Research Article

Effect of Heavymetals on Growth and Root Essential oil of *Vetiveria zizanioides* Grown under Jorhat Condition, Assam

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ABSTRACT

A first hand exploratory pot experiment under the agroclimatic condition of Jorhat, Assam, India has been carried out to understand the impact of heavy metals (Pb, Cu, Cd, Zn and Ni) on associated oil content, biomass and phytotoxic behavior of Vetiveria zizanioides L. On the measured dose of heavy metal application at 250, 500 and 1000mg/kg, observations were carried out up to 120 days (60,90 and 120days) after plantation of vetiver slips. Decrease in biomass content was observed, on application of higher dose (1000mg/kg), along with the decrease in oil yield without any phytotoxic implications on the crop at 120days. The impact of Pb and Cd on oil content is much more prominent than Cu, Ni and Zn. Valerenol, the major oil component was observed at 27% in controlled plant however, it decreases to 19% and 17% on application of Pb and Cd respectively. The maximum accumulation of heavy metal on root then shoot with low translocation ratio probably causes an effect on metabolism and thereby reduced the synthesis of oil content with stunted growth. Moreover, the higher rate of accumulation of heavy metals in vetiver grass justifies the effectiveness of use of the plant for phytoremediation under existing agroclimatic condition of Assam.

Keywords: *Vetiveria zizanioides*, Heavy metals, Phytoremediation, Essential oil, Valerenol.

INTRODUCTION

Heavy metals are important environmental pollutants and many of them are toxic even at very low concentration[1].The primary sources of the pollutants are the burning of fossil fuels, the mining and smelting of metaliferous ores, municipal wastes,fertilizers, pesticides and sewage[2]. Heavy metals accumulated in soil can affect flora, fauna and human living in the vicinity or downstream of the contaminated sites. Soil polluted with heavy metals is one of the world's major environmental problem posing significant risks to human health, often requiring soil remediation practices. Heavy metals are generally bound to organic and inorganic soil constituents or alternatively present as insoluble precipitates, a large proportion of metal contaminants are unavailable for root uptake by field grown plants[3]. The methods of increasing heavy metal contaminants bioavailability in soil and its transport to plant shoots are vital to the success of phytoremediation in the field[4]. Phytoremediation is considered as cost effective, plant based technology and expected to have significant economic, aesthetic and technical advantages over traditional engineering techniques[5,6,7]. Recently, many high – biomass plants have been used in phytoremediation studies. Among them the Brassicaceae (Mustard) family, to which most metal hyper-accumulator species belong, represents a potential and promising source of plants. The other plants like corn, peas, sunflowers, ragweeds and golden rods have also been successfully used in chelate assisted phytoextraction studies [8,9]. However root systems of these plants are mainly located in the top 5-20 cm layers of soil and very few root systems can penetrate to deeper soil layers. Therefore the root systems of these plants are unable to absorb the heavy metals that may possibly have leached in soil profiles[10] .

Vetiver (*Vetiveria zizanioides* L.) is a tall (1-2m), fast growing, perennial grass belonging to the family Poaceae, is well known since ancient times for its essential oil contents and soil binding properties. It is native to India and yield an essential oil which is mainly used as fixative in perfumery and for blending in cosmetic and soap industry. It has long (3-4m), massive and complex root system, which can penetrate to the deeper layers of soil and also can tolerate extreme soil condition[11,12,13,14]. However, the use of vetiver grass in phytoremediation technology is not widely recognized owing to the lack of detailed investigations on its capacity to absorb contaminants and practical field application. Now a day in Assam, the result of increased tempo of industrialization and population growth has catalyzed the enhancement of this problem. The growth of industry together with its effluent disposal system causes an acute environmental destabilization in the existing ecosystem. Therefore, the present approach has been undertaken to investigate the use of vetiver grass grown in Jorhat, Assam, in context to understand its ability to tolerate and accumulate toxic metals like Copper, Nickel, Cadmium, Lead and Zinc by its root and shoot and also their impact on yield and quality of oil.

MATERIALS AND METHODS

Planting materials of the *Vetiveria zizanioides* were collected from the Experimental Farm of CSIR -North East Institute of Science and Technology, Jorhat, Assam. Garden soils were collected by digging to a depth of about 0 to 30cm from surface of a farm area. Representative portions of soils were separated out by conning and quartering and utilized for physicochemical analysis. The collected soils were then sun dried and removed their foreign particles and mixed with sand (1:1). Fifty earthen tubs of size 50cmx30cm, sufficient to contain 8kg of sand and soil mixture were used. Soils were then amended with nitrate salt of lead, cadmium, copper, zinc, and nickel to have soil with, 250, 500, 1000 mg kg^{-1} (w/w) of the heavy metals. Nitrate salts were preferred over other forms of salt because of their high solubility. Calculated amount of metal salts were first mixed thoroughly with 8 kg of sand-soil mixture over a polythene sheet, and then transferred to the earthen tub. Each treatment was replicated thrice. Five pots were also used as control (excluding heavy metal). The experiment was carried out in Green house. After the tubs were filled with heavy metal amended soil, the tubs were allowed to stand for one week so that the soil can settle down properly. Three healthy and freshly collected slips (stem portion cut into 15cm and root portion 5cm) were planted in each tub and poured water regularly.

Observations were made with respect to growth and development of grass at an interval of 60, 90 and 120days respectively from the date of plantation. The root and shoot heights were also recorded for the respective periods. However, considering the growth the root and shoot biomass were recorded only at 90 days of intervals.

Isolation of volatile Oil

The vetiver roots were removed and collected from each of the pots. The attached soil contents were removed and washed thoroughly and dried in room temperature for two days by cutting into small pieces. Root samples were then weighed and subjected to hydrodistillation in a Clevenger type apparatus for 16 hours. The distilled oil was collected and dried over anhydrous sodium sulphate and analyzed by GC and GC/MS. The percentage of oil recorded was reported in w/v.

Analysis of oil quality

Analysis of oil was carried out by a combination of capillary GC and GC/MS using Shimadazu GC 17A and GC-MS- QP5000 instruments. The capillary column used for analysis was a CP-Sil 5CB fused silica column, 25mx0.25mm, film thickness 0.25m. The initial oven temperature was held at 35^0C for 25min, then programmed at 5^0C/min to 28^0C, Split ratio,50:1; carrier gas, Helium at a flow rate of $30cm^3$/s. The injector and detector (FID) temperature were maintained at 280^0C. For GC/MS analysis, a quadruple mass analyzer with electron ionization (EI) system was used. The mass spectra acquired was recorded in the range of 10-400Da, with a scanning rate of 4spectra/s. The transfer line temperature was kept at 280^0C and the helium flow rate was $40cm^3$/s.

The percentage composition of oil was calculated from electronic integration measurements using FID detection without response factor correction. Liner retention indices of the component were determined relative to n-alkanes. The constituents of the oil were identified by matching their mass spectra and retention indices using NIST library search facility available with the instrument.

Analysis of Heavy metals through Atomic Absorption Spectroscopy

Estimation of heavy metal was made by Atomic Absorption Spectroscopy. The level of heavy metal was estimated for different plant parts and tub soil. For shoots and roots, estimations were made for 60, 90 and 120days. The plant samples specially the roots and shoots were washed vigorously under running water and finally with distilled water to remove all unwanted matter specially the soil and sand. The samples were initially dried in sunshade and then cut into small pieces followed by drying in oven at 70^0C till constant weight was obtained. Dried plant samples were ground and placed in Stoppard plastic bottles[15]. Required Heavy metal (Pb, Ni, Co, Zn and Cd) contents were then estimated by AAS after proper digestion.

Digestion of Plant material and soil

Microwave Digestion System Model- START-D, Milestone, Italy, was used for digestion. Soils samples collected from control tubs, sun dried by spreading in shallow non-metallic trays disaggregated and then ground in a agate mortar until all of it passed through 0.25mm sieve. Standard methodology as advocated by Jackson [16] and Sakata [17] was followed for digestion and analysis of soil samples. 0.5 gm of dried sample was added with 10ml concentrated Nitric acid and digested in a Microwave Digester at a temperature 200^0C for 20mins at 15 bar pressure. After digestion samples were filtered through filter paper and filtrate volume make up to 100ml by distilled water. The samples were then ready for analysis of heavy metals by Atomic Absorption Spectroscopy.

For digestion of plant materials 0.5gm root or shoot samples were added with 7ml concentrated nitric acid and then added 1ml of hydrogen peroxide, digested for 20mins at 200^0C, 28 bar pressure. The digested samples were than filtered through filter paper and filtrate volume make up to 100ml with distilled water in a 100ml volumetric flask. Samples were then subjected for heavy metal analysis by Atomic Absorption Spectroscopy (Model Analyst-100 Perkin Elmer Instruments, USA).

RESULTS AND DISCUSSION

The untreated soil under agroclimatic condition of Jorhat, Assam, used for the present experiment evidenced physicochemical characteristics with eighty percent of soils as sandy–clay loam having pH 5.5-6.0; organic matter 0.86%, nitrogen, phosphorus and potassium as 0.075%, 0.0006% and 0.0034% respectively. Likewise the area witnessed average temperature 16.72^0C to 31.17^0C and rainfall 0.5mm to120.4mm.

In the pot experiment with treated soil, the vetiver grass grew well and there were no visual sign of phytotoxicity in any of the treatments. High concentrations of heavy metals in contaminated soils do not affect plant growth[18]. Sand soil mixture (1:1) was selected for this study because it is low in organic matter, nutrients and heavy metal contents thereof has negligible interference with the treatment applied. Estimated average heavy metal concentrations in control soils (untreated) were Pb 0.11, Cu 0.35, Zn 1.25, Ni 0.19 and Cd 0.03mg/kg respectively. Growth parameters of different heavy metal treated vetiver plants at different age level were observed and shown in Table 1.Maximum root and shoot heights were observed at 120days on application of 500mg/kg of heavy metals. But at higher dose of application (1000mg/kg) of heavy metals have retarded the growth rate, without any observable phytotoxic implications. Likewise, the shoot and root biomass yield were found to be higher at 250mg/kg application compared to others. (Table 2). Variation of oil composition depending upon the agro-climatic conditions at different region has been well established. The agroclimatic condition of Assam is unique and composition of essential oil content of vetiver is still not precisely known. Oil yield was varying from 0.15% to 0.3% at different treatments (Table 2) whereas the oil yield in the control plant was 0.5% only. There was no significant difference in the composition of oil in the vetiver plant between control and treatment. Oil percentage in the treated plants was low as compared to control which might be related to decrease in root biomass of the crop and also the impact of heavy metals on plant growth interferes the enzyme activities which, prevents the absorption of essential nutrients. The chemical composition of root essential oil was almost unchanged in all the applications of Zn, Cu and Ni but yield was quantitatively more or less affected by Pb and Cd (Table-3). The major constituents of vetiver root oil comprise khusimol, khusinol, khusimene, alpha–vetivone, beta-vetivone [19]. But under the agro climatic condition of Jorhat, Assam, valerenol was found as the major component [20]. Quality of vetiver oil is seemed to be closely related to the metabolism of its root system in relation to the agroclimatic condition under which it is grown. The components like alpha-vetivone (2.6%) and zizanoic acid (4.6%) were found more in Pb contaminated soil compared to control. Quantitatively beta – vetivone compound was very low in Pb (0.03-0.08%) and Cd (0.05-0.06%) contaminated soils. However, the other major compounds were not affected by Cu, Zn and Ni treated soil. The percentages of the compounds like khusimol, khusimene, dehydro-valerenol and valerenol compound were decreased in Cd (16%, 0.31%, 9%, 14%) and Pb (17%, 0.35%, 8.9%, 19%) respectively. Cu, Zn and Ni are the essential elements for the growth of plant and are involved in several metabolic processes[21] . Pb and Cd may interfere in metabolic processes including the synthesis of essential oil. Elevated level of Pb in plants have shown significant impact on oil content and sometimes altered the chemical composition also as reported in dill, peppermint and basil essential oil [22]. At higher contamination levels, all contaminants caused reduction of oil yields and associated components. However, the oil yields can be improved by prolonging cultivation time and iterating the planting/harvesting cycle[23]. Vetiver oil production is closely related to plant metabolism that is affected by temperature. The role of Pb in the enhancement of oil yield in vetiver has been reported [24]. However, in contrast the enhancement of oil yield in the present findings showed decrease of oil yield in the crops. Wide variations in the yield of oil in vetiver grass have been reported [25,26,27,28].

The concentration of heavy metals in shoot and roots of vetiver harvested at 120days was considerably higher than that harvested at 60 and 90 days intervals which may be attributed due to the effect of dilution [18]. The level of heavy metals accumulated in roots and shoots are presented in Table -4,fig-1 which showed that Cu, Zn and Ni are evenly distributed in the plant parts. However, the concentrations of Pb and Cd were found to be much higher in root compared to shoot. The low rate of translocation of heavy metals makes vetiver grass an ideal plant for phytostabilization. The decreases in translocation rate with the increasing concentration of heavy metals in soils have clearly attributed the restricted accumulation of heavy metals within the root system under existing physico-chemical condition of soil [29]. Maximum accumulation of heavy metal on root then shoot with low translocation ratio probably causes an effect on metabolism and thereby resulting stunted growth. Moreover, the higher rate of accumulation of heavy metals in vetiver grass justifies the effectiveness in use of this plant for phytoremediation under existing agroclimatic condition of Assam.

CONCLUSION

The Brahmaputra Valley region of Assam which contains abundant natural resources poses a major environmental concern to the associated soil system affecting crops and vegetation due to the increasing tempo of industrialization and unattended environmental consequences. The findings of the present investigation attributed insight on effective application of vetiver grass, a commercial crop which can withstand such concerns endowed with heavy metal pollutants. Thus the vetiver grass could be a potential source for phytoremediation of such soils.

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Table 1: Root and shoot length of *Vetiveria zizanioides* grown on different concentration of heavy metals at different age level

Treatm-ent mg/kg	Av. root length (cm)	Sd	Av. Shoot length (cm)	Sd	Av. Root length (cm)	Sd	Av. shoot length (cm)	Sd	Av. root Length (cm)	Sd	Av. shoot Length (cm)	Sd
	60 days				90 days				120days			
Control	38	±2	106	±5.13	59	±5.57	122	±2.65	63.7	±3.21	125	±5
Pb/250	36.6	±4.37	107.3	±6.65	46.3	±3.51	113.7	±11.15	53.7	±5.13	119	±3.06
500	42.6	±3.06	114	±5.57	48.7	±2.08	118	±7	54	±2	122	±7.55
1000	30.3	±6.03	78.3	±3.51	36	±3.61	87	±3	40.3	±2.52	89.3	±3.05
Cu/250	32.6	±2.51	96.3	±1.53	41.6	±3.51	116.7	±6.11	48	±2	125.3	±4.51
500	42.7	±2.52	100	±1.73	49.6	±1.53	110	±4.58	53	±4.36	130.3	±4.04
1000	26.3	±1.53	67.6	±2.52	37.6	±2.52	75.7	±5.13	41	±3.6	76.7	±1.53
Zn/250	47.3	±1.15	103.7	±6.65	53.3	±4.5	125.3	±6.43	58	±2	128.3	±5.03
500	55.3	±3.51	119.7	±1.53	60.7	±2.52	128.6	±2.08	61.7	±3.21	128.3	±2.52
1000	30.3	±2.08	86.7	±7.76	37.3	±2.08	90.3	±9.07	45	±2	92.3	±6.66
Ni/250	41	±2.65	107	±5.57	44	±3.46	116.3	±4.04	51	±4.6	121	±2.65
500	44.3	±3.51	108	±6.08	49	±1	116.3	±5.68	51	±1	118.7	±6.11
1000	35.6	±1.53	67.3	±4.51	39	±1	82	±9.13	41.3	±2.08	84.3	±1.53
Cd/250	33.3	±1.53	87	±3	37.7	±2.52	91.3	±2.52	27.7	±1.53	99.7	±1.53
500	29.7	±1.53	95.3	±7.73	41.3	±1.15	111	±2.64	45.3	±2.52	113.7	±5.13
1000	27	±1	71	±3	32	±2	69.3	±8.14	30	±2	50	±2

sd . standard deviation

Table 2: Root and Shoot Biomass and root essential oil of *Vetiveria zizanioides* with different treatment of Heavy Metal

Treatment	Average root weight (gm) 3plants/pot	Standard deviation	Average shoot Weight (gm) 3plants /pot	Standard deviation	Root essential oil (%)
Control	66.2	±1.07	216.2	±3.87	0.5
Pb/250	62.2	±2.46	198.5	±3.3	0.28
500	54.2	±0.9	196.4	±0.6	0.27
1000	33.7	±1.65	107.1	±5.86	0.2
Cd/250	47.8	±0.49	109.5	±1.44	0.24
500	31.4	±0.93	97.4	±1.53	0.2
1000	21.4	±0.9	43.1	±2.20	0.15
Zn/250	64.7	±1.48	195.5	±0.66	0.33
500	63.3	±0.85	193.7	±1.23	0.32
1000	47.4	±0.70	176.9	±1.59	0.25
Cu/250	63.7	±1.46	173.6	±3.02	0.32
500	46.4	±2.77	169	±0.8	0.28
1000	27.3	±2.40	72.8	±2.46	0.18
Ni/250	61.8	±1.42	191.8	±2.02	0.31
500	49.2	±1.93	160.3	±3.0	0.28
1000	33.5	±1.76	78	±0.73	0.24

Sd = standard deviation

Table 3:Composition of vetiver oil of different heavy metal treatment plant

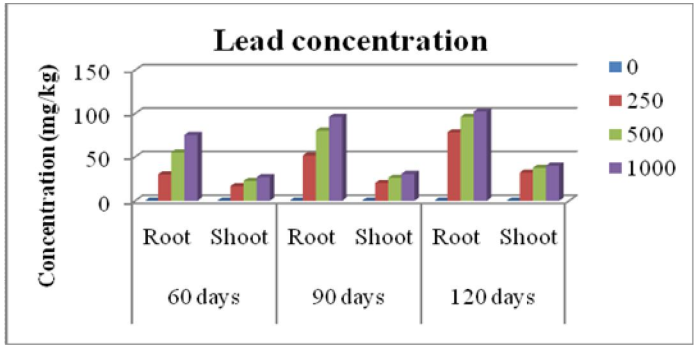
Compound	Control	Pb			Cu			Zn			Cd			Ni		
Treatment	0	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
Terpenin-4-ol	0.18	0.15	0.15	0.25	0.18	0.17	0.17	0.18	0.18	0.18	0.17	0.16	0.14	0.18	0.17	0.172
5-epiprezizane	0.48	0.30	0.32	0.20	0.40	0.42	0.38	0.43	0.43	0.43	0.25	0.23	0.35	0.38	0.37	0.38
Naphthalene	0.19	0.2	0.22	0.21	0.18	0.18	0.18	0.16	0.17	0.17	0.16	0.15	0.14	0.17	0.17	0.17
Khusimene	**0.61**	**0.33**	**0.35**	**0.34**	**0.56**	**0.52**	**0.49**	**0.46**	**0.46**	**0.48**	**0.21**	**0.29**	**0.31**	**0.52**	**0.49**	**0.48**
α – muurolene	0.29	0.24	0.29	0.28	0.28	0.29	0.29	0.28	0.28	0.28	0.14	0.11	0.08	0.24	0.22	0.25
α – amorphene	0.38	0.41	0.40	0.37	0.38	0.37	0.38	0.31	0.35	0.32	0.09	0.14	0.18	0.37	0.37	0.37
γ- amorphene	1.03	1.03	1.04	1.04	0.99	0.98	0.98	0.88	0.88	0.89	1.02	1.0	0.97	1.02	1.03	1.02
Calacorene	0.26	-	0.09	0.10	0.16	0.17	0.19	0.22	0.29	0.26	0.08	0.05	0.03	0.19	0.14	0.16
Valencene	0.35	0.04	0.02	0.02	0.23	0.24	0.27	0.20	0.27	0.30	0.30	0.31	0.30	0.31	0.35	0.35
Khusimone	**1.35**	**1.09**	**1.1**	**1.25**	**1.3**	**1.32**	**1.31**	**0.97**	**1.23**	**1.24**	**0.60**	**0.85**	**0.90**	**1.32**	**1.32**	**1.35**
α- longipinene	0.43	0.20	0.21	0.21	0.30	0.28	0.28	0.21	0.32	0.35	0.06	0.02	--	0.30	0.35	0.41
δ-selinene	0.27	0.08	0.03	0.01	0.06	0.07	0.07	0.08	0.10	0.12	0.04	0.01	0.01	0.05	0.02	0.01
δ –cadinene	2.93	2.1	2.5	2.32	2.8	2.86	2.9	2.4	2.46	2.49	2.12	2.13	2.16	0.27	2.76	2.82
Khusimol	**19.09**	**17.4**	**17.2**	**17.2**	**19.0**	**18.6**	**18.9**	**18.1**	**18.2**	**18.9**	**16.2**	**16.8**	**16.9**	**19.0**	**19.2**	**19.0**
Iso-khusinol	4.12	3.1	3.4	3.9	2.5	2.8	2.83	4.1	3.9	3.72	3.20	3.12	3.5	3.89	3.9	3.92
Epigizanol	5.73	5.1	5.26	5.4	5.2	5.31	5.32	4.7	4.85	4.90	4.90	4.98	5.2	5.1	5.3	5.36
β- epizizanol	2.39	1.4	1.33	1.34	2.1	2.11	2.10	2.1	2.28	2.86	2.13	2.26	2.34	2.24	2.31	2.33
Dehydrovaler enol	**11.12**	**8.67**	**8.94**	**8.9**	**9.6**	**9.94**	**9.7**	**11.0**	**11.05**	**11.0**	**10.2**	**9.43**	**9.51**	**9.92**	**10.9**	**11.2**
Valerenol	**27.49**	**19.8**	**18.2**	**18.4**	**26.1**	**26.8**	**25.8**	**26.9**	**27.0**	**26.5**	**14.8**	**14.6**	**14.6**	**26.5**	**26.8**	**26.4**
Nootkatone	2.25	1.0	0.9	1.1	1.8	1.85	1.92	2.1	2.14	2.17	0.80	0.82	0.82	2.1	2.12	2.13
Zizanoic acid	**3.53**	**4.60**	**4.58**	**4.6**	**2.9**	**2.93**	**2.95**	**3.1**	**3.26**	**3.28**	**1.16**	**1.17**	**2.16**	**3.3**	**3.34**	**3.10**
α – vetivone	**1.59**	**2.60**	**2.61**	**2.62**	**1.51**	**1.53**	**1.54**	**1.43**	**1.48**	**1.48**	**1.52**	**1.55**	**1.58**	**1.5**	**1.51**	**1.48**
δ – vetivone	**0.42**	**0.03**	**0.06**	**0.08**	**0.42**	**0.42**	**0.43**	**0.42**	**0.43**	**0.45**	**0.05**	**0.06**	**0.06**	**0.43**	**0.43**	**0.42**

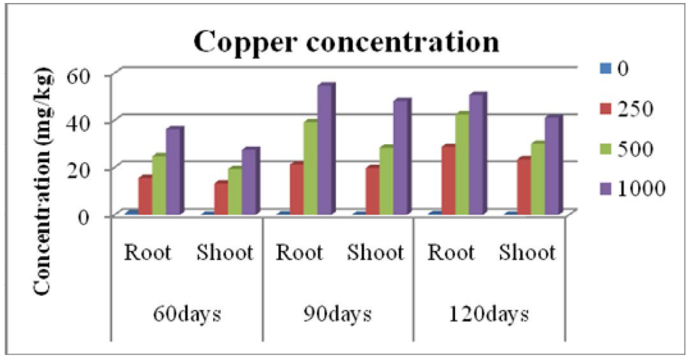
Table 4: Heavy metal concentration in root, shoot and translocation rate of *Vetiveria zzanioides* with different age level

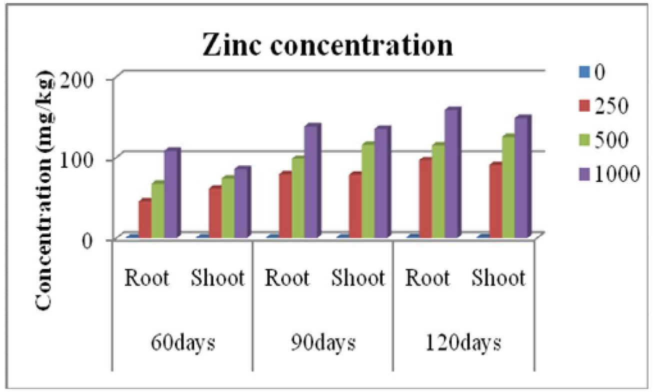
Treatment	Concen-tration mg/kg	60days			90days			120days		
		Root	Shoot	T.R.	Root	Shoot	T.R.	Root	Shoot	T.R.
Control	0	ND	ND		ND	ND		0.03	ND	
Pb	250	30.15	16.75	0.56	51.65	20.26	0.39	78.62	32.10	0.41
	500	55.12	22.63	0.41	80.72	26.17	0.32	96.25	37.69	0.39
	1000	75.65	26.95	0.36	96.25	30.69	0.32	102.35	40.13	0.39
Control	0	0.17	0.14	0.82	0.24	0.18	0.75	0.35	0.27	0.77
Cu	250	15.63	13.26	0.85	21.25	19.80	0.93	28.69	23.45	0.82
	500	24.75	19.35	0.8	39.25	28.32	0.72	42.50	30.13	0.7
	1000	36.31	27.41	0.8	54.66	48.13	0.9	50.67	41.15	0.81

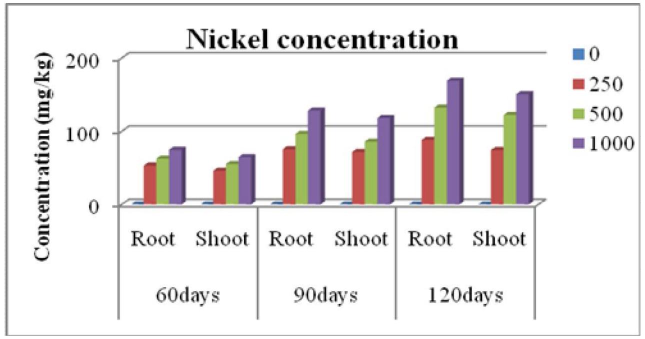
Control	0	0.95	0.86	0.91	0.98	0.93	0.95	1.22	1.05	0.86
Zn	250	45.63	61.25	1.34	78.85	78.20	1.0	96.50	90.21	1.0
	500	67.12	73.61	1.1	98.24	115.65	1.18	115.00	125.26	1.09
	1000	108.25	85.23	0.79	138.4	135.25	0.98	158.63	148.60	0.94
Control	0	0.09	0.07	0.78	0.11	0.09	0.82	0.14	0.2	0.86
Ni	250	53.16	46.13	1.0	75.21	71.56	0.95	87.81	74.15	0.84
	500	62.50	55.20	0.8	96.50	85.50	0.89	132.25	122.31	0.92
	1000	74.53	64.50	0.86	128.2	118.20	0.92	168.75	150.50	0.89
Control	0	ND	ND		ND	ND		ND	ND	
Cd	250	15.60	10.16	0.65	21.25	12.61	0.6	24.62	15.15	0.62
	500	20.25	12.75	0.6	28.24	15.30	0.54	30.13	16.35	0.54
	1000	32.14	19.23	0.6	41.25	21.39	0.52	54.66	24.75	0.45

ND – not detectable









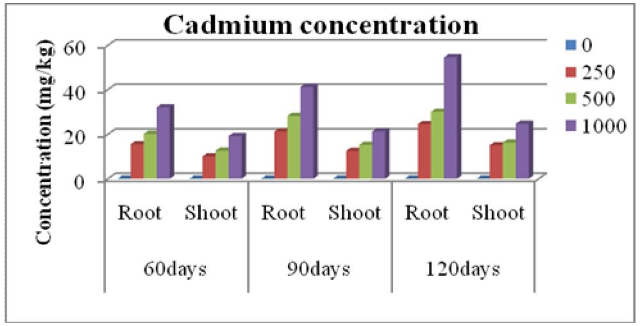


Fig. 2: Concentration of heavy metal on root/shoot of vetiver plant growth

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