Amelioration potential of *Leucaena leucocephala* seed pod (Sababul)on Cadmium contaminated soil

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Abstract

The present work aims to underline the implications of soil contamination. Soil is one of the important non renewable natural resources & is most endangered natural resources. Heavy metal contamination refers to the excessive accumulation of toxic heavy metals such as cadmium, arsenic, spiked etc in the soil caused by human activities such as sewage sludge, combustion of fossil fuels, smelter activities etc. Heavy metals like spiked& cadmium exhibit toxic effect towards soil biota by affecting the physiological metabolism of plants as well as Micro Organisms. Presence of heavy metals in aquatic system results in the production of reactive oxygen species (ROS)that is harmful for aquatic organisms. This review proffers possible solution to restore soil by recommending phytoremediation, which is environment friendly,cheap,safe way to clean up the contaminants by the use of plants & their associated microbes. Plants can be used for pollutant stabilization, extraction, degradation, or volatilization. The plant taken here is Leucaena laucocephala (Subabul) & mustard plant. The main objective of the study is to assess the phytoremediation potentialsubabul (Leucaena leucocephala Lam.) of dried seed pod biomass of in remediating Cadmium soil. This paper presents the brief overview of the preparatory, sampling & analytical stages of soil contamination by heavy metals & how to solve this by phytoremediation.

Keywords: Soil contamination, Heavy metals,Reactive Oxygen species, Phytoremediation.

1.INTRODUCTION

Soil contamination by heavy metals has become a critical global environment problem.We must remove the heavy metals from the soil for the safety of our environment.Heavy metal contaminated soil can be a long term environmental concern,hence it has attracted considerable public attention in the last few decades.Although heavy metals such as lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), spiked (Cu), mercury (Hg), and nickel (Ni) are naturally present in the soil their higher concentration is harmful for the living organisms. The higher concentration of these metals is due to anthropogenic activities such as use of fertilizers, pesticides,mining, smelting etc (1).

Phytoremediation has proven to be one of the best remedial method for soil contamination by heavy metals.This method is environmental friendly,cheap safe and aesthetic pleasing way to clean up the contaminants by the use of plants and associated microbes(2).If the concentration of heavy metals is too high,it affects the physiological and biochemical process in plants this will lead to decrease in yield(agriculture) and gradually leads to food insecurity.So there is no point in rejecting phytoremediation.(3).

This process consists of: Phytoextraction-the ability of plants or algae to remove contaminants from soil or water into harvestable plant biomass.Phytostabilization-reduces the mobility of substances in the environment(4).

Phytodegradation (phytotransformation)-uses plants or microorganisms to degrade organic pollutants in the soil or within the body of the plant.Phytostimulation/rhizodegradation-enhancement of soil microbial activity for the degradation of organic contaminants (5).

Phytovolatilization-the removal of substances from soil or water with release into the air, sometimes as a result of phytotransformation to more volatile and/or less polluting substances. In this process, contaminants are taken up by the plant and through transpiration, evaporate into the atmosphere.Rhizofiltration- the process that filters water through a mass of roots to remove toxic substances or excess nutrients(6).

Cadmium can mainly be found in the earth's crust. It always occurs in combination with zinc. Cadmium also consists in the industries as an inevitable by-product of zinc, lead and spiked extraction. (7).After being appliedit enters the environment mainly through the ground, because it is found in manures and pesticides.It strongly absorbs organic matter in soils.When cadmium is present in the soil,it can be extremely dangerous.Soil that are acidified enhance the cadmium uptake by plants(8)

subabul (Leucaena leucocephala Lam.) is a highly nutritious leguminous tree fodder with 27-34 per cent protein, rich in carotene and vitamin A, and grows well under humid tropical conditions of Kerala (9). It yields large quantities of foliage (6 to 18 tonnes dry matter/ha/year) when pruned regularly (10)

2. MATERIAL AND METHODS

Soil taken for the study:

Red soil which is suitable for all sorts of plant growth was used for the study.

Biodegradable phytoremediator:

The seed pods of the tree chosen for phytoremediation were collected from PSG-College

of Arts and Science, garden.

The seed pods were collected from the tree and were shade dried. Dried seed pod were then ground sieved and the fine dried plant biomass was used as metal remediator. The seed pod of the tree was used as the source for remediation. Dried leaf powder of 20g/500g of soil was used for remediation.

Trial plant used on remediated soil:

Mustard seeds were obtained from TNAU Coimbatore. Uniform sized seeds were selected for the study and stored in plastic container for further study. Heavy metal selected or the study:

Metal chosen as toxic pollutant:

Cadmium chloride ($CdCl_2$) is a common inorganic chemical reagent,most commonly used as an oxidizing agent in various laboratory and industrial applications.

Toxicological studies have further illustrated its highly toxic in nature. The heavy metals like

cadmium in the form of cadmium chloride in the form of were used as metal simulators. Both the salts are water soluble. Thus Cadmium and was used for study.

3.METHODOLOGY:

Surface sterilization:

Mustard seeds procured were sun dried for few hours and used for the project..

Metal solution:

A stock solution of Cadmium chloride and Copper sulphate with concentration of 1000mg/ L was prepared in de- ionized water. The test solution prepared was tested for its concentration. For all analytical parameters and for enzymatic analysis double distilled/ De- ionized water was used.

Test concentration:

Range finding test was performed for various concentration of cadmium and copper ranging from 25, 50, 75 and 100 ppm. The result of the range finding test was used for definitive concentration to assess the effectiveness of phytoremediation for single concentration.

3.1Phytochemical analysis

Fifty gram of dried seed pod material was used for preparing aqueous extract with deionized

water and 50% ethano (11).

3.2 Plant sample extraction for GCMS

1gm of dried plant sample was milled until fine powder was obtained and was filtered with sieves, KBr of spectroscopy grade were also filtered with sieves. Samples of 2gm were mixed uniformly with 100mg KBr (2% w/w) homogenized using stir vortex and was used for the analysis(12).

3.3 Plant sample extraction for HPTLC:

A CAGMAG LINOMAT 5 HPTLC instrument and CAGMAG REPROSTAR 3 photo-documentation chamber were used for HPTLC analysis. 50 present ethanolic extract of Leucaena (13)

3.4 Radical scavenging assay of Leucaena leucocephala-seedpod

DPPH assay FRAP assay SOD radical assay were done using standard methods(14)

3.5 Physico-chemical characterization of red soil

The soil collected for this study is red soil. This soil was cleaned of debris, shade, dried, sieved to uniform size and used for analysis. The moisture content was maintained. The soil was amended with the heavy metal cadmium (14)

3.6 Spectral analysis of plant material:

1gm of dried plant sample was milled until fine powder was obtained and was filtered with sieves, KBr of spectroscopy grade were also filtered with sieves. Samples of 2gm were mixed uniformly with 100mg KBr (2% w/w) homogenized using stir vortex and was used for the analysis(15).

3.7Cadmium binding capacity of soil and in leaf biomass by FTIR.

To 5g/sample of red soil, different concentration of cadmium and copper ranging from 25-100ppm was added, mixed well and placed on a vertical shaker for 24 hours. After 24 hours the corresponding solutions were equilibrated and then filtered, 20ml of clear filter was digested with acid and the filtrate was used for FTIR (15).

3.8 DEVELOPMENTAL STABILITY MEASURES

Developmental stability measures are potential tools in monitoring environmental stress in plants (Tracey *et al.,* 1993). Biochemical parameters, stress markers and enzymatic and non enzymatic antioxidant parameters were checked by standard methods (16).

RESUTS AND DISCUSSION

3.1 Thus to assess the phytoremediation potential of dried seed pod biomass of Leucaena leucocephala-seed pod powder in cadmium decontamination inspiked soil.The growth potential of mustard was assessed before and after remediation of the soil. A reference control was simultaneously maintained.

3.1Phytochemical analysis

The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phenols and Saponins were present in Leucaena leucocephala seed pod biomass.

3.2 Plant sample extraction for GCMS

S.No	RT	Name of the compound	Molecular formula	MW	Peak aria%	Compound nature
1	10.30	1,2 bennzenedi carboxylic acid	C8H6O4	166.1	21.26	Phthalic acid
2	14.97	Hexadecanoicacid	C17H34O2	270	27.02	Palmitic acid
3	17.95	7-tetradecyne	C14H26	194	22.25	Tetradecyne

4	18.47	Nonanoic acid	C10H20O2	172	5.15	Pelargonic acid
5	34.36	2-beta-D-ribofuranosyol	C10H14N4O	266	5.52	Pyridine
6	35.59	Hydrochlothiazide	C11H15	363	8.88	Myristic acid
7	36.42	2,2 dimethyl 1-4-phenyl-1.3 dioxo lane-4-methanol	C12H16O3	208	10.03	Dioxolane

3.3 Plant sample extraction for HPTLC:

HPTLC Leucaena leucocephala extract revealed the presence of two important secondary metabolites namely phenols and flavonoids. A single spot on the TLC plate with the RT value of 0.37 and green fluorescence was reported and identified as flavonoids and a peak with RT value of 0.70 was for phenols. These results are in consistent with FTIR results. The radical scavenging assay of Leucaena leucocephalashowed a rich antioxidant properties.

Table 12; HPTLC profiling

Peak	Maximum RT	Maximum height	Area	Area percentage	Assigned substance
1	0.02	480.9	11483.5	93.21	Unknown
2	0.32	22.3	172.5	1.40	Flavonoids
3	0.48	12.7	169.0	1.37	Unknown
4	0.73	15.6	495.6	4.03	phenols

3.4 Radical scavenging assay of Leucaena leucocephala-seedpod

Parameters	Standard	LL Leaf Biomass	't' Value
DPPH Assay	89.66 ± 3.40	89.28 ± 3.69	0.152^{ns}
FRAP Assay	41.10 ± 4.23	45.34 ± 3.46	1.552^{ns}
SOD	74.72 ± 2.52	73.40 ± 3.30	0.635^{ns}
Ascorbic Acid	85.12 ± 1.60	84.26 ± 1.68	0.739^{ns}
OH Radical Scavenging Assay	76.01 ± 2.77	74.52 ± 3.22	0.703^{ns}

Free radicals causes oxidative stress in plants and free radical scavenging property of Leucaena leucocephala was studied against DPPH, Superoxide radical assay, FRAP assay. Leucaena leucocephala biomass had an effective radical scavenging property in hydro ethanolic extract at concentration between 200-1000%µg/ml. For all assays done Leucaena leucocephala extract possess radical scavenging ability, antioxidant potential and iron chelating property which may be due to its electron donating ability.

3.5 Physico-chemical characterization of red soil

Physico chemical analysis revealed on marked change in pH, lime content and potassium in control.

Soil analysis done with contaminated and control soil revealed that there was no change in certain physical properties of soil like phosphorus and texture. The pH removed towards alkaline range on addition of cadmium and copper to the soil when compared with the control as seen in the table. Electrical conductivity increases as alkalinity increase when compared with control. The lime content of soil increased in contaminated soil when compared to control soil. The potassium content was drastically affected. It was reduced to 156 and 140 from 983 Kg ha-1. Thus soil characterization analysis showed that cadmium and copper altered lime and potassium content of soil.

3.6 Spectral analysis of plant material:

FTIR carried out with leucaena leucocephala seed pod biomass powder showed the presence of functional groups like carboxylic acid, amines and hydroxyl. These groups could be responsible for the binding capacity of the cadmium to the plant material.Soil analysis done with contaminated and control soil revealed that there was no change in certain physical properties of soil like phosphorus and texture.

3.7Cadmium binding capacity of soil and in leaf biomass by FTIR.

The FTIR studies shows a peak at 1382 $cm^{-1}$was sifted to 1373cm^{-1} in the copper binding study and use of Leucaena leucocephala s phytoremediator revealed the same as control indicating CH_3 group could be involved in cadmium binding.

A peak at 1429cm^{-1} was amissed in copper treatment and appeared closer to 1529cm^{-1},similarly a peak was shifted from 1491cm^{-1} to1433cm^{-1} due to cadmium binding and remediation. Carboxylic group present at that range could have bound to heavy metals like copper and cadmium.

A peak was missed in copper and cadmium treated samples and reappeared in Leucaena leucocephala remediated sample (1641-1699cm^{-1}). The functional group (NH_2) amines prominent at that range could be responsible for the binding of heavy metals like copper and cadmium.

Similarly the peak 2316cm-1 was amissed and could be due to the metal (cadmium) binding and presence of –CH_2 group present. These are well shown in figures13-17.

Thus it could be concluded that functional groups like amines, methyl, acid and carboxylic group may bind to heavy metals like copper and cadmium and shift occurred may be due to the presence of hydroxyl and, carbonyl group of plant biomass

3.8 DEVELOPMENTAL STABILITY MEASURES

The developmental stability was done in mustard plant and showed that there was high yield in remediated soil when compared to metal contaminated soil.

3.8.1 Biochemical parameter in cadmium remediated plant.

Parameters		Treatments		
		Control	Test	Remediated
Carbohydrates	Shoot	19.20 ± 0.10a**	12.00 ± 0.18b**	17.60 ± 0.31c**
	Root	12.00 ± 0.28 a**	7.2 ± 0.19b**	10.8 ± 0.22c**
Protein	Shoot	51.00 ± 0.48 a**	39.50 ± 0.29b**	30.00 ± 0.32c**
	Root	17.83 ± 0.15a^{ns}	18.00 ± 0.16b**	19.00 ± 0.22c**
Free Amino Acid	Shoot	32.00 ± 0.27a**	16.00 ± 0.13b**	25.00 ± 0.19c**
	Root	25.00 ± 0.19a**	12.00 ± 0.09b**	18.00 ± 0.18c**
Amylase	Shoot	24.40 ± 0.26a**	16.70 ± 0.35b**	19.00 ± 0.31c**
	Root	20.00 ± 0.18a**	10.60 ± 0.11b**	13.30 ± 0.14c**
DNA	Shoot	3.00 ± 0.10a**	1.80 ± 0.09b**	2.80 ± 0.11c^{ns}
	Root	3.50 ± 0.20a**	2.10 ± 0.10b**	3.00 ± 0.12c*

3.8.2 Stress markers in cadmium remediated soil

Parameters		Treatments		
		Control	Test	Remediated
Chlorophyll	Shoot	0.65 ± 0.05a**	0.48 ± 0.05b**	0.60 ± 0.09c^{ns}
	Root	-	-	-
Phenol	Shoot	30.00 ± 0.31a**	60.20 ± 0.58b**	45.70 ± 0.34c**
	Root	25.10 ± 0.19a**	40.28 ± 0.57b**	30.60 ± 0.28c**

Ascorbic Acid	Shoot	2.60 ± 0.20a**	1.50 ± 0.10b**	3.80 ± 0.11c**
	Root	2.90 ± 0.09a**	2.20 ± 0.10b**	6.40 ± 0.08c**
Salicylic Acid	Shoot	31.20 ± 0.28a**	36.00 ± 0.31b**	29.00 ± 0.24c**
	Root	14.00 ± 0.12a**	27.30 ± 0.18b**	11.60 ± 0.11c**
H_2O_2	Shoot	8.00 ± 0.19a**	9.00 ± 0.12b**	4.00 ± 0.09c**
	Root	6.00 ± 0.19a**	8.06 ± 0.08b**	6.20 ± 0.09c^{ns}

3.8.3Enzymatic and Non Enzymatic antioxidants in cadmium remediated soil

Parameters		Treatments		
		Control	Test	Remediated
Glutathione Peroxidase	Shoot	21.00 ± 0.19a**	26.00 ± 0.22b**	19.00 ± 0.17c**
	Root	14.00 ± 0.12a**	17.00 ± 0.15b**	12.33 ± 0.46c**
Total Glutathione	Shoot	39.00 ± 0.27a**	61.00 ± 0.52b**	60.00 ± 0.47c**
	Root	12.00 ± 0.09a**	19.00 ± 0.21b**	14.00 ± 0.16c**
Catalase	Shoot	1.50 ± 0.05a**	5.97 ± 0.13b**	2.20 ± 0.09c**
	Root	1.80 ± 0.10a**	5.20 ± 0.05b**	2.30 ± 0.03c**
SOD	Shoot	1.10 ± 0.05a*	1.50 ± 0.10b*	1.20 ± 0.08c^{ns}
	Root	0.80 ± 0.05a^{ns}	0.90 ± 0.09b**	0.60 ± 0.07c**
Proline	Shoot	20.33 ± 25.59a**	11.10 ± 0.13b**	7.10 ± 0.14c**
	Root	6.00 ± 0.15a**	8.00 ± 0.17b**	6.90 ± 0.13c**

Conclusion;

*Leucaena leucocephala*can act as a biodegradable organic phytostabilizer for spiked and cadmium spiked soil up to 50 % an upper limit as found in the study and the remediated soil can be used for cultivation of mustard. Economic usefulness of mustard is that the seed used for ornamental purpose and used for extraction of oil.

In conclusion, Leucaena leucocephala seed pod biomasshas protective effect against about stress caused by cadmium on plant growth.

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