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Comparative Study of Lead Removal by *Mirabilis jalapa* and *Datura inoxia*

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ABSTRACT

A study was carried out to investigate the potential of Mirabilis jalapa and Datura inoxia for phytoremediation of lead contaminated soils. Experiments were carried out in order to investigate the effect of lead on growth, leaf pigments and metal accumulation ability of selected plant species. The experiments consisted of 5 treatments in which lead concentration varied form 0 - 100mg/kg-1 [0ppm(TC1), 25ppm(TC2), 50ppm(TC3) 75ppm(TC4), and 100ppm(TC5)]. Selected plant species were grown for a period of 45 days after seedling in pots containing 5 kg of soils. Growth performance, leaf pigments (chlorophyll a, chlorophyll b and carotenoids) and metal accumulation were estimated after 45 days in root and shoot of plant using AAS. Lead concentration in soil after phytoremediation by Datura inoxia and Mirabilis jalapa were 5.59, 9.83, 5.89 and 10.98; 4.50, 8.99, 11.50 and 19.6 in TC1, TC2, TC3 and TC4 soils respectively. Concentration of lead in soil in all treatments after phytoremediation by datura inoxia was decreased between 80-90% and in Mirabilis jalapa it was 72-78%. Results indicated that both plants species could be effective accumulators for phytoremediation of lead wheras, the potential of Datura inoxia was more than Mirabilis jalapa for phytoremediation of lead contaminated soils.

Keywords: *Datura inoxia*, translocation factor, AAS, leaf pigments.

INTRODUCTION

Metal contamination has harmful effect on biological systems and does not undergo biodegradation. Rapid industrialization led to geometrical rise in the level of air, water, space and noise. One of the major concerns in India is the increasing level of land pollution largely due to the uncontrolled disposal of industrial effluents [1]. Toxic metals cadmium, chromium, copper, nickel, lead and mercury etc. are non-degradable and differentiated from other pollutants, since they cannot be biodegraded but can be accumulate in living organisms, thus causing various diseases and disorders even in relatively lower concentrations [2]. Lead was found to be acute toxic to human beings when present in high amounts. Since Lead is not biodegradable, once soil has become contaminated, it remains a long-term source of lead exposure. Soil can be contaminated with Pb from several other sources such as industrial sites, from leaded fuels, old lead Plumbing pipes, or even old orchard sites in production where lead arsenate is used. Lead accumulates in the upper 8 inches of the soil and is highly immobile. Contamination is long-term. Without remedial action, high soil lead levels will never return to normal [3].

Majority of homes built before 1978 contain lead-based paints. Degradation of old lead based paint in older homes and unsafe remodeling, sanding, or blasting of these homes can result in the accumulation of lead in the soil [4]. Lead contamination in the environment exists as an insoluble form, and the toxic metals pose serious human health problems namely, brain damage and retardation [5-6]. Lead toxicity leads to decreases in the percentage of seed germination, as well as growth, dry biomass of roots and shoots, disruption of mineral nutrition, reduction in cell division and inhibition of photosynthesis [7 -8]. The awareness of toxicological and ecological effects of toxic metals has attracted serious attention for decontaminating industrial waste waters prior to discharge into lands. Therefore, removal of these toxic metals from wastewaters prior to discharge into water and soil bodies is necessary in terms of environment and economic consideration. Therefore these contaminated soils need to be cleaned up for having a safe environment. There are some existing conventional methods of metal removal from industrial effluents are: chemical, precipitation, oxidation and reduction, membrane filtration technology, electrochemical precipitation, evaporation recovery and ion exchange resins. However these strategies have various limitations [9].

Therefore there is an urgent need to develop ecofreindly and sustainable alternatives that are not costly and are well suitable for small as well as commercial scales. Attention was given to phytoremediation, a promising clean up technology, using plants to remediate contaminated soils [10]. Which has already used for years [11]. Phytoremediation is environmental friendly method which utilises the uptake ability of plants for the removal of heavy metals from the soil-water environment. Phytoremediation is basically an assemblage of four different techniques, namely, rhizofiltration, which employ plants to clean various aquatic environments; phytostabilization, where plants are used to stabilize contaminated soil; phytovolatilization,where plants extract specific metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants extract metals from soil and translocate them to the shoots where they accumulate[12].Among all these techniques phytoextracton is best suited for remediation of contaminated soils.

The ideal plant species to remediate a heavy metal-contaminated soil would be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest [13]. Many investigations were conducted about phytoremediation of lead contaminated soils (Lasat et al., 2002; Yanqun et al., 2005; Chandra Sekhar et al., 2005; Li et al., 2005) using arabidopsis thaliana, sonchus asper, hemidesmus indicus, sedum alfredii.

In order to remove the pollutants using phytoremeidation technology, it is essential to identify a suitable plant species with high biomass. In this study *Datura inoxia* and *Mirabilis jalapa* were selected to evaluate their potential of phytoremediation.The selected plant species were exposed to different doses of lead and growth rate, biomass and photosynthetic apparatus were determined. The results obtained in the present study could be useful for understanding the role of *Datura inoxia* and *Mirabilis jalapa* in lead tolerance and detoxification strategy.

MATERIALS AND METHODS

The present study was designed to investigate the comparison of lead removal from polluted soil by *Mirabilis jalapa* and *Datura inoxia*.

(A) **(B)**

Figure 1 Schematic diagram of *Datura inoxia*(A) and *Mirabilis jalapa*(B)

Collection and characterization of soil:
The soil samples were collected form uncontaminated sites and air dried, mixed and sieved (2 mm) prior to determine the physicochemical characteristics.

Preparation of lead nitrate solution
The aqueous stock solutions of pb (II) were prepared by dissolving 1.3 g of Lead Nitrate from Fisher scientific (Ls6200) was dissolved in deionised water to give 1000 ml of the solution. The mixture was stirred by the stirring bar until all the lead was completely dissolved. The stock solutions were used to contaminate the soil to attain the desired concentrations of these heavy metal, i.e, 0, 25, 50, 75 and 100 mg/kg-1.

Sample collection:
The seeds of *Mirabilis jalapa* and *Datura inoxia* were collected form uncontaminated sites and sterilized for 30 minutes, washed with tap water and then sowed directly in to prepared soils 5 seedlings each plant species with similar size selected for phytoextraction experiment.

Experimental setup:
The experiment conducted using soil treated spiked with lead nitrate. Approximately 5 kg of soil was used in each pot for the pot study experiment. The soil in each pot was homogenized with salt. No external fertilizer was used in any of the pot studies. About 30 seeds were sowed in all the pots, 5 seedlings were maintained in each pot.
The experiment was designed to grow the plant with natural light and temperature to keep them under similar conditions. The species were harvested after 45 days from 3 replicates pots without damaging the roots. Plants were rinsed in distilled water to remove dust and taken to laboratory washed with demonized distilled water separated in to root and shoot parts, dried in oven at650C for 72 hours [14]. Each plant was separated in toot and shoot i.e., above ground biomass and growth performance of each was measured. 3 plants for each concentration were harvested after 45 days. Chlorophyll a, chlorophyll b and carotenoids in all test species were measured about 0.5 g of the material was weighed accurately, 80% acetone was added and ground with the help of a mortar and pestle until the material turned to colourless and solution is filtered using whatman filter paper no. 44 and diluted to 50ml with 80% acetone to read the values in UV-Vs spectro photometer [15].

Metal accumulation:
Metal accumulation is the total amount of metal present in different plant parts. Total metal concentration in soil and plant samples was determined by Mixed Acid digestion procedure [16]. From the oven dried

grinded plant and soil 0.5gm was accurately weighed and transferred mixture of 1ml g $HCl0_4$, 5 ml of Con.H_2So_4 and 0.5ml of Con.HNO_3 and digested slowly. The digestion was continued for 10-15 minutes after the appearance of white fumes, cooled diluted with DDW and Filtered using whatman filter paper No.44 and made up to 50 ml with DDW. A blank was prepared in similar manner. The filtrate was analyzed for the total lead in sample solution was quantified using AAS [16].

Statistical Analysis:
Bioaccumulation factor (BCF) and translocation factor (TF) were used to evaluate plant phytoextraction efficiency. BCF is defined as the ratio of metal concentration in plant shoots to that in soil. TF is determined by the ratio of metal concentration in plant shoots to metal concentration in roots. The significance of the present work has been analyzed by statistical validation in terms of standard deviation (SD) using SPSS 13.0 statistical software. Data were analyzed by one-way ANOVAs with least significant difference (LSD) to determine any significant differences between treatments ($P<0.005$).

RESULTS AND DISCUSSION

Physiochemical parameters of soil
The soil used for the experiments was sandy with 70% of sand, 20%of slit and 7% of clay contents. According to **Table 1** some selected soil characteristics as follows: PH :8.0, and it was determined by using a digital PH meter(systonics) [17], EC:0.116ms and it was determined in 1;2 soil and sediment water slurry using a digital conductivity meter(Elico cm 180) [17], Organic matter: 1.1% and it was determined by Walkley Black method [17], Available nitrogen: 0.05(mg/100g) [18], available phosphorous 0.6(mg/100gm) and it was determined by molybdenum blue method [18] and potassium 0.06% and it was determined by flame photometric method [18] and total and available lead concentrations were 4.0 mg/kg and 1.0 mg/kg respectively.

Table 1 Physiochemical properties of soil

Physio-chemical characteristics	
Colour	Reddish Brown
Gravel (%)	2
Sand (%)	70
Silt (%)	20
Clay	7
texture	Sandy
PH(1:2:5)	8.2
EC(mS/cm)	0.116
Chemical characteristics	
Organic matter (%)	1.1
N(mg/100g)	0.05
P(mg/100g)	0.6
K (%)	0.06
Heavy metals	

Available Lead(mg/kg)	4.0
Total Lead(mg/kg)	1.0

Comparative study of Growth performance and Leaf pigments in *Mirabilis jalapa* and *Datura inoxia*:

Growth performance

In both the plants four important parameters were focused which included root length, root dry matter, shoot length and shoot dry matter.

According to **Table 2** & **Figure 2(A and C)** average shoot length and root length of both the plants increased in all test concentrations as compared to control except in TC4 soils. In datura the maximum shoot length and root length were observed as 16.6cm in TC3 soils and 18cm in TC3 soils respectively. In *Mirabilis jalapa* the maximum shoot length and root length were observed as 18.7in TC3 soils and 5.9 in TC3 soils respectively.

Table 2 Comparison of growth studies of *Mirabilis jalapa* and *Datura inoxia*

Cd concentration in soil (mgkg-1	Shoot length	root length	shoot dry matter	root dry matter
Mirabilis jalapa				
C	14	4.5	0.9	0.13
TC1	16	5	1.02	0.4
TC2	18.2	3.5	1.04	0.31
TC3	18.7	5.9	1.7	0.35
TC4	4.5	3	1.5	0.2
Datura inoxia				
C	14.2	12.2	0.56	0.14
TC1	13.5	15	1.03	0.41
TC2	16.2	14	1.25	0.38
TC3	20.6	18	0.84	0.27
TC4	14.5	15.3	0.82	0.25

Results are means±SD(n=5)

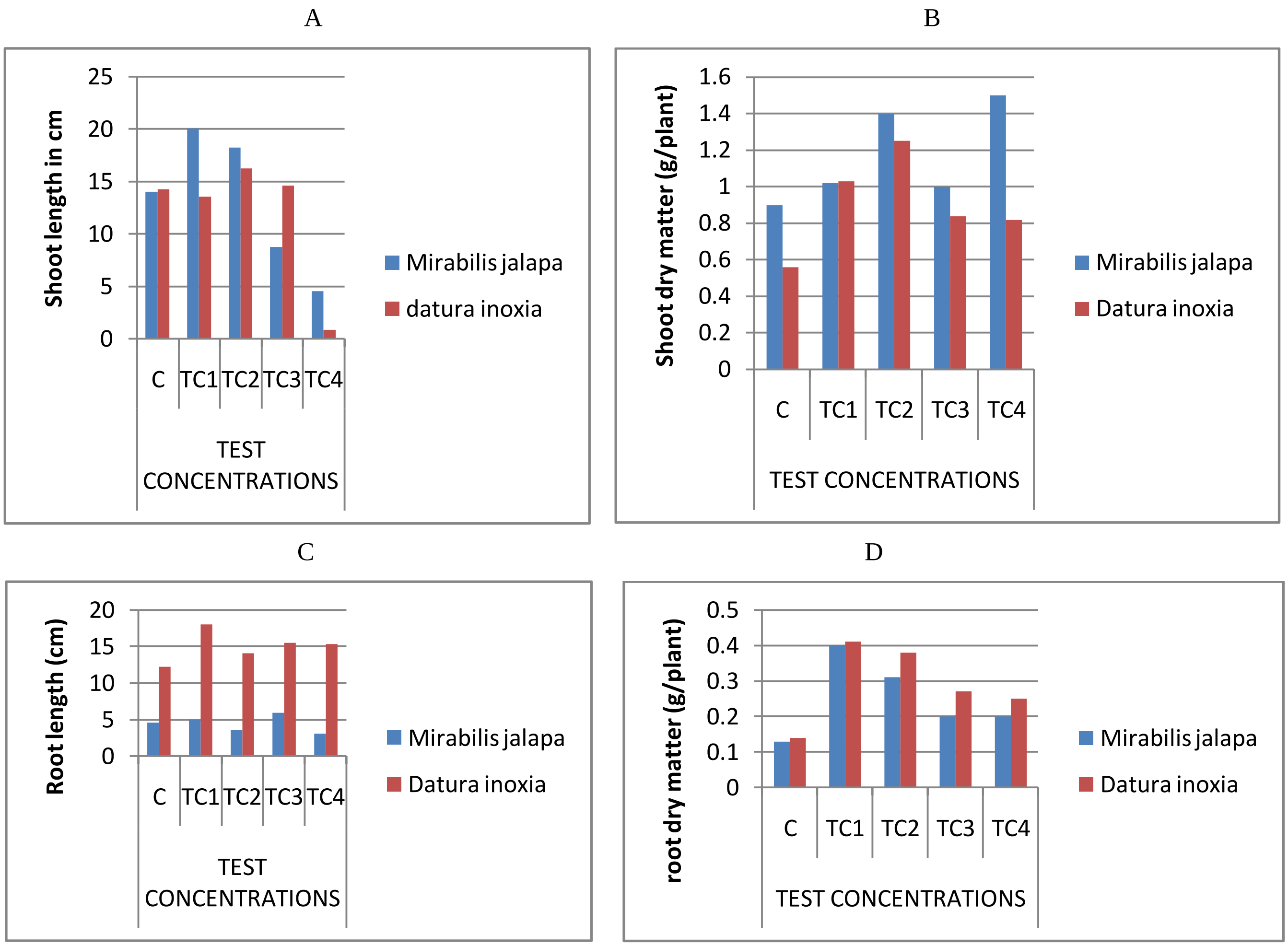


Figure 2. Shoot length (A), Shoot dry matter (B), root length (C) and root dry matter (D) of *Mirabilis jalapa* and *Datura inoxia* in different treatments levels of lead contaminated soils.

The results indicate that the increase metal content has shown no inhibitory effect on plant height. Maximum plant height observed by *datura inoxia* which has significantly greater than that of *Mirabilis jalapa*. Whereas according to the **Table2 & Figure 2(B&D)** root system showed reduction with increasing of concentration of metal in soils in mirabilis jalapa, with increasing levels of lead, the root dry matter decreased, due to concentration effect which inhibited the root growth.

Visible decrease in plant biomass with increase in concentration of lead metal in the soil was observed. It is in agreement with previous studies [19].

Leaf pigments:

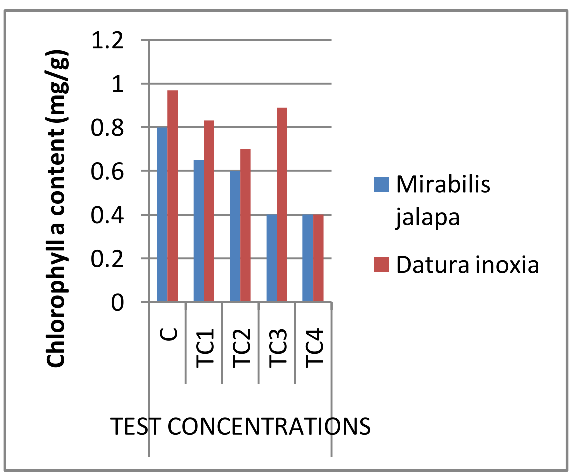
Chlorophyll a, chlorophyll b and carotenoids of *Datura inoxia* and *Mirabilis jalapa* for different treatments in pot study experiment shown in **Table 3& Figure 3 (A, B and C)**. Though root and shoot biomass was shown no inhibition on growth leaf pigments such as chlorophyll a, chlorophyll b and carotenoids **Fig. (3A, B &C)** were effected which was in agreement with previous studies [20]. In datura inoxia the maximum reduction observed in chlorophyll a, chlorophyll b and carotenoids were 0.4, 0.32 and 0.18 in TC4 soils respectively. In *Mirabilis jalapa* the maximum reduction was observed in chlorophyll a, chlorophyll b and carotenoids were 0.38, 0.28 and 0.17 respectively

Table 3 comparison of leaf pigments

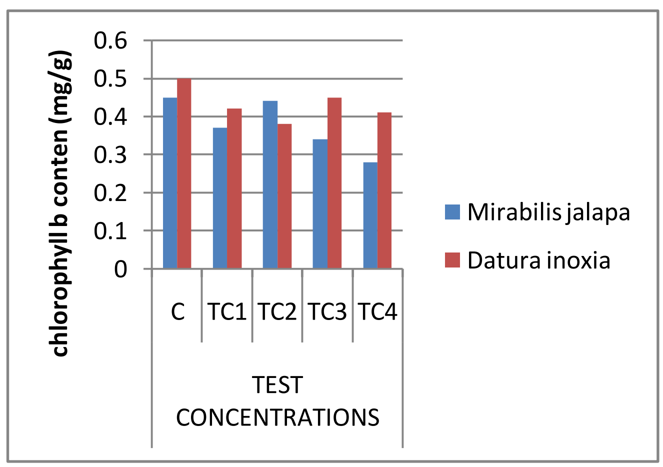
Pb concentration in soil (mg/kg-1	chlorophyll a	chlorophyll b	carotenoids
Datura inoxia			
C	0.97±0.004	0.5±0.008	0.45±0.003
TC1	0.83±0.003	0.42±0.007	0.3±0.004
TC2	0.7±0.001	0.38±0.001	0.25±0.001
TC3	0.62±0.005	0.36±0.004	0.2±0.002
TC4	0.4±0.002	0.32±0.006	0.18±0.003
Mirabilis jalapa			
C	0.8±0.008	0.45±0.006	0.35±0.006
TC1	0.65±0.006	0.37±0.006	0.22±0.005
TC2	0.6±0.004	0.34±0.007	0.2±0.004
TC3	0.4±0.003	0.34±0.001	0.18±0.003
TC4	0.38±0.002	0.28±0.002	0.17±0.002

Results are means±SD(n=5)

A



B



C

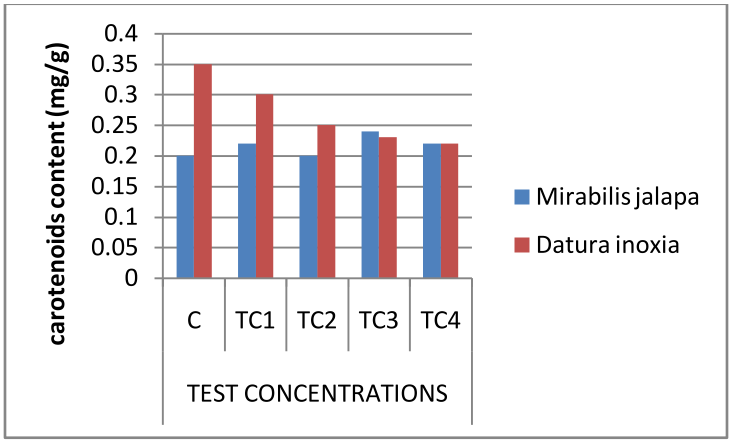


Figure 3. Chlorophyll a value (A), chlorophyll b value (B) and carotenoids (C) of *Mirabilis jalapa* and *Datura inoxia* in different treatments levels of lead contaminated soils.

The yellowish colour of the leaves and the reduction in leaf pigments as shown in fig are indication of lead accumulation which has caused chlorophyll degradation [21]. Summarizing the growth characteristics, it can be observed that root length and root dry matter and leaf pigments were greatly influenced by heavy metal stress. Lead is non essential heavy metal which is not known to have any metabolic function in plants and is toxic to plants at higher concentrations. It is in agreement with earlier studies.

Comparative study of metal extraction by *Mirabilis jalapa* and *Datura inoxia*

In *Datura inoxia* as shown in **Table 4 and Figure 4** maximum uptake of lead observed in roots as 25.62 mgkg-1 in TC3 soils, where as the maximum uptake of shoot was 31.72mgkg-1in TC3 soils respectively. Lead content was higher in shoots as compared that in areal parts. In *Mirabilis jalapa* the maximum uptake of lead observed 29.12 in roots in TC4 soils and 23.52 in shoots in TC4 soils respectively.

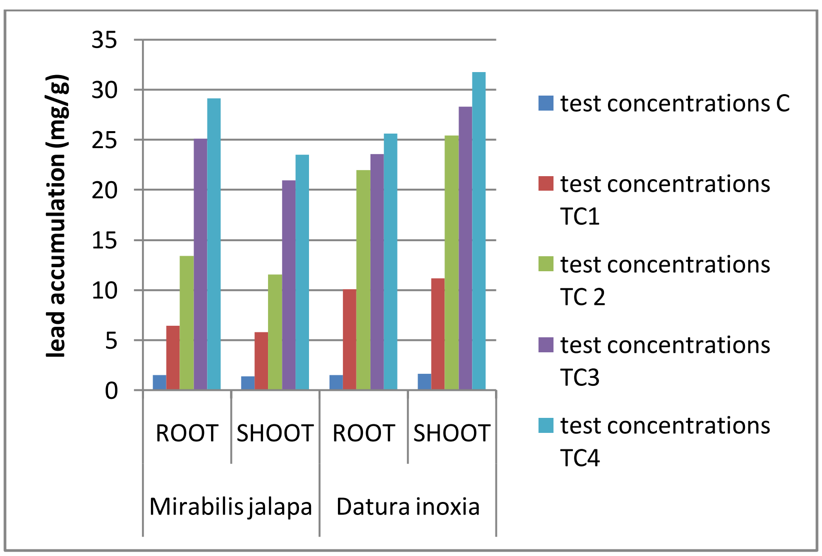


Figure 4. Lead accumulation in different treatments levels of lead contaminated soils by *Mirabilis jalapa* and *Datura inoxia*

Table 4 lead accumulation

Pb concentration in soil (mg/kg-1	Pb concentration		BCF		TF	% of removal
	root	shoot	root	shoot		
Datura inoxia						
C	1.53±0.002	1.66±0.006	1.36	1.48	1.09	75
TC1	10.06±0.006	11.18±0.009	1.8	2	1.11	80
TC2	21.98±0.009	25.45±0.003	2.24	2.59	1.16	83.2
TC3	23.56±0.005	28.27±0.003	4	4.8	1.2	90
TC4	25.62±0.005	31.72±0.002	2.33	2.89	1.24	82.1
Mirabilis jalapa						
C	1.55±0.005	1.42±0.003	0.94	0.87	0.92	60.5
TC1	6.48±0.002	5.83±0.005	1.44	1.3	0.9	72
TC2	13.42±0.003	11.59±0.005	1.49	1.29	0.86	70.5
TC3	25.1±0.006	20.92±0.006	2.18	1.82	0.83	78
TC4	29.12±0.005	23.52±0.002	1.49	1.2	0.81	65

Results are means±SD(n=5)

Based on the results it is evident from the **Figure 4** *Datura inoxia* was more tolerant and higher accumulation of lead compared to *Mirabilis jalapa* and also proved that with the increase in concentration of the heavy metal exposure to the plant bioaccumulation of the plant increased. In both the plants lead accumulation by the plants with increase in all test concentrations.
According to **Table 4** lead was recorded in the order of 1.659, 4.50, 8.99, 11.50 and 19.56mgkg-1in soil after phytoremediation by *Mirabilis jalapa* and it was in order of 1.125, 5.59, 9.83, 5.89 and 10.98 mgkg-1 after phytoremediation by *Datura inoxia* in C, TC1, TC2, TC3 and TC4 respectively.

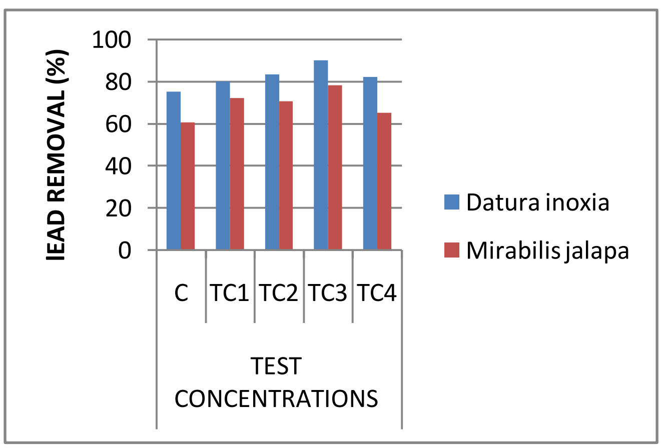


Figure 5. Lead removal percentage in different treatments levels of lead contaminated soils by *Mirabilis jalapa* and *Datura inoxia*

Based on **Table 4 & Figure 5** the lead concentration was reduced after phytoremediation by *Mirabilis jalapa* in order of 60.5%, 72%, 70.5%, 78% and 65% in C, TC1, TC2, TC3 and TC4 respectively. The results indicate that maximum reduction of lead concentration was in TC3 soils.

Based on **Table 4 & Figure 5** the lead concentration was reduced after phytoremediation by *Datura inoxia* in order of 75%, 80%, 83.2%, 90% and 82.1% in C, TC1, TC2, TC3 and TC4 respectively. The results indicate that maximum reduction of lead concentration was in TC3 soils.

BCF and TF

To evaluate the efficiency of lead phytoextraction in plants, BCF and TF were calculated. Results indicate based on the **Table 4** the bioconcentration factor of *Mirabilis jalapa* in shoot and root ranged in between 0-87–1.82, 0.94-2.18 respectively. The maximum biocentration factor value observed in TC3 soils in both root and shoot. Whereas, in *Datura inoxia* there are higher bioconcentration values in shoot and root were 1.48- 4.80, 1.36 – 4.00 respectively. In both of the plant species the maximum bioconcentration factor value observed in TC3 soils. The BCF values in both the plants were higher than 1.0 under different test concentrations, suggesting that both plants have a stable feature of lead accumulation.

The average Translocation factor values of *Mirabilis jalapa* and *Datura inoxia* were found to be in order of 0.81 – 0.90, 1.09 – 1.24 respectively. In *Datura inoxia*, maximum translocation factor values observed in TC4 soils and in *Mirabilis jalapa*, the maximum value found in control. In *Mirabilis jalapa* as the TF values were lower than 1.0, they indicate the limited ability of lead to translocte from roots and shoots. Where as in *Datura inoxia* all the TF values were higher than 1.0 which indicates strong potential to remedy lead contaminate sites. Plants with BCF and TF greater than one (TF and BCF>1) have the potential to be used in phytoextraction. Besides plants with (BCF>1 and TF<1) have the potential for phytostablization [22]. By comparing the BCF and TF, the ability of different plants in taking up metals from soils and translocating them to the shoots can be compared. [23].

Results indicated that *Datura inoxia* was suitable for phytoextraction and *Mirabilis jalapa* was suitable for phytostabilisation. However phytostabilisation could be a desirable property, as metals would not enter in to the food chain via herbivores and then avoid potential risk to the environment. Therefore plants with TF less than 1 should be used in order to realize the metal and reduce the metal dispersion through the grazing animals.

CONCLUSIONS

This study was conducted to determine the potential for metal accumulation. Based on the results obtained in this it can be concluded that compared to control, there was a grater accumulation of lead in both plants. This study showed that *Mirabilis jalapa* could be effective phytostabilisation and *Datura inoxia* could be used for phytoextraction and effective for phytoremediation lead from soils. Build of heavy metals such as lead in *Datura inoxia* and *Mirabilis jalapa* induces stress and chlorophyll loss. Soil contamination with heavy metals may also cause change in composition of soil, microbial community, adversity effect on soil characterization [24].

Based on results it was revealed that datura effectively removed over 90% of lead in TC3 soils, where as *Mirabilis jalapa* removed 78% in TC3 soils. Based on the results, tolerance and accumulation properties, both plants expressed tolerance to lead pollution. Whereas BCF and TF values revealed that though the BCF values in both plants were higher than 1.0, TF values in *Datura inoxia* higher than 1.0, where as in *Dirabilis jalapa* lesser than 1.0. Which indicates *datura inoxia* can efficiently remove lead from contaminated sites and could be a promising hyper accumulator for lead contaminated sites.

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