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Enrichment coefficient and translocation factors of Fe and Cu in weeds growing in Sandila Industrial Area, Hardoi District, Uttar Pradesh, India

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ABSTRACT

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Bioaccumulation of Fe and Cu in various weeds growing on industrial wasteland soil, rich in these metals, was investigated in different seasons, viz. summer (May), rainy (September) and winter (January). The wasteland soil was investigated with higher Bulk Density (BD), Water Holding Capacity (WHC), Electric Conductivity (EC), Organic Carbon (OC) and major nutrients, NPK than the fertile soil in the area. There were little seasonal differences in May, September and January 2008. The soil contained (N= 487.23 - 492.01 $\mu g\ g^{-1}$, P= 619.00 - 635.35 $\mu g\ g^{-1}$, K= 928.13 - 968.67 $\mu g\ g^{-1}$, Fe= 1600.00 - 1729.21 $\mu g\ g^{-1}$, Cu= 56.25 - 61.91$\mu g\ g^{-1}$) in 14 to 20 weeds were accruing in different seasons on the wasteland soil and the bioaccumulation of Fe and Cu in roots and shoots of natural flora was determined in three seasons. Metal removal from wasteland soil and waste substrates is most effective with the use of *Bothriochloa pertusa*, *Saccharum munja*, *Saccharum spontaneum* and *Cyperus rotundus*, because these plants were accumulating significantly higher amount of metals in all three seasons.

Key-words: Bioaccumulation, weed flora, industrial wasteland, metal, Sandila Industrial Area, Uttar Pradesh, India.

INTRODUCTION

Development is an irreversible process and necessary for the economic and social development. India is now trying to become a developed country by 2020 and has got immense scope for further industrial growth (Dutta & Mukherjee 2010). The anthropogenic inputs associated with industrial practices, mineral exploration, industrial processes and solid waste management are important contributors to heavy metal contamination of natural ecosystem (Cunningham et al. 1995). The industrial effluents often contain large quantity of toxic heavy metals. These metals are non-biodegradable, persistent and can be differentially toxic to microbes (Giller et al. 2009), plants (Singh et al. 2007, Bauddh & Singh 2009, 2011, Ghavri & Singh 2010, Sharma et al. 2010), animals (Rainbow 2007) and human beings (Butkus & Baltrenaite 2007, Lim & Schoenung 2010). The main advantage associated with study of plants including crops, is their ability to accumulate

metals, if grown on metal polluted land or irrigated with polluted water (Ghavri et al. 2010, 2013, Ghavri & Singh 2012). Thus, plants serve as a good tool for phytoremediation. However, determination of the nature of toxicity, distribution of toxicants and level of accumulation in different plant parts would be essential before selection and cultivation of plants for phytoremediation (Barman et al. 2000, Kumar et al. 2009, 2012). However, not all plants are equally resistant to all types of pollutants in the environment. It appears that the plant resistance against a particular toxicant is also dependent on the cyto-genetic makeup of the particular species.

Thus, study of physicochemical parameters of the wasteland soil including occurrence of certain metals will provide an account of soil properties to be reclaimed. Therefore, physicochemical properties of the wasteland soil including certain metals were analyzed to assess toxicity potential of the wasteland soil of Sandila Industrial Area (SIA) in different seasons.

MATERIAL AND METHOD

Site description: The study area is located at Sandila industrial area, Uttar Pradesh, India. Sandila lies between 26° 53" and 27° 46" North latitudes and between 79° 41" and 80° 46" East longitudes. Certain industries, viz. cotton mill, vegetable oil mill, steel industry (all closed now) have discharged, and safe yeast industry, milk powder factory, chemicals factory, etc. (still operating) are discharging their effluents in the water and soil of this area. The soil was completely barren at the time of sampling during January, May and September of 2007 and 2008. The selected area was uniform in topography and was submerged with rainwater during September – November.

Sampling: On the basis of the existing information about agriculture, we selected 8 locations for soil sampling. The plant samples were also collected from these locations. Composite soil samples were collected from 0 to 20 cm soil layer, mainly from the root zone (rhizosphere) from each site. In total, plant samples, including roots, shoots, were collected in summer, 2008. The plants for this study were based on the samples collected in summer (Table 1).

Soil analysis: After transportation to lab, large stones and plant debris were removed from the soil samples, air dried at room temperature for 6 d, and sieved through a 2-mm mesh. The concentrations of metals in soils were determined in the Environmental Sciences Laboratory of Babasaheb Bhimrao Ambedkar University, Lucknow, India. For the metal analysis, soil samples were dried in oven to constant weight and digested in conc. HNO_3 and $HClO_4$ in 5:1 (v/v) ratio at low temperature till a white residue

Table 1. Characterization of SIA Wasteland Soil. All the values are means of three replicates ± S.D., (t test), $p < 0.05$ *, $p < 0.01$**)

Soil properties	Control soil	SIA Wasteland soil	Range (January to September 2008)
Bulk Density(g cc^{-1})	1.01±0.05	2.15± 0.15**	2.05 - 2.15
Water holding capacity (%)	50.22±4.28	62.21±2.51	62.21 - 66.19
pH	7.99±0.35	8.52± 0.74	8.15 - 8.52
EC (dS m^{-1})	0.47±0.02	2.34±0.05**	2.04 - 2.34
Organic Carbon (%)	0.64±0.06	9.04±0.54**	9.04 - 10.14
Organic matter (%)	1.10 ±0.08	15.58 ±0.72**	15.58- 17.48
N (μg g^{-1})	145.10±10.25	487.23±15.24**	487.23 - 492.01
P (μg g^{-1})	175.12±10.23	635.65±17.45**	619.00 - 635.35
K (μg g^{-1})	179.23±8.25	928.13±94.25**	928.13 - 968.67
Fe (μg g^{-1})	402.21±12.23	1729.58±126.25**	1600.00 - 1729.21
Cu (μg g^{-1})	25.23±1.23	56.25±6.78**	56.25 - 61.91

was obtained. Double distilled water was used to maintain final known volume (Fritioff & Greger 2007).The samples were analyzed using VARIAN AA-240-FS make Fast sequentional AAS with flame (FAAS).

Plant analysis: After collection, the plants were segregated into shoots and roots. Plant shoots were rinsed thoroughly in deionized water while roots were properly washed with tap water and finally with deionized water to remove all visible soil particles. The washed plant samples were oven-dried at 70°C for 48 hours to a constant weight. The dry weights were determined and the samples were ground. Sub-samples (1.0 g) were subsequently digested with 15 ml ultrapure mixture of concentrated $HNO_3/HClO_4$ (3:1; V/V) on a thermo block. After cooling down, the suspensions were filtered and filtrate was adjusted to 50 ml with double deionized water. Concentrations of metal in the digested samples were analyzed using VARIAN AA240FS make Fast sequential AAS with flame (FAAS).

Metal translocation factor and enrichment coefficient: Root-to-shoot translocation factor was described as the ratio of metals in plant shoot to that in plant root, while enrichment coefficient (R) was calculated as follows:

R = C above ground /C soil (1); Where, C above ground and C soil represents the metal concentrations in the above ground parts of the plant and soil on dry weight basis, respectively. Enrichment coefficient basically depends on the soluble fraction of metals and organic matters in soils (Khan et al. 2006).

Statistical method: All treatments were replicated for six times (n=6). Results were analyzed using One-way ANOVA DMRT test (SPSS statistical package and MS excel). The difference between treatments were considered significant at $P<0.05$.

Quality control: Reagent blank and standard reference soil and plant materials were included to verify the accuracy and precision of the digestion and subsequent analysis procedure. The instruments were calibrated daily with calibration standards and the relative percent differences between the five-point calibration and the daily calibrations were < 20% for all of the garget analyses.

RESULTS AND DISCUSSION

Soil properties and metal concentration

Soil texture influences the physical properties like Bulk Density (BD), Water Holding Capacity (WHC) and nutrient availability. The bulk density of wasteland soil was 2.15± 0.15, which was higher than that of BBA University campus soil used as control. The water holding capacity of SIA soil was 62.21±2.51 in comparison to 50.22 ± 4.28 observed for the control soil (Table 1). Soil pH plays an important role in maintaining the availability of nutrients and thus affects the plant growth. The pH of SIA was slightly alkaline 8.52±0.74 than that of the BBAU campus soil (pH 7.99± 0.35). Electric Conductivity (EC) of the SIA soil was 2.34±0.02 dS m^{-1}, which was about 5 fold higher than that of the control soil (Table 1).

Organic matter (OM) is an important component of the soil fertility, which provides nutrients to the vegetation after decomposition. The SIA soil had about 20 fold higher % organic carbon (% OC) than that of the garden soil. It appears that the SIA soil had about 2 fold higher bulk density, 5 fold higher EC and a slightly higher pH and % WHC and about 20 fold higher % OC as compared to that in the control soil. The % organic carbon in wasteland soil indicates higher accumulation of organic substances in the soil. The total N, P and K were 487.23±15.24 µg g-1, 635.65±17.45 µg g^{-1}, and 928.13±94.25 µg g^{-1} respectively.

Fe concentrations in plants

Metal concentrations in plants vary with plant species (Alloway et al. 1990). Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport crosses the plasma membrane of root epidermal cells. Under normal growing conditions, plants can potentially accumulate certain metal

ions an order of magnitude greater than the surrounding medium (Kim et al. 2003). The results showed a great variability of Fe concentrations according to weed species (Table 2). The highest Fe concentration was measured in the sample of *Cynodon dactylon* (661.495±77.25 µg g^{-1}d wt in plant), while the lowest concentration was detected in the sample of *Saccharum munja* (354.755±50.87 µg g^{-1}d wt in plant. The concentrations of Fe in plant shoots were ranged from 211.03±33.11 to 406.29±63.75 µg g^{-1}d wt with an average value 296.92 µg g^{-1}d wt, whereas in plant root ranged 414.44±77.86 to 869.66±163.65 µg g^{-1}d wt with an average value 618.95 µg g^{-1}d wt (Table 2). The weed plants accumulated Fe concentration in the following order: *Cynodon dactylon* > *Cynodon arcuatus* > *Bothriochloa pertusa* > *Dichromena colorata* > *Cyperus difformis* > *Dactyloctenium aegyptium* > *Digitaria ciliaris* > *Pogonatherum paniceum* > *Paspalidium flavidum* > *Cymbopogon cambogiensis* > *Cyperus rotundus* > *Saccharum spontaneum* > *Eragrostis burmanica* > *Saccharum munja*.

Iron (Fe) is an essential micronutrient for plants and it is not toxic, if available in a low quantity in rhizosphere. Fe is needed for chlorophyll biosynthesis and function, energy transfer, and for plant cell metabolism being constituent of certain enzymes and proteins, mainly that involved in N_2 fixation and plant respiration (Jeong & Connolly 2009, Kong & Yang 2010). However, iron can induce toxicity if available in high concentrations in soil solution as it can promote deficiency of other essential nutrients by restricting their uptake (Lin & Wun 1994). If taken by the plants in excessive amounts Fe may also inhibit the activity of several enzymes and subsequently may lead to disturbances of the cellular metabolism (Juwarkar et al. 2008, Marschner et al. 2011). Iron delivered from the root to the shoot via the xylem as a ferric iron–citrate complex (Tiffin et al. 1973). While the molecular mechanisms involved in iron transport into leaf cells remain unclear (Hell & Stephan 2003). It has been reported that ferric chelate reductase activity is detectable in leaves of sunflower (Guardia & Alcantara 1996) and *Vigna unguiculata* (Bruggemann et al. 1993). A recent study examined the fractionation of stable iron isotopes taken up from the soil in various aerial portions of bean, pea, soybean, maize, oat, wheat etc (Guelke & Von Blanckenburg 2007). Tiffin et al. (1973) reported that translocation of Fe from soybean cotyledons to other parts of the plants could occur, and it was suggested that a proteinase is involved in releasing Fe from the Ft protein shell.

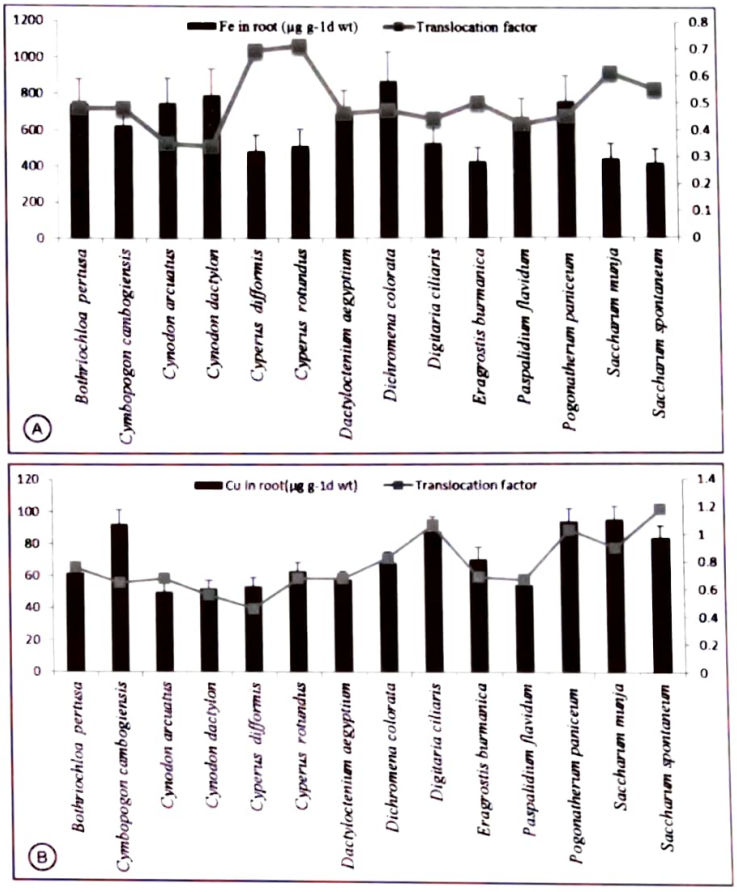
Table 2. Concentration of Fe in plant and plant shoots and plant roots (µg g^{-1} dwt). All the values are means of three replicates ± S.D., one way ANOVA DMRT test at $P > 0.05$

Plants names	Plants (µg g^{-1}dwt)	Shoot (µg g^{-1}dwt)	Root (µg g^{-1}dwt)
Bothriochloa pertusa	593.575±79.17^{ab}	357.93±54.97^{abc}	744.21±137.66^{abc}
Cymbopogon cambogiensis	460.505±66.54^{cd}	301.2±46.25^{bcde}	625.16±115.64b^{cdefg}
Cynodon arcuatus	595.69±73.65^{ab}	263.55±40.47^{def}	748.22±138.40^{abcd}
Cynodon dactylon	661.495±77.25^{a}	268.38±41.21^{def}	792.13±146.52^{abc}
Cyperus difformis	532.455±57.68^{bc}	334.45±52.48^{abcd}	482.34±89.22^{def}
Cyperus rotundus	423.425±61.45^{de}	363.01±56.96^{ab}	508.52±94.06^{def}
Dactyloctenium aegyptium	511.97±68.65^{bc}	315.76±49.55^{bcde}	689.13±129.68^{bcdef}
Dichromena colorata	536.12±21.26^{bc}	406.29±63.75^{a}	869.66±163.65^{a}
Digitaria ciliaris	492.19±55.54^{cd}	233.68±36.67^{ef}	526.06±99.00^{cdef}
Eragrostis burmanica	356.65±26.23^{e}	211.03±33.11^{f}	421.03±79.23^{ef}
Paspalidium flavidum	478.23±67.47^{cd}	271.08±42.54^{cdef}	651.19±122.33^{abcde}
Pogonatherum paniceum	488.24±67.47^{cd}	340.06±52.22^{abcd}	759.54±142.69^{abcd}
Saccharum munja	354.755±50.87^{e}	268.07±41.17^{bcde}	439.71±82.60^{ef}
Saccharum spontaneum	359.99±46.39^{e}	227.69±34.97^{ef}	414.44±77.86^{f}

Cu concentrations in plants

The bioavailability and, toxicity potential of a toxic metal ion in the soil depends on its concentration in the soil solution and the ability of soil to release the trace metal ions from the solid phase to replenish those removed from the soil solution by plants (Sheldon & Menzies 2005). There is an inherent tendency of plants to take up toxic substances including the heavy metals, which are subsequently transferred along the food chain. Use of polluted land or water for cultivation of crops mainly accounts for decrease in the overall productivity and results in contaminated food grains and vegetables, which adversely affects human health. Table 3 present the values of Cu contents in native and cultivated plants (d wt). The results showed that the variability of Cu according to weeds.

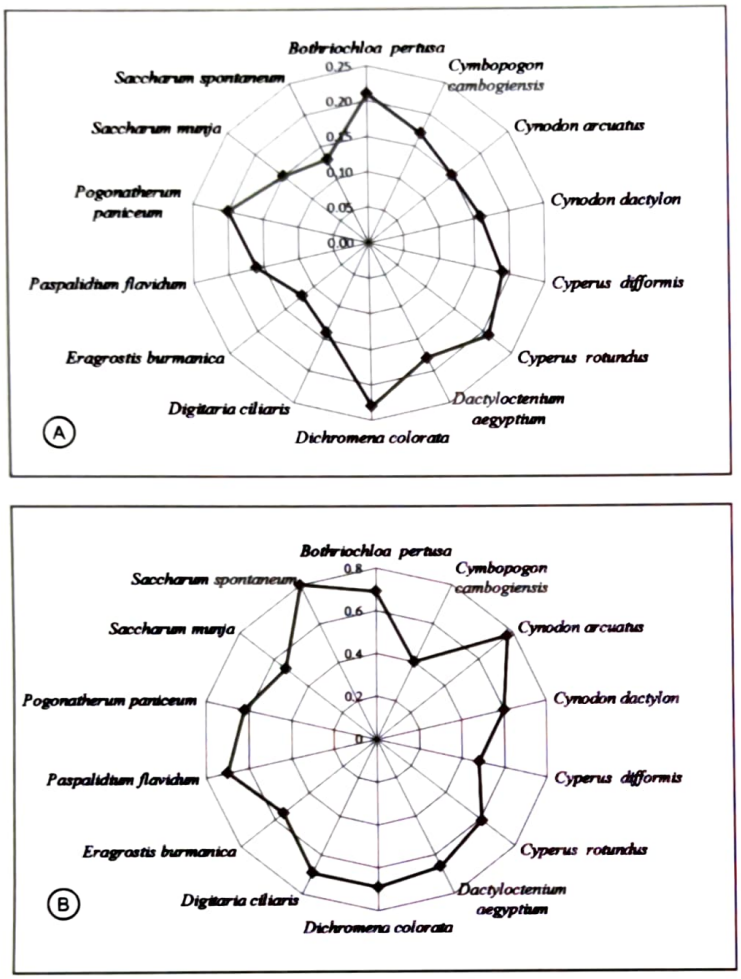
Results showed the maximum copper was found in *Digitaria ciliaris* whereas minimum in *Cynodon dactylon*. Cu concentrations of 6.4–160 mg kg^{-1} in the plant biomass were reported by Stoltz and Greger (2002), which were lower than those in our research. Maximum copper concentration in shoot part was found in *Saccharum spontaneum* and minimum in *Cyperus difformis*, however, in root part maximum copper concentration was found in *Saccharum munja* and



Text-figure 1. Translocation trends of metals from root to shoot. A. Iron; B. Copper.

Table 3. Concentration of Cu in plant and plant shoots and plant roots (µg g^{-1} dwt). All the values are means of three replicates ± S.D., one way ANOVA DMRT test at $p > 0.05$

Plants names	Plant (µg g^{-1}dwt)	Shoot(µg g^{-1}dwt)	Root(µg g^{-1}dwt)
Bothriochloa pertusa	59.955±5.47^{bc}	42.65±7.92^{efe}	61.83±5.96^{bc}
Cymbopogon cambogiensis	61.235±4.71^{bc}	36.67±6.81^{def}	92.23±8.89^{a}
Cynodon arcuatus	55.44±4.93^{bc}	38.42±7.14^{def}	50.2±4.84^{c}
Cynodon dactylon	40.91±4.04^{d}	31.5±5.85^{ef}	52.28±4.8c
Cyperus difformis	46.595±3.3^{d}	25.68±4.77^{f}	53.67±5.02^{c}
Cyperus rotundus	54.23±4.9^{bc}	38.2±7.09^{def}	62.96±5.34^{bc}
Dactyloctenium aegyptium	59.42±4.88^{bc}	38.02±7.06^{def}	57.93±4.91^{bc}
Dichromena colorata	52.25±4.56^{bc}	46.89±8.71^{cdef}	67.97±6.76^{b}
Digitaria ciliaris	85.075±7.71^{a}	60.08±11.16^{ab}	87.63±9.44^{a}
Eragrostis burmanica	65.45±5.89^{ab}	38.57±7.16^{def}	70.16±7.56^{b}
Paspalidium flavidum	57.3±4.87^{bc}	37.91±7.04^{def}	54.19±5.84^{d}
Pogonatherum paniceum	78.25±6.25^{b}	58.14±10.80^{abc}	93.86±7.96^{a}
Saccharum munja	90.15±6.5^{a}	50.65±9.41^{bcde}	95.02±8.06^{a}
Saccharum spontaneum	80.22±8.53^{a}	66.48±12.35^{a}	83.41±7.31^{a}



Text-figure 2. Enrichment factor in weeds plants. A. Iron; B. Copper.

minimum in *Cynodon arcuatus*. The weed plants accumulated Cu concentration in the following order: *Saccharum munja* > *Digitaria ciliaris* > *Saccharum spontaneum* > *Pogonatherum paniceum* > *Eragrostis burmanica* > *Cymbopogon cambogiensis* > *Bothriochloa pertusa* > *Dactyloctenium aegyptium* > *Paspalidium flavidum* > *Cynodon arcuatus* > *Cyperus rotundus* > *Dichromena colorata* > *Cyperus difformis* > *Cynodon dactylon*. Copper (Cu) is an essential redox-active transition metal that is involved in many physiological processes in plants because it can exist in multiple oxidation states. Under physiological conditions, Cu exists as Cu^{2+} and Cu^{+}. It acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Marschner 1995, Haque et al. 2009). Cu ions act as cofactors in many enzymes such as Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, amino oxidase, laccase, plastocyanin and polyphenol oxidase. At the cellular level, it also plays an essential role in signaling of transcription and protein trafficking machinery, oxidative phosphorylation and iron mobilization (Cuypers et al. 2011). Thus, plants require Cu as an essential micronutrient for normal growth and development; when this ion is not available plants develop specific deficiency symptoms, most of which affect young leaves and reproductive organs. The redox properties that make Cu an essential element also contributes to its inherent toxicity (Rosselli et al. 2003). Redox cycling between Cu^{2+} and Cu^{+} can catalyze the production of highly toxic hydroxyl radicals, with subsequent damage to DNA, lipids, proteins and other biomolecules (Halliwell & Gutteridge 1992). Thus, at high concentrations, Cu can become extremely toxic causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth (Marschner 1995).

Fe and Cu translocation factor

In this study, none of the plant species showed metal concentrations >1000 mg kg^{-1} in the shoots (Text-figures 1A and 1B), i.e. none of them are hyperaccumulators (Baker & Brooks 1989). However, the ability of these plants to tolerate and accumulate heavy metals may be useful for phytostabilization. Translocation factors (TF) can be used to estimate plant's potential for phytoremediation purpose. A plant's ability to translocate metals from the roots to the shoots is measured using the TF, which is defined as the ratio of metal concentration in the shoots to the roots. Enrichment occurs when a contaminant taken up by a plant is not degraded rapidly, resulting in an accumulation in the plant. The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable plant parts, i.e. shoots. By comparing TF, we can compare the ability of different plants

in taking up metals from soils and translocating them to the shoots. Tolerant plants tend to restrict soil–root and root–shoot transfers, and therefore have much less accumulation in their biomass, while hyper accumulators actively take up and translocate metals into their aboveground biomass.

Translocation factors higher than 1 indicated a very efficient ability to transport metal from roots to shoots, most likely due to efficient metal transport systems (Zhao et al. 2002). The translocation factors for Fe ranged from 0.34 to 0.71, with the highest value in the sample of *Cyperus rotundus* and the lowest for *Cynodon dactylon*. The results indicated that the translocation factors of plant species were lower than 1 in this study (Text-figure 1A). Low translocation of Fe from roots to shoots possibly owes negative effect on weeds. Fe can be toxic to photosynthetic activity, chlorophyll synthesis and antioxidant enzymes if available in higher amount (Kim et al. 2003). Baker and Brooks (1989) also discussed the restriction of metal uptake by plants from contaminated soils and the presence of exclusion mechanisms in plant species. Translocation factors for Cu ranged from 0.46 to 1.18, with the highest value in the sample of *Saccharum spontaneum* and the lowest in *Cyperus difformis*. The results indicated that the translocation factors of plant species were higher than 1 in this study (Text-figure 1B).

Fe and Cu enrichment coefficient

The enrichment coefficient values of all studied samples for Fe are given in Text-figure 2. Enrichment coefficients varied between 0.12 and 0.23, but always <1. The maximum value (0.23) was observed for *Dichromena colorata*, while minimum (0.12) for *Eragrostis burmanica*. Enrichment coefficient is an important factor when considering the phytoremediation potential of a plant species (Zhao et al. 2003).

At present, there are rules for hyper accumulator. One of the standards is enrichment coefficient, the enrichment coefficient > 1 shows a special ability of the plant to absorb from soils and transport metals and store them in their above-ground part (Baker & Brooks 1989, Brown et al. 1995). But some research indicates that although the enrichment coefficient < 1, some plant was heavy metal hyper accumulator (Zu et al. 2005). The enrichment coefficient values of all studied samples for Cu are given in Table 3. Enrichment coefficients varied between 0.40 and 0.80, but always < 1. The maximum value (0.80) was observed for *Saccharum spontaneum*, while minimum (0.40) for *Cymbopogon cambogiensis*. This process uses the ability of plant roots to change environmental conditions via root exudates. Plants can immobilize heavy metals through absorption and accumulation by roots, adsorption onto roots, or precipitation within rhizosphere. This process reduces metal mobility and leaching into ground water, and also reduces metal bioavailability for entry into the food chain. One advantage of this strategy over phytoextraction is that the disposal of the metal-laden plant material is not required (Susarla et al. 2002). Almost all collected plant species showed heavy metals concentration higher than the normal or phytotoxic levels. These results may indicate that plant species growing on the site contaminated with the heavy metals were tolerant of these metals. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanism. In general, all the three heavy metals occurred at elevated levels in plant biomass collected from the site. Normal phototoxic concentrations of Cu, as reported by Levy et al. (1999), were 20–100 mg/kg.

CONCLUSION

On the basis of present study, it is concluded that *Saccharum spontaneum* has great potential value for phytoremediation of Cu contaminated soils because of the fact that the Cu translocation factor for *Saccharum spontaneum* was 1.18. Similarly, two weeds species (*Pogonatherum paniceum* and *Digitaria ciliaris*) also showed the phytoremediation potential for Cu contaminated soils, with Cu translocation factor 1.03 and 1.07. In this study, the Cu accumulation capacity of 3 hyperaccumulators was found as *Saccharum*

spontaneum > *Digitaria ciliaris* > *Pogonatherum paniceum*. Among the 14 plant samples, no plant species were identified as Fe hyperaccumulators.

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