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Screening of Plants for the Phytoremediation of Zinc from Dir Lower, Khyber Pakhunkhwa, Pakistan

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Abstract

Zinc can cause fatigue and dizziness. Its higher concentration induce observable toxicity symptoms in plant leaves as chlorosis and necrosis and reducing elongation of central root while promoting lateral roots formation. Changes in the concentration of macronutrients and reduction of water content in tissues are the other common toxicity effects of zinc. Ecological risk assessments often rely on toxicity thresholds for agronomic species, which may differ from those of restoration species. In the present research work sixty one wild plant species belongs to thirty families were collected and analyzed for the concentration of zinc metal. Present study was conducted to find out the uptake potential of zinc metal (Zn) of some plants grown in the research area and to screen plants for the phytoremediation of this metal.The total concentration of zinc in sixty one sites (soil), roots and shoots was found in the range of 3.07- 90.3, 10.13-56 and 3.87-90.9 mg/kg dry weight basis (DW) respectively. The highest zinc contents were present in the root of *Medicago minima* (L.) L. (56), *Cerastium glomeratum* Thuill. (50.2), *Geranium rotundifolium* L. (42.2) and in the shoot of *Plantago lanceolata* L. (90.9), *Sanguisorba minor* Scop. (79.8) as well as *Erigeron canadensis* L. (67.03). None of the plant species was identified as hyperaccumulator for zinc metal but based on Bioconcentration factors (BCFs), Translocation factors (TFs) and Bioaccumulation Coefficients (BACs) values most of the species showed feasibility for the phytoextraction and phytostabilization of zinc metal. *Plantago lanceolata* L., *Sanguisorba minor* Scop. *and Erigeron canadensis* L. are suggested for the phyto-extraction while *Medicago minima* (L.) L. *and Cerastium glomeratum* Thuill. are suggested for the phytostabilization of Zinc.

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Introduction

The pollution of heavy metals causes potential environmental risks. However several metals are essential but all of them are poisonous at higher concentration as by formation of free radicals they cause oxidative stress. A basic reason that why metals may be poisonous is that they can disrupt the function of pigments and enzymes by replacing their necessary metals as well as render the land unsuitable for the growth of plants and destroy the biodiversity (Malayeri *et. al.,* 2008). Soils may be polluted with zinc due to the use of fertilisers, urban composts or sewage sludge, emissions from municipal waste incinerators, the metal smelting industry, residues from metalliferous mining and other anthropological activities (Zhao *et al.,* 2003). Over dosage of zinc can cause fatigue and dizziness. (Hess and Schmid, 2002). The concentrations of zinc higher than 300 mg kg−1 in leaf induce observable toxicity indications (Marschner 1995) such as chlorosis and necrosis (Di Baccio *et al.,* 2009; Todeschini *et al.,* 2011). Zinc toxicity is evident in the roots, as reducing elongation of central root and promoting lateral roots formation (Potters *et al.,* 2007). Changes in the concentration of macronutrients in tissues and reduction of water content in tissues are the other common toxicity effects of zinc (Sagardoy *et al.,* 2009). Many physicochemical and biological methods have been applied to decrease the effects of contaminants mainly heavy metals (Sheoran *et al.,* 2011).The physico-chemical procedures of soil remediation provide the soil incompatible for the plant growth because these procedures seriously change biological activities; like remove useful microbe's i.e. beneficial bacteria, fungi, fauna and mycorrhiza in the process of removal of metals from the soil (Khan *et. al.,* 2015).Phytoremediation is basically a green technology which is the use of plants and related soil microorganisms to decrease the toxic effects or concentrations of pollutants in the environments (Greipsson, 2011). It can be used for the elimination of organic pollutants (such as, polychlorinated biphenyls, polynuclear aromatic hydrocarbons and pesticides), radionuclides and heavy metals. In addition it is a cost-effective, novel, efficient, eco-friendly, solar-driven and in situ applicable remediation approach (Singh and Prasad, 2011; Vithanage *et al.,* 2012). It has been recommended as a sustainable and reasonable in situ method to assist in the renovation of soils polluted by metalloids and metals without negative effects on the properties of soil (Mahdavian *et al.,* 2015).

Accumulators are the plants which can concentrate metals in their aerial parts. This suggests that accumulators are characterized by foliage to root concentration ratio greater than one. The root metal uptake and transport are in balance in accumulator plants while excluders limit metal transport to shoot from root and are not able to regulate the uptake of metals. Baker considered the relation between heavy metals in the plant shoots and soil and verified that accumulator's takes up high magnitudes of metals in roots and transports them to aerial parts with a logarithmic relation between the concentration of metal in soil and in shoot. He verified that shoot metal concentrations in excluders were constant and low over a varied range of soil concentration up to a certain soil value above which unrestricted transport occurs while indicators reflect soil metal concentration. This means that accumulators can be efficiently used in phytoextraction processes whereas excluders in phytostabilization (Baker, 1981).

Sufficient data about the sensitivity of non-agricultural and wild plant species to heavy metals would help land managers to make proper decisions when planning the renovation of zinc polluted soils. Much of the investigations related to zinc phytotoxicity thresholds in plants has been carried out with agricultural species. There are little findings about the properties of high zinc levels on those non-agricultural plant species suitable for revegetating and restoring zinc polluted soils (Paschke *et. al., 2005).*

In the present research work sixty one wild plant species belongs to thirty families were collected and analyzed for the concentration of zinc metal. Purpose of the study was to find out the uptake potential of zinc metal (Zn) of some plants grown in the research area and to screen plants for the phytoremediation of this metal (Zn). Zinc metal were analyzed in the soil of the root zone, in root and shoot of each plant. Phytoremediation potential of the analyzed plants grown in their natural habitats were evaluated by the calculation of Bioconcentration Factor (BCF), Translocation Factor (TF) and Bioaccumulation coefficient (BAC).

Materials and methods

Study area

Lower Dir is one of the 26 districts in the Khyber Pakhtunkhwa province of Pakistan. The district was formed in 1996, when the district of Dir was divided into Upper and Lower Dir. Timergara city is the district headquarters and largest city.

It mainly comprises the terrain drained by the Panjkora River and its affluents. Dir takes its name from the name of a village, Dir, which served as capital of the state during the Nawabs era Dir (princely state). It has District Swat in the East, Afghanistan on the West, Upper Dir on North-West and Malakand on the south. Pashto is the main spoken language of the population, followed by Kohistani and Gujri. Plants and soil for the analysis of zinc metal was collected from Timergara and its surrounding village's district Dir lower (Figure 1).

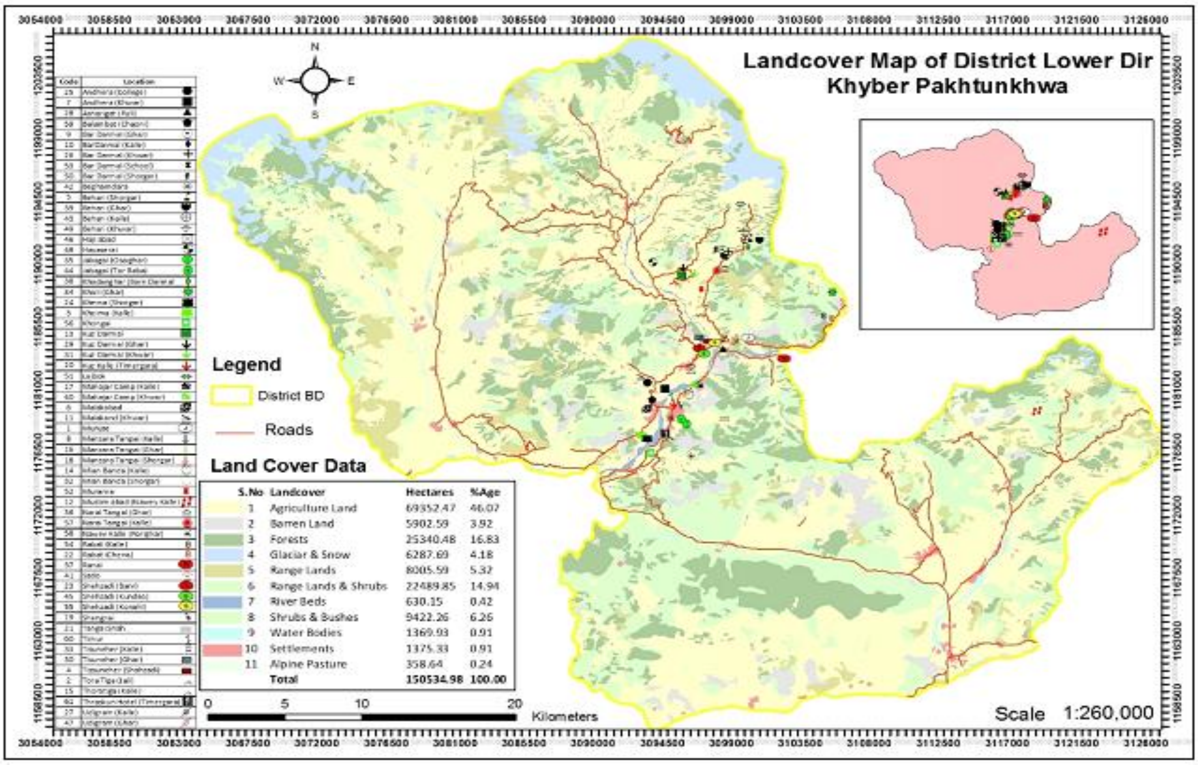

Fig. 1. Land Cover Map of District Dir Lower.

Collection of plants and soil from the study area

Sixty one plant species were collected from different locations of District Dir Lower, Khyber Pakhtunkhwa, Pakistan. Soil were also collected from the root zone of each collected plant. The Collected plants were identified with the help of Flora of Pakistan or by matching with the already preserved specimens at the Herbarium of Islamia College University Peshawar. The correct scientific names of the collected plants was confirmed using the plant list. After identification, each plant was separated into roots and shoots. These plant parts were analyzed for the concentrations of zinc using Atomic Absorption Spectroscopy (AAS).

Analysis of zinc in soil samples

The collected soil of the root zone of each plant were analyzed for the background concentrations of zinc. Zinc metal in the soil were determined according to Sharidah (1999): 5g sample of the soil were taken in a 100 mL beaker. 3 mL of 30% H_2O_2 was added to it. This was left undisturbed for 1 hour until the vigorous reaction ceased. Then 75 mL of 0.5 M HCl solution was added to it and heated on hot plate for 2 hours. The digest was filtered through a Whatman filter paper. The filtrate was used for the determination of zinc by atomic absorption spectrometry. The analysis was conducted in triplicate. Results were shown as mean ± SD.

Analysis of accumulated zinc in plant samples

For this purpose, each plant part was thoroughly washed with tape water and then with distilled water in order to remove dust and soil particles. The clean plant parts (roots and shoots) were dried in an oven at 105°C for 24 hours. Then the plant samples were grinded with the help of pistil and mortar. The powder was digested according to Awofolu (2005): 0.5 g sample of the plant part was taken into a 100 mL beaker. 5 mL concentrated (65%) HNO_3 and 2 mL $HClO_4$ were added to it and heated on hot plate until the digest became clear. The digest was allowed to cool and then filtered through a Whatman filter paper. The filtrate was collected in a 50 mL volumetric flask and diluted to the mark with distilled water. The filtrate was used for the analysis of zinc by AAS. As mentioned previously, each experiment was run in triplicate. Results were shown as mean± SD.

Bioconcentration Factor (BCF), Translocation factor (TF) and Bioaccumulation coefficient (BAC).

Bioconcentration factor (BCF), translocation factor (TF) and Bioaccumulation coefficient (BAC) of the collected plants were determined according to equation a, b and c and the overall feasibility of the plants for the phytoremediation of zinc metal was evaluated. (Zhuang *et al.,* 2007; Padmavathiamma and Li, 2007; Adesodun *et al.,* 2010; Malik *et al.,* 2010; Nazir *et al.,* 2011).

BCF=Concentration of zinc in soil / Concentration of zinc in root………… (a)

TF= Concentration of zinc in root / Concentration of zinc in shoot……….. (b)

BAC= Concentration of zinc in shoot / Concentration of zinc in soil……….(c)

Results and discussion

Concentration of zinc metal in soil

It is clear from the data of table 1 that the concentration of zinc in the soil of sixty one sites varies in the range of 3.07- 90.3 mg/kg. The concentration of zinc in the soil of the studied sites were found in the order: site 27 (90.3) > site 59 (15.92) > site 61 (14.81) > site 2 (14.13) > site 60 (13.35) > site 6 (12.12) > site 21 (11.28) > site 15 (10.75) > site 25 (10.29) > site 3 (9.73) > site 24 (9.47) > site 50 (8.78) > site 23 (8.58) > site 20 (8.39) > site 30 (7.93) > site 19 (7.71) > site 36 (7.68) > site 51 (7.22) > site 5 (6.72) ≥ site 41 (6.72) ≥ site 46 (6.72) > site 45 (6.49) > site 28 (6.4) > site 58 (6.39) > site 17 (6.37) > site 7 (6.31) > site 14 (6.3) > site 8 (6.22) > site 54 (6.17) > site 56 (6.07) > site 39 (6.01) > site 37 (5.94) > site 12 (5.91) ≥ site 22 (5.91) > site 42 (5.81) > site 53 (5.62) > site 47 (5.59) > site 18 (5.53) > site 16 (5.42) ≥ site 34 (5.42) > site 32 (5.41) ≥ site 55 (5.41) > site 48 (5.38) > site 57 (5.27) > site 40 (5.17) > site 52 (5.02) > site 26 (4.98) > site 43 (4.83) > site 10 (4.78) > site 49 (4.7) > site 29 (4.61) > site 38 (4.58) > site 4 (4.54) > site 1 (4.5) > site 33 (4.09) > site 44 (4.01) > site 9 (3.81) ≥ site 35 (3.81) > site 13 (3.7) ≥ site 31 (3.7)> site 11 (3.07). Results indicates that the lowest concentration of zinc was recorded in site 11 (3.07) while that of highest in site 27 (90.3). According to Kabata-Pendias and Pendias (1984), the sufficient Zn concentration in soil is between 25 and 150 mg kg-1. Degryse and smolders, (2006) found that the average zinc concentration in the upper horizon were 9 mg/kg in the unpolluted field and 71 mg/kg in polluted field.

It is clear from the data of table 1 that in the soil of eleven sites the concentration of zinc is higher than 9 mg/kg.

Table 1. Concentration of zinc in different parts of the plants, in soil and bioconcentration factor, translocation factor as well as bioaccumulation coefficient.

S. No.	Family	Plant Species	Site	Concentration of Zinc mg/kg (DW)			Bioconcentration Factor, translocation Factor and Bioaccumulation coefficient		
				Soil	Root	Shoot	BCF	TF	BAC
1.	Amaryllidaceae	*Allium griffithianum* Boiss.	1.	4.5 ±0.01	21.2 ±0.2	26.8 ±0.26	4.71	1.26	5.96
2.	Apiaceae	*Torilis leptophylla* (L.) Rchb.f.	2.	14.13 ±0.01	38.2 ±0.35	14.17 ±0.25	2.7	0.37	1
3.	Apocynaceae	*Catharanthus roseus* (L.) G. Don	3.	9.73 ±0.07	14.1 ±0.2	21.13 ±0.99	1.45	1.5	2.17
4.	Aspleniaceae	*Asplenium dalhousiae* Hook.	4.	4.54 ±0.05	24.87 ±0.4	14.1 ±0.44	5.48	0.57	3.11
5.	Asteraceae	*Artemisia japonica* Thunb.	5.	6.72 ±0.03	13.73 ±0.2	24 ±0.2	2.04	1.75	3.57
		Artemisia vulgaris L.	6.	12.12 ±0.02	18.27 ±0.21	20.97 ±0.21	1.51	1.15	1.73
		Calendula arvensis Boiss.	7.	6.31 ±0.02	19.2 ±0.36	3.87 ±0.15	3.04	0.2	0.61
		Cirsium vulgare (Savi) Ten.	8.	6.22 ±0.03	17.13 ±0.25	19.1 ±0.2	2.75	1.12	3.07
		Cousinia buphthalmoides Regel	9.	3.81 ±0.03	10.13 ±0.15	14.17 ±0.25	2.66	1.4	3.72
		Erigeron canadensis L.	10.	4.78 ±0.03	14.8 ±0.17	67.03 ±0.15	3.1	4.53	14.02
		Filago hurdwarica (Wall. ex DC.) Wagenitz	11.	3.07 ±0.02	29.8 ±0.35	13.17 ±0.21	9.71	0.44	4.29
		lactuca dissecta D.Don	12.	5.91 ±0.02	14.97 ±0.31	9.37 ±0.35	2.53	0.63	1.59
		Himalaiella heteromalla (D.Don) Raab-Straube	13.	3.7 ±0.02	13.73 ±0.21	11.2 ±0.3	3.71	0.82	3.03
		Silybum marianum (L.) Gaertn.	14.	6.3 ±0.03	24.6 ±0.92	12.13 ±0.32	3.9	0.49	1.93
		Xanthium strumarium L.	15.	10.75 ±0.08	26.2 ±0.53	11.6 ±0.36	2.44	0.44	1.08
6.	Boraginaceae	*Nonea edgeworthii* A. DC.	16.	5.42 ±0.02	28.3 ±0.5	9 ±0.4	5.22	0.32	1.66
		Onosma hispida Wall. ex G. Don	17.	6.37 ±0.04	24.7 ±0.4	12.8 ±0.2	3.88	0.52	2.01
7.	Brassicaceae	*Arabidopsis thaliana* (L.) Heynh.	18.	5.53 ±0.02	39.27 ±0.31	23.07 ±0.95	7.1	0.59	4.17
		Isatis tinctoria L.	19.	7.71 ±0.02	11.77 ±0.23	15.03 ±0.15	1.53	1.28	1.95
		Stellaria media (L.) Vill.	20.	8.39 ±0.05	32.67 ±0.9	11.23 ±0.31	3.48	0.34	1.33
		Sisymbrium irio L.	21.	11.28 ±0.04	24.1 ±0.2	18.47 ±0.55	2.14	0.77	1.64
8.	Buxaceae	*Sarcococca saligna* (D.Don)	22.	5.91	31.07	23.83	5.26	0.77	4.03

		Muell.-Arg. in DC., Prodr.		±0.02	±0.12	±0.4			
9.	Cannabaceae	*Cannabis sativa* L.	23.	8.58 ±0.03	25.8 ±0.4	26.7 ±0.36	3.01	1.04	3.11
10.	Caryophyllaceae	*Cerastium glomeratum* Thuill.	24.	9.47 ±0.06	50.2 ±0.4	14.2 ±0.36	5.3	0.28	1.5
11.	Crassulaceae	*Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A. Berger	25.	10.29 ±0.05	32.67 ±0.5	30.3 ±0.53	3.17	0.93	2.95
		Rosularia adenotricha (Wall. ex Edgew.) C.-A. Jansson	26.	4.98 ±0.02	16.13 ±0.31	14.4 ±0.6	3.24	0.89	2.89
12.	Euphorbiaceae	*Euphorbia helioscopia* L.	27.	90.3 ±0.2	11.5 ±0.1	28.27 ±0.25	0.13	2.46	0.31
13.	Fabaceae	*Argyrolobium stenophyllum* Boiss.	28.	6.4 ±0.01	26.07 ±0.23	18.87 ±0.35	4.07	0.72	2.95
		Medicago lupulina L.	29.	4.61 ±0.03	23.93 ±0.42	16.57 ±0.15	5.19	0.69	3.59
		Medicago minima (L.) L.	30.	7.93 ±0.04	56 ±0.5	27.87 ±0.61	7.06	0.5	3.52
		Vicia sativa L.	31.	3.7 ±0.02	26.33 ±0.42	15.93 ±0.15	7.12	0.61	4.31
14.	Geraniaceae	*Geranium rotundifolium* L.	32.	5.41 ±0.02	42.2 ±0.53	17.77 ±0.32	7.8	0.42	3.28
15.	Iridaceae	*Iris germanica* L.	33.	4.09 ±0.05	15.2 ±0.2	7.87 ±0.35	3.71	0.52	1.92
16.	Ixioliriaceae	*Ixiolirion tataricum* (Pall.) Schult. & Schult. f.	34.	5.42 ±0.03	15.97 ±0.06	8.83 ±0.31	2.95	0.55	1.63
17.	Lamiaceae	*Ajuga integrifolia* Buch.-Ham.	35.	3.81 ±0.02	14.03 ±0.35	12.6 ±0.26	3.68	0.9	3.31
		Phlomoides superba (Royle ex Benth.) Kamelin & Makhm.	36.	7.68 ±0.03	19.7 ±0.2	10.77 ±0.25	2.57	0.55	1.4
		Micromeria biflora (Buch.-Ham. ex D.Don) Benth.	37.	5.94 ±0.05	13.13 ±0.31	14.9 ±0.2	2.21	1.15	2.51
		Marrubium vulgare L.	38.	4.58 ±0.03	16.77 ±0.31	13.17 ±0.38	3.66	0.79	2.88
		Rydingia limbata (Benth.) Scheen & V.A.Albert	39.	6.01 ±0.02	14.63 ±0.15	10.33 ±0.25	2.43	0.71	1.72
		Salvia moorcroftiana Wall. ex Benth.	40.	5.17 ±0.02	32.17 ±0.38	12.23 ±0.25	6.22	0.38	2.37
		Teucrium stocksianum Boiss.	41.	6.72 ±0.03	19.87 ±0.15	22.43 ±0.4	2.96	1.13	3.34
18.	Papilionaceae	*Astragalus pyrrhotrichus* Boiss.	42.	5.81 ±0.03	14.13 ±0.23	29.27 ±0.42	2.43	2.07	5.04
19.	Plantaginaceae	*Plantago lanceolata* L.	43.	4.83 ±0.04	26.1 ±0.26	90.9 ±0.36	5.4	3.48	18.81
20.	Plumbaginaceae	*Limonium macrorhabdon* (Boiss.) O. Kuntze, Rev. Gen.	44.	4.01 ±0.02	10.43 ±0.21	16.93 ±0.4	2.6	1.62	4.22
21.	Polygalaceae	*Polygala abyssinica* R.Br. ex Fresen.	45.	6.49 ±0.06	24.73 ±0.21	23.8 ±0.26	3.81	0.96	3.67
22.	Polygonaceae	*Emex spinosa* (L.) Campd.	46.	6.72	15.03	21.74	2.24	1.45	3.24

				±0.03	±0.12	±0.32			
		Persicaria glabra (Willd.) M. Gómez	47.	5.59 ±0.04	21.17 ±0.21	14.3 ±0.7	3.79	0.68	2.56
23.	Pteridaceae	*Cheilanthes pteridoides* C. Chr.	48.	5.38 ±0.02	31.47 ±0.5	13.2 ±0.26	5.85	0.42	2.45
		Pteris cretica L.	49.	4.7 ±0.01	16.73 ±0.12	10.43 ±0.35	3.56	0.62	2.22
24.	Ranunculaceae	*Delphinium uncinatum* Hook.f. & Thomson	50.	8.78 ±0.03	24.07 ±0.25	26.2 ±0.6	2.74	1.09	2.98
		Delphinium suave Huth	51.	7.22 ±0.03	26.93 ±0.4	14.7 ±0.2	3.73	0.55	2.04
		Ranunculus arvensis L.	52.	5.02 ±0.03	28.27 ±0.31	14.47 ±0.42	5.63	0.51	2.88
25.	Rosaceae	*Duchesnea indica* (Jacks.) Focke	53.	5.62 ±0.02	19.7 ±0.2	23.13 ±0.42	3.51	1.17	4.12
		Sanguisorba minor Scop.	54.	6.17 ±0.03	17.03 ±0.12	79.8 ±1.15	2.76	4.69	12.93
		Rosa macrophylla Lindl.	55.	5.41 ±0.02	20.4 ±0.4	14.27 ±0.42	3.77	0.7	2.64
26.	Scrophulariaceae	*Verbascum Thapsus* L.	56.	6.07 ±0.02	10.13 ±0.15	18.93 ±0.15	1.67	1.87	3.12
		Wulfeniopsis amherstiana (Wall. ex Benth.) D.Y. Hong	57.	5.27 ±0.03	20.07 ±0.21	25.87 ±0.31	3.81	1.29	4.91
27.	Solanaceae	*Solanum nigrum* L., Sp. Pl.	58.	6.39 ±0.03	23.07 ±0.35	14.57 ±0.45	3.61	0.63	2.28
28.	Thymelaeaceae	*Daphne mucronata* Royle	59.	15.92 ±0.07	13.07 ±0.21	21.63 ±0.15	0.82	1.66	1.35
29.	Urticaceae	*Urtica pilulifera* L.	60.	13.35 ±0.05	34.13 ±0.42	18.43 ±0.45	2.56	0.54	1.38
30.	Verbenaceae	*Verbena officinalis* L.	61.	14.81 ±0.16	16.3 ±0.26	19 ±0.1	1.1	1.17	1.28

Concentration of zinc in soil and plant parts is shown as mean (n=3) ±SD, Bioconcentration Factor (BCF) = Conc. Of Zinc in root ÷ Conc. Of Zinc in Soil, Translocation Factor (TF) = Conc. Of Zinc in Shoot ÷ Conc. Of Zinc in root, Bioaccumulation Coefficient (BAC) = Conc. Of Zinc in Shoot ÷ Conc. Of Zinc in root.

Concentration of zinc in the roots of the analyzed plants

The concentration of Zinc was found in the roots of the plant in the range of 10.13_56 mg/kg. Its concentration in the roots of all the analyzed plants was found in the order: *Medicago minima* (L.) L. (56) > *Cerastium glomeratum* Thuill. (50.2) > *Geranium rotundifolium* L. (42.2) > *Arabidopsis thaliana* (L.) Heynh. (39.27) > *Torilis leptophylla* (L.) Rchb.f. (38.2) > *Urtica pilulifera* L. (34.13) > *Stellaria media* (L.) Vill. (32.67) ≥ *Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A. Berger (32.67) > *Salvia moorcroftiana* Wall. ex Benth. (32.17) > *Cheilanthes pteridoides* C. Chr. (31.47) > Sarcococca *saligna* (D.Don) Muell.-Arg. in DC., Prodr. (31.07) > *Filago hurdwarica* (Wall. ex DC.) Wagenitz (29.8) > Nonea *edgeworthii* A. DC. (28.3) > Ranunculus *arvensis* L. (28.27) > *Delphinium suave* Huth (26.93) > *Vicia sativa* L. (26.33) > *Xanthium strumarium* L. (26.2) > *Plantago lanceolata* L. (26.1) > *Argyrolobium stenophyllum* Boiss (26.07) > Cannabis *sativa* L. (25.8) > *Asplenium dalhousiae* Hook. (24.87) > *Polygala abyssinica* R.Br. ex Fresen. (24.73) > *Onosma hispida* Wall. ex G. Don (24.7) > *Silybum marianum* (L.) Gaertn. (24.6) >

Sisymbrium irio L. (24.1) > *Delphinium uncinatum* Hook.f. & Thomson (24.07) > *Medicago lupulina* L. (23.93) > *Solanum nigrum* L., Sp. Pl. (23.07) > *Allium griffithianum* Boiss. (21.2) > *Persicaria glabra* (Willd.) M. Gómez (21.17) > *Rosa macrophylla* Lindl. (20.4) > *Wulfeniopsis amherstiana* (Wall. ex Benth.) D.Y. Hong (20.07) > *Teucrium stocksianum* Boiss. (19.87) > *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. (19.7) ≥ *Duchesnea indica* (Jacks.) Focke (19.7) > *Calendula arvensis* Boiss. (19.2) > *Artemisia vulgaris* L. (18.27) > *Cirsium vulgare* (Savi) Ten. (17.13) > *Sanguisorba minor* Scop. (17.03) > *Marrubium vulgare* L. (16.77) > *Pteris cretica* L. (16.73) > *verbena officinalis* L. (16.3) > *Rosularia adenotricha* (Wall. ex Edgew.) C.-A. Jansson (16.13) > *Ixiolirion tataricum* (Pall.) Schult. & Schult. f. (15.97) > *Iris germanica* L. (15.2) > *Emex spinosa* (L.) Campd. (15.03) > *lactuca dissecta* D.Don (14.97) > *Erigeron canadensis* L. (14.8) > *Rydingia limbata* (Benth.) Scheen & V.A.Albert (14.63) > *Astragalus pyrrhotrichus* Boiss. (14.13) > *Catharanthus roseus* (L.) G. Don (14.1) > *Ajuga integrifolia* Buch.-Ham. (14.03) > *Artemisia japonica* Thunb. (13.73) ≥ *Himalaiella heteromalla* (D.Don) Raab-Straube (13.73) > *Micromeria biflora* (Buch.-Ham. ex D.Don) Benth. (13.13) > *Daphne mucronata* Royle (13.07) > *Isatis tinctoria* L. (11.77) > *Euphorbia helioscopia* L. (11.5) > *Limonium macrorhabdon (Boiss.)* O. Kuntze, Rev. Gen. (10.43) > *Cousinia buphthalmoides* Regel (10.13) ≥ *Verbascum Thapsus* L. (10.13).The permissible limit of Zinc in plants recommended by WHO is 50 mg/kg (Shah *et al.,* 2011). Results showed that the concentration of Zinc in the roots of *Medicago minima* (L.) L. (56) and *Cerastium glomeratum* Thuill. (50.2) is higher than that of this permissible limit while in the roots of the rest of the plants its concentration is less than that permissible limit.

Concentration of zinc in the shoots of the analyzed plants

The concentration of zinc in the shoots of the analyzed plants were found in the order: *Plantago lanceolata* L. (90.9) > *Sanguisorba minor Scop.* (79.8) > *Erigeron canadensis* L. (67.03) > *Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A. Berger (30.3) > *Astragalus pyrrhotrichus* Boiss. (29.27) > *Euphorbia helioscopia* L. (28.27) > *Medicago minima* (L.) L. (27.87) > *Allium griffithianum* Boiss. (26.8) > *Cannabis sativa* L. (26.7) > *Delphinium uncinatum Hook.f. & Thomson* (26.2) > *Wulfeniopsis amherstiana* (Wall. ex Benth.) D.Y. Hong (25.87) > *Artemisia japonica* Thunb. (24) > *Sarcococca saligna* (D.Don) Muell.-Arg. in DC., Prodr. (23.83) > *Polygala abyssinica* R.Br. ex Fresen. (23.8) > *Duchesnea indica* (Jacks.) Focke (23.13) > *Arabidopsis thaliana* (L.) Heynh. (23.07) > *Teucrium stocksianum* Boiss. (22.43) > *Emex spinosa* (L.) Campd. (21.74) > *Daphne mucronata* Royle (21.63) > *Catharanthus roseus* (L.) G. Don (21.13) > *Artemisia vulgaris* L. (20.97) > *Cirsium vulgare* (Savi) Ten. (19.1) > *verbena officinalis* L. (19) > *Verbascum Thapsus* L. (18.93) > *Argyrolobium stenophyllum* Boiss (18.87) > *Sisymbrium irio* L. (18.47) > *Urtica pilulifera* L. (18.43) > *Geranium rotundifolium* L. (17.77) > *Limonium macrorhabdon* (Boiss.) O. Kuntze, Rev. Gen. (16.93) > *Medicago lupulina* L. (16.57) > *Vicia sativa* L. (15.93) > *Isatis tinctoria* L. (15.03) > *Micromeria biflora* (Buch.-Ham. ex D.Don) Benth. (14.9) > *Delphinium suave* Huth (14.7) > *Solanum nigrum* L., Sp. Pl. (14.57) > *Ranunculus arvensis* L. (14.47) > *Rosularia adenotricha* (Wall. ex Edgew.) C.-A. Jansson (14.4) > *Persicaria glabra* (Willd.) M. Gómez (13.3) > *Rosa macrophylla* Lindl. (14.27) > *Cerastium glomeratum* Thuill. (14.2) > *Torilis leptophylla* (L.) Rchb.f. (14.17) ≥ *Cousinia buphthalmoides* Regel (14.17) > *Asplenium dalhousiae* Hook. (14.1) > *Cheilanthes pteridoides* C. Chr. (13.2) > *Filago hurdwarica* (Wall. ex DC.) *Wagenitz* (13.17) ≥ *Marrubium vulgare* L. (13.17) > *Onosma hispida* Wall. ex G. Don (12.8) > *Ajuga integrifolia* Buch.-Ham. (12.6) > *Salvia moorcroftiana* Wall. ex Benth. (12.23) > *Silybum marianum* (L.) Gaertn. (12.13) > *Xanthium strumarium* L. (11.6) > *Stellaria media* (L.) Vill. (11.23) > *Himalaiella heteromalla* (D.Don) Raab-

Straube (11.2) > *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. (10.77) > *Pteris cretica* L. (10.43) > *Rydingia limbata* (Benth.) Scheen & V.A.Albert (10.33) > *lactuca dissecta* D.Don (9.37) > *Nonea edgeworthii* A. DC. (9) > *Ixiolirion tataricum* (Pall.) Schult. & Schult. f. (8.83) > *Iris germanica* L. (7.87) > *Calendula arvensis* Boiss. (3.87). WHO's recommended limit of zinc in plants is 50 mg/kg (Shah *et al.,* 2011). It is clear from the data that in three plants *Plantago lanceolata* L. (90.9), *Sanguisorba minor* Scop. (79.8) and *Erigeron canadensis* L. (67.03) the concentration of zinc is above the permissible limit while in the shoot of the rest of the plants its concentration is below this limit.

Bioconcentration Factor (BCF) of the analyzed plants for zinc

Bioconcentration factor (BCF) was calculated as metal concentration ratio of plant roots to soil (Yoon *et al.*, 2006; Malik *et al.,* 2010; Nazir *et al.,* 2011). The calculated bioconcentration factor (BCF) of all the plants was in the order: *Filago hurdwarica* (Wall. ex DC.) Wagenitz (9.71) > *Geranium rotundifolium* L. (7.8) > *Vicia sativa* L. (7.12) > *Arabidopsis thaliana* (L.) Heynh. (7.1) > *Medicago minima (L.) L.* (7.06) > *Salvia moorcroftiana* Wall. ex Benth. (6.22) > *Cheilanthes pteridoides* C. Chr. (5.85) > *Ranunculus arvensis* L. (5.63) > *Asplenium dalhousiae* Hook. (5.48) > *Plantago lanceolata* L. (5.4) > *Cerastium glomeratum* Thuill. (5.3) > *Sarcococca saligna* (D.Don) Muell.-Arg. in DC., Prodr. (5.26) > *Nonea edgeworthii* A. DC. (5.22) > *Medicago lupulina* L. (5.19) > *Allium griffithianum* Boiss. (4.71) > *Argyrolobium stenophyllum* Boiss (4.07) > *Silybum marianum* (L.) Gaertn. (3.9) > *Onosma hispida* Wall. ex G. Don (3.88) > *Polygala abyssinica* R.Br. ex Fresen. (3.81) ≥ *Wulfeniopsis amherstiana* (Wall. ex Benth.) D.Y. Hong (3.81) > *Persicaria glabra* (Willd.) M. Gómez (3.79) > *Rosa macrophylla* Lindl. (3.77) > *Delphinium suave* Huth (3.73) > *Himalaiella heteromalla* (D.Don) Raab-Straube (3.71) ≥ *Iris germanica* L. (3.71) > *Ajuga integrifolia* Buch.-Ham. (3.68) > *Marrubium vulgare* L. (3.66) > *Solanum nigrum* L., Sp. Pl. (3.61) > *Pteris cretica* L. (3.56) > *Duchesnea indica* (Jacks.) Focke (3.51) > *Stellaria media* (L.) Vill. (3.48) > *Rosularia adenotricha* (Wall. ex Edgew.) C.-A. Jansson (3.24) > *Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A. Berger (3.17) > *Erigeron canadensis* L. (3.1) > *Calendula arvensis* Boiss. (3.04) > *Cannabis sativa* L. (3.01) > *Teucrium stocksianum* Boiss. (2.96) > *Ixiolirion tataricum* (Pall.) Schult. & Schult. f. (2.95) > *Sanguisorba minor* Scop. (2.76) > *Cirsium vulgare* (Savi) Ten. (2.75) > *Delphinium uncinatum* Hook.f. & Thomson (2.74) > *Torilis leptophylla* (L.) Rchb.f. (2.7) > *Cousinia buphthalmoides* Regel (2.66) > *Limonium macrorhabdon* (Boiss.) O. Kuntze, Rev. Gen. (2.6) > *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. (2.57) > *Urtica pilulifera* L. (2.56) > *lactuca dissecta* D.Don (2.53) > *Xanthium strumarium* L. (2.44) > *Rydingia limbata* (Benth.) Scheen & V.A.Albert (2.43) ≥ *Astragalus pyrrhotrichus* Boiss. (2.43) > *Emex spinosa* (L.) Campd. (2.24) > *Micromeria biflora* (Buch.-Ham. ex D.Don) Benth. (2.21) > *Sisymbrium irio* L. (2.14) > *Artemisia japonica* Thunb. (2.04) > *Verbascum Thapsus* L. (1.67) > *Isatis tinctoria* L. (1.53) > *Artemisia vulgaris* L. (1.51) > *Catharanthus roseus* (L.) G. Don (1.45) > *verbena officinalis* L. (1.1) > *Daphne mucronata* Royle (0.82) > *Euphorbia helioscopia* L. (0.13). Fitz and Wenzel (2002) demonstrated that plants exhibiting BCF value less than one are unsuitable for the phytoextraction of metals. Results showed that the calculated bioconcentration factor of all the plants were greater than one except *Daphne mucronata* Royle (0.82) and *Euphorbia helioscopia* L. (0.13) whose BCF value is less than one.

Translocation Factor (TF) of the analyzed plants for zinc

Translocation Factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root (Cui *et al.*, 2007; Li *et al.*, 2007; Malik *et al.,* 2010; *Nazir et al.,* 2011).The calculated translocation factor of all the analyzed plants are shown in table 1 The translocation factors of the plants were found in the order: *Sanguisorba minor* Scop. (4.69) > *Erigeron canadensis* L. (4.53) > *Plantago lanceolata* L. (3.48) > *Euphorbia helioscopia* L. (2.46) > *Astragalus pyrrhotrichus* Boiss. (2.07) > *Verbascum Thapsus* L. (1.87) > *Artemisia japonica* Thunb. (1.75) > *Daphne mucronata* Royle (1.66) > *Limonium macrorhabdon* (Boiss.) O. Kuntze, Rev. Gen. (1.62) > *Catharanthus roseus* (L.) G. Don (1.5) > *Emex spinosa* (L.) Campd. (1.45) > *Cousinia buphthalmoides* Regel (1.4) > *Wulfeniopsis amherstiana* (Wall. ex Benth.) D.Y. Hong (1.29) > *Isatis tinctoria* L. (1.28) > *Allium griffithianum* Boiss. (1.26) > *Duchesnea indica* (Jacks.) Focke (1.17) ≥ *verbena officinalis* L. (1.17) > *Artemisia vulgaris* L. (1.15) ≥ *Micromeria biflora* (Buch.-Ham. ex D.Don) Benth. (1.15) > *Teucrium stocksianum* Boiss. (1.13) > *Cirsium vulgare* (Savi) Ten. (1.12) > *Delphinium uncinatum* Hook.f. & Thomson (1.09) > *Cannabis sativa* L. (1.04) > *Polygala abyssinica* R.Br. ex Fresen. (0.96) > *Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A. Berger (0.93) > *Ajuga integrifolia* Buch.-Ham. (0.9) > Rosularia *adenotricha* (Wall. ex Edgew.) C.-A. Jansson (0.89) > *Himalaiella heteromalla* (D.Don) Raab-Straube (0.82) > *Marrubium vulgare* L. (0.79) > *Sisymbrium irio L.* (0.77) ≥ *Sarcococca saligna* (D.Don) Muell.-Arg. in DC., Prodr. (0.77) > *Argyrolobium stenophyllum* Boiss (0.72) > *Rydingia limbata* (Benth.) Scheen & V.A.Albert (0.71) > *Rosa macrophylla* Lindl. (0.7) > *Medicago lupulina* L. (0.69) > *Persicaria glabra* (Willd.) M. Gómez (0.68) > *lactuca dissecta* D.Don (0.63) ≥ *Solanum nigrum* L., Sp. Pl. (0.63) > *Pteris cretica* L. (0.62) > *Vicia sativa* L. (0.61) > *Arabidopsis thaliana* (L.) Heynh. (0.59) > *Asplenium dalhousiae* Hook. (0.57) > *Ixiolirion tataricum* (Pall.) Schult. & Schult. f. (0.55) ≥ *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. (0.55) ≥ *Delphinium suave* Huth (0.55) > *Urtica pilulifera* L. (0.54) > *Onosma hispida* Wall. ex G. Don (0.52) ≥ *Iris germanica* L. (0.52) > *Ranunculus arvensis* L. (0.51) > *Medicago minima* (L.) L. (0.5) > *Silybum marianum* (L.) Gaertn. (0.49) > *Filago hurdwarica* (Wall. ex DC.) Wagenitz (0.44) ≥ *Xanthium strumarium* L. (0.44) > *Geranium rotundifolium* L. (0.42) ≥ *Cheilanthes pteridoides* C. Chr. (0.42) > *Salvia moorcroftiana* Wall. ex Benth. (0.38) > *Torilis leptophylla* (L.) Rchb.f. (0.37) > Stellaria *media* (L.) Vill. (0.34) > *Nonea edgeworthii* A. DC. (0.32) > *Cerastium glomeratum* Thuill. (0.28) > *Calendula arvensis* Boiss. (0.2). Translocation factor value greater than one indicates translocation of metal from root to above ground part (Jamil *et al.,* 2009). Results showed that the TF value of thirty eight plant species is less than one while that of twenty three plants is greater than one. Among the twenty three plants; *Sanguisorba minor Scop.* (4.69), *Erigeron canadensis* L. (4.53), *Plantago lanceolata* L. (3.48), *Euphorbia helioscopia* L. (2.46) and *Astragalus pyrrhotrichus* Boiss. (2.07) showed high TF value.

Bioaccumulation coefficient (BAC) of the analyzed plants for zinc

Bioaccumulation coefficient (BAC) was calculated as ratio of heavy metal in shoots to that in soil (Li *et al.*, 2007; Cui *et al.*, 2007; Malik *et al.,* 2010; Nazir *et al.,* 2011).The calculated Bioaccumulation coefficient of each plant species as clear from table 1 was found in the order: *Plantago lanceolata* L. (18.81) > *Erigeron canadensis* L. (14.02) > *Sanguisorba minor* Scop. (12.93) > *Allium griffithianum* Boiss. (5.96) > *Astragalus pyrrhotrichus* Boiss. (5.04) > *Wulfeniopsis amherstiana* (Wall. ex Benth.) D.Y. Hong (4.91) > *Vicia sativa* L. (4.31) > *Filago hurdwarica* (Wall. ex DC.) Wagenitz (4.29) > *Limonium macrorhabdon* (Boiss.) O. Kuntze, Rev. Gen. (4.22) > *Arabidopsis thaliana* (L.) Heynh. (4.17) > *Duchesnea indica* (Jacks.) Focke (4.12) > *Sarcococca saligna* (D.Don) Muell.-Arg. in DC., Prodr.(4.03)>*Cousinia buphthalmoides* Regel (3.72) > *Polygala abyssinica* R.Br. ex Fresen. (3.67) > *Medicago lupulina* L. (3.59) > *Artemisia japonica* Thunb. (3.57) > *Medicago minima* (L.) L. (3.52) > *Teucrium stocksianum* Boiss. (3.34) > *Ajuga integrifolia* Buch.-Ham. (3.31) > *Geranium rotundifolium* L. (3.28) > *Emex spinosa* (L.) Campd. (3.24) > *Verbascum Thapsus* L. (3.12) > *Asplenium* *dalhousiae* Hook. (3.11) ≥ *Cannabis sativa* L. (3.11) > *Cirsium vulgare* (Savi) Ten. (3.07) > *Himalaiella heteromalla* (D.Don) Raab-Straube (3.03) > *Delphinium uncinatum* Hook.f. & Thomson (2.98) > *Bryophyllum daigremontianum* (Raym.-Hamet & Perrier) A. Berger (2.95) ≥ *Argyrolobium stenophyllum* Boiss (2.95) > *Rosularia adenotricha* (Wall. ex Edgew.) C.-A. Jansson (2.89) > Marrubium vulgare L. (2.88) ≥ *Ranunculus arvensis* L. (2.88) > *Rosa macrophylla* Lindl. (2.64) > *Persicaria glabra* (Willd.) M. Gómez (2.56) > *Micromeria biflora* (Buch.-Ham. ex D.Don) Benth. (2.51) > *Cheilanthes pteridoides* C. Chr. (2.45) > *Salvia moorcroftiana* Wall. ex Benth. (2.37) > *Solanum nigrum* L., Sp. Pl. (2.28) > *Pteris cretica* L. (2.22) > *Catharanthus roseus* (L.) G. Don (2.17) > Delphinium *suave* Huth (2.04) > *Onosma hispida* Wall. ex G. Don (2.01) > *Isatis tinctoria* L. (1.95) > *Silybum marianum* (L.) Gaertn. (1.93) > *Iris germanica* L. (1.92) > *Artemisia vulgaris* L. (1.73) > *Rydingia limbata* (Benth.) Scheen & V.A.Albert (1.72) > *Nonea edgeworthii* A. DC. (1.66) > *Sisymbrium irio* L. (1.64) > *Ixiolirion tataricum* (Pall.) Schult. & Schult. f. (1.63) > *lactuca dissecta* D.Don (1.59) > *Cerastium glomeratum* Thuill. (1.5) > *Phlomoides superba* (Royle ex Benth.) Kamelin & Makhm. (1.4) > *Urtica pilulifera* L. (1.38) > *Daphne mucronata* Royle (1.35) > *Stellaria media* (L.) Vill. (1.33) > *verbena officinalis* L. (1.28) > *Xanthium strumarium* L. (1.08) > *Torilis leptophylla* (L.) Rchb.f. (1) > *Calendula arvensis* Boiss. (0.61) > *Euphorbia helioscopia* L. (0.31). Results shows that the BAC values of only two plant species; *Calendula arvensis* Boiss. (0.61) and *Euphorbia helioscopia* L. (0.31) are less than one while that of all other plants is greater than one. Only plant species with BCF, BAC and TF greater than one have the potential for the remediation process (Nazir *et al.,* 2011).

In general no plant species was identified as hyperaccumulator for zinc because in the above ground parts of all the plant species zinc content was found less than 3000 mg/kg dry weight basis but the bioconcentration factor (BCF), Translocation factor (TF) and Bioaccumulation coefficient value of the twenty one plant species were found greater than one. Metal indicators accumulate heavy metals in their aerial parts (Sheoran *et al.,* 2011). Translocation factor value greater than one indicates the translocation of the metal from root to above ground part, (Jamil *et al.,* 2009) and Yoon *et al.* (2006) reported that, only plant species with both BCF and TF value greater than one have the potential to be used for the phytoextraction of metals. *Plantago lanceolata* L., *Sanguisorba minor* Scop. and *Erigeron canadensis* L. are suggested for the phyto-extraction of zinc metal. Most of the plant species showed bioconcentration factor value greater than one but Translocation factor or Bioaccumulation coefficient value less than one. Metal excluders accumulate heavy metals from substrate into their roots but restrict their transport and entry into their aerial parts (Sheoran *et al.,* 2011; Malik and Biswas, 2012). Such plants have a low potential for metal extraction but may be efficient for phytostabilization purposes (Lasat, 2002; Barcelo and Poschenrieder, 2003). *Medicago minima* (L.) and *Cerastium glomeratum* Thuill. are suggested for the phytostabilization of Zinc.

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