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Biomass Growth and Remediation Efficiency of *Chrysopogon zizanioides (L.) Nash* for artificially contaminated Alluvial soil

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Abstract: Though Chrysopogon zizanioides (vetiver grass) has potential characteristics for remediation of contaminated soils, sediments, and water, but it has very limited application for the remediation of contaminated alluvial soil. The vetiver was planted to evaluate its biomass growth in artificially contaminated alluvial soil in Prayagraj, India for the purpose of its remediation. The vetiver was planted on alluvial soil mass artificially contaminated by two heavy metal salts, lead nitrate (Pb(II)) and potassium dichromate (Cr(VI)), for a period of 150 days. The vetiver grass, having 10cm root and 20cm shoot length, was planted in total 30 treatment boxes of dimensions 45cm x 45cm x 90cm, made of GI sheets. Four replicates (100, 300, 500, and 700mg/kg) of lead ($Pb(NO_3)_2$), four replicates (50, 100, 200, and 400mg/kg) of chromium ($K_2Cr_2O_7$), one mixed replicate (Cr-100+Pb-300), and one control have been selected. With increasing Cr-concentration in soil, vetiver shows a higher toxic effect, resulting in reduced biomass production with increased Cr-accumulation in roots. Whereas increasing Pb-concentration in soil, biomass production increases with minimum toxic effects. 65-75% of Cr and Pb were found to accumulate in root tissues and only 10-15% were translocated to aerial parts from roots tissues. The findings reveal that vetiver has good potential for rhizostabilization of Pb and Cr from polluted soils, minimizing the risk to contaminate groundwater and entering into the food chain as well as enhanced biomass of vetiver would be utilized for slope stabilization of embankments constructed on erodible soils and as a potential renewable energy source.
Keywords: Alluvial soil · Biomass · Chromium · Lead · Phytoremediation · Vetiver grass.

I. INTRODUCTION

Due to the increase in industrialization, urbanization, and change in lifestyle of human beings, the exploitation of natural resources had increased in an uncontrolled manner. The impact of which is that the environmental contamination has increased in the form of contamination of soil, air, and water which further has become a concern for public health and environmental risks. Different types of contaminants: inorganic, organic, and biological, cause adverse effects on human health, and the environment. Among these, heavy metals have received special attention of researchers due to their long persistence and acute toxic nature [1]. Heavy metals in the environment occur naturally and anthropogenically both ways. The anthropogenic activities involve the mining of metals, foundries, smelting, and other metal-based industries, leaching of metals from landfills, waste dumps, excretion, livestock, and chicken manure etc., runoffs, traffic, and agricultural activities [2]. According to a report of the United States Environmental Protection Agency [3], the most common metals found at polluted sites are in order: Pb, Cr, As, Zn, Cd, Cu, and Hg. The various impacts of heavy metals on plant life and human/animal health have been presented in Table 1. According to a report of the Central Ground Water Board of India, people in West Bengal and Bihar used to drink chromium and arsenic-contaminated water without knowing their harmful effects [4]. Inhalation, ingestion, and dermal contact are the main routes of exposure for the contaminants to impact the natural environment and human health [5].

Table 1. Impacts of heavy metals on animal and plant [6]

Contaminant	Impacts on	
	Human/animal health	Plant life
Cadmium (Cd)	Renal dysfunction, increased uric acid, Liver cirrhosis, lung cancer, itai-itai.	Brown margin to leaves, chlorosis, necrosis, cubed leaves, reduction in growth, purple coloration.
Chromium (Cr)	Kidney and liver damage, skin ulcer, cancer	Reduced seed emergence, stunted plant growth, and decreased biomass production.
Lead (Pb)	Affect the central nervous system, vomiting, immune reduction, loss of weight	Dark green leaves, stunted foliage, increased shoot biomass.

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Mercury (Hg)	Loss of smell sense, insomnia, mental deterioration, vomiting	Stunting the seeds and roots, chlorosis, browning of leaf tips, reduction biomass growth,
Copper (Cu)	Wilson's disease, jaundice, hypertension, neurological problem, paralysis.	Yellow and purple coloration, decreased growth of roots, chlorosis.

According to [7], in surface soil of Prayagraj municipal area, following heavy metals: Cr, Zn, Cu, Pb, and Cd have been identified. Lead (Pb(II)), mercury (Hg(II)) and chromium (Cr(VI)) are the most toxic metals which affect more than 30 million people globally [8]. Table 2 summarizes the acceptance criteria of heavy metals in soil and their regulatory limit.

Table 2.Criteria for heavy metals in soil and regulatory limits [9]–[12]

Elements	Range (mg/kg)	Regulatory limit (mg/kg)	Acceptance Criteria (mg/kg)
Arsenic	0.1-102	20	30
Cadmium	0.1-345	100	3
Chromium	0.005 – 3,950	100	6 (Cr(VI)) – 600 (Cr(III))
Copper	0.03 – 1,550	600	300
Lead	1.0 – 69,000	400	300
Zinc	0.15 – 5,000	1500	200

In environment, chromium exists in two forms: Cr(III) and Cr(VI), out of which, Cr(VI) is highly toxic and carcinogenic as per guidelines of World Health Organization [13]. Lead (Pb(II)) has also been listed as a potential carcinogenic source in 2021 report of toxic release inventory of United States Environmental Protection Agencies [14]. Therefore, heavy metals contamination of soil is emerging with higher rate due to modern life style, industrialization and urbanization. They move through the soil/water-plant habitat to human/animal and interfere with biological processes of their body. Unlike organic contaminants, the heavy metals do not degrade by itself therefore an effective cleanup remediation technology is necessary for their immobilization and minimization of their toxic effects [15]. Most of the remediation techniques for decontamination of heavy metals polluted soil/water currently available are very expensive, time consuming and environmentally not appropriate. The cost of remediation of contaminated sites through conventional methods will easily run into millions of dollars. India presently has a very limited experience on subject of remediation of contaminated sites. In recent decades, scientist and engineers have started to create cost effective technologies, which include use of green plant-based microorganism to clean the polluted areas [16]. Phytoremediation of contaminated sites by using green plants, a group of techniques (Fig. 1), is an economical and sustainable way to remediate these sites along with its aesthetic advantages and long-term applicability [17], [18].

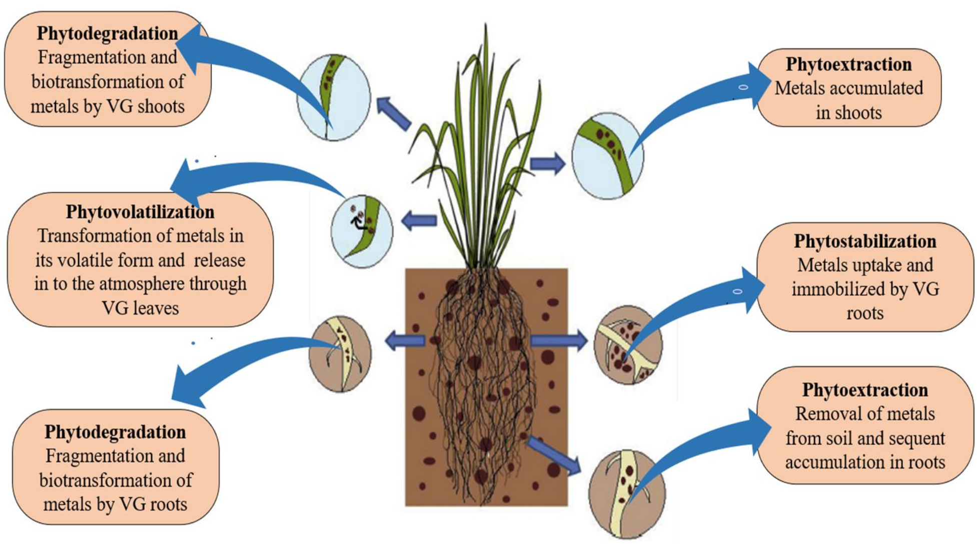

Fig. 1. Phytoremediation techniques through vetiver grass [19]

Phytoremediation avoids the excavation and transport of contaminated land, therefore, it reduces the economical consideration and risk of spreading the pollutants along with its potential to remediate the sites contaminated with different types of contaminants [20], [21]. Phytoremediation is a sustainable option for remediating contaminated environment in terms of cost, emission of greenhouse gases, oxides of nitrogen & sulphur, PM_{10}, energy consumption/expenditure, water and any kind of accidents/injury [22]. The phytoremediating plants should typically have characteristics such as (i) tolerant to high level of contaminants (ii) extract and accumulate higher amount of the contaminants within its tissues (iii) enhanced growth rate (iv) high biomass production, and (v) deep penetrating and fine structured root system [21]. *Chrysopogon zizanioides (L.) Roberty*, formerly *Vetiveria zizanioides (L.) Nash* (Vetiver grass),, belongs to Poaceae family, is a fast-growing and perennial species and is found effective for metal uptake from polluted soil due to its huge biomass production [23]. The massive, fine and complex structured root system of vetiver are rendering it to tolerate various adverse environmental conditions [24]. 6cm length of vetiver root had been reported as optimal length for its re-growth [25]. Vetiver grass has been used for soil and water conservation purposes and for steep slopes stabilization [26], [27]. It is originated and distributed mainly in India and tropical region of the world [28], [29]. In spite of many advantages, it has very limited applications for remediation of contaminated alluvial soil. The alluvial soil deposits are available in India and mainly cover the northern Gangetic plains, with availability up to 43% of total area. According to a report of central pollution and control board (CPCB) published in 2020, there are 280 contaminated sites in India, among which 43 are located in Uttar Pradesh state, which is highest among all Indian states [30]. Therefore, the objective of present study was to evaluate the growth parameters and extraction potential of vetiver grass in artificially contaminated alluvial soil, without using any organic or inorganic fertilizers.

II. STUDY LOCATION AND ITS GEOGRAPHY

This study has been carried out in the campus of Motilal Nehru National Institute of Technology Allahabad (25°44’N & 81°85’E), Prayagraj, Uttar Pradesh, India. Prayagraj is located in southern part of Uttar Pradesh, India with an altitude of 98m above MSL at the confluence of Ganga and Yamuna rivers. The area of Prayagraj is covered by a deep layer of alluvial soil, spread by the slow moving rivers of the Ganges system, which range from sandy to clayey loam. The monsoon season begins in early July and lasts till September every year. There was good precipitation at the end of this experiment, which was recorded between 250-300mm [31]. Fig. 2. shows the variation of the precipitation, temperature and humidity during entire period of this experimental study.

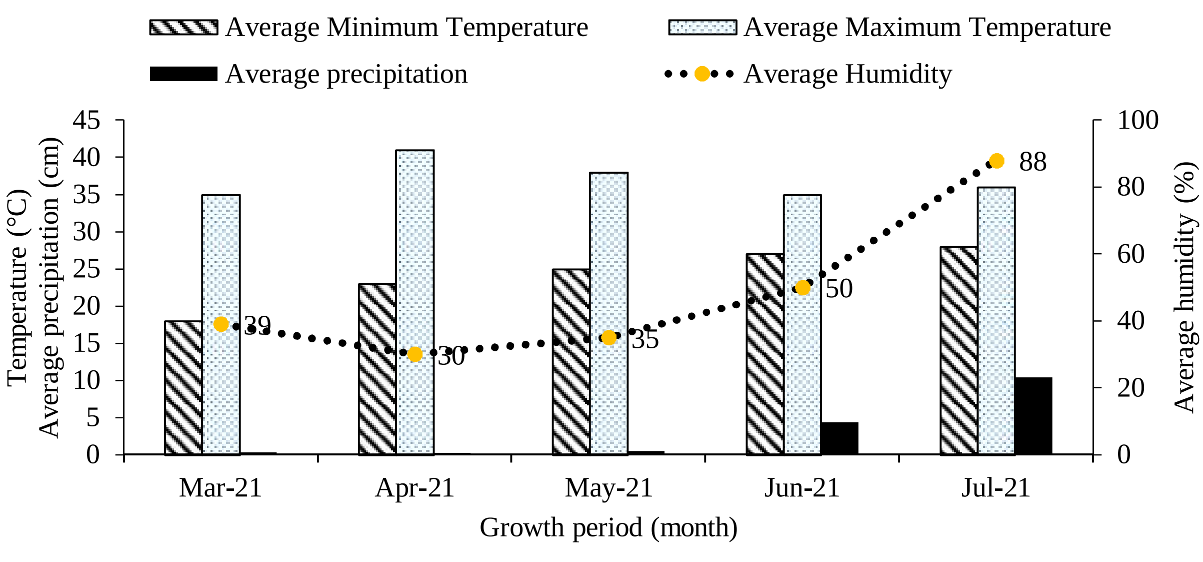

Fig. 2. Variation of temperature, humidity and precipitation during the experiment

III. MATERIALS AND METHODS

A. Materials

The virgin alluvial soil was collected from the campus laboratory garden of Motilal Nehru National Institute of technology Allahabad, Prayagraj at a depth 50 cm from top surface and passed through 10mm size of Indian standard sieve to remove stone, pebbles and geotechnical properties have been determined as per Indian standard guidelines (Table 3). The soil was classified as low compressible inorganic silt (ML) as per Indian Standard Soil Classification System (ISSCS).

The vetiver grass was bought from a nursery named Yuva Herbs, Chennai, Tamil Nadu, India (11°65'N & 79°75'E) and planted in the campus laboratory garden for their growth. For artificial contamination of soil, two heavy metal salts were used: potassium dichromate ($K_2Cr_2O_7$) for chromium and lead nitrate ($Pb(NO_3)_2$) for lead. A flow chart of materials and methods used for their characterization has been presented in Fig 3. The characterization of alluvial soil has been done to analyze the presence of heavy metals within soil before and after artificial contamination.

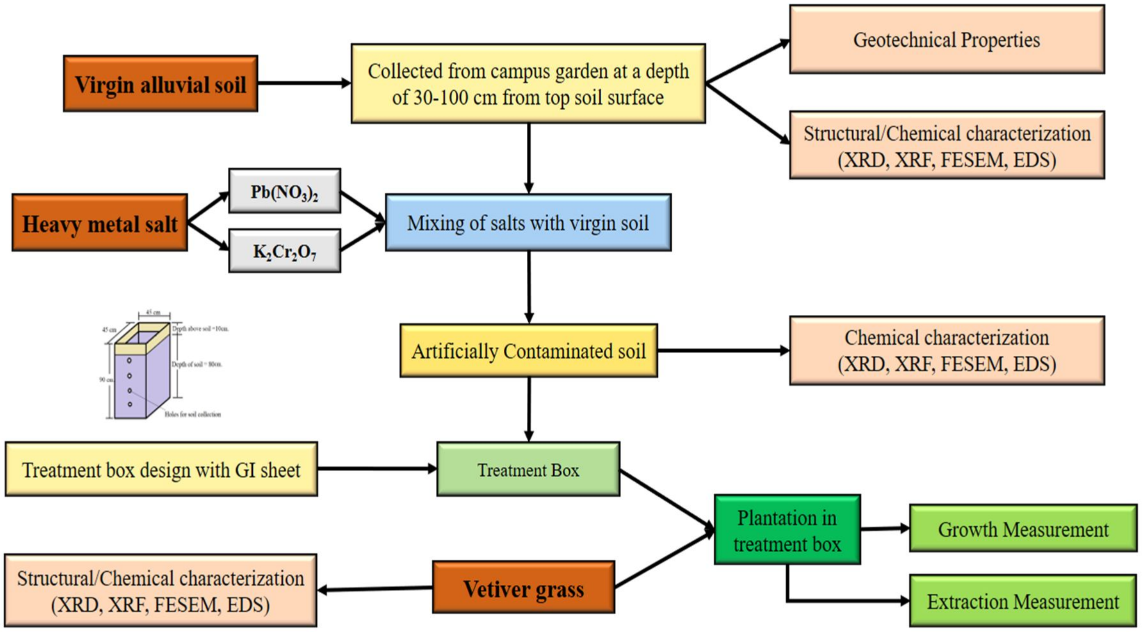

Fig. 3. Flow chart for material and their characterizations

The pH value of soil was found to be 7.65 ± 0.3. The field (water holding) capacity of garden soil was determined and found to be 43% [32].

Table 3. Geotechnical properties of virgin soil

Parameters (unit)	Values	IS specification
Grain size analysis		
Gravel (%)	4.20	
Sand (%)	6.60	IS 2720 (Part 4): 1985
Silt (%)	65.99	
Clay (%)	23.21	
Liquid limit (%)	34	
Plastic limit (%)	24	IS 2720 (Part 5): 1985
Plasticity index (%)	10	
Soil classification	ML	IS 1498: 1970
Specific gravity	2.66	IS 2720 (Part 3/sec.1): 1980
Field density(kN/m^3)	13.24	IS: 2720 (Part-29): 1975
Natural water content (%)	4.35	IS 2720 (Part-2): 1980
Organic matter (%)	0.11	IS 2720 (Part 22): 1972
Permeability coefficient (k) (m/s)	1.01×10^{-7}	IS 2720 (Part-17): 2002

The crystalline nature of virgin soil has been observed by X-Ray Diffraction (XRD) pattern (Fig. 4) which shows the presence of kaolin (K), illite (I), montmorillonite (M), quartz (Q), calcite (C), and bornite (B). The crystal system of quartz was found to be hexagonal, white in colour, with density 26.09 kN/m^3, for calcite, it was rhombohedral, colourless with density 26.59 kN/m^3, and for bornite, it was cubic with density 49.93 kN/m^3.

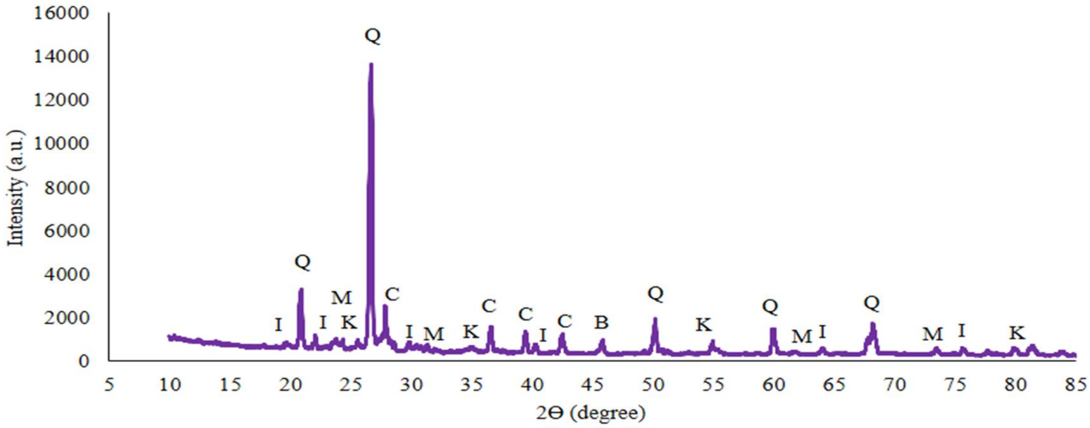

Fig. 4. XRD pattern of virgin soil

The Energy Dispersive X-ray (EDX) spectroscopy analysis of virgin soil had been carried out and oxides of Si, O, C, Al, Fe, K, Mg, Ti, and Na have been detected (Table 4).

Table 4. Chemical composition of virgin soil

S. No.	Oxides	% (w/w)
1	SiO_2	59.098
2	Al_2O_3	12.517
3	Fe_2O_3	3.939
4	K_2O	2.26
5	MgO	1.872
6	CaO	1.165
7	Na_2O	1.147
8	P_2O_5	0.194
9	TiO_2	0.722
10	NiO	0.003
11	MnO	0.09

IV. METHODOLOGY

The treatment boxes were designed to have sufficient base area and depth for the growth evaluation of the vetiver grass within 150 days of experiment. Based on literatures, it was decided to design Galvanized Iron (GI) sheet box with dimension 45cm x 45cm x 90cm, with 4 holes of diameter 2.54cm on one side for collecting the soil sample from different depths at different growth period. A total of 30 such boxes were designed for field study with three replicates for reproducibility of research data (Fig. 5).

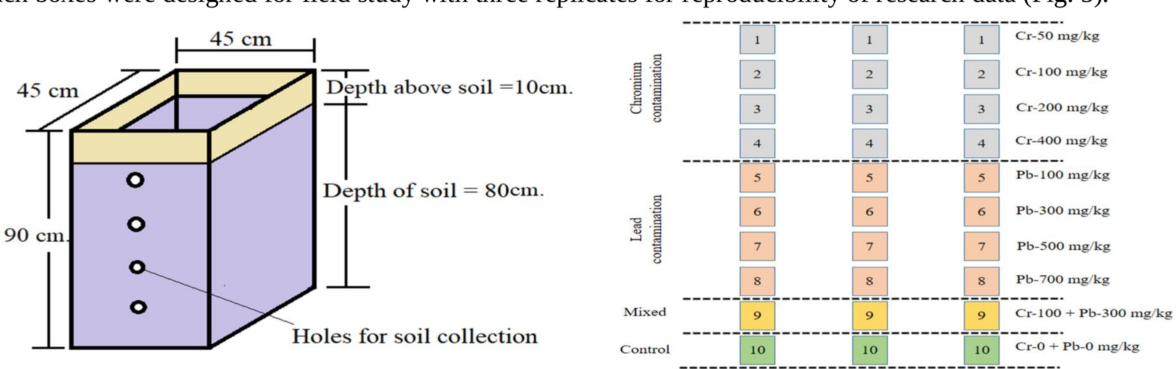

Fig. 5. Pictorial view of designed treatment box with three replicate

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For artificial contamination of soil, the salt of Lead ($(Pb(NO_3)_2)$) [33]–[37] and Chromium ($K_2Cr_2O_7$) [38]–[40] has been used. The concentrations of metal were finalized on the basis of toxicity of metals for plant growth, criteria provided in relevant literatures for Cr and Pb soil contamination [9]–[11], [41], [33]–[40]. A total of 10 GI treatment boxes were used, out of which four box for Cr and Pb contamination with four different concentration levels for each, one for mixed concentration and one for control (virgin soil) had been adopted and details have been presented in Table 5. The field study had been carried out with three sets and total 30 GI treatment boxes were used. Before keeping the soil in the box, the total weight of soil was divided into small amounts to ensure proper mixing of salts within the soil. The lead and chromium salts were added in the soil according to above concentration and thoroughly mixed by free hand to get homogenous contaminated soil. The contaminated soil was kept into the box, marked with number 01 to 10, and irrigated with tap water to ensure the field moisture within the soil in the box before plantation. The healthy plants of vetiver having 4-5 stems with overall diameter 4-5cm were cut down having 10cm root length and 20cm shoot length for plantation. The vetiver grass was planted in a square pattern of 3 x 3 on the top surface of the box to ensure the proper growth of root within the treatment box.

Table 5. Concentration of metals selected for artificial soil contamination

Treatment box	Metal salt used	Concentration used for artificial contamination	
		Chromium (Cr, mg/kg)	Lead (Pb, mg/kg)
1	Potassium Dichromate ($K_2Cr_2O_7$)	50	0
2		100	0
3		200	0
4		400	0
5	Lead Nitrate ($(Pb(NO_3)_2)$)	0	100
6		0	300
7		0	500
8		0	700
9	($K_2Cr_2O_7$) and ($(Pb(NO_3)_2)$)	100	300
10 (CU)	No metal salt	0	0

V. OBSERVATIONS AND RESULTS

After one week of plantation, the plant started to grow. Their growth was observed on an interval of one month and recorded. During the entire growth and development period, the vetiver grass continuously produced the tillers and the clump diameters were found to increase in all the treatment boxes.

A. *Growth of Vetiver Shoot*

Total biomass reported as dry weight per box, increased in all the treatment boxes over the entire duration of this experiment. As expected, the biomass production was lower in the contaminated soil than in control unit. When the plants started to grow, they generated leaves having dark green color. After one month of plantation, they started to grow with tillers made by the leaves. The length of the leaves was found to vary from 54 to 125cm, with thickness 0.6 - 0.7 cm. During this study, the length of shoot biomass was found to be 172 cm to 277cm with an average height of 111cm. Initially, during the first 60 -70 days, the color of leaves was observed to be dark green, the leaves were narrow and small in comparison to the control.

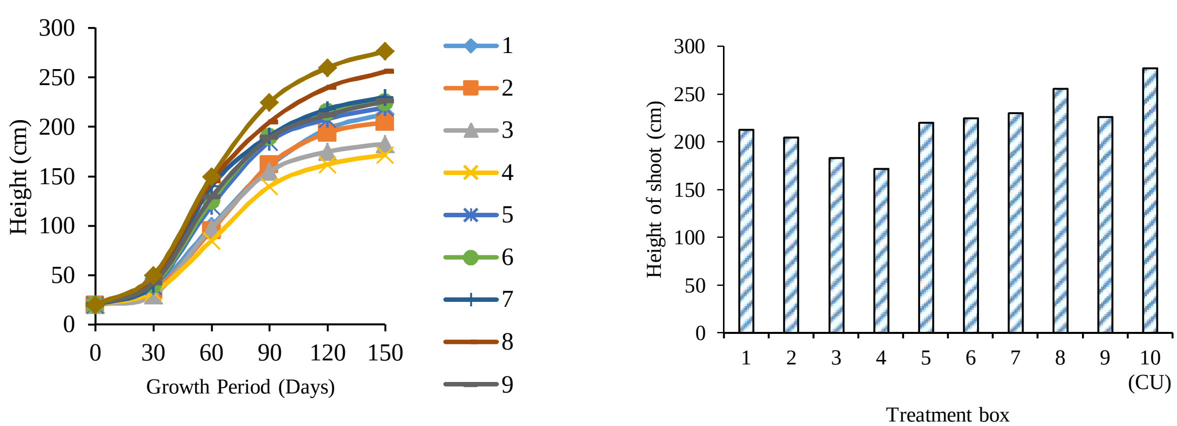

Fig. 6. (a) Growth of vetiver shoot during 150 days of growth period, (b) Maximum height of vetiver shoot after 150 days of growth period

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The color of leaves started to vary from dark green to light green with yellowish pigment in the Cr-200 mg/kg, Cr-400 mg/kg, Pb-100 mg/kg, Pb-300 mg/kg, Pb-500 mg/kg and with both Cr-100 + Pb-300 mg/kg. The growth of vetiver grass was found to be very healthy and thick with dark green leaves, high amount of shoot biomass and with maximum height in the Pb-700 mg/kg as compared to the control.

B. Growth of Stem in Vetiver

The experiment started with 4-5 stems in one clump, each having length of 20cm. Throughout the experimental duration, the number of tillers were increased by vegetative reproduction in all the treatment boxes. At the end of this experiment, the treatment box 1, 2, 3, 4, 5, 6, 7 and 9 having contaminated soil, showed almost equal stems growth (<40) but the treatment box 8 having Pb-700 mg/kg, produced the highest number of stems (68), whereas in virgin alluvial soil, the total stem growth was observed to be 70 tillers.

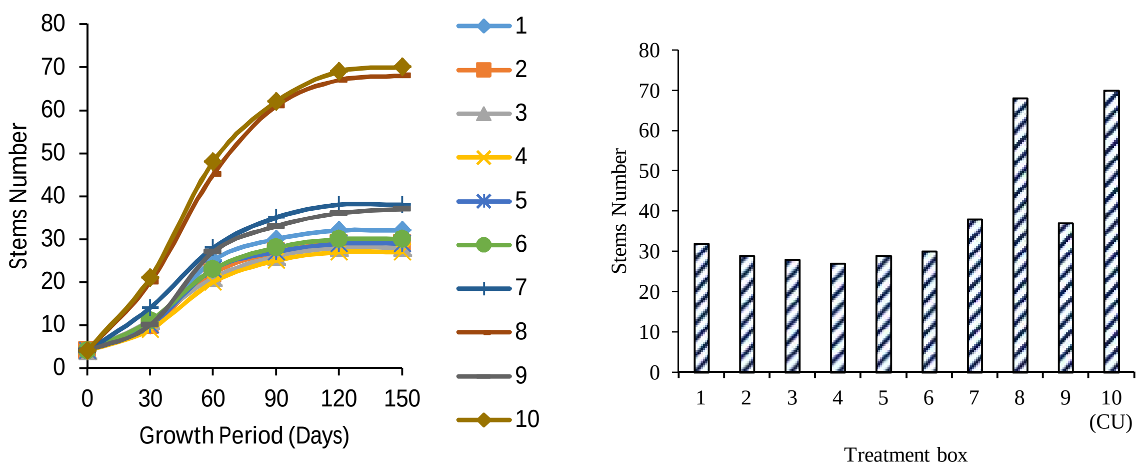

Fig. 7. (a) Growth of stems in vetiver during 150 days of growth period,
(b) Maximum number of stem produced after 150 days of growth period

C. Growth of vetiver leaves

During the entire duration of experiment, the treatment box 8 and control showed the highest growth rate of stems and stems with flowers and leaves. After the experiment, the treatment box with Pb-700 mg/kg showed dark green colored leaves with length 100 to 120cm, height of shoot 256cm, having 68 number of stems and 1109 number of leaves.

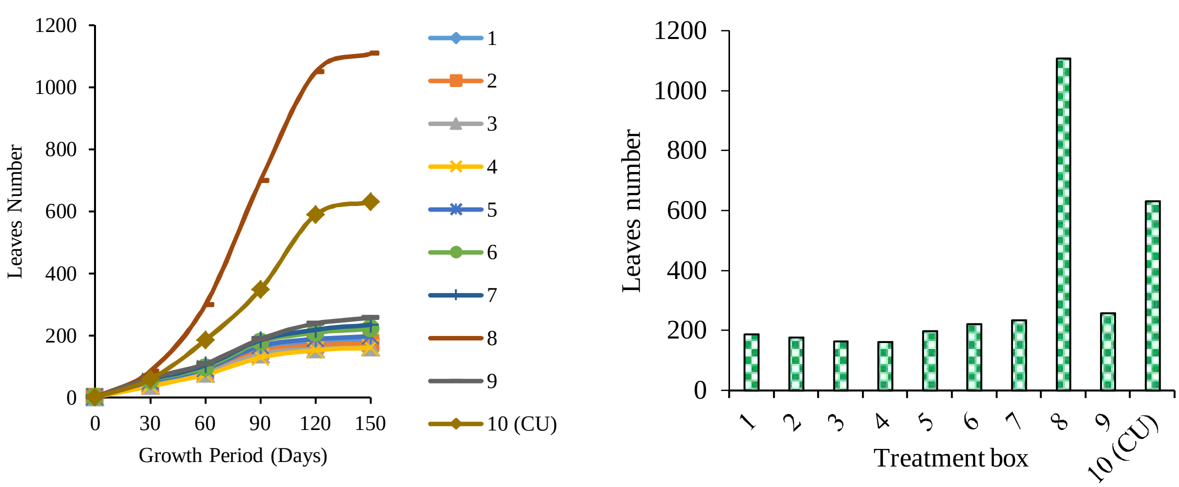

Fig. 8. (a) Growth curve of vetiver leaves during 150 days of plantation
(b) Maximum number of leaves after 150 days of growth period

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D. Growth of vetiver roots

The vetiver grass was planted initially with 10 cm root length. The thickness of the roots was found to vary between very fine to 4 mm diameter. The lengths of roots were between70cm and195cm with an average length of 105.3cm. The average green weight of root was measured to be 620.7 gram/box and the maximum root length was observed in virgin soil (control) with a length of 195cm.

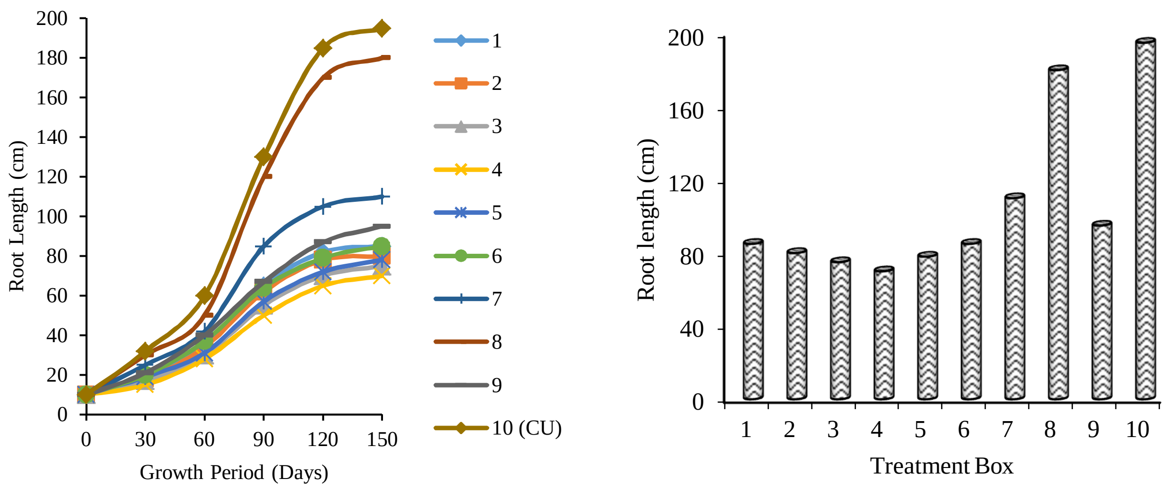

Fig. 9. (a) Growth curve for vetiver roots during 150 days of plantation
(b) Maximum root length of vetiver after 150 days of growth period

The roots with higher length in the contaminated soil made a round fibrous mesh with itself when it could not find the path to penetrate below.

E. Characteristics of vetiver roots

Vetiver has distinguished morphological features for its massive, finely structured root system with enhanced root hairs. The root characteristic was one of the most important reasons for studying of vetiver as a plant applied for phytoremediation.

Fig. 10. Roots of vetiver grass (a) Root in Pb-700 mg/kg box (b) Meshed, fine root

The roots of the vetiver below the top surface soil (0-4cm), were found to be very fine and meshed with each other creating a fine and fibrous net/wall like structure (Fig. 10) and bound the top soil very well, enabling the plant to retain water and moisture which creates a favorable environment to a diversity of micronutrients in the soil. The roots of the vetiver were very fine with an average diameter of 0.7mm and thickness of 3 to 4mm. The higher length feature of vetiver root system supports its survival under extreme drought conditions as it is able to utilize deep soil moisture. The roots system of vetiver has high power of penetration due to which it can penetrate through difficult soil, including asphaltic ground. The roots of the vetiver usually grow straight down without competing with neighboring vegetation; therefore, it is possible to establish the vetiver in agricultural lands. The horizontal spreading of root was in the range of 15–29cm with an average span of 23cm [42]. The average root growth measured was about 24cm wide. In the Cr-contaminated soil the growth of roots was considerably reduced in comparison to root growth in virgin soil whereas in Pb-contaminated soil it was found with enhanced growth of roots.

VI. EXTRACTION OF CR AND PB THROUGH VETIVER GRASS

After 150 days of growth period of vetiver grass in different treatment boxes, the vetiver grass were taken out from the box and washed with normal water to remove soil entrapped within roots. The remediated soil samples were collected for elemental analysis. The roots were separated from shoots and green weight of root and shoot for each box were determined and recorded, and after 7 days the air-dried weight of root and shoot were recorded. The root and shoot of each plants were then converted into small pieces with the aid of knife and then converted into powder form with the help of grinder and passed from 300µm IS sieve and stored. The XRF/EDX tests were carried to determine the elemental composition in contaminated soil, vetiver root, vetiver shoot and remediated soil. The phytoextraction ability of vetiver grass were calculated by translocation factor (TF) [43], and bioaccumulation factor (BF) [44] of vetiver root and shoot. Translocation of metals from root to shoot was measured by TF by using following equation:

$$\mathrm{TF} = \frac{Concentration\ of\ element_{Shoot}}{Concentration\ of\ element_{Root}} \tag{4.1}$$

The potential of the plants to extract metals in root and shoot with respect to metal concentration present in soil was measured by Bioaccumulation Factor (BF) as in following equations:

$$BF_{Shoot} = \frac{Concentration\ of\ element_{Shoot}}{Concentration\ of\ element_{Soil}} \tag{4.2}$$

$$BF_{Root} = \frac{Concentration\ of\ element_{Root}}{Concentration\ of\ element_{Soil}} \tag{4.3}$$

The removal efficiency [45] of Pb and Cr was calculated to determine the potential removal of contaminants from the contaminated alluvial soil mass by using following equation:

$$\text{Removal efficiency} = \frac{Concentration_{initial} - Concentration_{\ Final}}{Concentration_{Initial}} \text{ x } 100 \tag{4.4}$$

Table 6. Concentration of metals, bioaccumulation factor, translocation factor and removal efficiency of vetiver grass

Treatment box no. with added metal conc. in virgin soil	Concentrations of Cr and Pb (mg/kg)				Bioaccumulation Factor (BF)		Translocation Factor (TF)	Removal Efficiency of contaminants (%)		
	Contaminated Soil	Root	Shoot	Remediated Soil	Root	Shoot		Root	shoot	Total
1 (Cr-50)	100	505	58	27	5.05	0.58	0.17	505	58	73.00
2 (Cr-100)	149	840	152	38	5.64	0.52	0.19	563	52	74.49
3 (Cr-200)	247	700	148	69	2.83	0.6	0.29	283	60	72.06
4 (Cr-400)	448	480	226	91	1.07	0.51	0.21	107	51	79.69
5 (Pb-100)	134	90	36	38	0.67	0.27	0.44	67	27	71.64
6 (Pb-300)	334	296	67	102	0.89	0.2	0.23	89	20	69.46
7 (Pb-500)	552	265	104	162	0.48	0.19	0.25	48	19	70.65
8 (Pb-700)	730	567	158	203	0.78	0.22	0.28	78	22	72.19
9 (Cr-100) (Pb-300)	148	800	105	46	5.41	0.71	0.13	541	71	68.91
	334	207	21	101	0.62	0.06	0.1	62	06	69.76
10 (Cr-0) (Pb-0)	51	105	31	21	2.05	0.61	0.29	205	61	58.82
	36	21	9	15	0.58	0.25	0.42	58	25	58.33

VII. DISCUSSION

The green plants and grasses uptake not only macronutrients (Ca, K, Mg, N, P and S) to grow and complete their life cycle, but also uptake essential micronutrients (Cu, Fe, Mn, Mo, Ni and Zn) from soil. This mechanics seems to play role to extract heavy metals from soil. The presence of certain heavy metals may interfere their protein and photosynthesis, and may even cause membrane damage etc. Vetiver is a high-biomass plant due to C_4 photosynthesis efficiency which makes it capable of producing high biomass. Abundant growth of the roots of vetiver grass has been observed in the artificially contaminated alluvial soil. The subsurface plant tissues of vetiver grass grow vertically in the contaminated soil by creating a fine and extensive matrix for large surface area to absorb and extract the nutrient ions as well as heavy metals.

A. Effect of chromium (Cr(VI)) on vetiver growth

The presence of chromium within the soil affects the seed emergence in the plants, stunted plant growth and decreased dry matter production. With increasing Cr-concentration in soil, vetiver shows reduced germination percentage & reduced bud sprouting, decrease in root length & dry weight, decrease in root diameter & root hairs, reduction in vetiver shoot height and up to 50% reduction in yield with reduced number of flowers per plant. The reason behind this might be the toxic characteristics of chromium towards growth of vetiver, and the effect of potassium from K_2Cr_2O7 may not help in the growth of vetiver.

B. Effect of lead (Pb(II) on vetiver growth

A significant relationship was found between the different concentration levels of Pb-treatments in the analysis of growth of vetiver grass. By increasing the concentration levels of Pb, biomass growth of vetiver was found to be increasing with increased amount of biomass. The presence of Pb within the planting soil mass produces dark green leaves, stunted foliage, increased number of shoots. The number of tillers, height of biomass (shoot and root) was increased with higher concentration of lead and color of leaves were found to be vary from light to dark green with increased concentration. With increasing Pb-concentration, the vetiver shows enhanced germination percentage & bud sprouting, increase in root length & dry weight, increase in root diameter & root hairs, enhancement in vetiver shoot height and up to 110% enhancement in yield with respect to control with increased number of flowers per plant. The reason behind this might be the effect of nitrogen present in $(Pb(NO_3)_2)$, which may help the growth of biomass of vetiver grass as fertilizer.

C. Extraction potential of vetiver grass

Table 6 represents the accumulation, their translocation from root to above ground biomass and removal efficiency of vetiver grass. The accumulation of chromium was found to be more in the roots of the vetiver grass as compare to initial soil concentration, while in the Pb-contaminated soil, the extraction of Pb was more in roots but less than initial soil concentration [37], [38], [46], [47]. The reason of high accumulation of Cr in root of vetiver could be because Cr is immobilized in the vacuoles of the roots cell, which may be natural toxicity of the grass [38]. Very less amount (5-20%) of Cr and Pb were translocated to shoot from the amount absorbed by vetiver roots [47]. The removal efficiency of vetiver for Cr was found nearly 70-75% and for Pb it was found to be 65-70% [33]. The vetiver was found efficient to reduce the degree of contamination in remediated soil. The micro structural analysis of cross section of vetiver shoot and root was taken with the help of Nova NanoSEM-450 instrument, and given in Fig. 11.

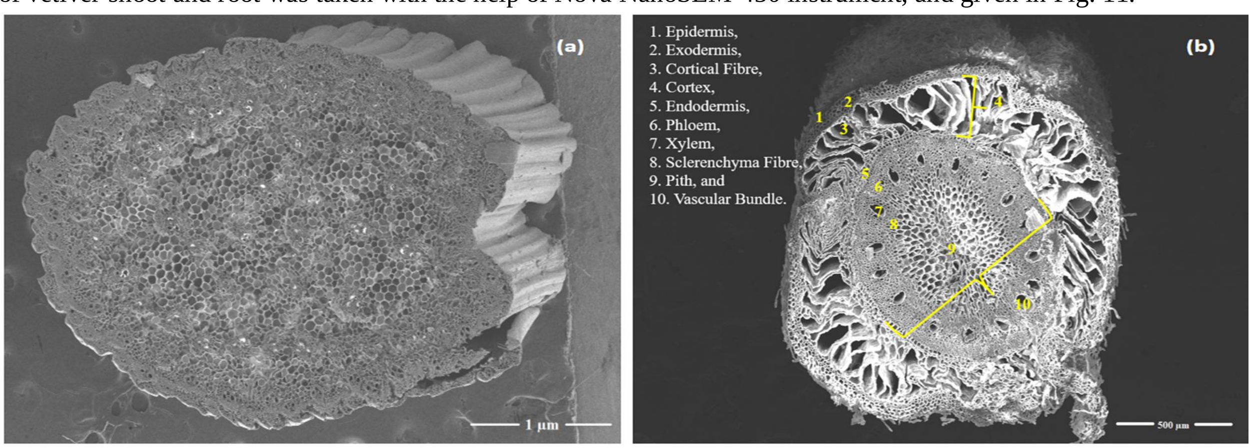

Fig. 11. FESEM micrographs of transverse cross-section of vetiver (a) shoot, & (b) root

The root cross section of vetiver had been found to have epidermis, exodermises, cortical fiber, cortex, endodermis, phloem, xylem, sclerenchyma fiber, pith, and vascular bundle. The central part of root system such as sclerenchyma and pith, was responsible for uptake and store the ingredients and metals within its vascular tissues [47]. The epidermis to the xylem parts of the root system help in the formation of uptake mechanism for its nutrients and metals [25], [47]. The metal ions sequestered inside the vacuoles may move forward into the stele and enter into the xylem stream through the root structures [48] and subsequently are translocated to the shoots and leaves through xylem vessels [49].

VIII. CONCLUSIONS

The biomass growth of vetiver (*Chrysopogon zizanioides (L.) Nash*) in alluvial soil has been investigated. Two heavy metal salt, $K_2Cr_2O_7$ and $Pb(NO_3)_2$, were used to contaminate the soil artificially. Based on the experimental study and discussion, following are the main conclusions.

1) Exponential growth of vetiver was observed during 150 days of plantation in artificially contaminated alluvial soil by Cr and Pb. The vetiver growth was observed highest between 60 to 90 days of plantation.
2) Chromium (Cr(VI)) shows the toxic effects on growth of vetiver with decreased survival rate, reduced mass production, lesser number of leaves, stems (tillers), stems with flower and the color of the leaves were varying from light green to light yellowish. In the treatment box number-4 (Cr-400 mg/kg), the vetiver was planted with 9 clumps out of which only six survived with reduced biomass growth.
3) Lead (Pb(II)) shows the positive effect on mass growth of vetiver. In all the Pb-treated soil, the vetiver had 100% survival rates. With increase in Pb-concentration in soil, the biomass growth was also enhanced in terms of number of leaves, stems and more stem with flowers. The color of leaves varied from light green to dark green. In the treatment box number-8 (Pb-700 mg/kg), highest mass growth was observed in terms of increased number of leaves, stems, stems with flowers, and height of shoot and roots. The overall growth of vetiver (shoot & root length, stems, leaves, weight of biomass) was found in decreasing order with increasing the concentration level of Cr, while in case of Pb the growth of vetiver was found to be increasing with increasing the concentration levels of Pb in soil.
4) In the combined contamination of Cr and Pb, enhanced growth of biomass was observed as compared to Cr-contaminated soil and reduced as compare to Pb-contaminated soil and virgin soil. The reduction was observed in terms of height (length) and mass of shoots and roots, and the number of leaves and stems. The growth was higher in case of Pb-contaminated soil as comparison to Cr-contaminated soil.
5) The huge biomass growth of vetiver in Pb-contaminated soil, makes it a potential candidate for stabilization of embankment constructed with erodible soils or soils which are prone to high erosion.
6) The structure of the roots in contaminated and uncontaminated alluvial soil was found to be massive, fibrous and with enhanced roots hairs. The average growth of roots in virgin soil was found to be approximately 1.3cm/day which was reduced in contaminated soil. The roots of the vetiver below the top soil surface (0-4cm), were found to be very fine and meshed with each other creating a fine and fibrous net/wall like structure and bound the top soil very well, enabling the plant to retain water and moisture. The massive and fibrous roots systems ensure high contact surfaces area for soil particles and contaminants resulting in efficient phytoremediation of contaminated soil. In the Cr-contaminated soil the growth of roots was considerably reduced in comparison to root growth in virgin soil whereas in Pb-contaminated soil, enhanced growth of roots was observed.
7) Cr and Pb both were found to accumulate more in the root of vetiver grass and a small amount, almost 15-20% of Cr and Pb in soil was translocated to shoot, above ground part of vetiver grass.
8) A comparison of vetiver biomass growth in Cr(VI) and Pb(II) contaminated soil indicates that the effects of $Pb(NO_3)_2$-contamination lead to better growth of vetiver however in $K_2Cr_2O_7$-contaminated soil, the growth was appreciably reduced due to higher accumulation of chromium within its root tissues. A little amount of Cr and Pb were translocated from vetiver root to shoot. For higher biomass growth in lead nitrate contaminated soil, the nitrogen associated with the salts might be in support of biomass growth as a fertilizer which was not observed in case of potassium dichromate due to presence of potassium. The results indicate that the vetiver grass is a potential candidate for rhizostabilization (phytoremediation) of Cr and Pb from contaminated soil. By the rhizostabilization of contaminants, vetiver grass was found effective to reduce the risk of contaminants to move downward (groundwater contamination) and minimize the associated human health risk due to toxicity of Cr and Pb through food chain. The higher biomass growth of vetiver in contaminated soil makes it a suitable candidate for erodible soil stabilization, carbon sequestration, and for higher renewable biomass energy production through pyrolysis.

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Supplementary Information Not applicable.

Data availability The data used to support the findings of this study are available from the corresponding author upon request.

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