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COMPARITIVE LEAD UPTAKE AND RESPONSES OF FIVE DIFFERENT PLANT SPECIES GROWN ON LEAD CONTAMINATED SOIL

D. REENA NAVAROJA AND M. KANCHANA*
Department of Botany, P. S. G. R. Krishnammal College for Women, Coimbatore - 641 004, T.N.
E.mail: kanchana74@gmail.com

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***Corresponding author**

ABSTRACT
The present study was focused on identifying the fast growing plants with lead absorbing capacity. The seeds of five different plants species viz., French bean, Cosmos, Marigold, Gomphrena and Soybean were treated with different concentrations of lead nitrate. The concentrations of lead accumulated in the roots by species were: Soybean (6.905ppm), French bean (10.36ppm), Cosmos (6.98ppm), Marigold (3.73ppm) and Gomphrena (4.37ppm). Among the five species evaluated, French bean is the most suitable species for phytoextraction of lead from soil if the root biomass is harvested. On the other hand, Marigold absorbed the smallest amount of lead in its roots.

INTRODUCTION

Phytoremediation has emerged recently as a promising, cost effective, environmental friendly, aesthetically pleasing approach to the conventional engineering based remediation methods for extracting heavy metals from moderately polluted agricultural soils. The practicability of using vegetation as a viable, cost-effective alternative to clean up metal-contaminated soils depends largely on the identification and selection of plant species that possess the ability to accumulate metals, while producing high biomass using current crop production and management practices. Plants serve as primary source of carbon for the remainder of life forms on earth and, as such, can act as vectors for contaminant introduction into the food chain. Considering their relative position in many natural food chains, metal accumulating plants are directly or indirectly responsible for a large proportion of the dietary uptake of toxic heavy metals by humans and animals. Although some heavy metals are required for life's physiological processes (e.g., components of metalloenzymes), their excessive accumulation in living organisms is always disadvantageous. Generally, toxic metals cause enzyme inactivation, damage cells by acting as antimetabolites or form precipitates or chelates with essential metabolites. Therefore, this experiment was conducted to compare five plant species in terms of their ability to tolerate toxic levels of lead (Pb) and accumulate lead in shoots and roots.

MATERIALS AND METHODS

The soil was collected from garden and mixed with farm yard manure, potash, sand, vermicompost. Five different plant species were collected.

Common name	Binomial name	Family
Soybean	*Glycine max*	Fabaceae
French bean	*Phaseolus vulgaris*	Fabaceae
Cosmos	*Cosmos caudatus*	Asteraceae
Marigold	*Calendula officinalis*	Calenduleae
Gomphrena	*Gomphrena agrestis*	Amaranthaceae

Seed collection: The seeds of five different plants species viz., french bean, cosmos marigold, gomphrena and soybean were purchased from local market. The seeds were kept for germination prior to sowing.

Seed sowing: The potted plants were kept for lead treatment after the shoots raised around 5cm. Regular watering was done for all the treated and untreated plants for a week.

Lead treatment: The lead solution was prepared in different concentrations such as, 2.5μg, 5μg, 7.5μg and 10μg respectively. 10 mL of each solution were added to the 5 different plant species at normal temperature. An untreated control plant was maintained for each plant species. The same procedure was continued for another 15 days. The data has been colleted in each plant by regular observation.

Collection of roots: After 15 days, the lead treatment was stopped and plants shoots height were measured. The roots of each sample were colleted and the length of roots were measured and tabulated. The collected samples were kept ready for atomic absorption spectrophotometry.

Analysis of atomic absorption spectrophotometry: The sample roots were digested and lead uptake was measured through AAS.

Heavy metal analyses: Lead contents of each tissue were extracted using previously described nitric acid-hydrogen peroxide procedures (Pratt, 1965) with slight modifications. Briefly, 40mL of 50% aqueous nitric acid were added to a representative 1- to 2-g sample of ground plant tissue. The acidified sample was heated to 95C, refluxed for 15 minutes without boiling and then allowed to cool. Another 10mL of 50% aqueous nitric acid were added and the sample was again heated and refluxed for 30 minutes. The heated sample was allowed to cool, and then completely oxidized in 5mL concentrated nitric acid. The oxidized solution was allowed to evaporate to approximately 5mL without boiling. To initiate the peroxide reaction, 2mL of deionized, distilled water and 3mL of 30% hydrogen peroxide were added to the concentrated digestate and then were heated until effervescence subsided. Another 7mL of 30% hydrogen peroxide were added continuously in 1mL aliquots as the digestate was again heated. The digestate was heated until effervescence was minimal and its volume reduced to approximately 5mL. After cooling, the final digestate was diluted to about 100mL with deionized, distilled water. The digestate was filtered through a filter paper (Whatman No. 1) and the final volume was adjusted to 100mL with deionized, distilled water.

Lead contents of each sample were quantified using inductively coupled atomic absorption spectroscopy and expressed as μg lead/gram dry weight of plant tissue

RESULTS

The five species tested exhibited differential sensitivity to lead. Compared to the untreated controls, lead-treated Soy bean, French bean and Gomphrena were the most sensitive showing 50% reduction in leaf area, respectively. Marigold appeared be tolerant to 2.5μg of lead. Moreover, lead-treated, especially Soybean and French bean, showed purplish or anthocyanin pigmentation of leaves, which developed 14 days after the initial lead treatment.

Shoot growth in Soybean and French bean was inhibited by 52% and 35%, respectively, in plants with lead added to soil. Marigold and Cosmos were least affected by lead, with a shoot biomass reduction of 0 % (Table 1).

Roots of all lead-treated plants were purplish, in contrast to the dirty white roots of untreated plants (data not shown). Aside from the visual differences in color, root growth of all lead-treated plants was retarded, compared to the controls. Gomphrena showed the greatest sensitivity to lead, showing a 75% reduction in root biomass (Fig. 1). Soybean and Cosmos, on the other hand, exhibited the least sensitivity to lead (Table 2).

Table 1: Shoot length of control and treated plants

S.no	Plant samples	Shoot length (cm) Control	0.5μg	10μg
1	Soybean	13	9	3
2	French bean	19	6	4
3	Cosmos	6	6.5	6.5
4	Marigold	8	7	7
5	Gomphrena	4.5	2	3

Table 2: Root length of control and treated plants

S.no	Root samples	Root length (cm) Control	0.5μg	10μg
1	Soybean	4.5	3	11
2	French bean	3	3	2.5
3	Cosmos	3.5	5	6.5
4	Marigold	5.3	9.5	4
5	Gomphrena	3.4	2.5	dead

Table 3: AAS Analysis of lead concentration in each root sample

Samples (Plant)	Weight	Concentration of Pb (ppm)
Soy bean	0.25 ± 0.12[a]	6.905 ± 0.95[b]
French bean	0.025 ± 0.007[b]	10.36 ± 4.17[a]
Cosmos	0.015 ± 0.001[c]	6.98 ± 1.55[b]
Marigold	0.025 ± 0.001[b]	3.73 ± 1.43[d]
Gomphrena	0.01	4.37 ± 0.75[c]
CD (5%)	0.002	0.125

Values are mean ± SD of three samples in each group; A means followed by a different superscript letter are not significant at 5% level

Table 4: AAS analysis of untreated control plant and soil samples

S.No.	Samples	Sample weight (g)	Concn. of lead(ppm)
1.	Soil Control	0.5	1.857
2.	Plant Control	0.5	0.234

The concentrations of lead accumulated in the roots (Table 3) by species was: Soybean (6.905ppm), French bean (10.36ppm), Cosmos (6.98ppm), Marigold (3.73ppm) and Gomphrena (4.37ppm). Among the five species tested, French bean accumulated the greatest amount of lead in its roots. On the other hand, Marigold absorbed the smallest amount of lead in its roots.

The control soil sample was also analysed and it shows 1.857 ppm of lead concentration. The control plant of each plant species were analysed and shows an average of 0.234 ppm of lead concentration (Table 4).

DISCUSSION

The attainment of phytoextraction in an environmental cleanup effort depends to a large degree on the identification of suitable plants that not only concentrate metals to levels that would inhibit growth of most species, but demonstrate prolific growth in response to an well-known agronomic or horticultural practice. Such plentiful growth produces the necessary biomass to extract large amounts of metals per hectare that are commonly encountered in most contaminated sites.

Stunting is a recurrently observed growth response in a wide range of plants grown in metal-laden soils (Foy, Chaney and White, 1978). The stunting or reduced shoot biomass (Table 1) and decreased root biomass of lead-treated plants can be due to a specific toxicity of the metal to the plant, antagonism with other nutrients in the plant, or inhibition of root penetration in the soil. In this study, root penetration was not hindered since plants were grown in relatively small volumes of porous, artificial soil media. Although the nutrient solution and aqueous lead nitrate were applied separately, it is possible that the stunting and anthocyanin pigmentation in leaves of lead-treated plants can be ascribed to a deficiency of an element like phosphorous. Lead had been shown to form insoluble complexes with phosphorous (Johnson and Proctor, 1977; Johnson *et al.,* 1977). Similar anthocyanin pigmentation and inhibited growth have been recently noted in a corollary greenhouse study involving Indian mustard treated with 500 μg/mL lead (Daniels-Davis, 1996). While analyze the studies of Daniels Davis (1996) experiment, shoot and root biomass of lead-treated plants were reduced 6% and 44%, respectively compared to the untreated controls. The differential growth responses of the various species to lead suggest that phytotoxic mechanism of lead involve different biochemical pathways in different plant species. The exact nature of these mechanisms was not investigated in this study, but is currently being pursued in our laboratory.

Results from this study demonstrated that although all five species accumulated lead, they exhibited differential ability to take up lead from solid media (*e.g.,* perlite/vermiculite) and to transport and concentrate lead in their shoots or roots (Table 1 and 2) Although French bean accumulated the greatest amount of lead in its roots, the shoots to be analyzed for its lead accumulation. The metal-scavenging ability of the three species such as, French bean and Cosmos and Soybean is not surprising since some wild species have been found to grow on metalliferous soils and accumulate large amounts of heavy metals in their roots and shoots (Baker and Brooks, 1989). It must be noted however, that although French bean showed the ability to accumulate lead, its sensitivity to high levels (10μg) of lead as evidenced by its reduced biomass, may limit its potential in any phytoextraction effort.

Tight binding of lead to soils and plant material at least partially explains the relatively low mobility of this metal in soils and plants. Lead binding to clay and organic matter and its inclusion in insoluble precipitates make a significant fraction of lead unavailable for root uptake by field-grown plants. While plants are known to concentrate lead in the roots, lead translocation to the shoots is normally very low (Jones *et al.,* 1973; Jones *et al.,* 1973a; Malone *et al.,* 1974). Actively growing roots provide a barrier which restricts the movement of lead to the above-ground parts of plants (Jones *et al.,* 1973; Jones *et al.,* 1973a). Further study is to be extended for analyzing the accumulation metal in the shoot will give more information.

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