Journal of
Environmental and Applied Bioresearch
Published online 20 June, 2016 (http://www.scienceresearchlibrary.com)

Vol. 04, No. 02, pp. 43-48
ISSN: 2454 7557

Research Article

Open Access

QUANTITATIVE DETERMINATION OF HEAVY METAL POLLUTION IN SOIL AND DIFFERENT PLANT PARTS OF *THEVETIA PERUVIANA* FOR PHYTOACCUMULATION DETERMINATION AT DIFFERENT LOCATIONS IN KURUKSHETRA CITY, HARYANA

***Neelu Sood[1] , Jasmine Kaur[2] and Gajendra Singh[3]**

[1]College of Pharmacy, Pt. B.D.S. University of Health Sciences, PGIMS, Rohtak-124 001; India
[1, 2] Department of Botany, Kurukshetra University, Kurukshetra-136 119; India
[3]College of Pharmacy, Pt. B.D.S. University of Health Sciences, PGIMS, Rohtak-124 001; India,

Received: 02 May, 2016 / Accepted : 29 May, 2016

Abstract

Considering the present scenario of urban environment pollution, there is a dire need for the improvement in approach of planting trees and other plant species. Ornamental and medicinal plants having pollution solving ability should be included in the plantation plan. Remediation of heavy metal contaminated sites using chemical or physical techniques is the most challenging task. Phytoremediation can be used as an alternative remediation and cleaning up technique. The aim of our research was to identify the research plant, *Thevetia peruviana* as a phytoremediation agent which may associate an important biomass production with an effective absorption and translocation of heavy metals. The amount of heavy metals present was determined using ICP-Auto Analyzer. Selected sites were evaluated for pollution level scale by applying Pearson Correlation test. Positive correlation between the degree of pollution and the concentration of heavy metals was found as the value of 'r' for Lead was 0.847 and for Chromium 'r' was equal to 0.860. Among the analyzed heavy metals, Pb had the highest concentrations in all the sampling sites while Ni had the lowest concentration whereas Cr was found in between the two. *Thevetia peruviana* plant showed maximal phytoremedial property with respect to Chromium followed by Lead.

Keywords: Heavy Metals, ICP-Auto Analyzer, Phytoremediation, Polluted Sites, *Thevetia peruviana*.

*Corresponding authors: neelusood61@yahoo.com

Introduction

The quality of life on the planet Earth is dependent on the quality of environment. Over exploitation of spaces, increasing number of automobiles and demographic pressure are some factors which are causing a threat to the environment. Earlier, the plantations in urban areas were done for their aesthetic value but now it is a necessity to mitigate urban environmental pollution (Anonymous, 1981; Pokhriyal *et al.,* 1986). All countries are concerned to handle the problems associated with contaminated sites. Cairney (1993) estimated number of such sites is significant and the problem is voluminous. Examination of the influence and transmittance of pollutants in the form of physiological effects in food chain from the plant-animal- man is an important topic for the research of biological amplification of trace elements (Menzel, 1965; Leiterer *et al*., 1997). There is a dire need to completely destroy the pollutants if possible or at least to transform them to harmless substances. Phytoremediation can prove a better option. Proper planning of planting trees i.e. selection of pollution-tolerant and dust-scavenging trees and shrubs should be done for bioremediation of contaminated land (Leiterer *et al*., 1997; Amusan *et al.,* 2005).

Phytoremediation is an emerging tool that uses plants to remove contaminants from soil and water (Kumar *et al.,* 1995). For the remediation of heavy metals from the soil, phytoextraction (also known as phytoaccumulation) should be used in which plants are employed to accumulate contaminants into the soil and aboveground shoots or leaves (Kumar *et al,* 2013).

The research plant i.e. *Thevetia peruviana* was identified at the Department of Botany, Kurukshetra University, Kurukshetra. The accredition number provided was KUK/BOT/IPS-19. The present study was carried out in order to find out if the research plant can help in phytoremediation. To achieve this, soil adjoining the plant, roots and leaves of the plant were analyzed by ICP-Auto Analyzer for the content of heavy metals present in the soil and plant parts. Due to its high detection sensitivity and simultaneous multi-electron capability, ICP-Auto Analyzer is particularly suitable for the trace analysis of a large number of elements in very low concentration (Leiterer *et al*, 1997). An analytical procedure that was accurate, precise and as effective as possible for the quantitative determination of Cr, Pb and Ni in soil and plant samples by means of ICP-Auto Analyzer was developed.

MATERIAL AND METHODS

Study Area

Division of sites:

Pertaining to the research work, contaminated soil area was divided into three sites i.e. non-polluted, moderately polluted and highly polluted site. The criterion for selecting different sites was the constant exposure of the particular site to automobile expulsion. The sites were categorized according to Table 1.

Fig 1: Non-polluted site, Moderately Polluted site and Highly polluted site(from left to right)

Table 1: Criteria for division of sites

Sites	Description	No of vehicles passing through that area per minute
S_1	Non-Polluted	1-2
S_2	ModeratelyPolluted	10-20
S_3	Highly Polluted	20-30

Investigation Procedures:

Sample Collection and Treatment:

- Soil Samples: 3 samples were collected from each site. To determine the heavy metal content in soil, 10g of 15 cm deep soil was collected from each sampling site and it was sieved to remove pebbles and debris. The samples were then air-dried and passed through 10 and 20 mesh sieves and preserved.
- Plant Samples: 3g of leaves and roots of the plant were collected from the respective sites. From these sites the prominent ground feeder species *Cynodon dactylon* (grass), growing in the vicinity of the plant (within 3 m) was also collected. The samples were then air-dried and processed. The samples were first digested in digestion mixture i.e. conc. HNO_3 and double distilled H_2O in the ratio of 5:1 for the removal of organic matter. The mixture was then centrifuged at 5000 rpm for 15 minutes. The supernatant was filtered with Whatmann filter paper no. 42 and then diluted.

Assay: The samples were analyzed by ICP-Auto Analyzer in Central Soil and Water Testing Laboratory, Karnal (Haryana). The content of Cr was observed at 283.5 nm, Pb at 220.3 nm and Ni at 221.6 nm.

Statistical Analysis: For statistical analysis, the samples were classified according to the sites and the value of Pb, Ni and Cr detected from the soil. In order to quantitatively analyze and confirm the relationship among different sites (S_1, S_2 and S_3) and heavy metal content, a Pearson’s correlation analysis was applied to dataset. The results were confirmed using Evan’s Table (Table 2).

Table 2: Relationship between strength and value of “r”

Value of ‘r’	Strength of relationship
0.00-0.19	Very weak
0.20-0.39	Weak
0.40-0.59	Moderate
0.60-0.79	Strong
0.80-1.0	Very strong

RESULTS AND DISCUSSION

With the increasing population, the demand for resources is increasing and the available land for cultivation is decreasing. Available land has to be divided in two areas-one for growing crop plants and the other for the growth of medicinal plants. Crop plants cannot be grown in polluted and heavy-metal loaded soil as it may harm the food chain but medicinal plants having the potential of phytoremediation can be grown in such soils. Heavy metal stress may help in the elicitation of phytoconstituents. One of the main advantage of phytoremediation is that of its relatively low cost compared to other remedial measures (Cunningham *et al.*, 1997; Waoo *et al.*, 2014). *Thevetia peruviana* is one of the many such plants which is of medicinal value and may prove as a phytoremediating plant for certain heavy metals.

Table 3: Comparison of heavy metal content in soil samples of different sites.

Sites	No of samples	pH	Content of heavy metal(ppm)		
			Pb	Ni	Cr
Non Polluted (S_1)	3	7.0-8.5	0.3500-0.8500	n	0.0250-0.0580
Moderately Polluted (S_2)	3	7.1-8.0	1.0150-1.6000	n	0.0750-0.0900
Less Polluted (S_3)	3	7.1-8.2	1.7500-1.9000	n	0.1700-0.1750
Average Value		7.5	0.1875		0.1150

n = negligible

The following trends were observed after quantitative determination of the samples:

Non-Polluted Site (S_1):

Soil = Pb > Cr > Ni

Roots = Cr > Pb > Ni

Leaves = Cr > Pb > Ni

Adjoining grass = Cr > Pb > Ni

Polluted Sites (S_2 and S_3):

Soil = Pb > Cr > Ni

Roots = Cr > Ni ≈ Pb

Leaves = Cr > Pb > Ni

Adjoining grass = Cr > Pb > Ni

It was assumed that polluted sites contain more amounts of heavy metals and it was confirmed by the results too and also verified by statistical analysis. Fig 2 shows that the amount of Pb and Cr in soil in different sites increases in the order- $S_1 < S_2 < S_3$. However, Ni was found in negligible amounts.

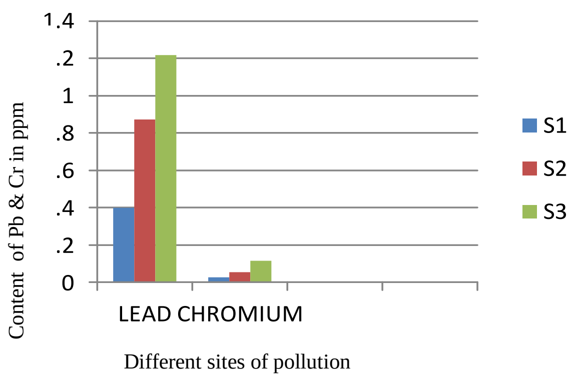


Fig 2: Variation in amount of Lead and Chromium present in soil of various sites.

Correlation Analysis:

The significant relationship between concentration of heavy metals and extent of pollution was further proved by performing Pearson Correlation Analysis (Tables 5 & 6). It is clear that the amount of heavy metals, Pb and Cr are significantly correlated with the extent of pollution (i.e. the number of vehicles passing through the selected sites). Both Chromium and Lead show positive correlation with the number of vehicles passing through that area in one minute. As the number of passing vehicles increased, pollution also increased as indicated by the values of Chromium and Lead in soil samples. Further, statistically it was also verified as the Pearson Correlation Coefficient 0.847 in case of Lead and 0.860 in case of Chromium. Both these results indicate a strong positive correlation (Tables 5 & 6)

Table 4: Concentration of Lead and Chromium in soil of different sites.

S. No .	Sites	No. of vehicle per min	Amount of Lead (ppm)	Amount of Chromium (ppm)
1	Non polluted	0	0.3576	0.0290
2	Non polluted	1	0.5164	0.0502
3	Non polluted	2	0.8406	0.0576
4	Moderately polluted	10	0.7540	0.0616
5	Moderately polluted	12	0.8507	0.0764
6	Moderately polluted	15	0.8977	0.0696
7	Highly polluted	20	0.9332	0.0796
8	Highly polluted	22	1.0168	0.1726
9	Highly polluted	27	1.7748	0.1780

Table 5: Pearson correlation among content of Lead and the no. of vehicles (extent of pollution)

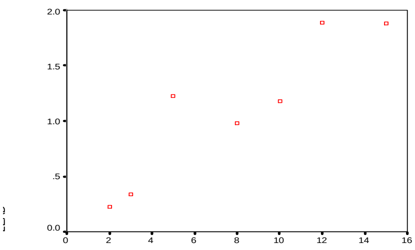
		LEAD	Number of vehicles
LEAD	Pearson Correlation	1	.847(**)
	Sig. (2-tailed)	.	.004
	N	9	9
NO. OF VEHICLES	Pearson Correlation	.847(**)	1
	Sig. (2-tailed)	.004	.
	N	9	9

**** Correlation is significant at the 0.01 level (2-tailed).**

Table 6: Pearson correlation among content of Chromium and the no. of vehicles (extent of pollution)

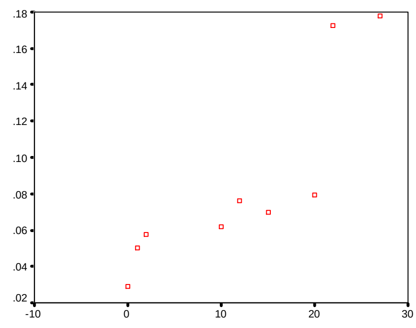
		CHROMIUM	Number of vehicles
CHROMIUM	Pearson Correlation	1	.860(**)
	Sig. (2-tailed)	.	.003
	N	9	9
NO. OF VEHICLES	Pearson Correlation	.860(**)	1
	Sig. (2-tailed)	.003	.
	N	9	9

** Correlation is significant at the 0.01 level (2-tailed).



Note: X axis indicates the number of vehicles passing from selected site and Y axis indicates the concentration of Lead (in ppm) in soil samples

Fig 3: Scatterplot showing relationship between amount of Lead and the number of vehicles(extent of pollution)



Note: X axis indicates the number of vehicles passing from selected site

Y axis indicates the concentration of Lead (in ppm) in soil samples

Fig 4: Scatterplot showing relationship between amount of Chromium and the number of vehicles(extent of pollution)

The findings from this study indicate that determination of heavy metal content in soil and prediction of its relationship with the polluted site by means of statistical methods can be a strong tool for monitoring the extent of pollution in agricultural and non-agricultural soils. The observed scatter plot (Figs 3 & 4)suggests a definite relationship between number of vehicles and concentration of heavy metals with larger number of vehicles (i.e. larger degree of pollution) tending to be associated with larger concentrations of heavy metals. In the present study, the value of ‘r’ (Pearson’s Correlation Coefficient) for the selected heavy metals is-

Pb	0.847
Cr	0.860
Ni	negligible

The value of ‘r’ ranges from $-1 \leq 0 \leq +1$. The closer the value is to +1 or -1, stronger is the linear correlation. So, there appears to be a positive correlation between the degree of pollution and the concentration of heavy metals.

We can also describe the strength of correlation using the guide that Evans (1996) suggests for above value of ‘r’ (Table 2). As the value of ‘r’ in the present study is between 0.80-1.0, it is confirmed from the table that the two variables have a very strong positive correlation. The value of ‘r’ was calculated using SPSS-11.5 for windows.

Status of Lead in soil and different plant parts:

Amount of Lead in polluted soil was greater than non-polluted soils. Lead accumulation showed an increasing trend from S_1 to S_3 Moreover, amount of Lead penetration in polluted sites followed the order-

Soil > Roots > Leaves ≈ Adjoining grass

This could be due to aerial pollution and automobile expulsion that soil absorbed more amount of Lead than other plant parts (Figs 2 & 5).

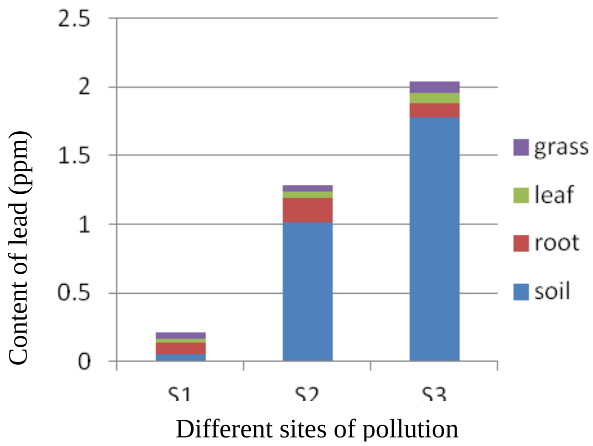


Fig 5: Amount of Lead in different plant parts from different sites.

Status of Nickel in soil and different plant parts:

As Nickel content in soil in all the sites resulted to be of negligible quantity, so it can be assumed that due to automobile expulsions, soil was not contaminated with Nickel and random low concentration of Ni and random low concentration of Ni in roots of *Thevetia peruviana* and grass species would have been because of the constitution of the two species. Therefore, it can be inferred that *Thevetia spp.* is not playing any significant role in bioremediation of the contaminated soil for Ni or the values were inadequate for some inference to be drawn (Fig 6).

Status of Chromium in soil and different plant parts:

From S_1 and S_2 sites, soil was slightly loaded with Cr but maximally polluted. S_3 (highly polluted site) was nearly four times more contaminated than S_1 (less polluted site). Grass accumulated maximal Cr in S_3 site as compared to S_1 and S_2 sites (nearly four times the S_1 site). *Thevetia* roots accumulated nearly two times more Cr in S_3 sites in comparison to S_1 and S_2 sites. *Thevetia* leaves also accumulated Cr in variable low concentrations in all the three sites. Cr accumulation followed an increasing trend from $S_3 < S_2 < S_1$ (Fig 7).

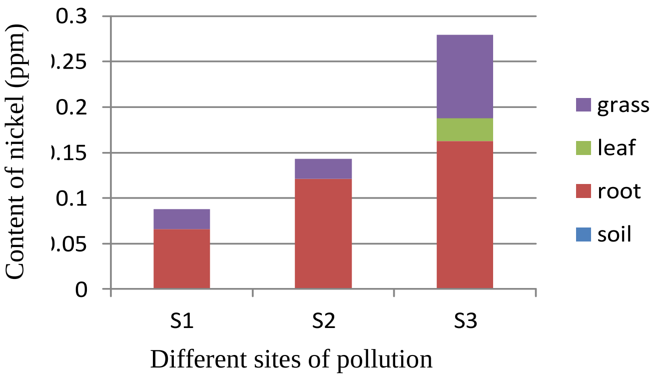


Fig 6: Amount of Nickel in different plant parts from different sites.

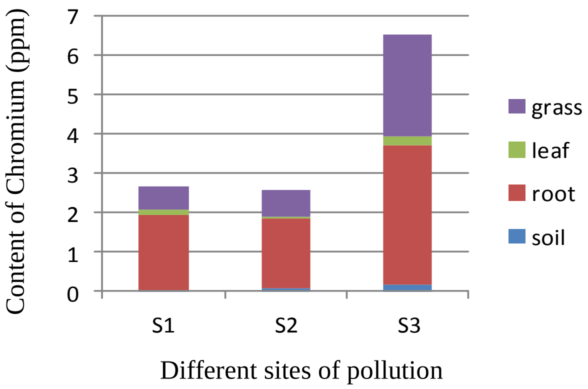


Fig 7: Amount of Chromium in different plant parts from different sites.

Among the three heavy metals undertaken Lead has been found to occur at highest concentration in all the sites. Pb tends to accumulate in the surface ground layer and its concentration decreases with soil depth (de Abreu *et al.*, 1998). It is easily taken up by the plants from the soil and is accumulated in different organs. Plants growing near highways are usually exposed to more Lead than other localities (Paivoke, 2002). Moreover, highway dividers were selected as most polluted site i.e S_3. The amount of Lead was found in the increasing order $S_1 < S_2 < S_3$ (Fig 2). Lane and Martin (1977) demonstrated that roots have an ability to take up significant quantities of Lead simultaneously restricting its translocation to above ground parts. In the research plant also, Lead was more localized to roots only (Fig 5).

The grass as groundcover species also absorbed Lead to a smaller extent than the select plant but still it may prove harmful as the ground flora is regularly chopped off and used as fodder. The grass growing at polluted sites should be burnt and disposed off properly so that Lead does not enter into the food chain. Though concentration of Lead was more in soil samples in the order of $S_3 > S_2 > S_1$, but Chromium absorption was preferred over Lead by both *Thevetia peruviana* and the adjoining grass species (*Cyanodon dactylon*). Our reports of higher accumulation of Pb in roots as compared to shoots were supported by Jones *et al*, 1973; Verma and Dubey, 2003; Seregin and Ivanov, 1997.

High amount of heavy metals triggers the formation of secondary metabolites. *Thevetia peruviana* can be grown at dividers as it is easy to grow, requires less amount of water and it has phytoremediation potential. So, *Thevetia peruviana* can be selected as a model system for phytoextraction. i.e. the use of plants to remove contaminants from soil by the accumulation of contaminants in plant tissue. The results were supported by Patidar *et al.* 2016 who suggested that *Thevetia neriifolia* is the resistant species which can tolerate vehicular pollution so it should be planted along heavily polluted roadside. *Thevetia peruviana* showed very significant trend in Cr uptake from soil and competitively more than grass growing on the adjoining land. Roots could extract Cr very efficiently. Hence, *Thevetia peruviana* can be selected as a model system for rhizoextraction.

Conclusion

The present study has revealed the ability to rejuvenate the contaminated environments effectively. Due to the abundance of natural plant resources, phytoremediation is the most powerful tool against industrial pollution. It is much more cost-effective method since plants self act as a bioreactor. In this concern, *Thevetia peruviana* is very important plant. As the result indicates, plants growing near highways i.e. highly polluted sites are usually exposed to more heavy metals than other localities. Presence of heavy metals triggers the production of secondary metabolites. This approach can prove useful in case of *Thevetia peruviana* which absorbs significant amount Lead and Chromium from affected soils thus helping in rhizoextraction. It can prove a more significant phytoremediating agent for Chromium loaded soils than Lead loaded soils as the absorption of Chromium is preferred by both *Thevetia peruviana* and the ground fodder. In this way, wastelands or polluted lands can be massively used for the production of medicinal plants secreting high amount of secondary metabolites. The grass as groundcover species also absorbed heavy metals especially Lead, to a smaller extent than the select plant but still it may prove harmful as the ground flora is regularly chopped off and used as fodder. The grass growing at polluted sites should be burnt and disposed off properly so that Lead does not enter into the food chain.

Acknowledgement:

Authors are thankful to Central Soil and Water Testing Laboratory, Karnal (Haryana) for providing the instrumentation facility for ICP.

References

Amusan AA, Ige DV, Olawale R (2005). Characteristics of soils and crops' uptake of metals in Municipal Waste Dump Sites in Nigeria. J. Hum. Ecol. 17:167-171.

Anonymous (1981). A Guide to tree planting. Parks and Recreation Department, Ministry of National Development, Singapore.

Cairney T(1993). Contaminated Land, Blackie, London p:4

Cunningham SD, Shann JR, Crowley DE and Anderson TA (1997). Phytoremediation of contaminated water and soil. In: E.Kruger et al. (ed.) Phytoremediation of soil and water allocainants. ACS Symp. Ser. 664. Am. Chem. Soc., Washington, DC. p. 2.

de Abreu CA (1998). Distribution of Lead in the soil profile evaluated by DTPA and Mehlich-3 solutions. Bragantia., 57: 185-192.

Jones LHP, Clement CR, Hopper MJ (1973). Lead uptake from solution by perennial ryegrass and its transport from roots to shoots. Plant Soil 38: 403-414.

Kumar PBAN, Dushenkov V, Motto H and Raskin I (1995). Phytoextraction: The use of plants to remove heavy metals from soils. Environ. Sci. Technol., 29: 1232-1238.

Kumar SR, Arumugam T, Anandakumar CR, Balakrishan S and Rajavel DS (2013). Use of Plant Species in Controlling Environmental Pollution- A Review. Bulletin of Environment, Pharmacology and Life Sciences. 2: 52-63.

Lane SD, Martin ES (1977). A histochemical investigation of Lead uptake in Raphanus sativus. New Phytol. 79: 281-286.

Leiterer M, Einax JW, Loser C and Vetter A (1997). Trace analysis of metals in plant samples with inductively coupled plasma-mass spectrometry. Fresenius J Anal Chem. 359: 423-426.

Menzel RG (1965). Soil-plant relationships of radioactive elements. Health Phys. 11: 1325-1332.

Paivoke AEA (2002). Soil Lead alters phytase activity and mineral nutrient balance of *Pisum sativum*. Environ. Exp. Bot. 48:61-73.

Pokhriyal TC and SubbaRao BK (1986). Role of forests in mitigating air pollution. Indian For., 112: 573-582 (1986).

Patidar S, Bafna A, Batham AR and Panwar K (2016). Impact of Urban Air Pollution on Photosynthetic pigment and Proline content of plants growing along the A.B.road Indore city, India. Int. J. Curr. Microbiol. App. Sci. 5(3): 107-113.

Seregin IV, Shipgun LK, Ivaniov VB (2004). Distribution and toxic effects of cadmium and Lead on maize roots. Russ. J. Plant Physiol. 51:525-533.

Verma S, Dubey RS (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164: 645-655.

Waoo AA, Khare S and Ganguly S (2014). Comparitive in-vitro Studies on Native Plant Species at Heavy Metal Polluted Soil Having Phytoremediation Potential. International Journal of Scientific Research in Environmental Sciences 2: 49-55.