Biochemical and Heavy Metal Analysis of selected Plant Species collected from Solid Waste Dumping Site, Rawalpindi

Master of Philosophy

In

Environmental Biology

BY

Sadia Rashid

DEPARTMENT OF PLANT SCIENCES,
FACULTY OF BIOLOGICAL SCIENCES,
QUAID-I-AZAM UNIVERSITY
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APPROVAL CERTIFICATE

This is to certify that the dissertation entitled **"Biochemical and Heavy Metal Analysis of selected Plant Species collected from Solid Waste Dumping Site , Rawalpindi."** submitted by **Sadia Rashid** is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan, as satisfying the dissertation requirement for the degree of M. Phil in Environmental Biology.

Supervisor: _____

Dr. Riffat Naseem Malik

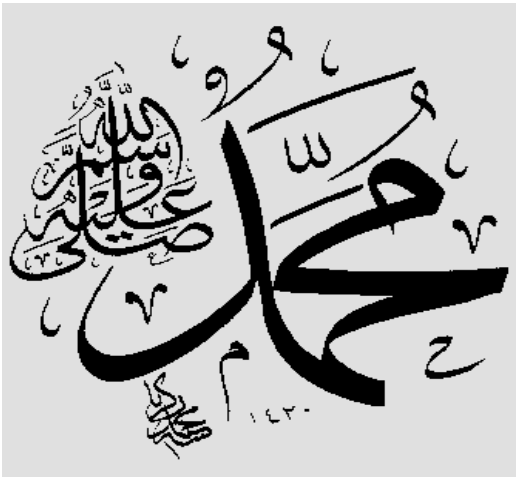
External Examiner: _____

Chairperson: _____

Prof. Dr. Asghari Bano

Date: _____

DEDICATED TO



My Most Beloved MOTHER

&

Affectionate FATHER

Who taught me the first word to speak,
first alphabet to write & first step to take,
Who are always in my mind & in my heart

&

To my most dearest, caring
and loving Siblings and friends

ACKNOWLEDGEMENT

All prayers for Almighty Allah, the most merciful and beneficent, without whose consent and consecration nothing would ever be imaginable. I am absolutely beholden by my Lord's generosity in this effort. Praises be to Holy Prophet for He is a beacon as I pace on in my life and work.

I feel great pleasure in expressing my ineffable thanks to my encouraging, inspirational and cool minded supervisor **Dr. Riffat Naseem Malik** for his sense of devotion, creativity, affectionate criticism and keen interest in my work; it was because of his inspiring guidance and dynamic cooperation during entire study program that I could complete this manuscript.

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I convey my thanks from the deepest of my heart to my very caring friends Tayebba Mustaq and Saba firdous. Words do not come out easy for me to mention the feelings of obligations towards my affectionate parents. My father proved the ocean of love, care for me in which I saturated myself. In life he is the man who tells me what life is, how to pass it, how to go through the problems, etiquettes and the every aspect of my life is incomplete without him. I am most earnestly appreciative to my mother for the strenuous efforts done by her in enabling me to join the higher ideals of life. I am grateful to my parents, brothers and sisters for their financial and moral support, patience and prayers they had made for my success. May **ALLAH ALMIGHTY** infuse me with the energy to fulfill their noble inspirations and expectations and further edify my competence.

Sadia Rashid

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LIST OF ABBREVIATIONS

Cr	Chromium
Ni	Nickel
Fe	Iron
Cu	Copper
Mn	Manganese
Zn	Zinc
˚C	Centigrade
Pb	Lead
Cd	Cadmium
Co	Cobalt
K	Potassium
Na	Sodium
Mg	Magnesium
Ca	Calcium
Chl	Chlorophyll
G	Gram
HCl	Hydro Chloric acid
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
HNO_3	Nitric acid
$HCLO_4$	Perchloric acid
TF	Transfer factor
Car	Carotenoids
BCF	Biological Concentration Factor
ROS	Reactive Oxygen Species
Can sat	*Cannabis sativa*
Cor did	*Coronopus didymus*

SW	Solid Waste
Tar off	*Taraxacum officinale*
Eup hel	*Euphorbia helioscopia*
Che alb	*Chenopodium album*
Mal cor	*Malvestrum coromandelianum*
Par hys	*Parthenium hysterophorus*
EU	European Union
WHO	World health organization

Abstract

The study was conducted to assess the degree of heavy metal contamination and impact of open solid waste dumping site, Rawalpindi on heavy metal contents of surface soils and wild medicinally important plants. Antioxidant enzymes such as Peroxidase (POD), Superoxide dismutase (SOD) and Catalase (CAT) were used as biomonitor of heavy metal contamination. Heavy metals (Fe, Cu, Ni, Cr, Mn, Co, Pb, Zn, Cd, Na, K, Ca, Mg) in soil and plant samples from solid waste dumping and control sites were analyzed by Flame Atomic Absorption Spectrophotometer (Varian FAAS-240). Heavy metal concentrations in solid waste soils were significantly higher as compared to control sites.

The heavy metals in soil from dumping site were in the order Co< Cd <Mn< Ni < Cr < Cu < Pb < Zn<Fe. Mean concentrations of Cd (4.7 mg/kg) and Ni (56.5 mg/kg) in soils from solid waste dumping sites exceeded permissible limits (50 mg/kg for Ni and 3.0 for Cd).

Cu concentration was highest in *M. coromandelianum* (23.55 mg/kg) and was above the permissible limits of 20 mg/kg. Relatively higher concentration of Ni in *T. officinale* (34.58 mg/kg), Cr in *C. album* (46.85 mg/kg), Zn in *P. hysterophorus* (24.73 mg/kg) and Pb in *C. sativa* leaves collected from dumping sites.

The enzymatic activities of POD, CAT, SOD and carotenoid contents, chlorophyll a, b, total chlorophyll (a+b) showed significant increase in plants collected from dumping sites. *T. officinale* showed greater BCF value in roots than leaves for Pb, Cr, Co, Cd, Cu and Fe indicating its suitability for phytostabilization. Higher TF (root to shoot) values of *P. hysterophorus* (1.52, 1.92, 1.58), *M. coromandelianum* (1.24, 2.13, 1.08) and *C. sativa* (1.03, 2.01, 1.14) for Pb, Cu and Zn at dumping sites highlighted its potential for phytoextraction.

Table 3.3. Heavy metal concentrations (mg/kg) in the collected leave and root samples from dumping and control site**.**

Metals	Parts	Control	Polluted	*P* values
Ni	**Leaves**	21.61±4.08	30.65±2.62	<0.001
	Roots	26.29±3.42	21.80±2.87	0.017
Cu	**Leaves**	14.24±3.26	19.61±2.81	0.004
	Roots	13.51±4.64	11.8±3.800	NS
Fe	**Leaves**	173.1±46.84	79.4±57.34	0.004
	Roots	133.0±68.68	177.0±42.2	NS
Cr	**Leaves**	23.7±5.05	43.18±4.23	<0.0001
	Roots	53.54±3.35	24.32±2.77	<0.0001
Mn	**Leaves**	13.74±1.93	13.63±0.36	NS
	Roots	13.96±0.59	13.03±0.61	0.010
Zn	**Leaves**	8.13±5.13	17.61±3.02	<0.001
	Roots	17.5±2.67	13.70±2.68	0.01
Cd	**Leaves**	0.46±0.08	0.534±0.06	NS
	Roots	0.69±0.10	0.455±0.07	<0.001
Co	**Leaves**	31.7±8.19	24.71±5.37	NS
	Roots	22.206±4.4	27.11±3.09	0.03
Pb	**Leaves**	18.74±6.42	28.09±3.64	0.003
	Roots	28.843±3.3	15.60±2.10	<0.0001
Mg	**Leaves**	208.9±81.2	190.1±53.4	<0.0001
	Roots	119.5±11.1	124.2±13.0	NS
Ca	**Leaves**	1056.8±192.8	1059.8±526.6	NS
	Roots	564.2±119.9	467.67±32.47	NS
Na	**Leaves**	443.7±44.9	1027.9±210.56	<0.0001
	Roots	1186.6±186.1	440.3±28.51	<0.0001
K	**Leaves**	413.6±205.3	550.6±369.1	NS
	Roots	284.7±164.0	248.04±103.5	NS

NS (not significant)

Table 3.4. Transfer factor (TF) of different metals from root to leaves in plant species from control and dumping site.

Species	Site	Ni	Cu	Fe	Cr	Mn	Zn	Cd	Co	Pb
Chenopodium album L.	Control	**1.14**	0.93	0.73	0.84	0.97	0.32	**1.09**	**1.53**	**1.02**
	Polluted	**1.08**	**1.42**	**1.20**	0.86	0.98	0.75	0.86	**1.24**	0.88
Coronopus didymus	Control	**1.09**	0.95	**1.42**	**1.00**	0.91	0.59	0.90	0.72	0.98
	Polluted	**1.20**	**1.43**	0.31	0.81	0.91	0.80	0.68	0.83	0.93
Cannabis sativa L.	Control	**1.19**	**1.41**	**1.06**	0.78	**1.05**	0.29	**1.36**	0.93	**1.13**
	Polluted	**1.41**	**2.13**	0.53	0.68	0.97	**1.14**	**1.06**	**1.24**	**1.03**
Euphorbia helioscopia	Control	0.88	**1.01**	0.84	0.74	**1.08**	0.49	0.88	**1.26**	**1.34**
	Polluted	**1.11**	**1.33**	0.46	0.76	0.94	**1.06**	0.69	**1.32**	0.97
Malvestrum coromandelianum	Control	0.66	**1.86**	0.79	**1.06**	0.82	0.53	**1.21**	0.92	0.93
	Polluted	**1.02**	**2.01**	0.40	0.66	0.96	**1.08**	0.61	**1.46**	**1.24**
Parthenium hysterophorus L.	Control	0.67	**1.92**	0.94	**1.36**	**1.38**	0.56	**1.13**	**1.51**	**2.41**
	Polluted	**1.39**	**1.52**	0.56	0.79	0.97	**1.58**	0.71	**1.02**	0.82
Taraxacum officinale	Control	**1.46**	0.83	**1.22**	**1.05**	**1.18**	**1.40**	0.70	**1.27**	0.82
	Polluted	**1.04**	**0.90**	0.82	0.85	**1.00**	0.82	0.87	0.76	0.78

Table 3.5. Biological concentration factor (BCF) of different metals of roots and leaves from dumping site.

Species	Parts	Ni	Cu	Fe	Cr	Mn	Zn	Cd	Co	Pb
Chenopodium album L.	**Leaves**	0.46	0.23	0.11	0.75	1.04	0.05	0.12	5.25	0.12
	Roots	0.43	0.16	0.09	0.87	1.06	0.06	0.14	4.21	0.13
Coronopus didymus	**Leaves**	0.46	0.19	0.1	0.82	1.15	0.06	0.15	4.52	0.12
	Roots	0.55	0.27	0.03	0.67	1.06	0.05	0.11	3.77	0.12
Cannabis sativa L.	**Leaves**	0.57	0.28	0.03	0.63	0.98	0.05	0.11	6.67	0.12
	Roots	0.4	0.13	0.06	0.92	1.01	0.04	0.1	5.35	0.12
Euphorbia helioscopia	**Leaves**	0.54	0.21	0.03	0.67	1.02	0.05	0.12	7.18	0.11
	Roots	0.49	0.16	0.07	0.88	1.06	0.04	0.17	5.44	0.11
Malvestrum coromandelianum	**Leaves**	0.53	0.33	0.03	0.62	1.00	0.05	0.09	4.79	0.12
	Roots	0.51	0.16	0.08	0.93	1.04	0.04	0.14	3.28	0.09
Parthenium hysterophorus L.	**Leaves**	0.57	0.29	0.03	0.65	1.00	0.07	0.10	6.55	0.14
	Roots	0.41	0.19	0.06	0.81	1.03	0.05	0.14	6.39	0.13
Taraxacum officinale	**Leaves**	0.61	0.31	0.14	0.72	1.05	0.05	0.13	4.46	0.11
	Roots	0.58	0.34	0.17	0.84	1.04	0.06	0.14	5.8	0.14

Table 3.6. Specie-specific concentrations of heavy metal in leaves and roots collected from dumping and control site.

Plants	Parts	Metals	Polluted	Control	*P*-value
Chenopodium album L.	**Leaves**	Cu	16.65 ±1.8	12.7 ± 2.3	0.03
		Cr	46.8 ±18	23.2 ± 6.2	0.04
		Zn	16.14 ±1.9	5.5 ± 3.3	0.001
	Roots	Cr	54.3±5.4	27.4±12.4	0.01
		Na	1207±592	437±99	0.04
Coronopus didymus	**Leaves**	Cu	19.2 ± 5.0	10.9 ± 2.6	0.02
		Mn	14.0 ± 1.2	12.4 ± 0.4	0.04
		K	347.6 ± 80	637.6 ±19	0.03
	Roots	Cr	51.5±10.74	22.7±7.9	<0.01
Cannabis sativa L.	**Leaves**	Zn	17.44±3.99	4.8±2.71	0.001
	Roots	Cr	40.1±15.2	20.2±5.4	0.002
Euphorbia helioscopia	**Leaves**	Fe	40.3±4.5	165.2±98	0.044
		Cr	42.1±14.3	18.5±3.52	0.01
		Zn	16.20±2.37	5.05±3.2	0.003
	Roots	Cr	54.9±3.1	24.7±7.30	<0.001
		Zn	15.27±0.62	10.2±2.08	<0.01
Malvestrum coromandelianum	**Leaves**	Cu	23.5±3.9	16.20±4.5	0.04
		Mn	13.3±0.62	11.4±0.16	0.001
	Roots	Cu	11.07±1.37	8.7±1.6	0.03
		Na	1193±614	396.7±51.7	0.041
		Cr	58 ±5.13	19.15±5.5	<0.0001
Parthenium hysterophorus L.	**Roots**	Cr	50.8±15.11	24.5±7.2	0.01
		Cd	0.66±0.20	0.31±0.12	0.02
		Pb	30±10.1	13.63±6.08	0.03
Taraxacum officinale	**Roots**	Cr	52.75±1.43	26.65±9.76	0.02

Table 3.7. Chlorophyll content (mg/g FW) in leaves from control and dumping site.

Plants	Sites	Chl a	Chl b	Car	Chl (a + b)	Chl a/b
Chenopodium album L.	**Control**	1.31±0.091	0.217±0.039	2.39±0.2	1.52	6.03
	Polluted	0.58±0.107	0.279±0.010	4.007±0.21	0.85	2.14
Coronopus dedymus	**Control**	0.90±0.081	0.304±0.064	2.58±0.25	1.20	2.96
	Polluted	1.20±0.136	0.364±0.031	4.00±0.34	1.56	3.33
Cannabis sativa L.	**Control**	1.82±0.162	0.456±0.098	4.52±0.21	2.27	3.99
	Polluted	1.99±0.22	0.560±0.044	4.89±0.19	2.55	3.55
Euphorbia helioscopia	**Control**	0.95±0.137	0.459±0.036	3.48±0.14	1.40	2.06
	Polluted	1.85±0.161	0.480±0.123	4.09±0.47	2.33	3.85
Malvestrum coronomandelianum	**Control**	0.81±0.361	0.545±0.075	4.41±0.894	1.35	1.48
	Polluted	2.18±0.145	0.641±±0.257	4.96±0.259	2.82	3.40
Parthenium hysterophorus L.	**Control**	1.47±0.214	0.607±0.171	4.15±0.11	2.07	2.42
	Polluted	2.65±0.183	0.686±0.248	4.28±0.15	3.33	3.89
Taraxacum officinale	**Control**	0.85±0.140	0.388±0.050	3.23±0.231	1.23	2.23
	Polluted	1.22±0.11	0.691±0.062	4.02±0.61	1.91	1.76

Table 3.8. Heavy Metal Concentration (mg/kg) in soil of control and dumping sites.

Sites	Cr	Ni	Fe	Cu	Co	Mn	Pb	Zn	Cd
Control	10.74±4.18	16.8±2.5	314.25±149.1	9.075±3.94	1.53±0.5	1.77±0.71	24.12±5.7	52.67±7.01	0.24±0.12
Polluted	62.525±13.5	56.51±23.07	1322.2±27.7	71.82±32.8	4.49±1.67	13.31±4.2	240.7±66.7	341.3±318.8	4.7±2.53

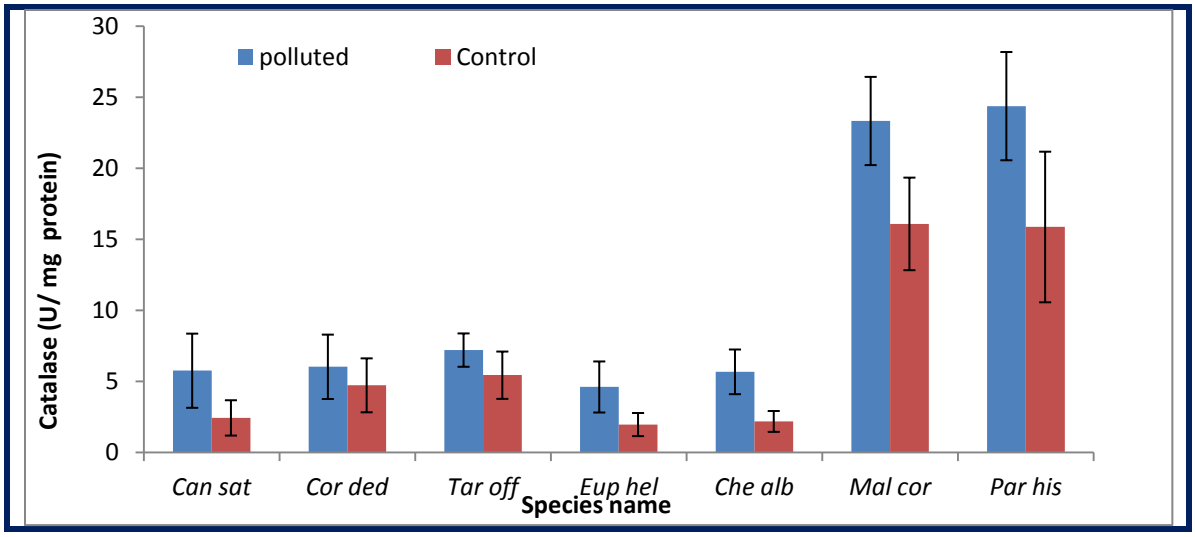

Fig. 3.2. Effect of different metals on CAT activity in leaves of different plant species from control and dumping site

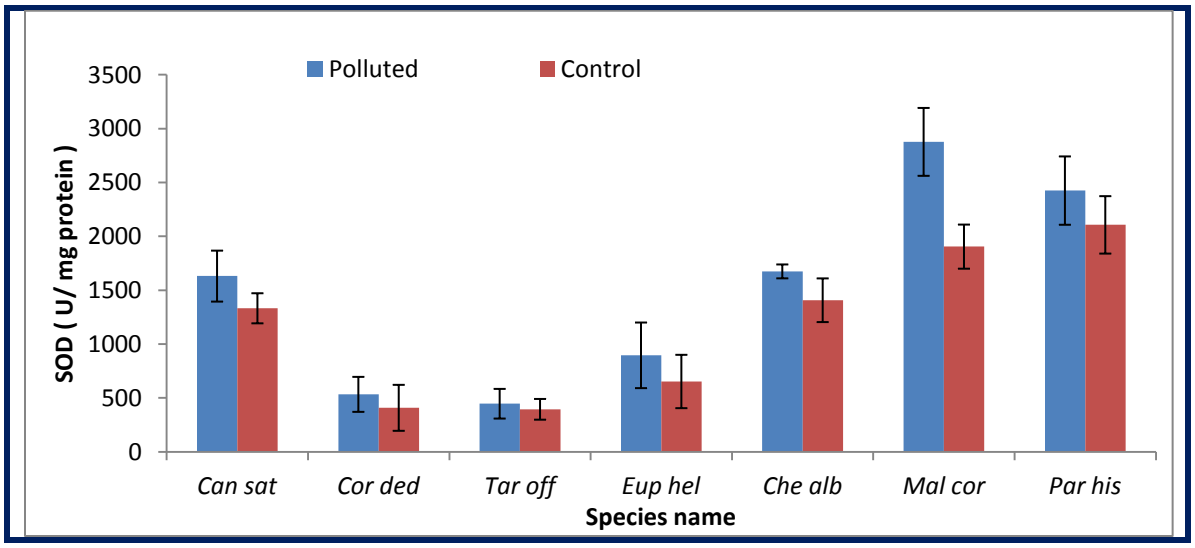

Fig. 3.3. Effect of different metals on SOD activity in leaves of different plant species from control and dumping site

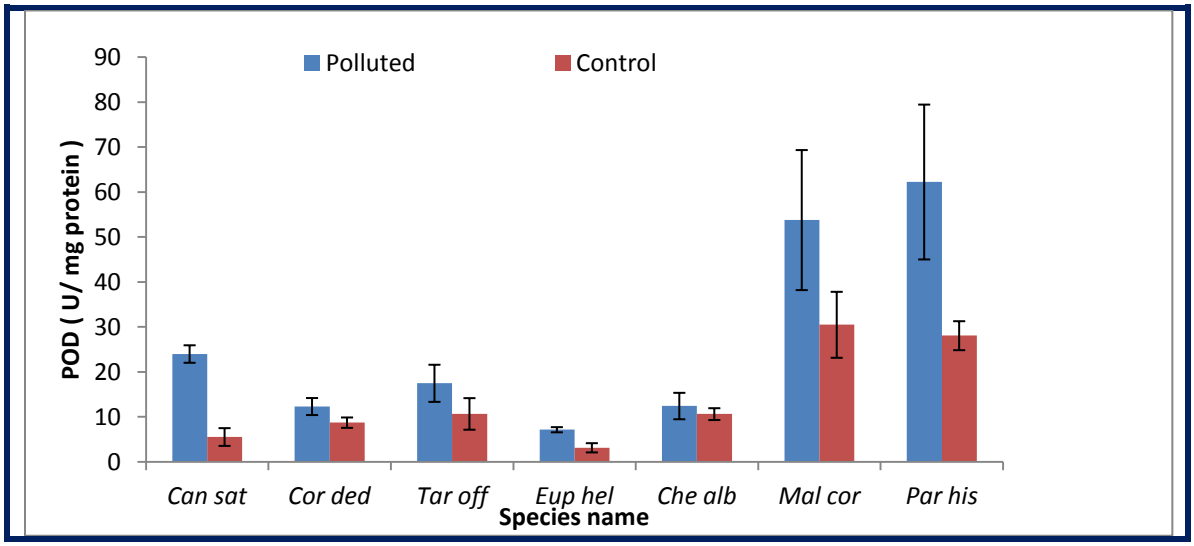

Fig.**3.4**. Effect of different metals on POD activity in leaves of different plant species from control and dumping site

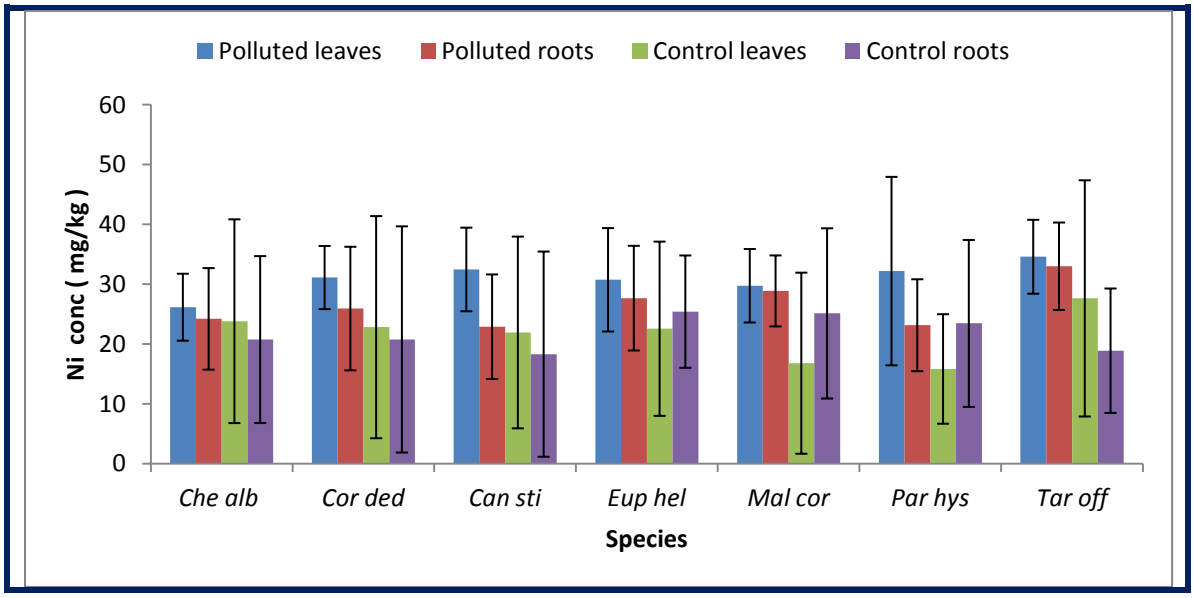

Fig. 3.5. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Ni.

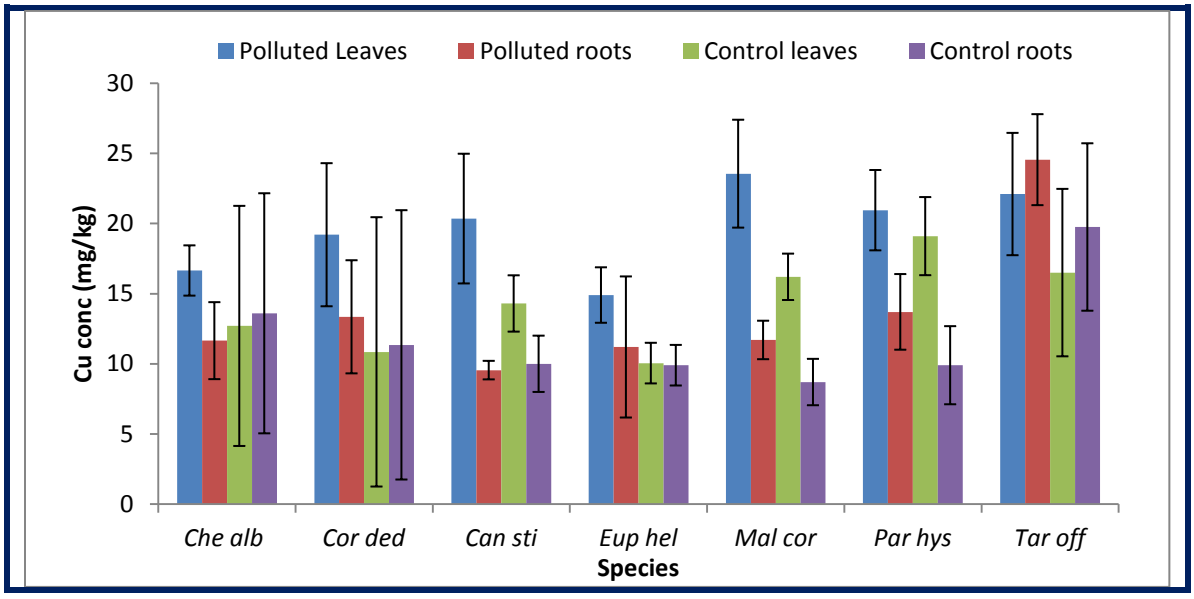

Fig. 3.6. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Cu.

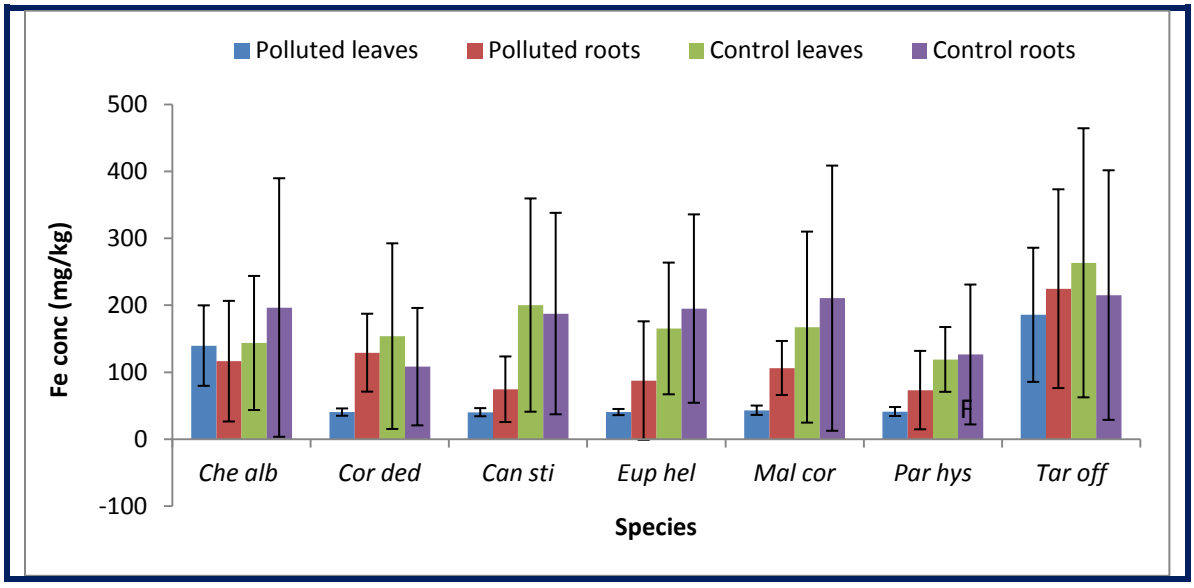

Fig. 3.7. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Fe.

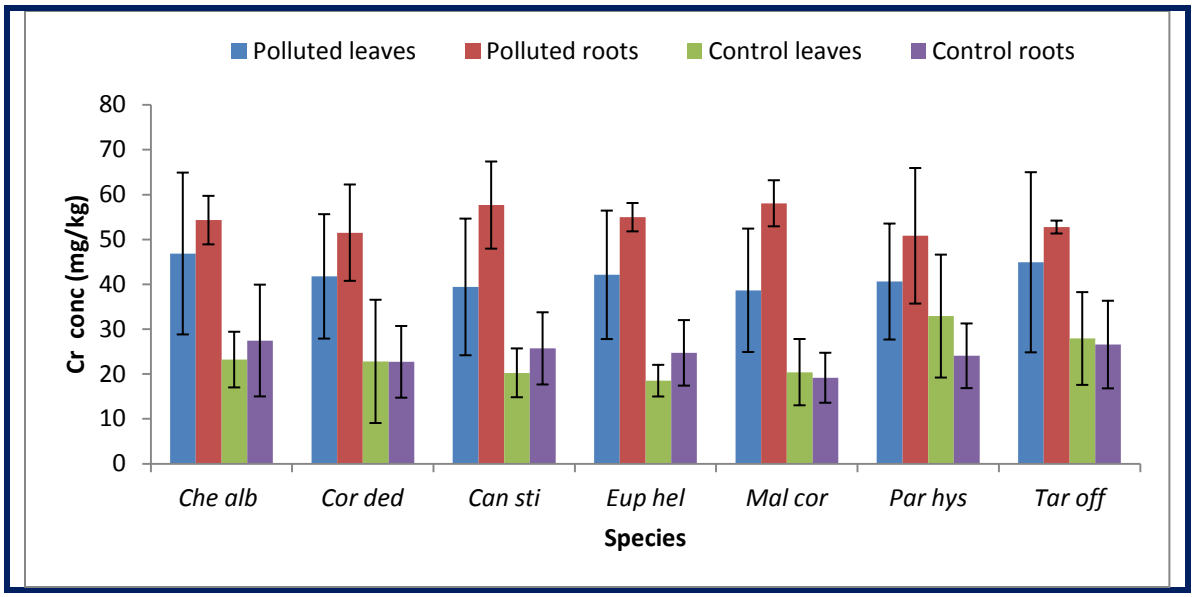

Fig. 3.8. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Cr.

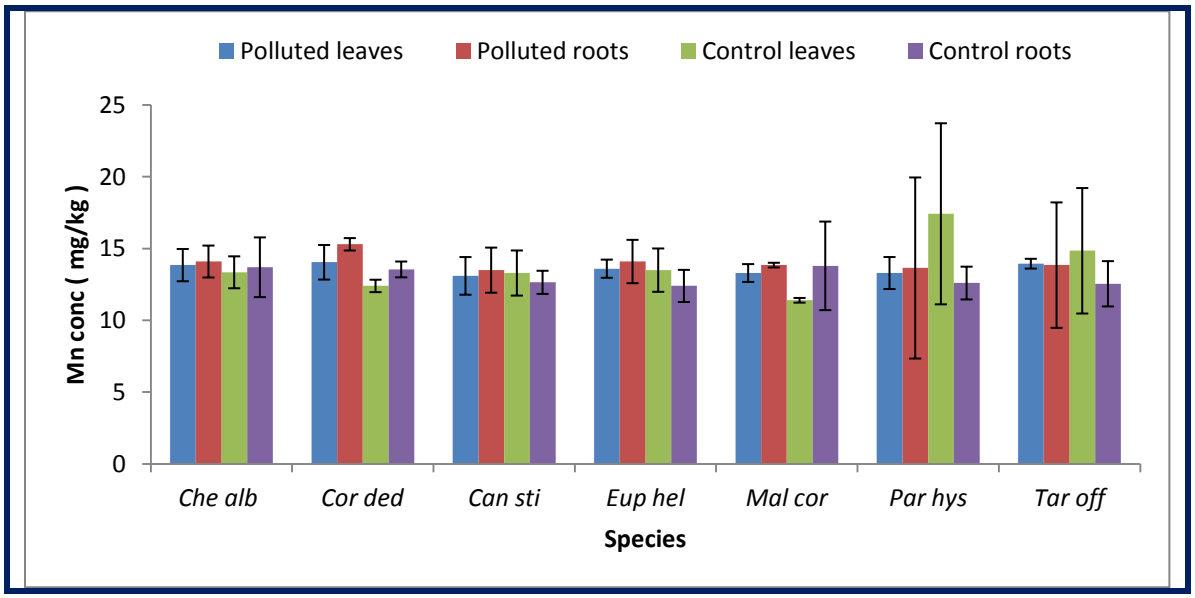

Fig. 3.9. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Mn.

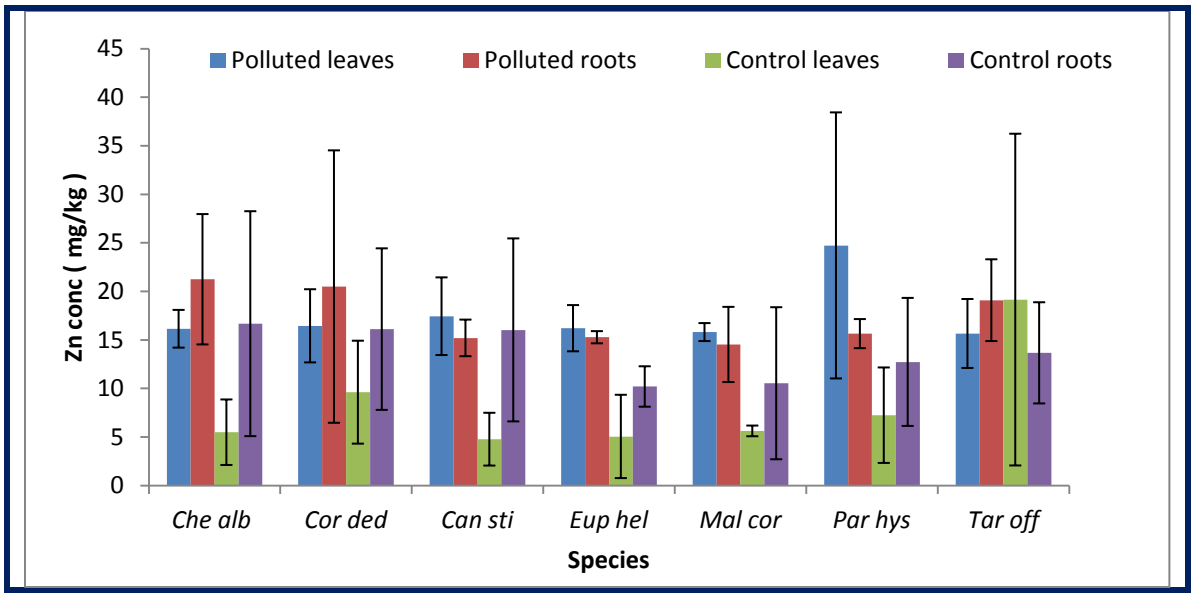

Fig. 3.10. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Zn.

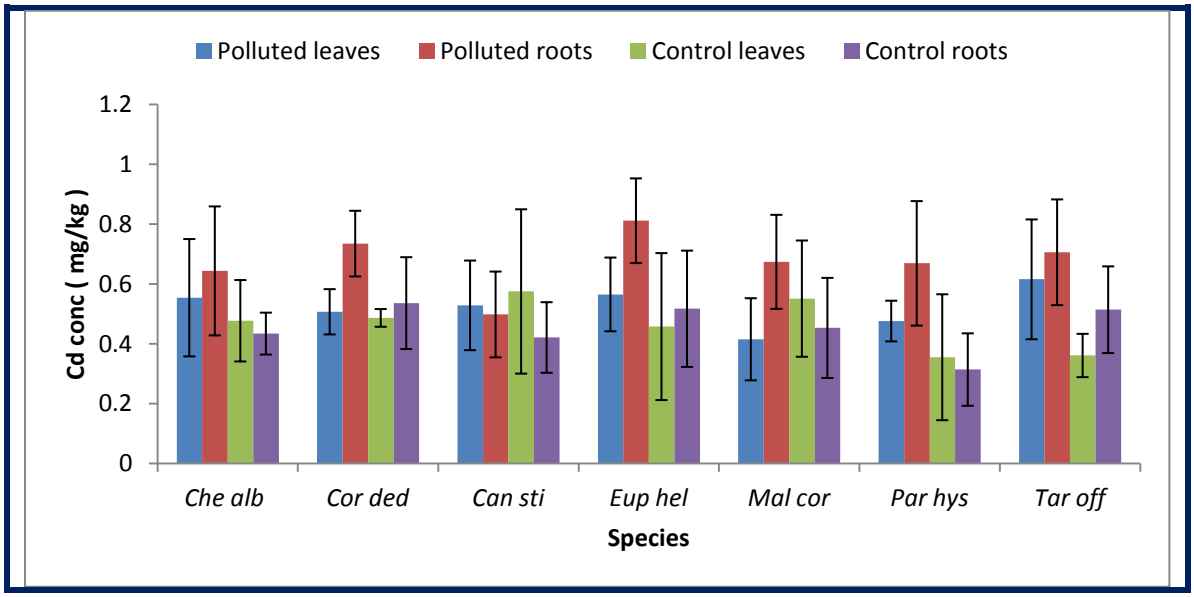

Fig. 3.11. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Cd.

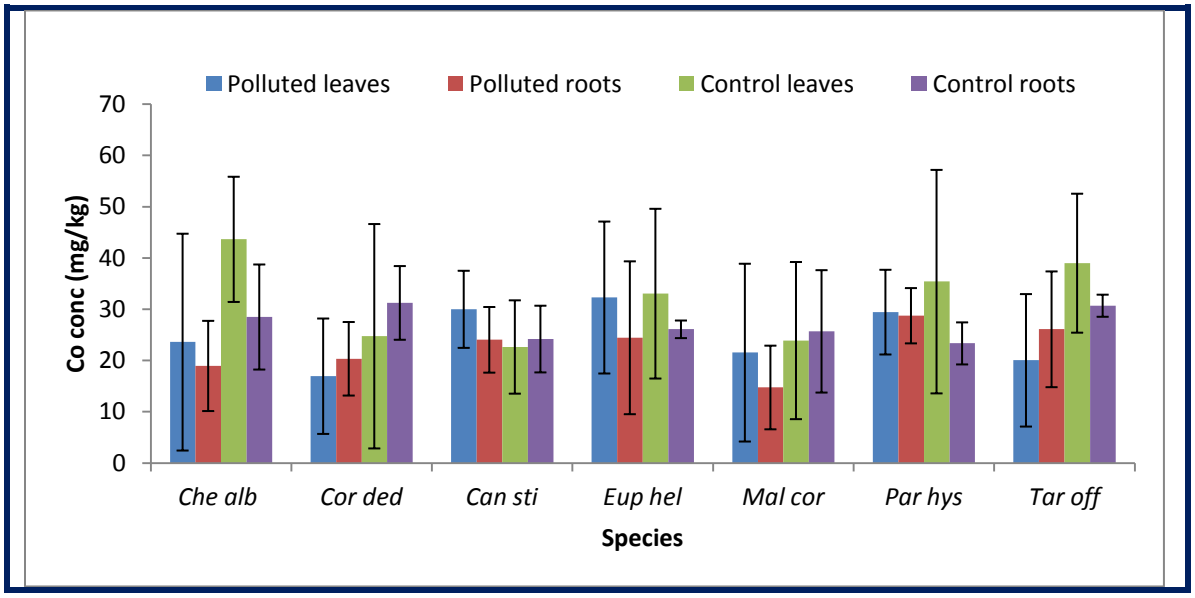

Fig. 3.12. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Co**.**

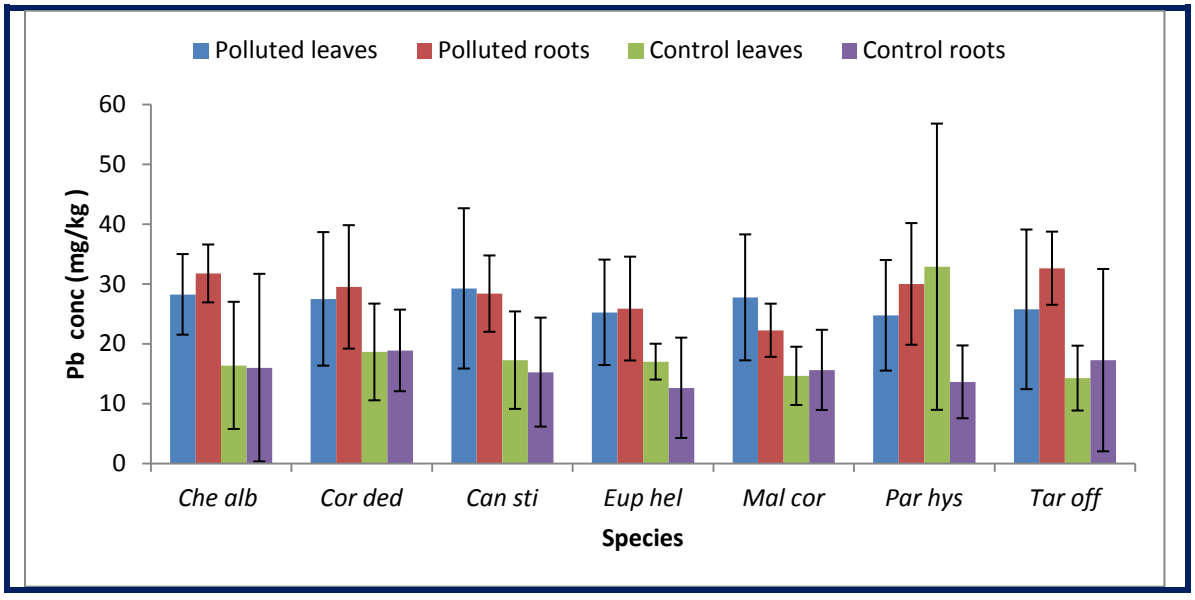

Fig. 3.13. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Pb.

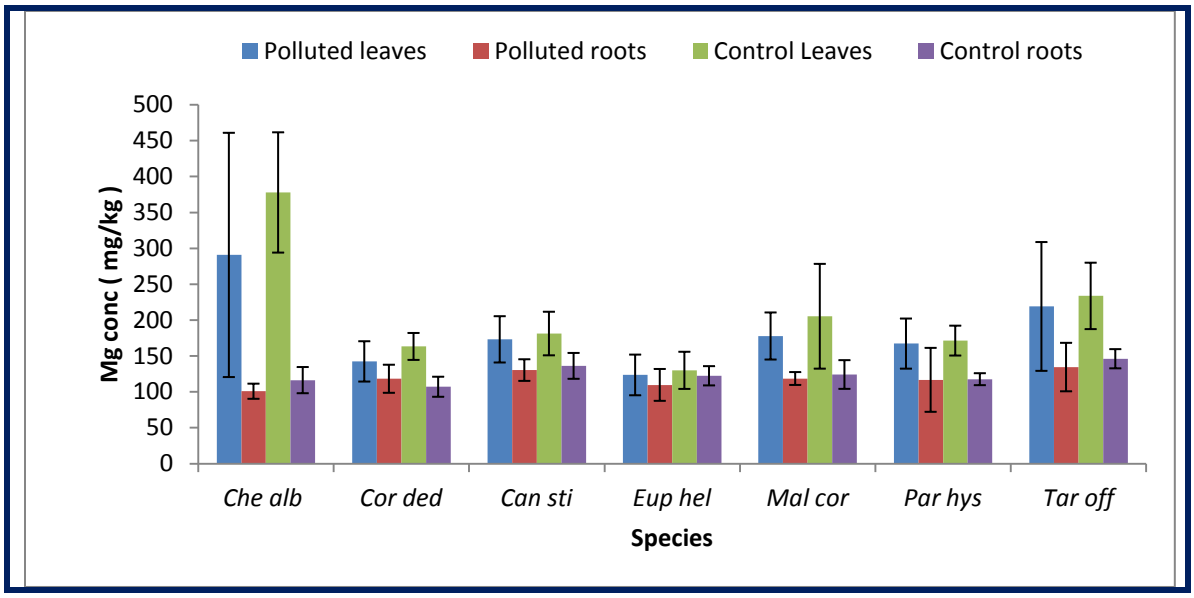

Fig. 3.14. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Mg.

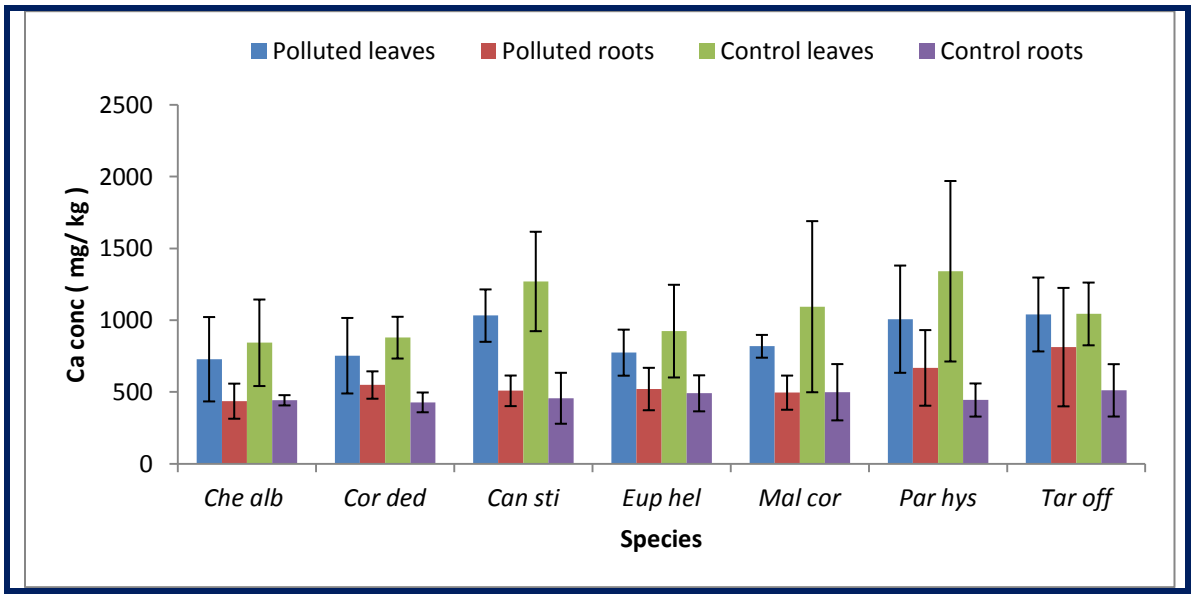

Fig. 3.15. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Ca.

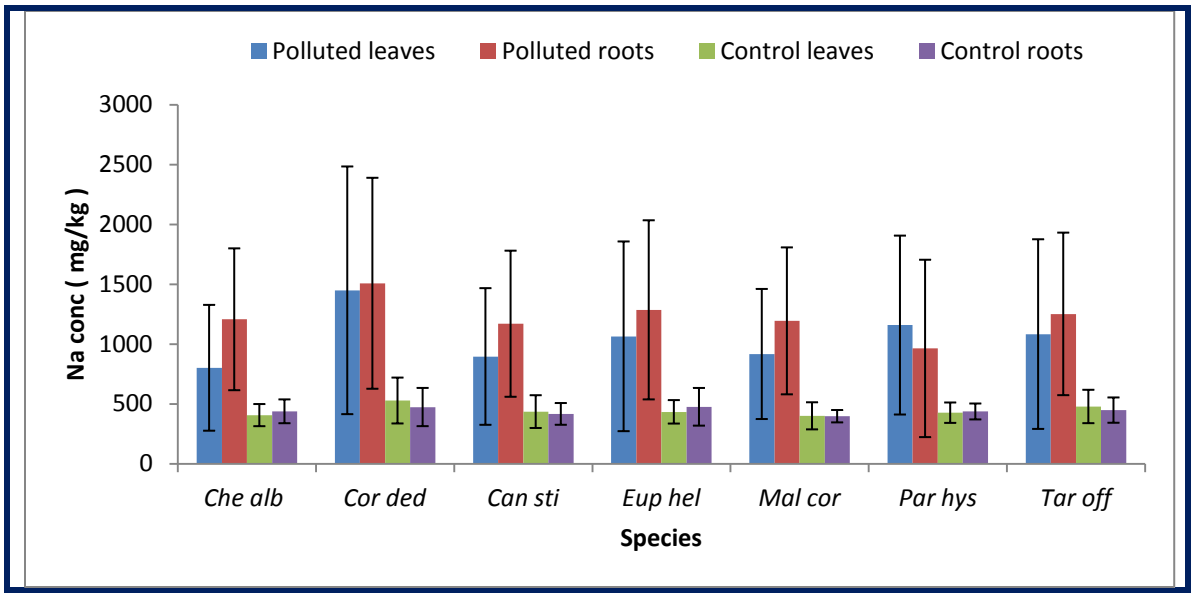

Fig. 3.16. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for Na.

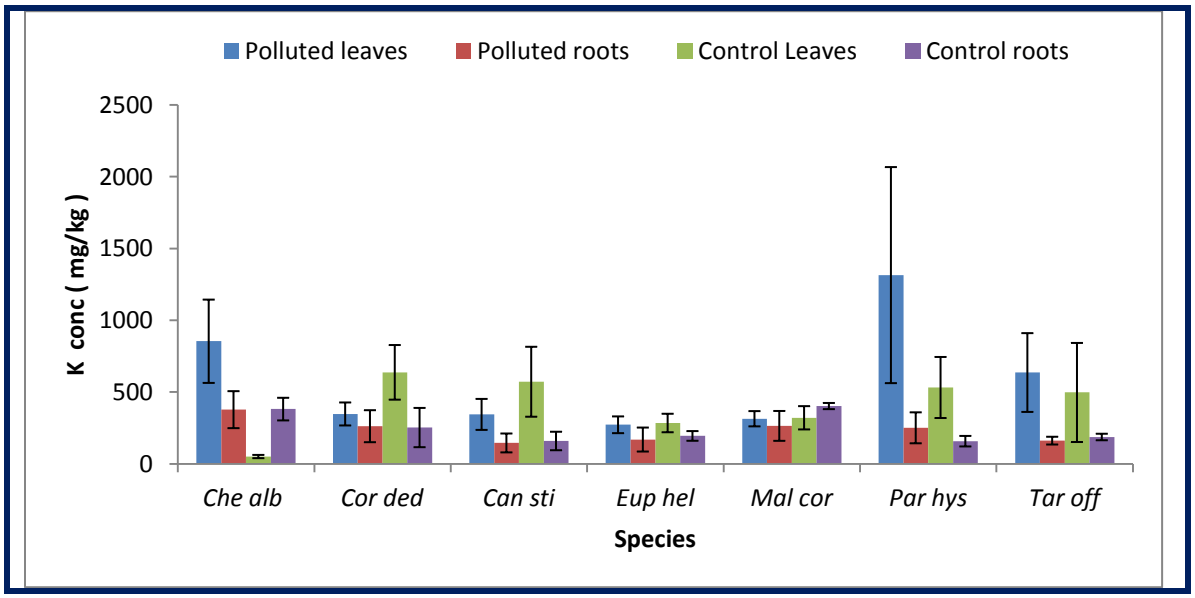

Fig. 3.17. Heavy metal concentration in the leaves and roots of plant species from control and dumping site for K.

Table 3.1. Heavy metals concentration (mg/kg) in leaves and roots of plants species from control site.

Species	Parts	Ni	Cu	Fe	Cr	Mn	Zn	Cd	Co	Pb	Mg	Ca	Na	K
Chenopodium album L.	**Leaves**	23.8±17.0	12.7±2.34	143.5±155.6	23.2±6.2	13.3±1.1	5.49±3.37	0.47±0.13	43.6±12.2	16.3±10.6	377.8±83.8	843.3±301.4	405.81±92.5	51.1±11.23
		7.0/46.0	9.2/14.2	34.2/364.4	15.6/30.8	12.2/14.4	2.36/8.94	0.3/0.58	30/58.8	7/29.5	306.7/489.8	562.2/1176.5	293.9/513.1	40.8/65.2
	Roots	20.7±13.9	13.6±8.6	196.5±193.2	27.4±12.5	13.7±2.0	16.6±11.5	0.43±0.07	28.5±10.2	16±15.6	116.3±18.2	442.7±35.7	437.5±99.5	381.5±78.8
		2.5/35.3	7/25.8	39/446.2	13.8/38.8	12.4/16.8	8.88/33.8	0.33/0.49	13.8/37.6	39.01/4	98.3/135.5	405.0/484.6	334.6/523.8	316.8/494.9
Coronopus dedymus	**Leaves**	22.8±18.5	10.8±2.6	153.7±138.7	22.8±13.7	12.4±0.4	9.61±5.3	0.48±0.02	24.7±21.8	18.6±8.0	163.2±18.7	878.9±145.4	527.8±191.6	637.6±190.3
		4.2/47.1	7.2/13	34/308.6	11.4/39	12/13.1	5.68/17.0	0.46/0.52	5.4/56.2	9/27.5	142.2/183.6	672.2/1008.9	345.2/774.2	393.5/827.6
	Roots	20.7±18.9	11.3±9.5	108.1±87.7	22.7±7.9	13.5±0.5	16.11±8.3	0.53±0.1	31.2±7.1	18.8±6.8	107.1±13.9	428.04±68.9	473.2±159.6	252.9±136.7
		4.6/44.5	3.4/25.2	34.4/232.6	12.8/31.2	13/14.2	10.4/28.3	0.41/0.74	25.2/39.8	12.5/28.5	92.0/125.8	348.4/515.44	330.8/676.1	129.0/396.5
Cannabis sativa L.	**Leaves**	21.9±16	14.3±3.9	200.2±159.4	20.2±5.4	13.3±1.5	4.77±2.71	0.57±0.27	22.6±9.1	17.2±8.1	181.2±30.3	1270.4±346.3	435.1±137.0	572.0±243.2
		7.2/39	10.2/19.6	37.4/391	15.6/28	11.6/15.4	1.12/7.34	0.33/0.95	14.4/32.4	5.5/23	148.6/221.3	910.9/1584.7	269.1/547.7	416.4/935
	Roots	18.3±17.1	10±2.0	187.5±150.5	25.7±8.0	12.6±0.8	16.03±9.4	0.42±0.11	24.2±6.5	15.2±9.1	136.1±18.0	456.6±177.4	415.9±90.9	159.3±64.3
		3.2/42.3	7.8/11.8	42/349.2	14.2/32.4	12/13.8	6.1/27.84	0.31/0.58	14.8/29.8	4.5/24	113.8/155.5	311.4/715.04	331.2/525.9	107.6/248.9
Euphorbia helioscopia	**Leaves**	22.5±14.5	10.0±3.7	165.2±98.4	18.5±3.5	13.5±1.5	5.05±4.28	0.45±0.24	33.0±16.5	17±3	129.9±25.8	924.5±322.9	433.3±98.0	284.6±64.4
		2.4/35.9	6.6/14.8	34.6/269.2	14.2/22.6	12.4/15.6	0.34/8.78	0.09/0.62	11.6/49.4	13.5/19.5	108.5/161.3	546.4/1335.9	352.9/572.5	189.6/329.5
	Roots	25.4±9.3	9.9±1.4	194.9±140.8	24.7±7.3	12.4±1.1	10.2±2.08	0.51±0.19	26.1±1.7	12.6±8.3	122.4±13.4	491.1±125.4	475.2±157.4	194.5±33.8
		11.4/31.4	8.2/11.6	37/350	17/34.6	11.4/13.8	7.1/11.52	0.33/0.78	24.8/28.6	0.5/18.5	107.3/139.0	358.2/610.8	323.0/618.4	149.4/225.7
Malvestrum coronomandelianum	**Leaves**	16.7±17.4	16.2±4.5	167.2±142.7	20.4±7.3	11.4±0.16	5.625±0.5	0.55±0.19	23.9±15.3	14.6±4.8	205.3±73.0	1094.7±596.1	399.7±113.2	320.9±81.1
		3.4/41.5	10.6/21.4	35.6/307.6	12.2/30	11.2/11.6	4.8/5.92	0.42/0.84	3.8/40	8.5/20	148.7/309.5	716.3/1984.3	286.2/550.3	226.8/424.7
	Roots	25.1±14.2	8.7±1.6	210.5±198.1	19.1±5.5	13.8±3.0	10.5±7.82	0.45±0.16	25.7±11.9	15.6±6.7	124.1±20.0	498.8±196.10	396.7±51.7	402.7±21.5
		4.75/37.7	6.4/10.01	39.2/496.4	13.6/26	11.8/18.4	3.6/21.72	0.27/0.6	19.2/43.6	8/24.0	107.1/150.6	334.12/756	347.6/465.6	370.9/417.6
Parthenium hysterophorus L.	**Leaves**	15.8±9.1	19.1±5.6	118.95±48.4	32.9±13.7	17.4±6.3	7.24±4.91	0.35±0.21	35.4±21.7	32.8±23.9	171.4±20.7	1341.7±628.8	426.0±85.2	532.1±212
		4.7/25.45	11/23.4	57.4/171.4	13/43.6	11.6/24.4	0.88/12.8	0.16/0.56	11.4/59.2	15/25	147.1/189.3	739.8/1885.6	316.0/516.5	373.1/832.72
	Roots	23.4±13.9	9.9±2.7	126.3±104.5	24.0±7.2	12.6±1.14	12.7±6.59	0.31±0.11	23.3±4.1	13.6±6.0	117.6±8.32	444.4±115.4	436.2±66.4	158.0±36.7
		7.51/37	7.2/12.4	33.6/258.8	16.2/33.2	11.6/14.2	8.3/22.42	0.21/0.48	19.6/27	7.5/21.5	107.4/126.3	318.1/593.9	348.2/506.2	128.0/206.5
Taraxacum officinale	**Leaves**	27.6±19.7	16.5±4.3	263.4±321.0	27.9±10.3	14.8±4.3	19.1±17.0	0.36±0.07	39±13.5	14.2±5.4	233.6±46.27	1044.2±218.6	477.9±139.4	497.2±345
		8.55/53.4	13.2/22.6	35.8/720.4	20.2/42.2	12.4/21.4	3.14/42.4	0.27/0.42	25.4/56.4	7/19.5	167.3/269.15	860.3/1321.0	279.1/598.9	39.7/838.3
	Roots	18.8±10.4	19.7±5.9	215.1±186.5	26.5±9.7	12.5±1.5	13.6±5.2	0.51±0.14	30.7±2.15	17.2±15.2	146.1±13.37	511.8±182.4	447.6±105.8	187.2±22.6
		4.45/27.8	10.8/22.8	43.6/385.8	16.6/36.8	11.2/14.2	9.08/18.8	0.38/0.67	28.8/33.2	5/38.5	128.7/161.41	343.7/771.1	331.3/572.2	161.1/214.3

Table 3.2 . Heavy metals concentration (mg/kg) in leaves and roots of plants species from dumping site.

Species	Parts	Ni	Cu	Fe	Cr	Mn	Zn	Cd	Co	Pb	Mg	Ca	Na	K
Chenopodium album L.	**Leaves**	26.15±5.6	16.65±1.8	139.6±151.5	46.85±18.	13.85±1.1	16.145±1.9	0.5±0.2	23.6± 21	28.25±6.7	290.8±170.1	728.72±293.8	801.4±525.9	854.1±407.6
		18.5/30.9	14.4/18.6	43/364.2	22.6/63.8	12.4/15	13.66/18.4	0.3/0.8	0.59/46.4	21/36.5	146.84/533.1	451.6/1097.7	303.3/1460.9	39.2/2123.0
	Roots	24.2±8.5	11.65±2.7	116.3±157.6	54.3±5.4	14.1±0.9	21.245±6.7	0.6±0.2	18.95±8.7	31.75±4.8	100.8±10.5	436.3±122.2	1207±592.8	377.7±128.8
		16.7/31.7	9.6/15.6	35.4/352.8	50.2/62.2	13.2/15.2	13.2/29.3	0.4/0.9	10.8/27.8	25/36.5	86.47/110.5	338.8/615.2	429.3/1871.4	253.85/548.4
Coronopus dedymus	**Leaves**	31.1±5.2	19.2±5.1	40.25±5.4	41.7±13.9	14.05±1.2	16.45±3.8	0.5±0.0	16.9±11.2	27.5±11.1	142.4±28.1	753.5±263.1	145.2±1035.4	347.6±80
		26.5/38.3	14.6/26.4	35.8/48.2	22.4/54.8	12.4/15.2	11.26/19.6	0.4/0.6	1.8/27.4	15.5/40.5	103.95/171.2	508.28/1126.6	390.3/2740.8	265.7/433.6
	Roots	25.9±10.3	13.35±4.03	129±151.8	51.5±10.7	15.3±1.4	20.49±14	0.7±0.1	20.3±7.1	29.5±10.3	118.2±19.5	548.9±95.4	1508.0±881.9	262.4±111.4
		10.45/31.6	10/18.4	36.6/355	35.6/59.2	13.6/17	12.56/41.4	0.64/0.8	13.6/27.6	15.5/38	92.3/134.24	468.36/667.1	549.7/2494.6	173.07/424.6
Cannabis sativa	**Leaves**	32.46±6.9	20.35±4.6	40.1±6	39.4±15.2	13.1±1.3	17.44±4	0.52±0.1	30±7.5	29.3±13.4	173.16±32.2	1032.5±182.5	896.0±571.3	344.5±107.8
		22.55/38.5	15/24.6	35.4/48.6	17.6/52.2	12/15	14.24/23.1	0.32/0.7	20.8/37	10/41.01	133.63/201	801.6/1238.9	311.9/1474.2	201.5/460.8
	Roots	22.88±8.7	9.55±0.7	74.4±49.	57.65±9.7	13.5±0.6	15.205±1.8	0.4±0.1	24.0±6.4	28.37±6.3	130.32±15	508.5±106.5	1169.7±610.6	145.7±65.7
		10.95/31.9	8.8/10.4	36.4/146.2	43.8/66	12.8/14.2	13.56/17.9	0.41/0.7	15.8/31.4	19.5/33.5	118.32/150.5	395.6/610.4	352.3/1676.7	90.17/237.9
Euphorbia helioscopia	**Leaves**	30.73±8.6	14.9±1.9	40.3±4.6	42.1±14.3	13.6±0.6	16.205±2.3	0.56±0.1	32.3±14.8	25.25±8.8	123.59±28.3	774.4±160.4	1064.6±792.9	272.2±58.3
		21.75/39.2	13/17.6	35/44.8	23/56.8	12.8/14.2	14.72/19.7	0.40/0.7	11/42.4	14/33	85.72/149.7	565.32/952.8	297.7/2050.6	204.8/328.2
	Roots	27.66±8.7	11.2±5.02	87.35±88.6	54.95±3.1	14.1±1.6	15.27±66.7	0.81±0.1	24.4±14.9	25.87±8.7	109.6±22.2	521.0±147.8	129.92±748.7	169.4±83.4
		20.35/39.2	6.8/18.4	36.8/220	50.4/57.6	11.8/15.6	14.72/16	0.61/0.9	13.8/45.8	14/33.5	84.8/138.4	351.44/703	409.7/2240.1	89.99/246
Malvestrum coronomendalinum	**Leaves**	29.73±6.1	23.55±3.8	43±6.9	38.6±13.7	13.3±0.6	15.805±0.9	0.41±0.1	21.5±17.3	27.±10.5	177.81±32.7	818.8±79.3	916.8±543.7	314.39±53.1
		20.65/34.2	19.6/28	37.4/53.2	19/49.2	12.4/13.8	14.86/16.6	0.26/0.5	0.4/38	13.5/38.5	140.7/220.5	708.5/883.2	330.8/1420.5	277.3/390.4
	Roots	28.875±5.9	11.7±1.37	106.2±122.2	58.05±5.1	13.85±0.7	14.53±3.8	0.67±0.2	14.7±8.1	22.25±4.4	118.5±8.9	495.7±118.9	1193.8±614.1	264.4±104
		20.05/32.6	9.8/12.8	40.6/289.2	51.4/63	12.8/14.4	11.5/19.9	0.52/0.8	7.4/26.4	18.5/28	110.9/131.4	334.96/621.2	375.4/1751.5	137.69/351.1
Parthenium hysterophorus L.	**Leaves**	32.1±15.8	20.95±2.9	41.1±6.7	40.6±12.9	13.3±1.2	24.73±13.7	0.57±0.6	29.4±8.2	24.75±9.2	167.2±34.9	1007.7±373.5	1158.6±747.9	1315.1±752.6
		16.55/47.3	16.8/23.2	34/48.8	22.4/50.4	11.8/14.4	12.52/44.3	0.40/0.6	19.2/39.4	18.5/38.5	120.1/204.5	682.12/1533.8	350.5/1837.8	580.4/2106.2
	Roots	23.13±7.9	13.7±2.7	73.1±58.61	50.8±15.1	13.65±0.6	15.64±1.5	0.66±0.2	28.7±5.3	30±10.17	116.7±44.5	668.4±263.4	963.3±741.4	251.1±107.8
		13.75/30.5	10/15.8	36.4/160	28.8/61	13.2/14.6	13.5/16.9	0.43/0.9	23.2/34.4	17/40.5	71.83/166.2	453.64/1043	244.0/1775.3	115.8/379.3
Taraxacum officinale	**Leaves**	34.58±6.2	22.1±4.4	185.6±277.6	44.9±20	13.95±0.4	15.66±3.5	0.61±0.2	20.0±12.9	25.7±13.3	219±89.7	1040.4±257.4	1083.2±792.8	636.3±274.4
		28.3/40.95	18.6/27.6	39.8/602	18.2/66.6	13.6/14.4	11.56/20.22	0.42/0.8	5/33.8	7.5/39.5	95.26/301	760.8/1333.5	283.2/1817.8	226.1/795.4
	Roots	33±7.3	24.55±3.2	224.7±148.5	52.75±1.4	13.85±0.9	19.09±4.2	0.7±0.17	26.1±11.2	32.62±6.1	134.54±32.6	813±412.6	1251.9±679.4	161.8±27
		23.45/40.05	20.6/28	41.6/377	51.2/54.6	12.8/15	15.34/23.18	0.5/0.96	9.6/33.6	25/38.5	108.94/182.6	428.7/1193.5	382.2/2005.9	123.3/185.8

1. Introduction

1.1. Background

Being one of the most important aspects of urban development linked with rapid population increase, solid waste disposal, its management and threats related to human health and environmental quality has gained importance in developing countries. In most of the developing countries, the municipalities lack financial resources and skills to cope with this crisis. Large amount of solid waste is produced by human settlements and practices related to collection, processing and disposing of solid wastes are considered least efficient in developing countries and solid waste is disposed off in empty plots, topographic depressions, along roads, railway lines, streets, drains and open sewers that may lead to environmental deterioration and loss of natural resources (Adila *et al.,* 2008). According to one estimate, solid waste generation rate is approx., 1.3 billion tons/day with an annual average increase rate of 3.7% per capita (Beede and Bloom 1995).

Pakistan has a population of 160 million, with 35% people living in urban areas. 54 % of them live in ten major cities (GOP, 1996). During the last few decades, migration from rural to urban areas has increased. The major cities are generating large amounts of solid waste which is increasing annually with the respective population growth. The average rate of waste generation in major cities varies from 1.89 kg/house/day to 4.29 kg/house/day (Pak-EPA, 2005) and 60% of the solid waste generated is generally collected and brought to dumping sites and rest of the 40% remains uncollected. Solid waste generated in urban areas of Pakistan is estimated as 55,000 tons/day (JICA, 2005).

The main hurdle to proper solid waste management in Pakistan include lack of reliable data and research, shortage of trained manpower, inadequate legal and regulatory cover, poor institutional and administrative arrangements, shortage of equipment, financial and technical difficulties and a serious shortage of competent private operators (Koica – World Bank, 2007).

Open dumping is one of the most common practice in Pakistan and dump sites are often set to fire to reduce the volume of accumulating solid waste, thus adding to the air pollution and causing environmental deterioration (Rehan *et al.,* 1998). At present, there

are no landfill regulations or standards that provide a basis for compliance and monitoring. Only 60% of the total waste generated in major cities is collected and disposed off in open dumps and collection of solid waste is quite irregular and limited to high income areas only.

Local authorities are handling and disposing solid waste often without any reliable procedures and knowledge of the serious problems such as contamination of organic and inorganic contaminants to the underground water reservoirs. There is no segregation of solid waste material and all organic and inorganic waste is being dump at open places.

Metals in solid waste dumps exist in various forms either as the pure metal or alloyed with various other metals. Heavy metals can be categorized into urban-industrial aerosols, liquid and solid wastes from animal and man, mining, industrial and agricultural chemicals (Gerard *et al.,* 1996). Solid waste increases heavy metal concentration in soil, underground water and wild plants growing in the dumping sites and may be toxic to man, livestock or other animals that depend on the plants, water or soil for food, drinking or shelter (Gad and Zaghloul, 2007; Hsu *et al.*, 2006; Kakulu *et al.*, 2001). Through inhalation of dusty soil toxic heavy metals can be taken directly by man and animals. Ideriah *et al.,* (2005) reported that the soil around solid waste dumps contain different concentrations of heavy metals depending on the topography, run-off , type of wastes and level of scavenging. The solid waste dumps takes a long time to decompose naturally so it not only degrades the physical environment but also impacts the natural environment aesthetically and health wise (Ogbonna *et al.,* 2007).

1.2. Impact of heavy metals on wild medicinally important plants

The effects of heavy metals on ecosystems have been studied widely and various field experiments have been conducted to study the remediation of heavy metal polluted sites. Its the natural tendency of plants to take up heavy metals along with toxic substances that are then transferred to food chain. The advantage of studying such plants is their ability to accumulate metals, if they are grown on heavy metal polluted land or irrigated with polluted water. Such plants are a good tool for phytoremediation. Distribution of toxicants from roots to upper parts of the plants, level of accumulation in

different plant parts and determining the nature of toxicity is necessary before selection and cultivation of such plants for phytoremediation (Barman *et al.*, 2000a, 2001b).

Due to toxic effects of heavy metals most of the plant species cannot survive in polluted sites and have negative impact on plant physiological activities like photosynthesis, gaseous exchange etc. (Wong, 2003). Previous studies have shown that plants can attain resistance against toxicants including heavy metals, depending upon the various ecophysiological factors in time and space (Ray *et al.,* 1988). However, all plants do not show equal resistant to various pollutants because plant resistance against a particular toxicant is also dependent on the cyto-genetic makeup of the species. As compared to other plants, wild species possess stress resistant properties, and can maintain growth even under adverse conditions. Wild species have an extensive adaptive capacity, and play an important role in water and soil conservation (Wei *et al.*, 2003) thus phytoremediation is a promising technology for remediating contaminated soils by growing such plants which are metal hyper accumulators (Brooks *et al.*, 1998).

There are many wild plants that are known for their medicinal value and they are being used for herbal medicine. Many phytochemical and pharmacological studies have been carried out on various wild medicinal plants. Our study focuses on the presence of trace elements in these plants which are growing naturally on dumping sites and could be either dangerous or useful to the humans who are consuming these plants or animals that feed on these medicinal plants.

The accumulation of heavy metals by plants grown in polluted soil have been reported by Okoronkwo *et al.*, (2005 a, b) where the author reported Pb, Ni, Cr, Cd and As present in the soils and also the uptake of Pb, Ni and Cd in the plant parts harvested from an abandoned solid waste dump soils in Umuahia, South-Easten part of Nigeria. Nwoko and Egunojobi, (2002) studied lead contamination of soils and vegetation in an abandoned battery factory site in Ibadan, Nigeria, and reported the presence of Pb in the tissues of plants, with roots showing higher Pb concentration than shoots in most cases.

1.3. Using Biomarkers as an Indicator of heavy Metal Pollution

It is important to understand the basic principles as to how the pollutants are taken up by the plants and act at the cellular and tissue level**.** In order to measure the effect of pollutants on an ecosystem, biomarkers have attracted a lot of attention. The main principle of the biomarker approach is to analyse an organism's physiological response to pollutant exposure. This is because toxic effect manifests itself at the sub cellular level before it becomes obvious at higher levels of biological organization. The measurement of biochemical responses to chemical contaminants may serve to improve the assessment of biologically significant exposures to toxic chemicals and enhance the ability to assess the risk of effects on the health and survival of toxicant exposed populations (Rees, 1993).

Biomarker studies have also been conducted in many plants that are exposed to various environmental stresses (Vitoria *et al.*, 2001). Enzymes of the detoxification machinery can serve as important markers of environmental pollution (Filho *et al.*, 2001) and a good correlation with pollutant levels further strengthens their utility as biomarkers (Fernandes *et al.*, 2002). The use of antioxidant enzymes as biomarkers of heavy metal pollution has been reported by several investigators (Ahmad *et al.*, 2000; Geret *et al.*, 2002, 2003). Reports from various plant species reveal that heavy metals cause oxidative stress by mediating the activities of antioxidative enzymes (Cuyers *et al.*, 2000). It has been reported that heavy metal stress leads to sharp changes in the activities of certain enzymes like superoxide dismutase, peroxidase, catalase (Vitoria *et al.*, 2001; Patisska *et al.*, 2002; Shainberg *et al.*, 2000).

These enzymes play a key role in inhibiting the cellular damage produced by a wide variety of structurally diverse carcinogens and endogenous toxins (Ansher *et al.*, 1986). Environmental pollution could induce the overproduction of reactive oxygen species (ROS) in plants. These ROS species react with proteins, lipids and nucleic acids, and cause lipid peroxidation and inactivation of enzymes thus affecting cell viability (Dixit *et al.*, 2001; Singh *et al.*, 2006). To minimize the damaging effects caused by ROS, plants have evolved various enzymes like peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and nonenzymatic antioxidants such as

ascorbic acid (AsA), glutathione (GSH), non protein thiol (NPT), cysteine (Cys), and proline to reduce the damaging effects by scavenging different types of ROS (Singh *et al.*, 2006).

In plants physiological parameters were used as bioindicators of environmental pollution and it dates back to 1974 (Keller, 1974) in which POD activity in tree leaves was monitored. After that, metabolic parameters in plants were adopted as biosensors of atmospheric pollution by several researchers. For example, Puccinelli *et al.,* (1998) measured the POD activities in tree leaves in Turin and verified the possibility of POD activity in tree leaves as biomarkers for air pollution. Li *et al.,* (2003) detected POD and SOD activities in leaves of Ficus microcarpa and found that the enzyme activities were corresponding to the O3 pollution, demonstrating the practicability of enzyme activity in plants as a general bio-indicator of air quality.

All this demonstrated that toxic effect of pollution on organisms manifest itself at the subcellular level before it becomes apparent at higher levels of biological organization (Fatima and Ahmad, 2005). Therefore, it is practicable to use the subcellular metabolic parameters in plants as biomarkers for the monitoring of environmental pollution in the absence of visible symptoms (Koricheva *et al.*, 1997).

In Pakistan, there is no regular mechanism for the collection and disposal of solid waste generated from industries, hospital and agriculture activities. The increasing solid waste generation in many urban areas of Pakistan is a serious environmental problem. This is because of improper solid waste handling and disposal system. According to an estimated more than half of the waste generated in urban areas goes uncollected and can be considered as vast nutrient sinks, because unlike rural areas, household waste and commercial waste are not returned to production but contribute to urban pollution (Ogbonna *et al.*, 2007).

Like many cities in Pakistan, Rawalpindi faces problems of environmental sanitation such as improper disposal of waste near residential areas, poor waste collection and handling etc. It is a common feature to find huge solid waste dumpsites within residential areas and along some minor and major roads. Rawalpindi presents an example of fast growing urban solid waste management problem. In Rawalpindi major portion of

the solid waste is thrown by the residents into Nullah Leh. These practices have not only given rise to environmental problems but have also caused wide spread health hazards.

Rawalpindi has been experiencing tremendous growth in all spheres like traffic, population, urbanization and trade and so on because of being close to the capital city Islamabad. The solid waste dumping site, Misrial road occupies an area of about 300-400 acres and 800 tons of solid waste is brought here per day collected from the whole city that includes 80 tons of animal waste, 510 tons of domestic waste and 110 tons of waste from commercial establishments. The municipalities due to their inadequate resources, manages to collect only 40 percent of the total daily waste and the 60 percent remains uncollected and is unlawfully dumped into open spaces. Moreover, the transported garbage is dumped without any measures for sanitary land filling.

The open solid waste poses serious threat to public health through formation of stagnant ponds, clogging of drains and providing breeding grounds for flies and mosquitoes with consequent risks of malaria and cholera.

No previous data and scientific literature is available on solid waste dumps and wild medicinally important plants but majority of the research is on heavy metal content of soil and crops (Oluyemi *et al.*, 2008; Okoronkwo *et al.*, 2006; Amusan *et al.*, 2005). However, there is a lack of literature on metal uptake in wild medicinally important plants at solid waste dumpsites and potential human health risk via consumption of these plants because they are collected by local people and hakeems and brought to local market and used for herbal medicine. People in Pakistan still prefer to consume large amounts of herbal plants for various purposes, especially for healthy life. Therefore, the metal toxicity has attracted concern over safety of wild medicinally important plants.

This is first study to evaluate the levels of metal accumulation in different parts of seven wild medicinally important plant species, which are growing naturally at dumping site. This study is also expected to provide us clues for selection of accumulator plant species toward metals and to provide information on the influence of solid wastes on soil and metabolic parameters as an indicator for monitoring environmental pollution in plants at solid waste dumping sites. The information will guide in formulating

appropriate land-use management policy for improved climate change through such a unique ecosystem.

1.4. Objective of the study:

The main objective of this investigation was:

- To evaluate heavy metals concentrations in wild medicinal plants and associated soil of solid waste dumping site.
- To quantify the metabolic parameters in plants as bioindicators for environmental pollution.
- To identify heavy metals transfer pathway of selected wild medicinally important plants growing on dumping sites.

Following Medicinal Plants were used:

- *Taraxacum officinale* F.H. Wigg is commonly known as Dandelion (Bathur) belongs to the family compositae is distributed in the world as hawkweed. Its root is used as an important drug of herbal medicine and has been used as a remedy for liver complaints. Dandelion leaves are adjunct to treatments where enhanced urinary output is desirable. The plant is diuretic, stimulant, anti-biotic, anti-rheumatic, anti-spasmodic, tonic, hepatic, laxative and nutritive. (Evans *et al.*, 2005)
- *Cannabis sativa* L. has been used by man for over 5000 years and is known to have many uses. The seeds are a good source of oil due to its composition of unsaturated fatty acid. The oil has a range of uses including production of colours, lacquer and in the cosmetic industry. The seeds also provide a source of protein for man and animals and compounds of major interest to the pharmaceutical industry (Robinson, 1996).
- *Euphorbia helioscopia* L. belongs to family Euphorbiaceae is widely distributed throughout the world. Its juice is commonly applied to warts used to cure intolerable pain and associated with sore eyelids. The root is anthelmintic and the seeds mixed with roasted pepper are used in treatment of cholera. The herb is also

used for humid asthma, cardiac hay fever, bronchitis and urethritis (Swarbrick, 1997).

- *Coronopus didymus* L. belongs to the family Cruciferae. It is used in rheumatism. Plant extract is used for bone disorders and used to open locks among joints (Mahmood *et al.*, 2011).
- *Chenopodium album* L. belongs to the family Chenopodiaceae. A local dish saag is prepared from it. It removes thirst and used as emollients. Its seeds are used for unconsciousness and also to relive constipation. Cooked leaves are used in urinary trouble. For pile, cough and worm leaf extract is used while the whole plant is used as a laxative. Powder of dried stem is used to remove stone. Powder extract is best treatment against hepatitis, jaundice and other liver problem. *C. album* is useful for eye problems, cardiac disorders and respiratory tract diseases; for this purpose leaf extract is used orally (Mahmood *et al.*, 2011).
- *Parthenium hysterophorus* L. belongs to the family Asteraceae. Its juice gives strength to stomach and relief from constipation. Some people use it in fever. The powder of dried flowers is used against diabetes (Mahmood *et al*., 2012).
- *Malvastrum coromandelianum* L. belongs to family Malvaceae its leaves are used for carbuncles, decoction of leaves used to clean wounds; also used for dysentery, used for wounds and sores as diaphoretic. It also has antibacterial, antioxidant activity (Mahmood *et al.*, 2012).

Chenopodium album L.

Malvestrum coromandelianum L.

Taraxacum officinale F.H. Wigg.

Coronopus didymus L. (Sm.)

Plate 1.1. Wild medicinal plants selected for the present study.

Parthenium hysterophorus L.

Euphorbia Helioscopia L.

Cannabis sativa L.

Plate 1.2. Wild medicinal plants selected for the present study.

2. Material and Methods

2.1. Study area

Solid waste dumping site lies between 33°36'8.60" N and 33°36'27.36" N latitudes and 72°58'28.86"E -72°58'52.87"E Longitudes in Rawalpindi city was selected for detailed physicochemical study and heavy metals assessment in soil and wild plants.

The solid waste dumping site occupies an area of 11500 meters receives solid waste of 800 tons/day consisted of food materials papers, food cans, food waste, straws, wood, animal waste, rags and bones, leaves, grass, plastic, rubber, textile waste, metals, card board, metal scraps, used battery cells, and all sorts of various wastes.

In comparison, a control site a sub-urban area in vicinity of Quaid-i-Azam University, North of Islamabad was also selected which is located approximately 15 km from the city centre with low traffic density. This area is free from obvious sources of industrial and vehicular pollution and is on the outskirts of the city Islamabad and is away from most intense pollution zone, the city centre.

Plant sampling

Seven commonly occurring wild plant species viz., *Parthenium hysterophorus* L., *Malvestrum coromandelianum* L., *Coronopus didymus* L (Sm.), *Euphorbia helioscopia* L., *Taraxacum officinale* F.H. Wigg., *Chenopodium album* L. and *Cannabis sativa* L., growing naturally of medicinal importance were collected in triplicate from four different locations from each site. Plant samples were uprooted at maturity and separated into shoots and roots for estimation of metal contents. During plant sampling, it was ensured that different plant samples of each species had the same size, physiological age and appearance. Plant species collected were the most dominant species at this site. In the laboratory plant samples were washed thoroughly with running tap water to remove adhering substrate materials, rinsed twice with distilled water, separated into leaf, stem, and root parts with stainless steel scissors and were than air dried before oven dried at 65°C for 24 hr.

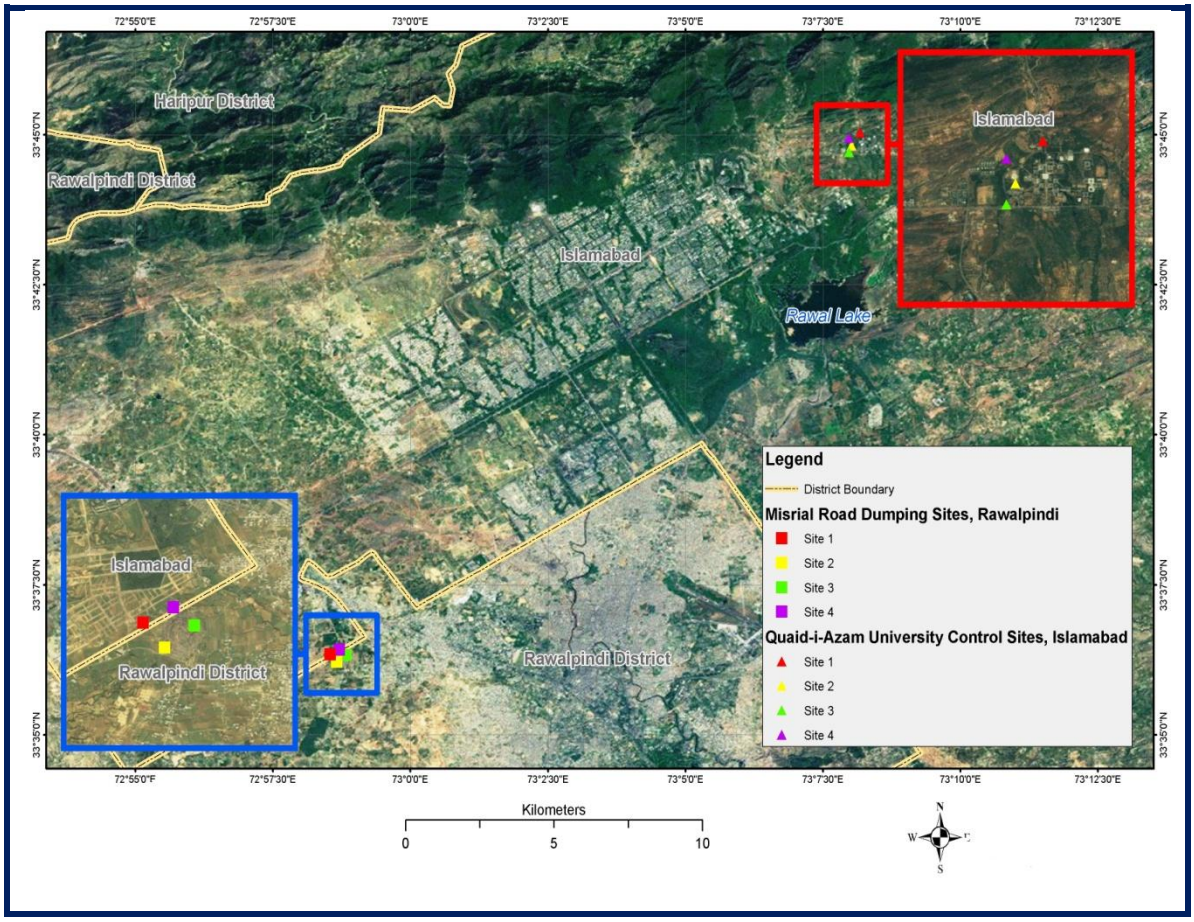

Fig 2.1. Map of the Study area showing four sampling locations from control and the dumping site.

Sampling Sites and Sample Collection

Plate 2.1. Waste Dumping site, Misrial road Rawalpindi selected for sample collection.

2.2. Soil sampling

From each site (dumping and control) **s**oil samples were also collected at the depth of 15 cm with the help of stainless steel auger. After removing the large size particles such as stick pebbles etc the sample was kept in proper labeled polythene bags and were transferred to laboratory for heavy metal analysis.

2.3. Chlorophyll and carotenoid content

Chlorophyll contents were evaluated by extracting the leaf material (0.05 g) in 10-cm^3 dimethylsulfoxide (DMSO) (Hiscox and Israelstam, 1979). Sample was heated at 65° C for 4 hours and absorbance of extracts was recorded at 665 and 645 nm. Chlorophyll contents were calculated as per standard method using Eq 1, 2 (Arnon 1949). The method of Lichtenthaler and Wellburn (1983) was used to estimate carotenoid content using Eq 3.

Chl. a (mg / g) = 1.07(OD663) - 0.09(OD645) ----------Eq 1

Chl. b (mg / g) = 1.77(OD645) - 0.280(OD663) ----------Eq 2

Carotenoid content = $A_{OD} \times 4$ ----------Eq 3

2.4. Antioxidant enzymes extraction

0.5g of leaves was grinded in 5ml of 50mM phosphate buffer placed in ice bath. Homogenate was centrifuged at 13000g at 4^oC for 20 min. The supernatant was used to measure SOD, POD, CAT activities.

2.4.1. Superoxide dismutase

SOD (EC 1.15.1.1) activity was assessed by following the method of Beauchamp and Fridovich (1971). The reaction mixture contained, 13mM methionine, 0.1mM EDTA, .002mM riboflavin and 0.075mM nitro blue tetra zolium salt (NBT) dissolved in 3ml of 0.05M sodium phosphate buffer (pH 7.8), 3ml of the reaction medium was added to 0.1ml of enzyme extract. The mixture was illuminated in glass test tubes. In light chamber illumination was started to start the reaction at 30°C for 1 hour. Identical solutions were kept under dark served as blanks. At 560nm the absorbance was read in the spectrophotometer against the blank. SOD activity was expressed as U/mg protein.

2.4.2. Peroxidase activity

POD activity was measured by following the method of Gorin and Heidema (1976). The assay mixture contained 0.1 ml enzyme extract, 1.35ml 100mM MES buffer (pH 5.5), 0.05% H_2O_2 and 0.1% phenylenediamine. Changes in absorbance were recorded at 485 nm for 3 min with the spectrophotometer. The activity of POD was presented as a change in OD485 and was expressed as U /mg protein.

2.4.3. Catalase activity

CAT activity was determined by the method of Goel *et al.* (2003). The 3ml reaction mixture contained 50mM phosphate buffer (pH 7), 15mM H2O2, 0.1ml enzyme extract. The decrease in H2O2 was followed as the decline in absorbance at 240nm, and activity of CAT was expressed as U/mg protein.

2.5. Soil and plant digestion procedure

Soil samples measuring 1g each were digested in 15 ml of aqua regia in 3:1 (HCL, HNO_3), left overnight and heated near about 150 $^{\circ}$C until brown fumes turnoff and additional 5 ml $HCLO_4$ (70-72%)is added and heated. Whatman filter paper No 42 (125mm) was used to filter the remaining solution and volume was raised up to the mark 50ml with double distilled water (FAO/SIDA, 1983).

A total of 10ml mixture of HNO_3 and $HCLO_4$ in 2:1 was added into 1 g plant sample which were left for overnight. It was heated for a movement until brown fumes was changed into white fumes. The solution was filtered over filter paper No. 42 (125 mm) by raising the remaining volume with de-ionized distilled water up to the mark 50ml and kept in plastic bottles at room temperature for heavy metal analysis.

2.6. Metal analysis

Thirteen Heavy metals i.e. Co, Cr, Cu, Fe, Na, Ni, Pb, K, Mn, Ca, Cd, Mg and Zn were analyzed in different samples of soil, and plants by Flame Atomic Absorption Spectrophotometer (Varian FAAS-240).

2.7. Transfer factor

Transfer factor, TF (Barman *et al.*, 2000; Gupta *et al.*, 2008) was calculated using Equation 4 to assess the relative translocation of toxic metals from root to other parts (leaves) of the plant species

$$TF = {[Metal]_{leaves}}/{[Metal]_{roots}} \text{----------Eq 4}$$

Biological Concentration Factor (BCF) was calculated using Eq 5 as metal concentration ratio of plants root to soil (Yoon *et al.*, 2006).

$$BCF = {[Metal]_{plant\ tissue}}/{[Metal]_{soil}} \text{------Eq 5}$$

2.8. Statistical analysis

All the data statistically represented in the form of means and standard errors of triplicates. Independent sample t test was used for significance of difference at $P<0.05$ to examine difference between the species metal concentrations in plant samples among the control and dumping sites. All statistical analysis were performed using Statistical package SPSS (version 13).

3. Results

3.1. Heavy metal content in soil from control and dumping site

The distribution of mean concentration elements present in the soils from control and dumping site are shown in Table 3. 8. Cd content in the dumping area is 4.7 mg/kg while Cu and Mn recorded were 71.82 mg/kg and 13.3 mg/kg respectively. Other values are 240.7 mg/kg for Pb and 341.3 mg/kg for Zn. Whereas Cr and Ni recorded were 62.52mg/kg and 56.5mg/kg respectively. While in the control area 24.12 mg/kg and 52.67 mg/kg was found for Pb and Zn, respectively. Cd and Cu showed 0.24 mg/kg and 9.07 mg/kg respectively. The profile of metal contents in the dumping area were Fe>Zn> Pb> Cu> Cr> Ni> Mn> Cd>Co.

3.2. Heavy metal concentration in leaves from dumping site

Total concentration of metals in plants leaves and roots from control and dumping site is given in Table 3.1 - 3.2 respectively. In plants from dumping site Cu concentration was highest in *M. coromandelianum* leaves 23.55 mg/kg, and lowest in *E. helioscopia* leaves 14.9 mg/kg. Ni concentration was highest in *T. officinale* leaves 34.58 mg/kg and lowest in *C. album* leaves (26.15 mg/kg) from dumping site. Whereas in case of Cr it was observed that Cr concentration was highest (46.85 mg/kg) in *C. album* leaves and lowest (38.65 mg/kg) in *M. coromandelianum* in dumping site. Whereas Fe concentration varied between 185.6 mg/kg in *T. officinale* leaves and 40.1 mg/kg in *C. sativa* leaves from dumping site. As in the case of Zn, *P. hysterophorus* showed the highest value i.e. 24.73 mg/kg and lowest was observed in *T. officinale* leaves 15.6 mg/kg. Maximum Pb content was recorded in *C. sativa* leaves from dumping site 29.2 mg/kg and minimum 24.7 mg/kg in *P. hysterophorus* leaves. *E. helioscopia* showed maximum value 32.3 mg/kg for Co and minimum was observed in *C. didymus i.e.* 16.95 mg /kg. The results indicated that accumulation of Pb, Co, Cu, Ni, Fe, Cr, Zn in none of the leaves of the plants species studied was more than 1000 mg/kg. However, it varied greatly among plant species.

Where as in case of macronutrients Mg concentration was highest (290.8 mg/kg) in *C. album* leaves and lowest in *E. helioscopia* leaves (123.9 mg/kg).Ca Highest value was recorded by *T. officinale* (1040 mg/kg) Leaves from dumping site. *C. didymus*

showed the maximum value for Na (1449.2 mg/kg). *P. hysterophorus* showed maximum value for K i.e. 1315 mg/kg.

3.3. Total metal concentration in leaves

Total mean concentration of metals in leaves and roots from control and dumping site is given in Table 3.3. Mean metal contents in dumping and control site leaves were 30.65, 19.61, 79.49, 43.18, 13.63, 17.61, 0.53, 24.71, 28.09 and 21.61, 14.24, 173.19, 23.7, 13.74, 8.13, 0.46, 31.77, 18.74 mg/kg for Ni, Cu, Fe, Cr, Mn, Zn, Cd, Co and Pb respectively. The order observed in dumping site leaves was Fe> Cr>Ni>Pb>Co>Cu>Mn>Cd. Whereas, the trend observed in control site was Fe>Co>Cr>Ni>Pb>Cu>Mn>Cd. Ni, Cr and Zn mean concentrations were higher in leaves from dumping site i.e. 30.65, 43.1, 17.61 mg/kg respectively where $P<0.001$. Mean Fe concentration was higher (173.19 mg/kg) in control site leaves. Cr, Cd, and Pb from control site showed higher mean concentration in roots i.e. 53.5, 0.69, 28.84 mg/kg respectively. Where as in case of roots from dumping site the order observed was Fe> Co>Cr>Ni>Pb>Zn>Mn>Cu. and in control site the trend was Fe>Cr>Pb>Co>Ni>Zn>Mn>Cu.

3.4. Transfer factor (roots to leaves) of plants species

The highest TF value was found for *P. hysterophorus i.e.* 1.92 for Cu and 2.41 for Pb in control site. Whereas in dumping site *M. coromandelianum* and *C. sativa* showed 2.01 and 2.13 for Cu respectively. The results (Table 3.4) in our study from dumping site indicated that number of plant species had TF>1 for Pb, Ni and Cu in comparison to other metals in particular Cu which was accumulated in *P. hysterophorus, M. coromandelianum and C. sativa* with TF values of 1.52, 2.01, 2.13 respectively. The results showed that it was easy for *P. hysterophorus* and *M. coromandelianum* to translocate four (Cu, Zn, Ni and Co) and five (Pb, Zn, Ni, Cu and Co) metals from roots to leaves. *C. album* (Ni, Cu, Fe, Co) , *C. didymus* (Ni, Cu, Fe, Co), *C. didymus* (Ni, Cu), *C. sativa* (Ni, Cu, Cd, Zn, Co, Pb), *E. helioscopia* (Ni, Cu , Zn ,Co), *T. officinale* (Ni, Mn) had TF values > 1 for the mentioned metals.

3.5. Biological concentration factor (BCF) of different metals of roots and leaves

All the species from the dumping site showed BCF greater than one in roots and leaves for Co and Mn given in Table 3.5. *M. coromandelianum, E. helioscopia, C. sativa, C. album, P. hysterophorus and T. officinale* from dumping site showed high BCF values for Cr in roots as compared to leaves. while *C. didymus* showed high value of BCF in their leaves than roots for Cr. *T. officinale* showed greater BCF value in roots than leaves for Pb, Cr, Co, Cd, Cu and Fe. All the species from the dumping site except *C. didymus* showed higher BCF values in leaves than roots for Ni. *C. album, M. coromandelianum, P. hysterophorus, E. helioscopia and T. officinale s*howed greater BCF value in roots than leaves for Cd.

3.6. Enzymatic activity in leaves from dumping site

SOD activity in leaves from dumping site was higher as compared to control site. And it was in the order of *M.coromandelianum >P. hysterophorus> C. stiva> C. album > E. helioscopia>C. dedymus> T. officinale*. Heavy metal stress resulted in increased POD activity in leaves from dumping site. The trend observed *was P. hysterophorus>M. coromandelianum>C. sativa>T. officinale>C. album> C. didymus > E. helioscopia*. CAT activity in leaves of *P. hysterophorus* and *M. coromandelianum* was highest in dumping site.

3.7. Copper, Lead and Cadmium absorption by roots and leaves from dumping site

The comparison of lead absorption by roots and leaves parts show that with the increase in lead in the soil from dumping site resulted in significant increase in lead absorption by roots and leaves. Increased lead levels in the soil led to increase in available lead in the soil from dumping site (Table 3.8). Table 3.2 shows the contents of lead and copper in roots and leaves from dumping site.

Generally the comparison between leaves in copper absorption shows that as soil copper content increased in dumping site the metal absorption by leaves from dumping site increased. Leaves from dumping site showed less concentration of lead in leaves as compared to roots. (Table 3.2) .whereas, Leaves from dumping site had more copper concentration than roots. From dumping site *M. coromandelianum* and *T. officinale*

leaves showed highest copper concentrations. Cd accumulated primarily in roots, and small amounts were transferred to leaves in dumping site. Higher percentage of Cd was translocated to the dumping leaves as compared to the leaves from control site. Cd concentration was highest in *T. officinale* leaves and in *P. hysterophorous*.

3.8. Chlorophyll Content in leaves from the dumping site

The level of carotenoid, total chlorophyll, chlorophyll *a*, *b* was higher in plants grown in dumping site as compared to control site. Chl a content was more in dumping site leaves in almost all species studied except for *C. album* leaves.

4. Discussion

4.1. Concentration of heavy metals in the soil at solid waste dumping site

The levels of toxic elements obtained in this study were compared with values reported in literature, Pb (240.7 mg/kg) level was found to be within a concentration range of 30-300 mg/kg reported by Kabata- Pendias and Pendias (1984) and the permissible level for soils recommended by USEPA (1986). Measured concentration of Ni (56.5 mg/kg), Cd (4.7 mg/kg) were found to be above the critical permissible concentration of 50 and 3.0 mg/kg, respectively, however, Cr (62.5 mg/kg) concentration was below the permissible concentration 400 mg/kg (provisional) as given by MAFF (1992) and EC (1986).

Results indicated that concentrations of Ni, Pb, Cd, and Cr were higher than those reported from waste dump in Umuahia, capital of Abia State in Southern Nigeria (Okoronkwo *et al.*, 2006). Similarly, the concentration of Cd was found lower than 1.85 - 8.65 and Pb higher than 42.05 - 60.85 mg/kg reported by Odukoya *et al.*, (2000). Anikwe and Nwobodo, (2002) have reported high level of heavy metals (Pb, Fe, Cu and Zn) in their study from municipal waste disposal site in Abakaliki, South eastern part of Nigeria. Uba *et al.*, (2009) has also reported both higher and lower values of Cu and Pb as compared to our values from various dumping sites in Zaria, Northern Nigeria.

The source of Zn in soil may be attributed to lubricating oil containers in which zinc is part of the many additives (Harrison *et al.*, 1981). The concentration of Zn (341.3 mg/ kg) in this study is similar to the one reported from Zaria, Northern Nigeria (Uba *et al.*, 2009) probably due to the composition and nature of the wastes. High concentrations of Zn in soil affect adversely the life processes of plants (Vysloužilová *et al.*, 2003)

According to Udosen *et al.*, (1990) the high concentration of Cd, Pb in soil may be due to anthropogenic sources such as spent batteries which are good sources of these elements (Chrysanthus *et al.*, 1996) as well as presence of fluorescent tubes, petroleum wastes, paints, plastics, metal smelting and refining, fossil fuel burning, application of phosphate fertilizers and sewage sludge contributes for Cd and Pb accumulation in soil (Kabata-Pendias, 2001).

Cr in soil may be because of Electroplating, industry, sludge, solid waste, tanneries (Knox *et al.*, 1999). Cr comes from anthropogenic sources such as solid wastes

and around 30% of Cr comes from plastic packaging such as colored plastic shopping bags (Jung *et al.,* 2006). Waste consisting of lead-chromium batteries, discarded plastic materials and empty paint containers are said to be huge source of Cr. Chromium compounds are also being used in the production of refractory steel, cleaning agents, catalytic manufacture (Shanker *et al.*, 2005). Ni has been introduced by nickel containing wastes or industrial solid waste brought to the dumpsite.

Even though heavy metal concentrations of Pb, Cr fell below the critical permissible concentration level and Cd, Ni above the critical permissible level , it seems that their persistence in the soils of the dump site may lead to increase uptake of these heavy metal by plants. Klock *et al.*, (1984) reported that plants can also accumulate relatively large amounts of these elements by foliar absorption. The uncontrolled input of heavy metals in soils results in metals accumulation in soil and that is very difficult to remove (Smith *et al.*, 1996). Thus the main problem is toxicity to the plants growing on the such contaminated soil and uptake by the plants.

4.2. Heavy metal content of plant (leaves)

Accumulation of metals varied greatly among plants species and uptake of an element by a plant is primarily dependent on plant species, soil quality and its inherent controls (Chunilall *et al.*, 2005). In soils metal solubility depends on pH, cations exchange capacity, organic carbon content and oxidation state of the system (Ghosh and Singh, 2005).Toxic metal contents in the foliage of plants of moderate tolerances to their surplus amounts has been reported by Kabata-Pendias and Pendias (1991) as Cr 5-20, Ni 10-100, Cd 5-30, Pb 30-300, Cu 20-100 and Zn 100-4000 mg/kg d.w.

Plants from dumping and control site showed significantly higher accumulation of toxic metals (Cr, Ni) in leaves and followed the order as leaves > roots. Concentrations from dumping site indicated that *M. coromandelianum (23.5 mg/kg)* and *T. officinale (22.1 mg/kg)* had slightly higher values of Cu in leaves than normal limit while the metal concentration was higher in leaves than roots. On the other hand, Cu concentration in leaves from the control site was below the permissible limits as compare to dumping site where it was found higher. Ni concentration was highest in *T. officinale* leaves 34.58 mg/kg and lowest in *C. album* leaves (26.15 mg/kg) from dumping site. Hussain and

Khan (2010) has reported lower values in *T. officinale* leaves collected from Peshawar valley as compared to the current study which are Cu (1.42 mg/kg), Cr (0.20 mg/kg), Ni (0.28 mg/kg) and Pb (4.96 mg/kg). In case of Cr it was observed that Cr concentration was highest (46.85 mg/kg) in *C. album* leaves and lowest (38.65 mg/kg) in *M. coromandelianum* at dumping site.

Present study showed that Pb accumulated mainly in leaves as compared to roots. Rosen (2002) has reported higher concentrations of Pb in leafy vegetables (e.g. lettuce). No toxic Pb content (over 30 mg/kg d.w.) was found in the examined plants. Lead level of *P. hysterophorus*, India has been reported as 39.08 mg/kg (Singh *et al.*, 2009) which is comparatively higher than the value reported in our study for *P. hysterophorus*.

Cd accumulated in roots, and small amounts were transferred to leaves in dumping site. Significantly higher percent of Cd was translocated to the dumping leaves as compared to the leaves from control site. Cd concentration was highest in *T. officinale* leaves and in *P. hysterophorous*. Linger *et al.*, (2002) has reported high values of Cd (3.92 mg/kg) and Ni (63.83 mg/kg) in *C. sativa* leaves growing on contaminated soil and these values are higher than the values reported in our study.

Presence of plant species in the dumping area with high level of Cu, Ni and Cr in leaves above the normal limits suggest their adaptation to contaminated soils and possibly developed mechanism for metal detoxification. In contrast to the present findings, several reports are available for poor translocation of Cr in upper parts of the plants (Singh *et al.*, 2004a, b). Hussain *et al.*, (2010) has reported Ni (28.60 mg/kg), Cu (16 mg/kg) and Pb (2.20 mg/kg) in *E. helioscopia* and these concentrations are much less than our study.

4.3. Transfer factor TF (root to leaves) of heavy metals

To evaluate the potential of plant species for phytoextraction and phytostabilization TF value>1 is used (Yoon *et al.*, 2006; Li *et al.*, 2007). The results indicated that *P. hysterophorus* had highest TF values for Pb (1.52), Cu (1.92), Zn (1.58) where as *C. sativa* and *M. coromandelianum* showed 1.03, 2.13, 1.14 and 1.24, 2.01, 1.08 for Pb, Cu and Zn. High root to leaves translocation of these metals indicate that these plants have vital characteristics to be used in phyto-extraction of Pb, Cu and Zn. Plant species with slow plant growth, shallow root system and small biomass production are

not recommended for phytoremediation. A sequence of decreasing TF values Cu> Co> Pb > Fe> Ni and > Cr was found for plant species. It is easy for plant species with TF >1 to translocate metals from roots to leaves than those which restrict metals in their roots. High metal accumulation indicates well develop detoxification mechanism based on sequestration of heavy metal ions in vacuoles, by binding them on appropriate ligands such as organic acids, proteins and peptides in the presence of enzymes that can function at high level of metalicions (Cui *et al.*, 2007) and metal exclusion strategies of plant species (Ghosh and Singh, 2005).

According to Ghosh and Singh (2005) phyto-extraction is a mechanism to remove the contamination from soil without destroying soil structure and fertility. The results in our study from dumping site indicated that number of plant species had TF >1 for Pb, Ni, Cu and Zn in comparison to other metals. The results showed that it was easy for *P. hysterophorus* and *M. coromandelianum* to translocate four (Cu, Zn, Ni and Co) and five (Pb, Zn, Ni, Cu and Co) metals from roots to leaves. *C. album* (Ni, Cu, Fe, Co) , *C. didymus* (Ni, Cu, Fe, Co), *C. didymus* (Ni, Cu), *C. sativa* (Ni, Cu, Cd, Zn, Co, Pb), *E. helioscopia* (Ni, Cu , Zn ,Co), *T. officinale* (Ni, Mn) had TF values > 1 for the mentioned metals.

The species which have ability of to tolerate and accumulate heavy metals are useful for phyto-stabilization and phyto-extraction (Yoon *et al.*, 2006). However, based on the present results *C. album* can be considered for phytoextraction for Ni, Cu, Fe and Co. Del-Río-Celestino *et al.*, (2006) considered *C. album* suitable for phytoextraction of Pb contaminated soils. TF value of Ni and Cu of *C. didymus* and *E. helioscopia* > 1 indicate their potential for phytoextraction .Transferring of heavy metals from root to other parts of a plant species can be very useful in biological monitoring of heavy metal contamination as well as selection of tolerant species.

4.4. Biological Concentration Factor (BCF) showing accumulation of metals in leaves and roots from dumping site

The roots ability to limit the contaminant mobility and bio-availability in the soils due to sorption, precipitation, complexation or metal valance reduction is called Phyto-stabilization (Ghosh and Singh, 2005). Heavy metals tolerant species with low TF from root to shoot and high TF from soil to roots can be used for phyto-stabilisation of contaminated soils. *M. coromandelianum, E. helioscopia, C. sativa, C. album, P.hysterophorus , T. officinale* from dumping site showed high BCF values for Cr in roots as compared to leaves indicating that these species are suitable for phytostabilization of soils contaminated with Cr. while *C. didymus* showed high values of BCF in their leaves for Cr indicating its suitability for phytoextraction of soils contaminated with Cr. Shanker *et al.*, (2005) has reported the movement of Cr in the upper parts and the availability of the Cr did not depend upon the soil properties and distribution of this element (Golovatyj *et al.*, 1999).

Whereas all the species showed BCF greater than one in roots and leaves for Co and Mn indicating that these species accumulate these metals in their roots and leaves. The plasma membrane takes up Mn+2 where it binds with malate in the cytoplasm , forming a complex Mn-malate and is transported through tonoplast membrane to the vacuole. Several other mechanisms may contribute to heavy metal tolerance depending on the type of metal and plant species (Memon *et al.,* 2001).

T. officinale showed greater BCF value in roots than leaves for Pb, Cr, Co, Cd, Cu and Fe showing that it is limiting metal mobility from roots to leaves once absorbed by roots of plants. The elevated concentration of heavy metals in roots of *T. officinale* and low translocation in above ground parts indicated its suitability for phyto-stabilisation of soils contaminated with these metals. All the species from the dumping site except *C. didymus* showed greater BCF values in leaves than roots for Ni indicating the movement of Ni from roots to leaves.

*C. album, M. coromandelianum, P. hysterophorus, E. helioscopia and T. officinale s*howed greater BCF value in roots than leaves for Cd. Zhao *et al.*, (2002) has reported rare hyperaccumulation of Cd in plants because of toxic effects of Cd (Anderson *et al.*, 2004) and may be due to the lack of unavailability of Cd, because of high affinity

of these metals to organic matter (Merritt and Erich, 2003). So, plants that are already growing on a soil contaminated with cadmium can be a better choice to grow on contaminated soils because such species are in fact showing metal tolerance.

The results indicated that none of the plant species were identified as hyperaccumulator because all species accumulated Pb, Cu, Zn, Ni, Co and Cr less than 1000 mg/kg (Baker & Brooks, 1989). However, based on TF and BCF values plant species were identified which have the potential for phytostabilization and phytoextraction. Most of the species were efficient to take up and translocate more than one metal from roots to leaves. Based on highest TF (root to shoot) values *P. hysterophorus, M. coromandelianum and C. sativa* can be used for phytoextraction of Pb, Cu and Zn. The results also find *P. hysterophorus* the most efficient species in translocating Pb, Cu, Ni, Zn, and Cu from roots to leaves from the dumping site. Whereas *T. officinale* can be used for the phytostabilization of soils contaminated with Mn, Fe, Ni, Cu and Cr.

4.5. Response of enzymes (SOD, POD, CAT) under toxic conditions resultant from heavy metals (Pb, Cd, Zn and Cu)

The presence of toxic metals in the cell leads to the formation of ROS (superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen collectively termed as ROS, which causes severe oxidative damage to different cell organelles and biomolecules such as nucleic acids, proteins, lipids and amino acids (Radotic *et al.,* 2000). As a defensive mechanism, antioxidative enzymes such as SOD, POD, CAT are correspondingly induced for removing free radicals. High antioxidant capacity prevents oxidative damage and improves the tolerance against oxidative stress established in contaminated environments. Our results highlighted enhancement in the activity of the three enzymes SOD, POD and CAT in the leaves of the studied plants collected from dumping site in response to Pb, Cu, Zn and Cd toxicity.

The increase in Cu, Pb , Zn and Cd concentrations in leaves from dumping site may be the possible reason for the increase in activity of the enzymes SOD, POD in *M. coromandalinum, P. hysterophorus* and *C. sativa.* and high CAT activity in *M. coromandelianum, P. hysterophorus* and *T. officinale* leaves. The increased SOD activity

leads to the over production of H2O2 to eliminate the toxicity of superoxide. Because these enzymes dismutase two O_2 – (peroxide) to water and oxygen (Cakmak and Horst, 1991). The higher SOD, POD, CAT activities in *M. coromandelianum* and *P. hysterophorus* indicate that the H_2O_2 scavenging mechanism is more effective in these two species as compared to other species. SOD activity alone cannot alleviate the burden of excess ROS. Peroxide is a highly toxic ROS and must be sequestered by the action of CAT and POD, which converts peroxide into oxide and oxygen (Mstte´ s, 2000). Since CAT activity coordinated with SOD activity plays a central protective role in eliminating H2O2 by breaking it down to form water and oxygen (Liang *et al.,* 2003). Thus, our results suggest that *P. hysterophorus* and *M. coromandalinum* may be more tolerant to heavy metals.

Pb increases the formation of oxygen reactive species in the plants and leads to oxidative stress in them, increase in antioxidant enzymes (SOD) has been reported in rice plants which were in a sandy medium containing 0.5 and 1 m mol Pb (NO 3)2 for 20 days (Sharma and Dubey, 2005) Which supports our study that constant exposure of plants in dumping site with Pb increases the activity of enzymes in them. Pb concentration was more in roots than leaves in almost all the plants from dumping site. Pb transfer into different organs of the plants, varies depending on species as it has been reported that the main proportion of absorbed Pb in the plant remains in roots. As we can see that Pb and Cu concentration in all these plants was more as compared to Control species. Cu transfer ratio was more in dumping site plants. And *M coromandalinum* showed highest TF value in dumping site. Thus high TF values of *P hysterophorus M coromandalinum* and *C sativa* for Pb and Cu support the fact that these metals may be responsible for maintaining high level of enzymatic activity.

As a redox metal, Cu participates in O_2 formation and subsequently in H2O2 and OH_ production via Fenton-reactions, both of which are well-characterized pathways of cellular injury by Cu and Fe (Imlay *et al.*, 1988). In previous studies it has been reported that *Datura stramonium* and *Chenopodium ambrosioides* elevated their antioxidative enzyme activities in response to Cu- toxicity (Mashhadi *et al*., 2007) Cu+2 reduces to Cu + in the presence of superoxide (O2-),and catalyze the formation processes of hydroxyl radicals (OH^o) from hydrogen peroxidase (H2O2) on the basis of Haber-

Viess reaction. Hydroxyl radical is the most strong oxidative radical in biological systems which is able to react with any biomolecule.

Cd and Zn are non-redox metals that do not participate in Fenton-reactions. However, they increase ROS production through indirect mechanisms, such as lipid peroxidation or disruption of the electron transport chain in chloroplast and mitochondria (Sharma and Dietz, 2008). The results from the dumping site indicated that plants had greater ability to accumulate Cd, primarily in roots, and to prevent the transfer of excess Cd to the leaves. Similar results were reported by Wang *et al.*, 2007. The increase in SOD activity has been reported in plant species exposed to toxic Cd concentrations (Shah *et al.*, 2001; Vitoria *et al.*, 2001; Qadir *et al.*, 2004). At very high Cd concentrations, Cd toxicity causes damage to tissue development and function by Wu *et al.*, (2003) who observed the increase in SOD, POD and CAT activities for barley genotypes at moderate Cd stress, and the decrease in enzymes activities at very high Cd concentration. In this study increased POD activity was observed in *M. coromandelianum* and *P. hysterophorus* which may be due to increased concentrations of Cd and Zn in these plants along with other heavy metals. and that there was a significant difference in POD activity in these two plants as compared to control plants. Our results are similar to those reported by Zhang *et al.*, (2007) where at higher concentration of heavy metals metal-tolerant mangrove specie Kandelia candel was able to maintain higher levels of POD activity than another mangrove specie Bruguiera gymnorhiza. Thus our study suggests that enhanced POD activity correlates with heavy metal exposure, and has protective role against oxidative stress. Higher POD activity than CAT suggests that POD can serve as a better defense tool to resist heavy metal-induced oxidative stress in plants.

All four of the heavy metals (Pb, Cu , Cd, Zn) could cause oxidative stress. Cu is able to facilitate the production of reactive oxygen species through a Fenton-like redox cycling mechanism (Halliwell and Gutteridge, 1984). Cd is able to enhance the formation of reactive oxygen species and promote cellular oxidative stress. Cd can compete with essential metals for protein-binding sites (Pruski and Dixon ,2002) leading to the release of Fe2+ and Cu2+ ions and causing increased production of reactive oxygen species and oxidative stress. Pb is also known to induce oxidative stress through over-production of reactive oxygen species (Verma and Dubey, 2003; Ruley *et al.*, 2004). Zn plays a very

important role in living organisms because of its antioxidant properties (Powell, 2000) however, an excess of Zn can cause severe effects on bio mass production, and results in oxidative stress. Enzymes such as SOD, CAT, and POD can be activated against reactive oxygen species in several organisms following heavy metal stress (Tripathi *et al.*, 2006).

4.6. Chlorophyll contents in leaves

Estimation of chlorophyll content is used to assess the impact of most environmental stresses because the change in the pigment content is connected to the visual symptoms and photosynthetic plant productivity. The level of total chlorophyll, chlorophyll *a*, *b*, carotenoid increased in plants grown in dumping site with the increase in heavy metal concentration as compared to control site. As a non-enzymatic antioxidant, carotenoid being a part of photosynthetic pigment plays an important role in the protection of chlorophyll pigment under stress conditions (Kenneth *et al.*, 2000) and thus acts as a defensive mechanism in plants to combat metal stress as in the case of present study.

Increase in chlorophyll content at dumping site may be due to the presence of essential metal ions in dumping site soil required for biosynthesis of chlorophyll. However, the increase in carotenoids in *M. coromandelianum* may be attributed to increased Cu concentration and indication of non-enzymatic antioxidant defense. In the present study, an increase in the carotenoid content was recorded in all the plants from dumping site which may be due to increased concentration of Cr, similar results are reported in plants treated with Cr (Vajpayee *et al.,* 2001). *T. officinale* showed highest concentration of Ni, Cu and Fe in leaves from dumping site which may be the possible reason for increased chlorophyll a, b content in *T. officinal*. Etiolated barley seedlings that were exposed to Ni and Fe with increased chlorophyll content has been reported by Shalygo *et al.,* (1999).

Our results are similar to those reported (Singh and Sinha, 2005) in the plant grown on tannery sludge amended soil, which may be due to the presence of essential metal ions in tannery waste required for chlorophyll biosynthesis. Increase in carotenoid content has also been reported in the plants grown on different amendment of tannery sludge (Singh *et al.*, 2004b) and on different amendments of fly ash (Sinha and Gupta, 2005).However,

degeneration of chlorophyll and carotenoid is observed in plants exposed to elevated concentrations of various heavy metals (Gallego *et al.*, 1996; Moustakas *et al.*, 1997).

Conclusions

The results showed that solid waste dumpsite is a major key player in metal contamination of soil. The heavy metals in soil were found to follow an increasing order of Co< Cd < Ni < Cr < Cu < Pb < Zn<Fe. The findings of this study show that wastes contributed to the levels of heavy metals in soils and wild medicinally important plants. Also the use of dumpsite as manure should be stopped to prevent possible transfer of toxic metals into the food chain. In general the results indicated that all the wild plants from solid waste dumping site accumulated Pb , Cu, Zn, Ni, Co. For all the tested metals Transfer factor (roots to leaves) varied from one metal to another and based on high TF these plants could be used for phytoextraction. Based on highest TF (root to shoot) values of *P. hysterophorus* (1.52,1.92,1.58), *M. coromandelianum* (1.24, 2.13,1.08) and *C. sativa* (1.03,2.01,1.14) can be used for phytoextraction of Pb, Cu and Zn at dumping sites. Plants with high antioxidant activity are more tolerant to oxidative stress. Since antioxidants are compounds capable of donating a single electron or hydrogen atom to reduce the opposite compounds. As a defensive mechanism *M. coromandelianum, P. hysterophorus,* and *C. sativa* can activate the antioxidant enzymes SOD, POD, CAT to counteract with toxic metals at dumping sites and can be used as an indicator for monitoring pollution.

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