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Phytoextraction of lead through *Helianthus annuus* and its effects on the growth of *Cajanus cajan*

Vijay Kumar Sinhal[1], Vipin Yadav[1] and Adarsh pandey[2]

1 Department of Botany, Maharaja Agrasen Mahavidyalaya Bareilly, UP

2 Department of Botany, S.S.(P.G.)College,Shajahanpur, UP

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CORRESPONDENCE

Vijay Kumar Sinhal, Department of Botany, Maharaja Agrasen Mahavidyalaya Bareilly, UP

E-mail:sinhal@rediffmail.com

EDITOR
Adarsh Pandey

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ABSTRACT

To evaluate the effects of Pb (Lead Nitrate) on the growth of *Cajanus cajan* and it`s remediation, the two varieties of *Cajanus cajan* (Var. UPAS-120 & Var. ICPL-151) were sown in two different set of fields. Both the set of field were irrigated with different doses of Pb (19, 20, & 21 mg lit^{-1}). In the second set of experiment the same varieties of *Cajanus cajan* were intercropped with *Helianthus annuus* to remediate the Pb from soil. A concentration dependent decrease was noticed in all the growth parameters (i.e. plant height, fresh & dry Weight of plants, chlorophyll content, and protein content) and biochemical parameters (enzymes like Nitrate reductase, Glutamine oxo glutarate amino transferase and Glutamate dehydrogenase, activities) studied but the proline content was increased with increase in concentration of Pb. The maximum reduction (% of control) was noticed under the influence of higher dose of Pb (21 mg lit^{-1}) in both the varieties of *Cajanus cajan*. Variety UPAS-120 was found to be more susceptible to Pb toxicity than variety ICPL – 151.

In second set of experiment (where *Cajanus cajan* was intercropped with *Helianthus annuus)* a significant recovery of the toxic effects of Pb was noticed in all the parameters studied. Atomic absorption spectrophotometer study suggested that the accumulation of Pb through *Helianthus annuus* grown in Pb treated soil was higher than non-treated soil. The results of the present investigation reveals that *Helianthus annuus* can be used as phytoremediator to reclaim the soil contaminated with Pb.

Introduction

The pollution of the environment with toxic metals has become a world wide problem, affecting crop yields, soil biomass and fertility, contributing to bioaccumulation in the food chain. Consequently, the use of plants contaminated with high levels of heavy metals for food, might pose a serious risk to human and animal health (Wang *et al.* 2003). Pb is hazardous at higher concentration and inhibits growth of plants (Bergmann 1992, Ali *et al.* 1999, Soares *et al.* 2001, Bekiaroglou & Karatagli 2002, Khudsar *et al.* 2004). The elevated level of heavy metals reduces the activities of enzymes like catalase, superoxide dismutage, guaiacol peroxidase, protease, nitrogenase, nitrate reductase etc. (Antipchuk *et al.* 2000, Panda and Khan 2003, Chhetri *et al.* 2004, Singh *et al.* 2005, Sinhal V.K. 2005, Sinhal *et al.* 2006). Photosynthesis and transpiration are also reduced by high concentrations of Pb (Van Assche *et al.* 1979) The observed reduction in growth is also a consequence of Pb interference with nutrient uptake (Chanay 1993, Kaya *et al.* 2000) and certain enzyme activities (Quariti *et al.* 1997).

Phytoremediation is a group of technologies that use plants to reduce, remove, degrade or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition usable for private or public applications. Phytoremediation can be ciassified into six different remediation methods: Phytoextraction, Phytostabilization, Phytodegradation, Phytovolatilization, Rhizofilterations, & Enhanced Rhizosphere Biodegradation. Among these methods, phytoextraction is a remediation method that uses plants to remove heavy metals from soil (Suresh & Ravishankar 2004, Solhi *et al.* 2005).

Thus present investigation has been under taken to assess the phytotoxicity of Pb on the growth of C*ajanus cajan* and *Helianthus annuus* has been chosen as a phytoremediator plant to remediate Pb from treated soil.

Materials and Methods

The seeds of the two varieties i.e. var. UPAS-120 & var. ICPL – 151 of *Cajanus cajan* and *Helianthus annuus* (var. Akash-333) were obtained from seed centre, Haldi, Pantnagar, India. The field was divided into two parts; one was for the evaluation of the effects of Pb on the growth of *Cajanus cajan* and second for phytoremediation experiment. The first part of the field was consists of 8 small plots, each having an area of 9 m^2 (3mx3m), four plots for each variety. Plots were arranged as follows : Control (irrigated with tap water), treatments A, B & C (irrigated with 19,20,& 21 mg lit^{-1} concentration of Pb separately).The second part of the field was consist of 6 small plots, each having an area of $9m^2$, 3 plots for each variety of *Cajanus cajan* which were intercropped with *Helianthus annuus* i.e., these plots were arranged as D, E & F (having *Cajanus cajan + Helianthus annuus* treated with 19,20 & 21 mg lit^{-1} concentration of Pb respectively). Prior to sowing, seeds were presoaked in distilled water for 8 hours to help in their easy from germination. The seeds were sown in June when the mean monthly temperature ranged 26^{o}C (minimum) to 41^{o}C (maximum) and were irrigated with different doses of Pb at 10, 15, 30 & 45 days of sowing.

To determine the effects of different doses of Pb (19, 20 & 21 mg lit^{-1}) on plant height, five plants were randomly selected from each treatment. The plants height was observed in 25 & 45 days old plants and was measured in centimeters. The fresh weight was measured in grams. For dry weight, plants were dried in oven at 60°C for 48 hours and measured in gms. Chlorophyll content was measured by the method of Arnon (1949), Protein content by the method of Lowery *et al.* (1951), NR activity by the method of Srivastava (1974), GOGAT activity by the method of Meers *et al.* (1970), GDH activity by the method of Wolf & Wiliams (1973) and Proline content was determined by the method of Bates *et al.* (1973). The amount of Pb accumulated (mg gm^{-1} dry wt.) by *Helianthus annuus* was determined by using Atomic Absorption Spectrophotometer (GBC Avanta S AAS, Australia) after samples were digested with concentrated HNO_3 + $HClO_4$.

One way ANOVA was carried out to compare the means of different treatments at 5% level of significance.

Results

The effects of different concentrations of Pb (19, 20 & 21 mg lit^{-1}) were noticed at 25 & 45 days of sowing on all the parameters studied. All the doses of Pb were found to have inhibitory effects on plant height (in cm.) but the maximum reduction was noticed in variety UPAS – 120 (32.30%) at highest does of Pb (21 mg lit^{-1}) in 45 days old plants. The maximum retardation i.e. 40.84% in fresh weight (in gms) and 43.15% in dry weight (in gms) was noticed in 45 days old plants in variety UPAS-120 with the highest does (21 mg lit^{-1}) of Pb. A concentration dependent decrease was also noticed in case of chlorophyll content (mg g^{-1} fresh weight), and the maximum reduction was noticed (43.28%) with higher does of Pb (21 mg lit^{-1}) in variety UPAS-120. All the concentrations of Pb significantly decreased the Nitrate reductase (in $NO_2^-hr^{-1}g^{-1}$ fresh weight) and Glutamine oxoglutarate amino transferase (μM NADPH oxi $min^{-1}g^{-1}$ fresh weight) activity, though, the highest reduction of 39.14% and 51.27% respectively were observed with the highest dose of Pb (21 mg lit^{-1}) in variety UPAS-120. Similarly Glutamate dehydrogenase activity (μM NADPH oxi $min^{-1}g^{-1}$ fresh weight) was also reduced to 42.64% under the influence of highest dose of Pb (21 mg lit^{-1}). All the concentrations of Pb also showed inhibitory effect on protein content (in mg g^{-1} dry weight) but the maximum reduction i.e., 47.30% was noticed with the higher dose of Pb (21 mg lit^{-1}) in variety UPAS-120 in 45 days old plants. (Fig.-1&2).

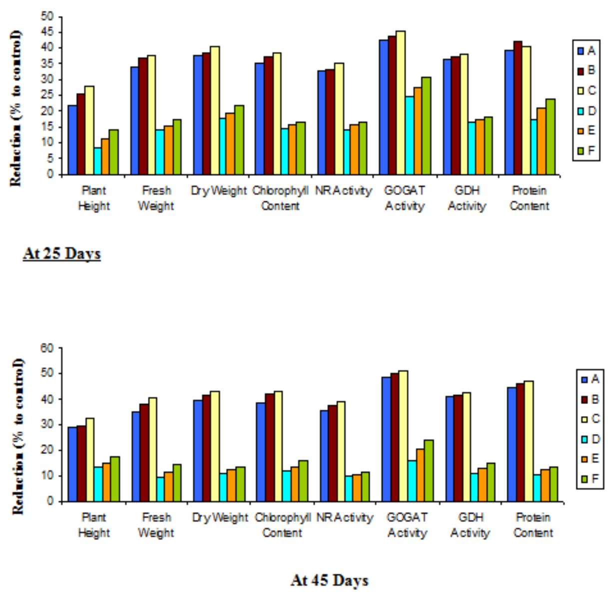
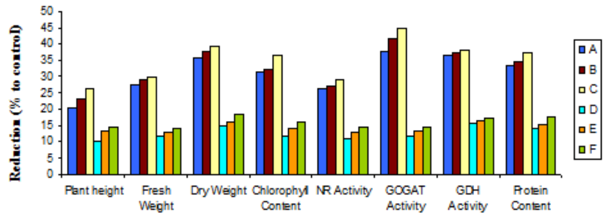


Fig.1: Effects of Pb on the growth of *Cajanus cajan* (UPAS-120) and its phytoremediation through *Helianthus annuus*

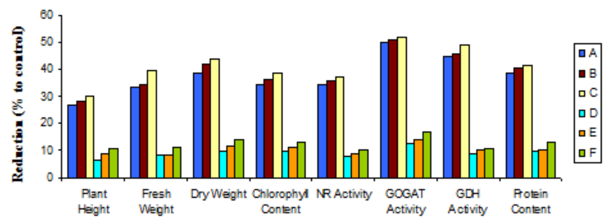
A = *Cajanus cajan* treated with 19 mg.lit^{-1} Pb Nitrate
B = *Cajanus cajan* treated with 20 mg.lit^{-1} Pb Nitrate
C = *Cajanus cajan* treated with 21 mg.lit^{-1} Pb Nitrate e
D = *Cajanus cajan* + *Helianthus annuus* treated with 19 mg.lit^{-1} Pb Nitrate
E = *Cajanus cajan* + *Helianthus annuus* treated with 20 mg.lit^{-1} Pb Nitrate
F = *Cajanus cajan* + *Helianthus annuus* treated with 21 mg.lit^{-1} Pb Nitrate

Results with reverse trend were noticed in case of proline content (μ moles g^{-1} fresh weight) i.e., a concentration dependent increase was noticed in both the varieties in 25 and 45 days old plants. The maximum proline content (.189 μ moles g^{-1} fr. wt.) was noticed with higher does of Pb (21 mg lit^{-1}) in variety UPAS-120 in 45 days old plants. (Fig.-6)

The recovery of phytotoxic effects of different doses of Pb (19, 20 & 21 mg lit^{-1}) on *Cajanus cajan* was achieved through phytoremediation with *Helianthus annuus*. In case of variety UPAS-120, the maximum recovery (% to reduction) through *Helianthus annuus* was noticed in plant height (53.23%), fresh weight of plants (73.39%), dry weight of plants (71.98%), chlorophyll content (68.88%), NR activity (73.08%), GOGAT activity (67.56%), GDH activity (72.42%) and protein content (77.15%) while in case of variety ICPL-151, the maximum recovery (% to reduction) was noticed in plant height (75.46%), fresh weight of plants (74.13%), dry weight of plants (75.41%), chlorophyll content (72.08%), NR activity (77.36%), GOGAT activity (75.10%), GDH activity (80.11%) and protein content (79.80%). (Fig. –3 & 4)



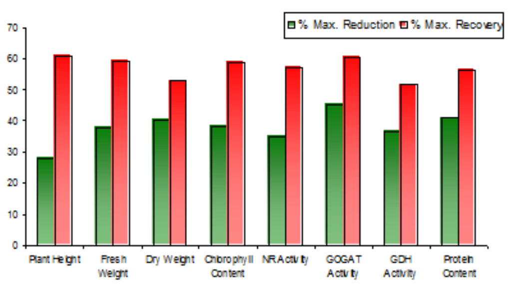
At 25 Days



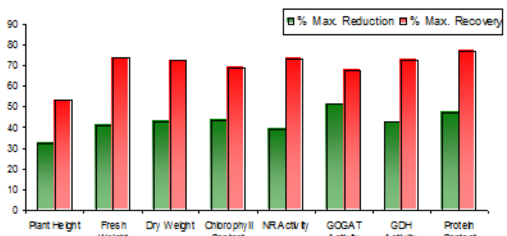
At 45 Days

Fig.2: Effects of Pb on the growth of *Cajanus cajan* (ICPL-151) and its phytoremediation through *Helianthus annuus*

A = *Cajanus cajan* treated with 19 mg.lit^{-1} Pb Nitrate
B = *Cajanus cajan* treated with 20 mg.lit^{-1} Pb Nitrate
C = *Cajanus cajan* treated with 21 mg.lit^{-1} Pb Nitrate
D = *Cajanus cajan* + *Helianthus annuus* treated with 19 mg.lit^{-1} Pb Nitrate
E = *Cajanus cajan* + *Helianthus annuus* treated with 20 mg.lit^{-1} Pb Nitrate
F = *Cajanus cajan* + *Helianthus annuus* treated with 21 mg.lit^{-1} Pb Nitrate



At 25 Days



At 45 Days

Fig.3: Effects of Pb on the growth of *Cajanus cajan* (UPAS-120) showing maximum reduction (% to control) and maximum recovery (% to reduction)

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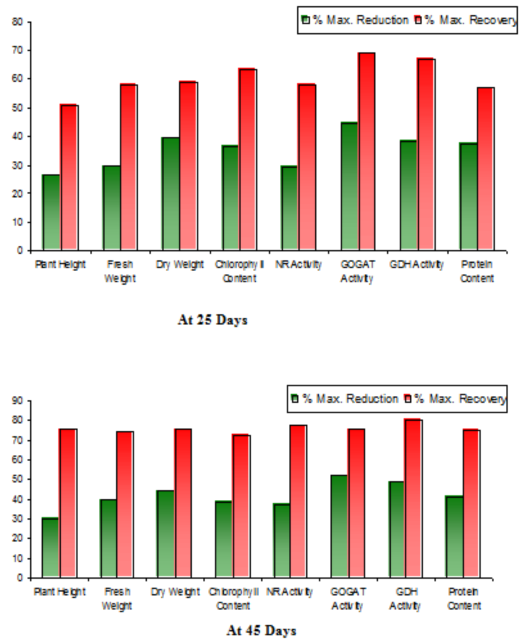


Fig.4: Effects of Pb on the growth of *Cajanus cajan* (ICPL-151) showing maximum reduction (% to control) and maximum recovery (% to reduction)

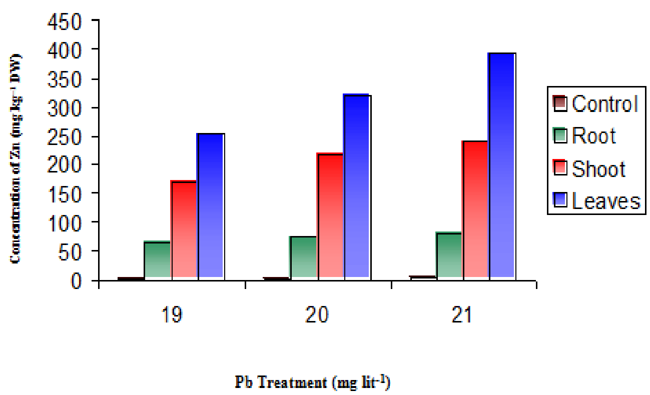
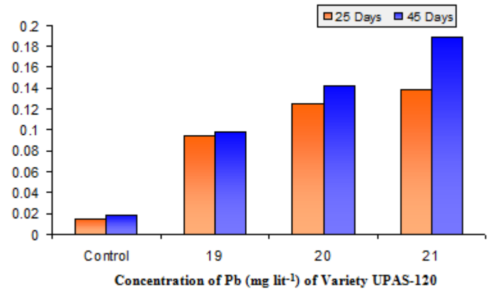


Fig.5: Comparison of amounts of Pb accumulated by different parts of *Helianthus annuus* (after 75 days of intercropping with *Cajanus cajan*)

Significant differences were obtained between concentrations of Pb in different parts of *Helianthus annuus* grown in contaminated soil as compared to its concentration in non-contaminated soil. The highest concentration of Pb was noticed in leaves (393.12 mg kg^{-1} DW) as compared to stem (240.23 mg kg^{-1} DW) and roots (80.15 mg kg^{-1} DW) under the influence of highest dose of Pb (21 mg lit^{-1}). (Fig.-5)

Discussion

In the present investigation Pb was found to be phytotoxic in nature at all the concentrations (19, 20, & 21 mg lit^{-1}) and significantly reduced the plant height of *Cajanus cajan*. Although Pb is essential for plant growth, its excess is inhibitive as was reported for a number of leguminous plants (Khudsar 1999), but at higher concentration it's interference with certain essential metabolic events of plant suggested that the inhibition of plant height under the influence of heavy metals is probably due to results of interference of these heavy metals with the respiratory activity and mobilization of reserves (Tripathi and Mohanty 1980, Alia *et al.* 1995, Jain *et al.* 2000, Khudsar 2004).



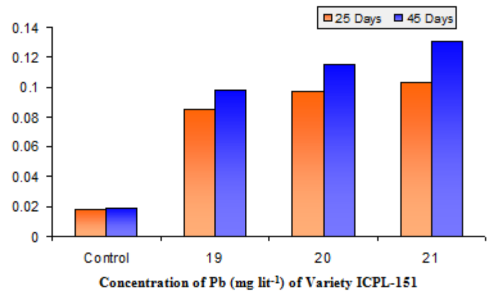


Fig. 6: Effects of Pb on proline content (μ moles g^{-1} fresh weight of leaves) of leaves in *Cajanus cajan*

Fresh and dry weight of plant was reduced significantly at every concentration of Pb (19, 20 & 21 mg lit^{-1}) whereas the maximum reduction with the higher dose of Pb (21 mg lit^{-1}) was noticed in variety UPAS – 120 as compared to variety ICPL-151. Decrease in fresh weight of plants was might be due heavy loss of moisture. The decrease in dry weight might be due to reduction in photosynthesis and chlorophyll 'a' synthesis as suggested by Okhi (1978), Joshi *et al* (1999), Sinhal (2005), Sinhal *et al.* (2006).

A concentration dependent decrease was also noticed in chlorophyll content. Prasad & Prasad (1987) suggested that heavy metal stress inhibits chlorophyll biosynthesis by interacting with sulphydryl (-SH) group of the two enzymes viz., δ-Amino laevulinic acid (ALA) dehydratase and Protochlorophyllide reductase, involved in chlorophyll biosynthesis.

The reduction of nitrate to nitrite by the enzyme nitrate reductase (N.R.E.C. 1.6.6.1) is a rate limiting step in overall nitrate assimilation and also controls the rate of protein synthesis in plants (Srivastava, 1980). The NR activity also reduced significantly under the influence of Pb treatments. The reduction in NR activity with Pb treatments may be due to it's strong affinity for –N and –S ligands (Woolhouse, 1983) and therefore they can bind to the active component of the enzyme such as histidine and sulphydryl groups (-SH) and can eventually inhibit NR activity (Van Assche & Clijsters 1990). The cause of inhibition of NR activity by heavy metals may be multifacial e.g. due to reduced supply of NADPH, disorganization of chloroplasts, less NO_3^- supply to the site of synthesis (Singh *et al.*,1998). The Pb concentrations were also found to have inhibitory effects on NR activity by Khudsar *et al.* (2004).

Concentration dependent decrease was also found in GOGAT activity. GOGAT is the main enzyme of GS-GOGAT pathway of ammonium assimilation in which NH_4^+ and glutamate form glutamine which converts into glutamic acid in the presence of enzyme GOGAT. The reduction in the assimilation of ammonium ions by heavy metals in cucumber plant (Burzynski and Buczek, 1998) may be due to intereference of metal ions with the active site of the ammonium assimilating enzymes.

Ammonia & 2–oxyoglutaric acid forms glutamic acid in the presence of enzyme GDH. The treatments of Pb significantly reduced GDH enzyme in both the varieties of *Cajanus cajan.* According to Miller *et al.* (1973) GDH enzyme contain free-SH (Sulphydryl) group at the active centre and it is well known fact that heavy metals show strong affinity for –SH group, therefore, Pb treatments (19, 20 & 21 mg lit^{-1}) adversely affected GDH activity. According to Tomar *et al.* (2000), the protein content under heavy metal stress may be affected due to (i) Enhanced protein hydrolysis resulting in decreased concentration of soluble proteins (Melinchuk *et al.*, 1982). (ii) Catalytic activity of heavy metals (Bhattacharya and Choudhari, 1997) and (iii) Protein Synthesis becoming reduced under all stress conditions. According to Chhetri *et al.* (2004) when heavy metal toxicity crosses the threshold, the protein level decreases and this might be due to the breakdown of protein synthesis mechanism at toxic concentration level of heavy metals or due to reduced incorporation of free amino acids into protein. Concentration of protein was significantly reduced in *Artemisia annua* leaves by Pb treatments (Khudsar *et al.*, 2004).

Reverse trend of results was noticed in case of proline content viz., a concentration dependent increase was noticed in proline content. The higher proline content was observed with the higher dose of Pb (21 mg lit^{-1}) in both the varieties of *Cajanus cajan.* The proline content accumulated in number of plants when they are subjected to salt or metal stress (Muthukumarsamy 1997). Proline has been shown to play an important role in ameliorating environmental stress in plants, including heavy metals stress (Surasak *et al.* 2002, Sharma & Dietz 2006). However, there has been much disagreement regarding the mechanism by which proline reduces heavy metal stress. Free proline has been proposed to act as an osmoprotectant (Delauney and Verma 1993, Taylor 1996), a protein stabilizer (Shan & Dubey, 1998), a metal chelator (Fargo & Muller, 1979), an inhibitor of lipid peroxidation (Mehta and Gaur, 1999) a hydroxyl radical scavenger (Smirnoff and Cumber, 1989). It is evident that there is no clear consensus regarding the mechanism by which proline reduces heavy metal stress.

The phytoremediation experiments, in which *Helianthus annuus* was intercropped with *Cajanus cajan*, were found to have a significant recovery of the reduction induced by different Pb treatments. Atomic Absorption spectrophotometer studies of *Helianthus annuus* reveals that a remarkable amount of Pb was accumulated by different parts of the *Helianthus annuus* but the highest accumulation was noticed in roots as compared to shoot and leaves. The accumulation of Pb through *Helianthus annuus* was also noticed by Herrero *et al.* (2003). Lesage *et al.* (2005), Solhi *et al.* (2005), Susan *et al.* (2006). Thus *Helianthus annuus* act as a powerful phytoremediator and hyper accumulator for Pb and makes the soil less toxic. Gratao *et al.* (2005) focused their attention on the physiological mechanism by which the metal is taken up, transported and sequestrated but they could know little about the genetic basis of hyper accumulation when compared with the genetic basis of metal tolerance (Pollard *et al.*, 2002). Oven *et al.* (2002) pointed out that hyper accumulation and detoxification includes chelating to organic acids or proteins (Martens *et al* .1996).

The overall view of the present investigation is that higher dose of Pb (21 mg lit^{-1}) exert higher toxic effects on plant growth and metabolism of *Cajanus cajan* which is further confirmed by presence of highest level of proline under the influence of higher dose of Pb (21 mg lit^{-1}). Proline is an effective indicator of heavy metal stress. Further the recovery of the reduction in growth and biochemical parameters, induced by Pb, could be achieved by the process of phytoremediation (through *Helianthus annuus*). Thus *Helianthus annuus* can be used as phytoremediator plant to remediate the soil contaminated with Pb.

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