Int.J.Curr.Microbiol.App.Sci (2018) *7*(9): 2261-2267

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 09 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.709.280

Estimation of Heavy Metal Accumulation in Seeds of *Leucaena leucocephala*, *Toona ciliata* and *Dalbergia sissoo* Collected from Different Sites in Baddi Barotiwala and Nalagarh (BBN) Industrial Area of Himachal Pradesh

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Keywords

Heavy metals, Seeds, Tree species, Icap Spectrophotometer

Article Info

Accepted:
16 August 2018
Available Online:
10 September 2018

A B S T R A C T

Heavy metal is a general technical term that applies to the group of metals and metalloids with density greater than 4±1 g cm^{-3} or elements having atomic weight more than 20. Heavy metals are considered toxic to living organisms and even trace metals that are considered essential for life can be toxic when present at excessive level, impair important biochemical process and also pose threat to human health, plant growth and animal life. The heavy metals, As, Cd, Cr, Ni and Pb were found accumulated in seeds collected from various sites. Higher concentrations of Pb were found in seeds of *L. leucocephala* collected from National Highway 21-A (2.650 mg/Kg), Baddi Barotiwala link road (1.35 mg/Kg) and Kalka Charnia link road (1.195 mg/Kg) against control (0.960 mg/Kg). Ni was recorded in higher quantities in seeds of *T. ciliata* and *D. sissoo* collected from roadside sites as compared to control.

Introduction

Heavy metal occurs as a natural constituent of earth's crust and is also released due to human activities. These also exist as ores in different chemical forms from which they are recovered as minerals (An *et al.,* 2004). Heavy metal is a general technical term that applies to the group of metals and metalloids with density greater than 4±1 g cm-3 or elements having atomic weight more than 20. Heavy metals have long biological half-lives and most of them are extremely toxic because of their solubility in water (Moya *et al.,* 1993). They are generally added to environment through automobile exhaust (Lagerwerff and Specht, 1970). Appreciable amount of gaseous and particulate heavy metals (Fe, Pb, Cr, Cd) are also added to the environment by industrial emission (Gupta *et al.,* 2011). Heavy metals are considered toxic to living organisms and even trace metals that are considered essential for life can be toxic when present at excessive level, impair important biochemical process

and also pose threat to human health, plant growth and animal life (Morrinson *et al.*, 1990). Deposition of lead on the vegetation growing along the road not only affects growth and germination but also causes a significant reduction in seed and fruit production of plants (Nassralla and Ali, 1985). Trees in cities are subjected to widespread pressure, which suppresses performance and shortens life span of trees (Gilbertson and Bradshaw, 1985).

Materials and Methods

The study was conducted in Baddi Barotiwala and Nalagarh (BBN) industrial area located in the foothills of Shiwalik hills. The study area lies on the border of Himachal Pradesh and Haryana states. Baddi, Barotiwala and Nalagarh area has emerged as a major industrial hub in Himachal Pradesh, situated in south west region of Solan and is about 45 km from Solan. The study area is situated at an altitude of 422-448 m above mean sea level and lies between 30^0 55' to 31^0 02' N latitude and 76^0 42' to 76^0 49' E longitude.

In order to study the vegetation distribution, amount of pollution and to select the study sites a survey was conducted in the region. Based on the survey four sites selected for study were as National Highway 21 A; in Baddi industrial area, Baddi-Barotiwala Link Road, Kalka-Charnia Link Road, Control; 200m away from road in Kalka-Charnia Link Road.

As control, the non-polluted agricultural area surrounding Kalka-Charnia link road was purposefully considered. Based on the vegetation distribution three commonly occurring tree species were selected in all the four sites for study. The tree species selected were *Toona ciliata* (Roem.) *Leucaena leucocephala* (Lamk.) and *Dalbergia sissoo* (Roxb.)

The seeds collected from selected sites of all the identified species were analysed for presence of heavy metals in the laboratory. The seed samples of different tree species were oven dried at temperature of 69°C for 10-15 days and then were powdered with the help of pestle and mortar. From each treatment 0.5 g of powder was measured and transferred to 100 ml conical flask separately. For its digestion, nitric acid and per chloric acid were mixed in the ratio of 3:1 and 10 ml of this mixture was added to each flask. The flasks were kept overnight for predigestion of samples. For digestion, flasks were placed on hot plate at temperature of 220°C for 2-4 hours. Digestion of samples was considered completed when transparent color appeared. Then these samples were kept in laboratory overnight and next day 10 ml of distilled water was added to each sample and filtered using whatman filter paper no. 1 and then samples were stored. After some days, total of 50 ml volume was made by adding distilled water in bottles and then again samples were filtered. The estimation of heavy metals was done by using Inductively Coupled Absorption Plasma Spectrophotometer (Model – icap 6300 DUO) of thermo scientific make.

Statistical analysis

The experiment was carried out at Department of Environmental Science, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan from April 2014 to April 2015. The experiment was planned as a completely randomized design with 5 replications.

Results and Discussion

Accumulation of heavy metals in the seeds of *L. leucocephala* collected from different sites

The quantities of As accumulated in the seeds of *L. leucocephala* collected from Kalka

Charnia link road, Baddi Barotiwala link road and National Highway 21-A were 0.018, 0.036 and 0.052 mg kg^{-1}, respectively, whereas, no As accumulation was found in seeds collected from control site. All the sites differed with each other with respect to As accumulation.

The accumulation of Cd in *L. leucocephala* seeds was 0.060, 0.050 and 0.036 mg kg^{-1} in National Highway 21-A, Baddi Barotiwala link road and Kalka Charnia link road, respectively, against 0.030 mg kg^{-1} in control. No significant differences in accumulation of Cd in seeds collected from different sites were observed.

The quantity of Cr accumulated in the seeds of *L. leucocephala* was lowest in control site (0.755 mg kg^{-1}) which was followed by Kalka Charnia link road and Baddi Barotiwala link road with 0.960 and 1.120 mg kg^{-1}, respectively, against highest accumulation of 1.125 mg kg^{-1} in National Highway 21-A (Table 1). The concentrations of Cr in seeds collected from Baddi Barotiwala link road were also at par with National Highway 21-A.

The highest accumulation of Ni in the *Leucaena* seeds were also recorded in in National Highway 21-A which was 0.705 mg kg^{-1} followed by Baddi Barotiwala link road and Kalka Charnia link road with 0.230 and 0.230 mg kg^{-1}, respectively, against 0.185 mg kg^{-1} of Ni accumulated in control site. Ni accumulation in *Leucaena* seeds was similar in Kalka Charnia link road and Baddi Barotiwala link road.

The accumulation of Pb in seeds collected from different sites was 2.650, 1.135 and 1.195 mg kg^{-1} in seeds collected from National Highway 21-A, Baddi Barotiwala link road and Kalka Charnia link road respectively, against 0.960 mg kg^{-1} in seeds collected from control site. Significantly highest concentration of Pb was recorded in seeds collected from National Highway 21-A.

Accumulation of heavy metals in the seeds of *T. ciliata* collected from different sites

The accumulation of As in the seeds of *T. ciliata* was significantly lowest in control site (0.005 mg kg^{-1}) followed by Kalka Charnia link road and Baddi Barotiwala link road with 0.035 and 0.050 mg kg^{-1}, respectively, against highest accumulation of 0.085 mg kg^{-1} in National Highway 21-A (Table 2). Accumulation of As in seeds collected from Kalka Charnia link road and Baddi Barotiwala link road was at par with each other. Significantly highest quantities of As were accumulated in seeds collected from National Highway 21-A.

The quantities of Cd accumulated in *T. ciliata* seeds were 0.315, 0.345 and 0.350 mg kg^{-1} in seeds collected from Kalka Charnia link road and Baddi Barotiwala link road and National Highway 21-A, respectively, against 0.275 mg kg^{-1} in control site. The accumulation of Cd in the seeds was highest in National Highway 21-A which was also at par with Baddi Barotiwala link road.

Similarly, the amount of Cr accumulated in seeds was also highest in National Highway 21-A (1.120 mg kg^{-1}), followed by Baddi Barotiwala link road and Kalka Charnia link road with 1.020 and 0.990 mg kg^{-1} of Cr accumulated against 0.850 mg kg^{-1} in control site. The concentrations of Cr in seeds collected from Baddi Barotiwala link road and Kalka Charnia link road were at par with each other.

The data on accumulation of Ni in *T. ciliata* seeds collected from different sites as presented in Table 2 revealed lowest concentration of Ni accumulated in seeds in control site i.e. 2.970 mg kg^{-1}.

Table.1 Accumulation of heavy metals in the seeds of *Leucaena leucocephala* Collected from different sites

Treatment	Heavy metals accumulated in seeds (mg kg^{-1})				
	As	Cd	Cr	Ni	Pb
National Highway 21-A	0.052	0.060	1.125	0.705	2.650
Baddi Barotiwala link road	0.036	0.050	1.120	0.230	1.135
Kalka Charnia link road	0.018	0.036	0.960	0.230	1.195
Control	0.000	0.030	0.755	0.185	0.960
$C.D_{(p=0.05)}$	0.015	N.S	0.010	0.011	0.019

Table.2 Accumulation of heavy metals in the seeds of *Toona ciliata* Collected from different sites

Treatment	Heavy metals accumulated in seeds (mg kg^{-1})				
	As	Cd	Cr	Ni	Pb
National Highway 21-A	0.085	0.350	1.120	3.200	2.155
Baddi Barotiwala link road	0.050	0.345	1.020	3.190	1.640
Kalka Charnia link road	0.035	0.315	0.990	3.080	1.620
Control	0.005	0.275	0.850	2.970	1.455
$C.D_{(p=0.05)}$	0.018	0.008	0.081	0.011	0.015

Table.3 Accumulation of heavy metals in the seeds of *Dalbergia sissoo* Collected from different sites

Treatment	Heavy metals accumulated in seeds (mg kg^{-1})				
	As	Cd	Cr	Ni	Pb
National Highway 21-A	0.200	0.230	1.455	3.045	1.930
Baddi Barotiwala link road	0.045	0.200	1.365	2.655	1.765
Kalka Charnia link road	0.015	0.125	1.255	2.360	1.580
Control	0.015	0.125	1.030	1.670	1.245
$C.D_{(p=0.05)}$	0.011	0.013	0.012	0.011	0.018

Table.4 Comparative concentrations of Pb and Cd in seeds of various tree species Collected from different sites

Sites	*L. leucocephala*		*T. ciliata*		*D. sissoo*	
	Pb	**Cd**	**Pb**	**Cd**	**Pb**	**Cd**
National Highway 21-A	2.650	0.060	2.155	0.350	1.930	0.230
Baddi Barotiwala link road	1.135	0.050	1.640	0.345	1.765	0.200
Kalka Charnia link road	1.195	0.036	1.620	0.315	1.580	0.125
Control	0.960	0.030	1.455	0.275	1.245	0.125
C.D $_{(p=0.05)}$	0.019	NS	0.015	0.008	0.018	0.013

Ni accumulated in Kalka Charnia link road, Baddi Barotiwala link road and National Highway 21-A was 3.080, 3.190 and 3.200 mg kg^{-1} respectively. The accumulation of Ni in seeds collected from National Highway 21-A and Baddi Barotiwala link road was at par with each other.

The accumulation of Pb in seeds collected from different sites was 2.155, 1.640 and 1.620 mg kg^{-1} in seeds collected from National Highway 21-A, Baddi Barotiwala link road and Kalka Charnia link road, respectively, against 1.455 mg kg^{-1} in seeds collected from control site. All the sites differed in accumulation of Pb in seeds with each other with highest accumulation in National Highway 21-A.

Accumulation of heavy metals in the seeds of *D. sissoo* collected from different sites

The amount of As accumulated in the seeds of *D. sissoo* was lowest in control site (0.015 mg kg^{-1}). As accumulated in seeds collected from Kalka Charnia link road, Baddi Barotiwala link road and National Highway 21-A was 0.015, 0.045 and 0.200, respectively (Table 3). The accumulation of As was highest in National Highway 21-A. As accumulated in Kalka Charnia link road was similar to control site.

The accumulation of Cd in *D. sissoo* seeds was 0.125, 0.200 and 0.230 mg kg^{-1} in Kalka Charnia link road, Baddi Barotiwala link road and National Highway 21-A respectively, against 0.125 mg kg^{-1} in control site. The accumulation of Cd was also highest in National Highway 21-A. The seeds collected from Kalka Charnia link road accumulated same quantity of Cd as in control site.

The accumulation of Cr in the seeds of *D. sissoo* was also highest in National Highway 21-A (1.455 mg kg^{-1}), followed by Baddi Barotiwala link road and Kalka Charnia link road with 1.365 and 1.255 mg kg^{-1}, respectively, against 1.030 mg kg^{-1} in control site (Table 3).

The data on accumulation of Ni in the seeds of *D. sissoo* collected from different sites as presented in Table 3 revealed lowest concentration of Ni in seeds collected from control i.e. 1.670 mg kg^{-1}.The seeds collected from National Highway 21-A, Baddi Barotiwala link road and Kalka Charnia link road accumulated i.e. 3.045, 2.655 and 2.360 mg kg^{-1} of Ni respectively. Ni accumulation in seeds differed significantly between sites.

The Pb accumulated in seeds of *D. sissoo* collected from different sites was 1.930, 1.765 and 1.580 mg kg^{-1} in seeds collected from

National Highway 21-A, Baddi Barotiwala link road and Kalka Charnia link road respectively, against 1.245 mg kg^{-1} in seeds collected from control site. The seeds collected from all roadside trees of *D. sissoo* accumulated higher quantities of Pb as compared to control with highest accumulation of Pb in National Highway 21-A (Table 4).

The lead (Pb) and Cadmium (Cd) are an important toxic pollutant added to the environment through auto-exhaust (Lagerwerff and Specht, 1970).

Higher concentrations of lead have been reported in plants growing in polluted areas (Asgar, 2004; Naveed *et al.,* 2010). Mahmood and Iqbal (1989) have reported that seeds of *A. lebbeck* and *D. sissoo* from polluted areas showed significant reductions in seed germination due to presence of lead.

In the studies also higher concentrations of lead have been observed in seeds of *L. leucocephala* collected from polluted areas than control (non-polluted areas). Highest lead accumulation in seeds was recorded in National Highway 21-A site, where reduction in seed germination was also highest.

These findings may validate the role of lead in affecting seed germination in roadside trees. The reductions in seed germination of *L. leucocephala* due to lead have also been reported by Iqbal *et al.,* 2001. Higher concentrations of nickel (Ni) have been recorded in seeds of *T. ciliata* and *D. sissoo* that may be attributed to industrial pollution as entire study area falls under important industrial hub of Himachal Pradesh.

Acknowledgements

The authors are thankful to Dr Y. S. Parmar University of Horticulture and Forestry Nauni, Solan, Department of Environmental Science for providing all the facilities to perform the study.

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How to cite this article:

Nitika Sharma and Mohinder Singh. 2018. Estimation of Heavy Metal Accumulation in Seeds of *Leucaena leucocephala*, *Toona ciliata* and *Dalbergia sissoo* Collected from Different Sites in Baddi Barotiwala and Nalagarh (BBN) Industrial Area of Himachal Pradesh. *Int.J.Curr.Microbiol.App.Sci.* 7(09): 2261-2267. doi: https://doi.org/10.20546/ijcmas.2018.709.280