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Phytoremediation of lead contaminated soil by using *sorghum bicolor*

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ABSTRACT

Heavy metal pollution in the soils is increasing due to the industrialization, urbanization and transportation. Lead pollution in the urban areas is increased due to increased number of vehicles on the roads. The heavy metal lead is a non-biodegradable and easily enters into the food chain. From the food chain, they accumulate into human bodies causing many health abnormalities. The present study deals with phytoremediation of soil by *Sorghum bicolor* (jowar) seeds. The ability of plants to extract the metals from the soil and ability of plant to move the metals to aerial parts of plant are expressed as Bioaccumulation factor ((BCF) and translocation factor (TF) respectively. The percentage removal of *Sorghum bicolor* plants are given in the study. The physicochemical characteristic of soils is evaluated before and after phytoremediation. Present study revealed that *Sorghum bicolor* is a phytoextractor and it is suitable phytoextraction process.

KEYWORDS

Phytoremediation;
Phytoextraction;
Sorghum bicolor (jowar) seeds;
Lead (Pb);
Bioaccumulation factor ((BCF) and translocation factor (TF).

INTRODUCTION

Soil is the natural medium for plant growth and it supplies nutrients to plants. Soil is a vital natural resource for our livelihood and it is a great resource of minerals, organic matter, water and energy[1-2]. It a gene pool for a huge variety of organisms. Industrialization and urbanization contaminated the soil by different heavy metals. Lead is considered as contaminant lead can persist in the environment for a long period and it causes adverse effect when it enters into water and soil. Lead is becoming a major pollutant in the urban areas due to emission of lead from the motor vehicles.

Phytoremediation is considered as innovative, eco-friendly, novel, economical, green technology for removal of heavy metals[3-5]. In the present study, phytoremediation is studied for removal of heavy metals from the soils. Jowar or sorghum is the major staple food grain grown in different parts of India and present investigation is under taken to study the phytoremediation through sorghum or jowar.

MATERIALS AND METHODS

Collection of soil sample

The soil sample used for present study was collected from agriculture fields of Ibrahimpatnam, which is 30 kms far from Hyderabad city. The soil samples were collected in plastic bags and physicochemical analysis was carried out in laboratory as per standard methods. This control soil sample was artificially polluted with desired amount of lead (Pb) and used as polluted soil samples. To find out the lead removal and phytoremediation efficiency of *Sorghum bicolor* the set of experiments were carried out with dump yard soil sample which was collected from Jawaharnagar dump yard which is near to secunderabad.

Selection of seeds

The seeds of *Sorghum bicolor* are selected for phytoremediation. 70 seeds of *Sorghum bicolor* are counted and selected based on floatation methods. The physicochemical characterization of soil was carried out before and after phytoremediation by using standard methods.

Phytoremediation experiments

Phytoremediation experiments were carried out in plastic trays in laboratory. For this purpose, 2 kg of soil samples are taken in plastic trays. The trays were divided into three group's i.e. dumping yard of Hyderabad (Jawahar nagar), artificially Lead (Pb)-contaminated with different concentration and control (to which no metal was added). The Pb was added to the soil as their water-soluble salt in the form of their aqueous solutions. The concentrations of lead added were 10 mg/2 kg, 20 mg/2 kg, 30 mg/2 kg, 100 mg/2kg, 150 mg/2kg, 200 mg/2kg, 250 mg/2kg and 300 mg/2kg. 70 seeds of sorghum were put into each tray. For each soil, sample three trays were used. Germination and growth of germination is studied[6].

Plant sampling and analysis

A seed was considered as germinated when root had emerged more than 2 mm. The number of germinated seeds per time was presented as seed germination rate. Germination percentage and tolerance indices determined by the following formula[7].

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Toleranceindices} = \frac{\text{Meanrootlengthof pollutedareaseeds}}{\text{Meanrootlengthof controlareaseeds}} \times 100$$

$$\text{Tolerance indices} = \frac{\text{Mean root length of polluted area seeds}}{\text{Mean root length of control area seeds}} \times 100$$

The inhibition of seedling growth was expressed according to the formula[8].

$$\text{Percentage of inhibition} = \frac{\text{length of control - length of test}}{\text{Length of control}} \times 100$$

Analysis of heavy metals in plants

For this purpose, each plant parts were thoroughly washed under tap water and then with distilled water in order to remove dust and soil particles. The clean plant parts were dried under sunlight for 48 hours. Then the samples were digested according to Awofolu (2005) 1g sample of the plant part was taken into a 100 ml beaker. 5 ml concentrated (65%) HNO_3 and 2 ml $HclO_4$ were added to it and heated on hot plate until the digest become clear. The digest was allowed to cool and then filtered through a whatman No.1 filter paper. The filtrate was collected in a 50 ml volumetric flask and diluted to the mark with distilled water[9]. The filtrate was used for the analysis of lead (Pb) by UV-spectrophotometer.

Calculation of bioconcentration and translocation factors

Bioconcentration factor (BCF) indicates the efficiency of plants in up taking heavy metals from soil and accumulating them into its tissues. It is a ratio of the heavy metal concentration in the plant tissue (root or shoot) to that in soil. It is calculated as follows[10]

$$BCF = \frac{C_{harvested\,tissue}}{C_{soil}}$$

Where $C_{harvested\ tissue}$ is concentration of the target, metal in the plant harvested tissue and C_{soil} is concentration of the same metal in soil. Bioconcentration factor can also be calculated in percent according to the following equation[11].

$$BCF\,(\%) = \frac{C_{plant\,tissue}}{C_{soil}}\,X\,100$$

Translocation factor (TF) indicates the efficiency of the plant in translocating the accumulated heavy metal from roots to shoots. It is a ratio of the concentration of the heavy metal in shoots to that in its root. It is calculated as follows[12-13].

$$TF = \frac{C_{shoot}}{C_{root}}$$

RESULTS AND DISCUSSION

Characterization of soil samples before Phytoremediation

The physicochemical parameters of collected soil samples were carried out by standard procedures and results are shown in Figure-1. From the Figure-1, it can be conclude that the amount of Lead, phosphate, nitrate nitrogen and nitrite nitrogen is very high in soil sample of dump yard compared with control soil sample. pH, Electro conductivity and salinity of the dump yard soil is lower compare with control sample. Lower amount of salinity and electro conductivity enhance the seed germination process compare with high amount of salinity.

Effect of lead concentration on seed growth response and germination

Germinated seeds were counted every other day until the time in which no germination was observed over three days ever after. Seeds were considered germinated when both the plumule and radical were extended to approximately more than 2 mm from their junction[14]. The growth response and germination of *Sorghum bicolor* under different concentrations of lead (Pb) shown in Figure-2, 3 & 4.

A graph was plotted by taking Pb concentration in soil on X- axis and percentage of seed germination on Y- axis and it is represented in Figure-4. From the Figure- 4 it can be concluded that the % seed germination is decreased with increase in lead (Pb) metal ion concentration. The growth response was observed in all different treatments and observed that the plants grown under higher concentrations (100 mg/2kg – 300 mg/2kg) were fall down and became dry after 10^{th} day, but the growth response at lower concentration was good. The results of growth response at lower concentration including control and dump yard soil sample has shown in Figure-5.

The experimental reports (Figure-4), concluding that, there is germination of seeds was observed in all soil samples at different concentrations of lead. The highest percentage of germination was observed in soil samples of dumping yard, reason behind this is the amount of nitrogen, phosphorus compounds were high. The soil samples rich in all micro and macro nutrients enhance the growth rate of *Sorghum bicolor* seed germination process[15].

The seed germination indicated that the percentage of germination decreased with increase in concentration of lead and after 15 mg/l of lead it is almost became constant. The decrease percentage of germination may be due to accelerated breakdown of stored food material in the seeds by application of lead to soils. It can also be attributed to alterations of selection permeability properties of cell membranes. The decrease in percentage germination may be due to loss of viability because of decreased energy generation by embryo.

The experimental data was tested with two way ANOVA (Analysis of Varience) using Graphpad prism version 6.04 software and the report concludes that there is significant change in % germination was observed in all led polluted soil samples with control soil sample ($p < 0.008$ to 0.005). From the statistical report with multiple comparisons with in polluted soils also shown significant change of % germination[15].

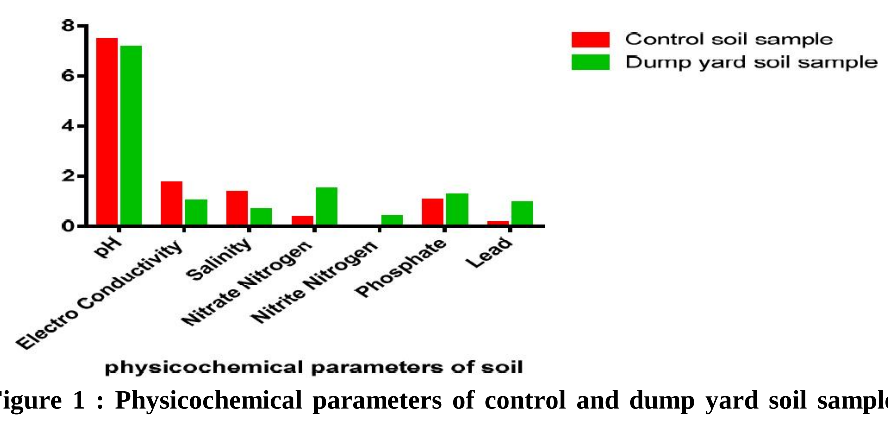

Figure 1 : Physicochemical parameters of control and dump yard soil samples

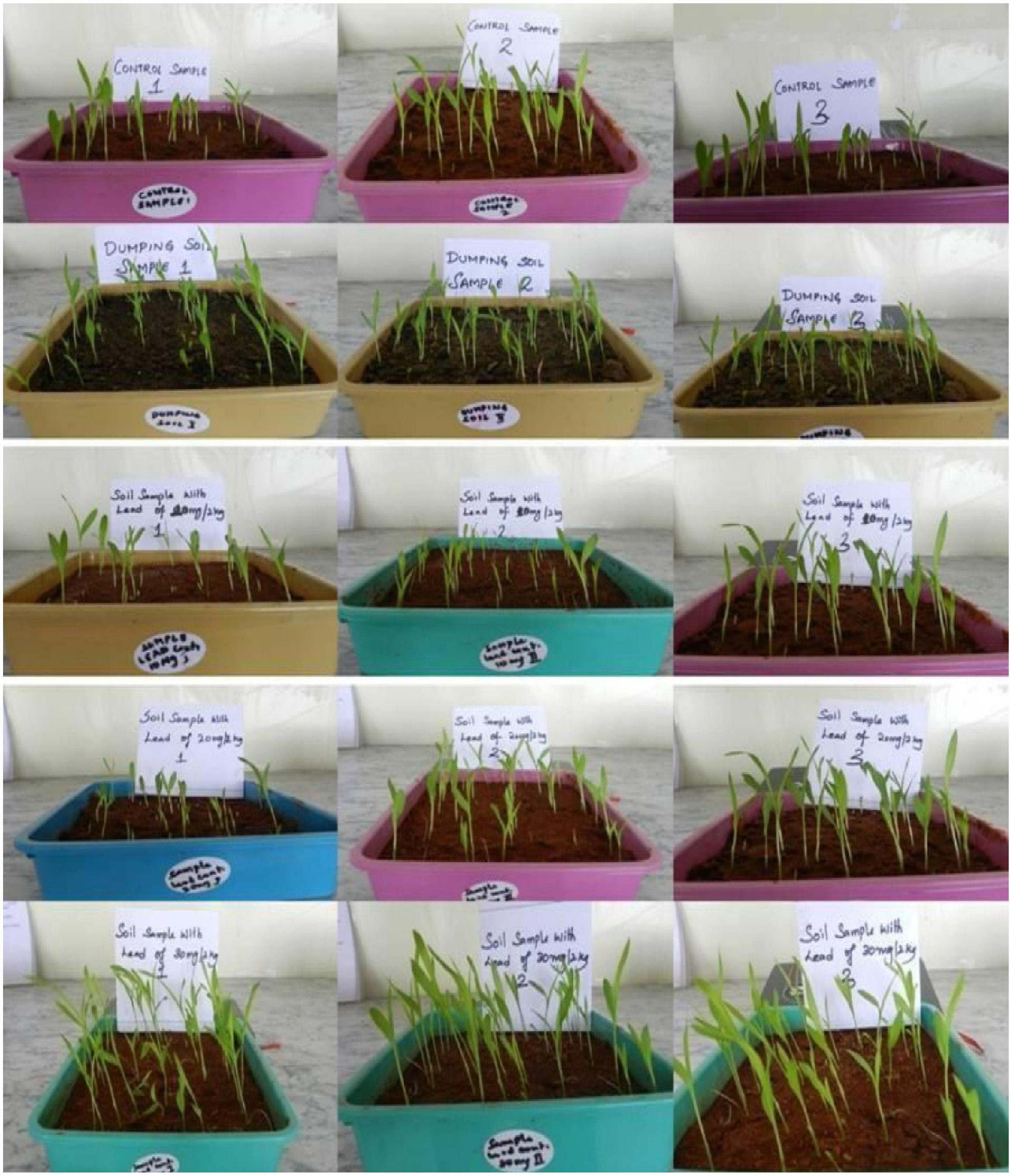

Figure 2 : Seed germination on 8th day at lower concentrations of lead

Phytoremediation of Lead (Pb) by *Sorghum bicolor*

From the Figure-6, it is observed that the percentage removal of lead in the planted soil decreased with increase in concentration. Lead is highly immobile in soil and solubility is less and it is not readily bio available to plants. The soil will not promote the lead uptake by plants as it is not bio available. Some plants have genetic capacity and ability to accumulate the metal but they must be bio available. In order to make the lead bio available to plants some chelating agents such as EDDS and EDTA can be add.

Physicochemical Analysis of Soil samples before and after seed Germination:

To find out the remediation efficiency of pollutant removal from soil with *Sorghum bicolor* the physicochemical analysis of soil samples were carried out before and after seed germination (14 & 21 day from seed germination). The change in pH,

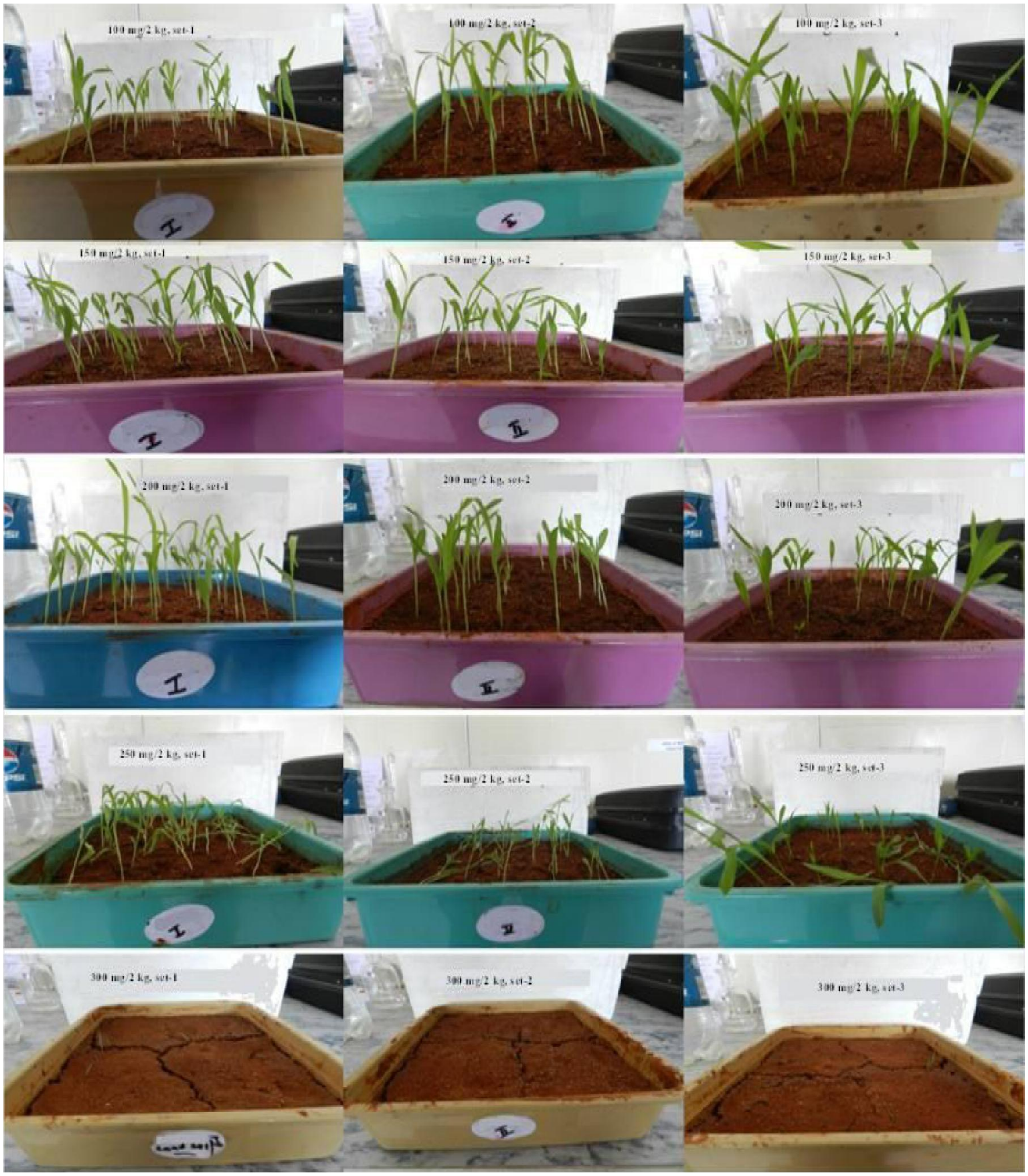

Figure 3 : Seed germination on 8th day at higher concentration of lead

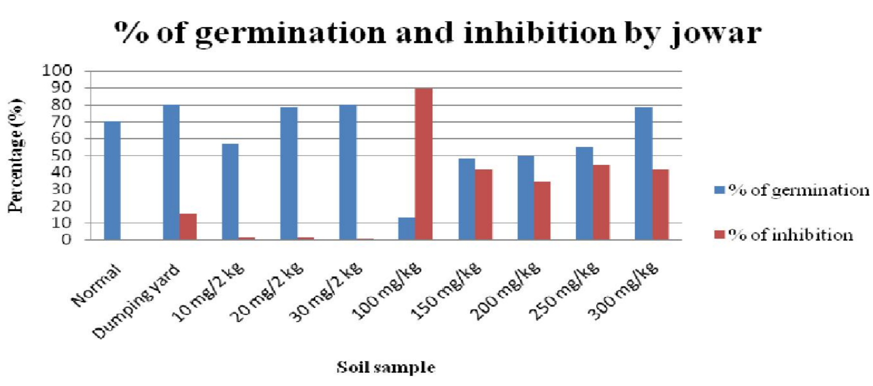

Figure 4 : Graph for percentage of germination and inhibition by *Sorghum bicolor* under different concentrations of lead (Pb)

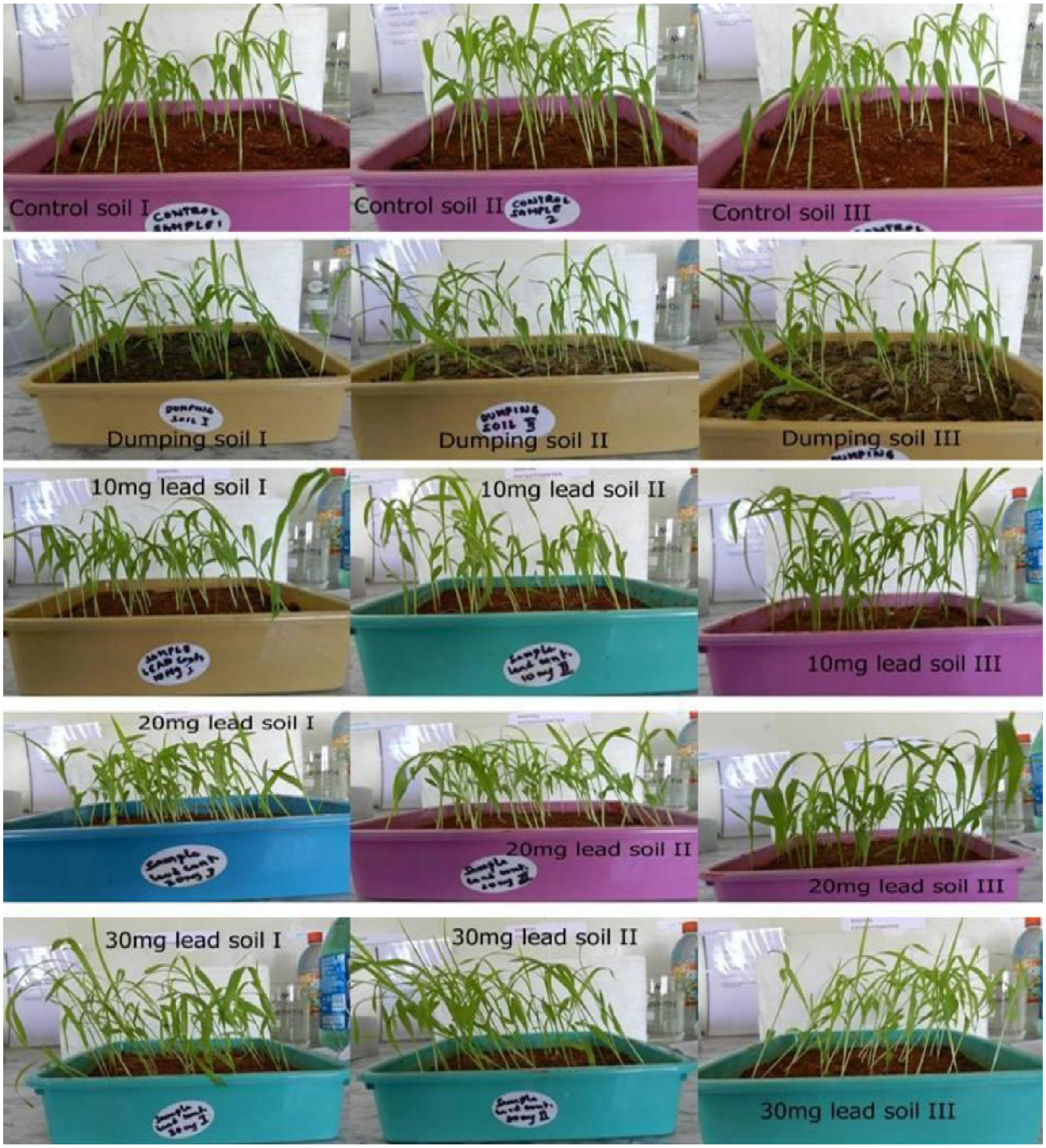

Figure 5 : Seed germination on 15th day at lower concentrations of Lead

Electro conductivity, Salinity, Nitrate Nitrogen, Nitrite Nitrogen, Phosphorus and lead concentration with both *Sorghum bicolor* seeds in all soil samples were shown in Figure-7 to Figure-9.

In the present study electrical conductivity, pH, Salinity, nitrate –nitrogen and nitrite-nitrogen, phosphate content in the soil samples are determined before and after phytoremediation. It is found from the studies that electrical conductivity, Nitrate nitrogen and Nitrite nitogen, Phosphate, are decreased after phytoremediation along with lead content. There is a significant change was observed decrease for lead in soil samples after 14 days of seed germination. The experimental results and amount of lead in soil, root and shoot were shown in table.

The electrical conductivity of the soils decreased during phytoremediation, which indicates active transportation of ions to the plant and absorption of dissolved solids by plant. The utilization of minerals of all soil samples increased which indicates a positive sign for cultivation. Nitrite –nitrogen decreased after phytoremediation, which indicates denitrification process. The study also suggests that plant species can assimilate different forms of nitrogen from the soil that indicates the favorable condition for phytoremediation. Nitrate-nitrogen is

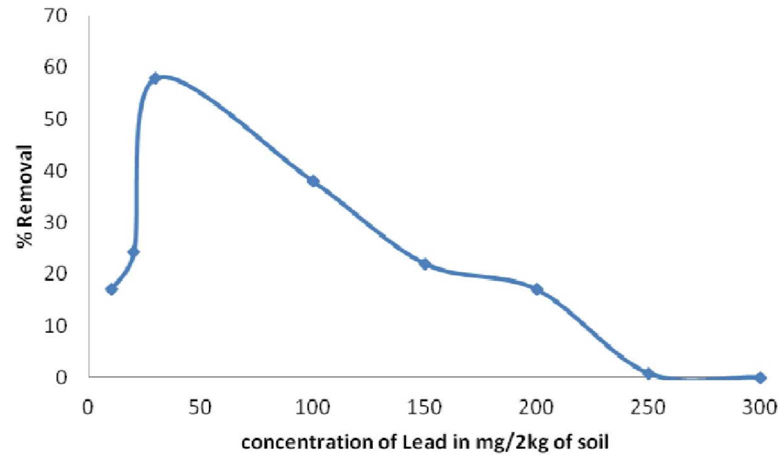

Figure 6 : The final percentage (%) removal of lead after 24 days

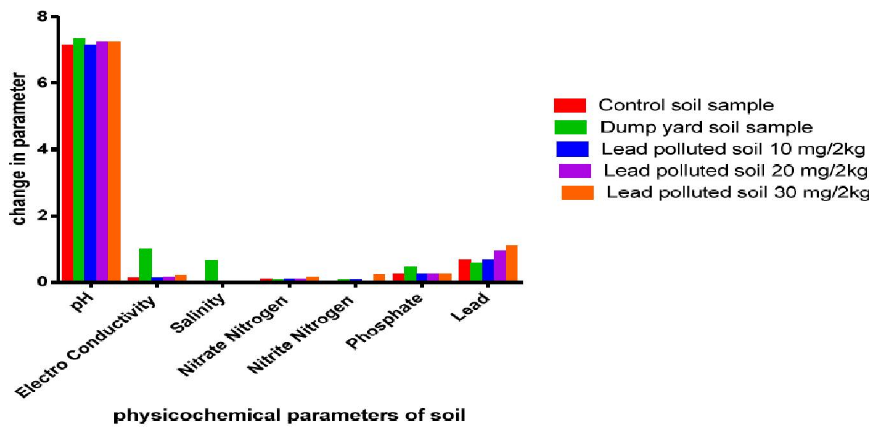

Figure7 : Change in Physico-chemical parameters of different soil samples after 14th day

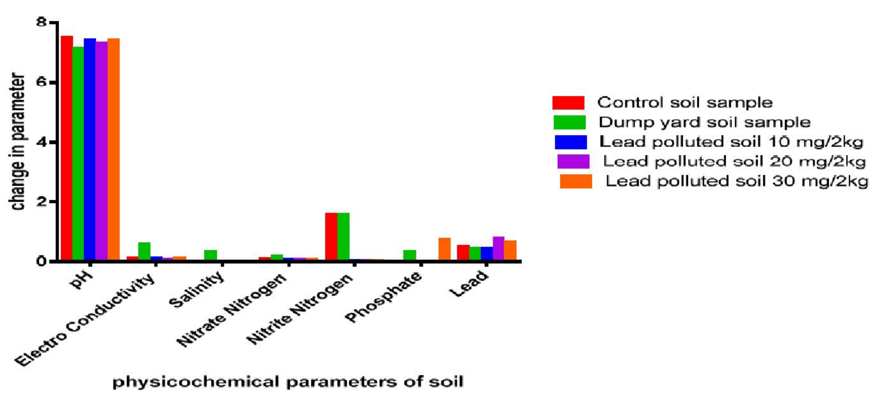

Figure 8 : Change in Physico-chemical parameters of different soil samples after 21st day at lower concentration

highly toxic to plants and zero in all the soil samples, which indicates the favorable condition for seed germination. The phosphate content in all the soil samples is very less and decreased during the phytoremediation process. Less amount of phosphate indicates the introduction of phosphate fertilizer in the soil and decrease in phosphate levels indicates the nutrient phosphate uptake by plants to perform biochemical pathways while germinating process.

The Present study revealed that *Sorghum bicolor* (jowar) is a great plant for phytoextraction of lead from contaminated soil. The translocation factor of lead decreased with the increase in lead content in the soil. The translocation factor was changed from 0.3 to 1. The results showed that TF<1 shows that lead can be effectively translocated from the shoots to the roots[16-19].

BCF factor in roots and shoots is very less. It is less than 1 and they are decreasing with the increase in concentration of lead. BCF values of roots are

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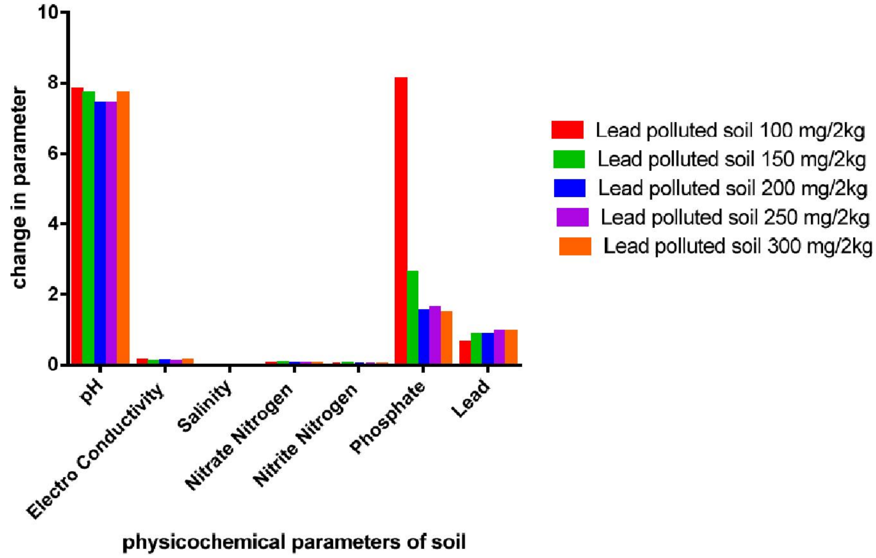

Figure 9 : Change in Physico-chemical parameters of different soil samples after 21st day at higher concentration

TABLE 1 : Bio concentration factor for roots and shoots of *sorghum bicolor*

.No	Initial concentration of Lead in Soil Samples	Amount of Lead in Shoots	BCF Values of Shoots	Amount of Lead in of Roots	B.C.F Values of Roots	% BCF values of Roots	%BCF Values of Shoots	TCF
1.	10 ppm / 2 kg	0.415	0.083	0.519	0.1038	8.3%	10.3%	0.799
2.	20 ppm/ 2 kg	0.623	0.0623	0.623	0.0623	6.2%	6.2%	1
3.	30 ppm/ 2 kg	0.623	0.0415	0.623	0.0415	4.1%	4.1%	1
5.	150 ppm/ 2 kg	0.415	0.002766	0.623	0.00415	0.27%	0.41%	0.666
6.	200 ppm/ 2 kg	0.311	0.001555	0.519	0.002595	0.15%	0.25%	0.599
7.	250 ppm/ 2 kg	0.311	0.001244	0.415	0.00166	0.12%	0.16%	0.44
8.	300 ppm/ 2 kg	0.415	0.00138	0.415	0.00138	0.13%	0.13%	0.32

greater than shoots. This would denote that higher amounts of lead were taken by the roots than lead. The plant is considered as hyper accumulator plant[20-22] and lead is mobile from shoots to roots.

The studies reveal that high metal concentration is toxic to the growth of plant indicating that the bioaccumulation factor decreases with the increases of metal concentration[23-24] and it indicates phytoextraction process. By comparing BCF and TF values, the ability of *Sorghum bicolor* (Jowar) plant in taking up metals from soils and translocation from shoots can be compared. Translocation factor is higher than bio concentration factor which indicates that *Sorghum bicolor* (Jowar) r plant is suitable for phytoextraction[25-30]

CONCLUSIONS

Sorghum bicolor has a potential to accumulate and extract the lead into roots, since the TF is less than 1. It is a phytoextractor and a promising plant for phytoremediation. The study implies and gives insight that they will not pose a health risk and they can be planted in lead contaminated soil. Phytoremediation is a long term process and it is not economical productive and socially acceptable, if it takes a long time (14 to 24 days).

At lower concentration, the ability to take up metals by *Sorghum bicolor* is feasible but at higher concentration the chelated assisted techniques can be employed successfully which may reduce the toxicity of lead.

Physic-chemical properties of soil were decreased after phytoremediation and they were within the range according to WHO guidelines

Lead content in the soil samples decreased with the increase in the period of cultivation time and optimum period is 14 days at lower concentration and 8 days for higher concentration. After 8 days cultivation time the falling down of shoots were observed at higher concentration and they become crispy after 10 days. This result concluding higher concentrations were showing more effect on biochemical pathway.

At higher concentration the plants could not survive due to increase in toxicity of lead (Pb)

The immobility of lead from soil matrix is very less which is indicated by the accumulation of lead in roots than shoots, which is a favorable conditions as it will not enter into the food chain as the seeds used for remediation studies were food grains

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