	76.05	77.05	75.63	75.21	77.15	4.55
	Dry Weight							
Roots	8.24	9.04	7.15	5.52	7.08	6.12	7.20	N.S
Shoot	73.15	73.69	71.13	71.97	68.70	61.01	69.82	N.S
Whole Plant	82.61	82.51	77.33	77.49	68.78	67.13	77.82	N.S

Lead Accumulation in Plant Tissues: The main characteristics of the primary soil and heavy metals (before treating) are shown in table 1. The soil in the pots had a silty loamy texture, with an average EC of approximately 3.80 ds.m-1, and they were slightly alkaline (pH=7.10), which means the pH conditions were suitable for plant growth (Table 1). After the treatment with the heavy metals and the removal of the plants, the total concentrations of Pb in leaves, stem, root and whole plants were measured separately. The results in figures 1 – 4 shows that the total concentrations for Pb heavy metal in plant parts were significantly different between the treated and control soils at some of the tested levels in Aqmar variety.

Pb Conc. (mg.Kg^{-1})	**Pb Concentraion in Soil (mg.Kg^{-1})**			**Bioconcentration factor of harvested tissues**		
	Background Conc	Concentration Added to soil	Total Concentration	BCF (Root)	BCF (Stem)	BCF (Leaf)
50	3.791	50	53.791	0.691	0.192	0.107
100	3.791	100	103.791	0.684	0.143	0.062
150	3.791	150	153.791	0.491	0.116	0.051
200	3.791	200	203.791	0.564	0.091	0.047
250	3.791	250	253.791	0.721	0.089	0.044
LSD				0.09	0.04	0.03

Pb Conc. (mg.Kg^{-1})	**Pb Concentraion in Soil (mg.Kg^{-1})**			**Translocation Factor**	
	Background Conc	Concentration Added to soil	Total Concentration	TF (Stem)	TF (Leaves)
50	3.791	50	53.791	0.249	0.133
100	3.791	100	103.791	0.221	0.091
150	3.791	150	153.791	0.234	0.101
200	3.791	200	203.791	0.161	0.077
250	3.791	250	253.791	0.112	0.061
LSD (0.05)				0.09	0.09

Lead Concentration of tested soil before and after treatment with lead

Test soil	**Dose Pb mg.Kg^{-1}**						**Mean**	**LSD (0.05)**
	0	50	100	150	200	250	-	-
Before treatment	3.790	3.790	3.790	3.790	3.790	3.790	3.85	-
After treatment	2.80	25.01	49.14	157.23	191.25	215.3	107.91	57.84

Translocation Index of sunflower plant in response to lead Concentrations

Pb Conc. (mg.Kg^{-1})	**Translocation Index (TI)**			
	TI (Roots)	TI (Stem)	TI (Leaves)	TI (Whole plant)
50	0.13	0.251	0.134	1.02
100	0.85	0.218	0.092	0.93
150	0.67	0.233	0.101	0.95
200	0.81	0.164	0.075	0.91
250	0.75	0.115	0.061	0.82
Mean	0.882	0.193	0.092	0.935
LSD (0.05)	0.14	0.09	0.09	0.14

Discussion:

The range of Pb available in the surface soil around the world is 9.0 - 84 mg kg−1 (Kabata-Pendias, 2011). Drinking water is one of the most common routes of intake of Pb in humans. Pb guidelines for air and drinking water are 0.5 µgm−3 and 10 µg l−1 (WHO, 2001, 2008). Excessive Pb concentration also causes a significant hazard to plants, animals and human beings (Shevyakova et al., 2008). It is getting attention because of the public interest for a green technology is preferred over conventional approaches. In addition, physical and chemical methods are costly and often alter the soil properties which are not suitable for agricultural purpose.

Phytoremediation ability of Cyamopsis tetragonoloba, Sesamum indicum, Tetraena qataranse, Calotropis procera, Salsola imbricata, Phragmites australis and Typha augustifolia have been investigated by several other researchers (Parveen et al., 2023) .Due to this ability of hyper-accumulating metals, sunflower is one of the potential candidates for phytoremediation (Al-Jobori et al. 2019).The fact that such plants acquire metals more frequently in the rhizomes as well as roots compared to the shoots or leaves is supported by TF values below one, which imply poor metal transport (Yoon et al., 2006).Sunflower plant biomass, which is metal accumulator and characterized as a phytostabilizer, can be investigated in terms of bioenergy potential after this process. From the current study, overall carotenoids content of 10th, 20th, and 30th day aged pigeon pea offsets had decreased when each the crops underwent exposure to higher $PbCl_2$ concentrations (25 µM/L, 50 µM/L, and 75 µM/L). Similar findings to those in the current study were made by (Saadaoui et al.,2022). Within developing as well as advanced nations, there are different ways to accumulate heavy metals found in food crops, such as atmospheric release, manure from livestock, the cultivation of crops through waste water and/or water that has been contaminated with metallopesticides or herbicides in particular phosphate-based fertilisers, as well as sewage sludge based supplements (Prabhat et al., 2019; Fasih et al., 2021).

There is also a need for studies that will increase the phytoextraction performance of sunflower by supplementing chelators such as ethylenediamine disuccinic acid (EDDS) and fertilization to increase plant biomass in order to increase the phytoextraction potential of Cu, Zn and Pb from the soil in mine sites. It has also been found that the sunflower (Helianthus annuus) is an excellent candidate for phytoremediation. The sunflower plant remediates heavy metal, and the harvested sunflower for biofuel production is a sustainable approach (T. Sultana et.al., 2019).Furthermore, metal stress conditions reduced chlorophyll and protein contents (P. Chauhan & J. Mathur., 2018;2020)Hence, the length of the sunflower showed stunted growth and a reduced leaf expansion compared to stem and root.(Al-Jobori and Kadhim et al.,2019). Observed that the highest Pb contents were obtained as 40.1 and 107 mg kg^{-1} DW in shoot and root respectively, by H. annuus plant grown on soil amended with 200 mg kg^{-1} of Pb (Alaboudi et al.,2018). A higher amount of accumulated Pb gets restrict within the roots whereas only a small fraction of Pb transport to aerial parts of the plants (Zhou et al., 2016; Kiran et al., 2017). In few studies reported that highest accumulation in the shoot rather than root of various sunflower germplasm (Zehra et al.,2019;2020).The activity of different enzymes inactivates when Pb binds to their SH-groups sites and increases the process of reactive oxygen species (ROS) production which leads to oxidative stress in the plants (Fahr et al., 2013;2018; Nas and Ali, 2018).

According to this study, sunflower (H.annus) is suitable for phytoextraction, phytostabilization, and identification as metal hyperaccumulators due to its rapid development, biomass, bioaccumulation coefficient, bioconcentration factor, and lead (Pb) translocation factor, as well as its unappealing nature to herbivores, which may prevent metal entry into the food web. More study is needed to understand the processes behind metal absorption and tolerance by plants.

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