PHYTOEXTRACTION OF HEAVY METALS FROM A DECOMMISSIONED TANNERY WASTE DISPOSAL AREA BY PIONEER HERBACEOUS PLANTS

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Abstract. A field survey of pioneer herbaceous plants growing on a decommissioned tannery waste disposal area in Hubei Province, China, was conducted to identify the species extracting heavy metals especially accumulating Cr in their tissues. The results show that the soil in the area was extremely contaminated with soil Cr range of 1300–3100 mg/kg, which was 13–30 times higher than that of unpolluted soil. Besides, 18 herbaceous species belonging to 11 families were found in the polluted area, among which 8 species, were identified as the dominant adaptive species with Cr > 200 mg/kg in their shoots and occupying dominant biomass. *Cynodon dactylon* L. was found to have the highest Cr concentration 774 mg/kg and 2335 mg/kg respectively in its shoot and root. The Cr bioaccumulation coefficients and translocation factors of dominant herbaceous plants both were lower than 1, indicating that the plants were possibly tolerant-stratified to the high Cr environment. *Cynodon dactylon* L. was considered as the suitable candidate for bioremediation of Cr-contaminated soils in the area.

Keywords: *chromium, soil contamination, phytoremediation, Cynodon dactylon L., field survey*

Introduction

Pollution of the biosphere with heavy metal has accelerated dramatically and has been attracting considerable public attention during the last century (Boularbah et al., 2006; Minkina et al., 2017). Removal of heavy metals from soil has been a subject of major concern to scientists for many years (Garbisu and Alkorta, 2001; Tong et al., 2020). The rapid development in economics together with the unplanned disposal of effluent from metallurgy, tanneries, electroplating, textile and other industries have increased the threat of soil pollution in China (Sun et al., 2016; Jia et al., 2019; Xiao et al., 2019; Zhang et al., 2020). For many years, China has remained one of the top countries with great tannery industry in the world, from which numerous hazardous tannery effluent and solid waste containing chromium and its compounds are introduced into natural ecosystems, resulting in an acute problem of chromium pollution in China (Xiao et al., 2019). Increasingly widespread heavy metal pollution has caused vast areas of land to become non-arable and hazardous for both wildlife and human populations. There is, therefore, an urgent need for research on the remediation of chromium polluted soil in tannery areas (Zhou et al., 2019).

Unlike many organic pollutants, heavy metals are persistent environmental contaminants, which cannot be chemically or biologically destroyed, and tend to accumulate in organisms, thereby eventually entering the food chains (Siegel, 2002). The restoration methods for heavy metal contaminated soil can be categorized into physical remediation (i.e, washing, extraction, solidification and stabilization of heavy metals), chemical remediation (i.e., chemical leaching, chemical fixation, electrokinetic remediation, vitrifying technology), biological remediation (i.e., phytoremediation, bioremediation and animal remediation) and cocktails of the above remediation methods (Rajendran et al., 2022). However, considering the cost effectiveness of remediation, the physical or/and chemical strategies face some challenges such as cost, machine and logistics (Dhaliwal et al., 2020). Furthermore, such physicochemical methods render the land useless as a medium for plant growth, as they remove all biological activity including useful symbiotic microbes such as nitrogen-fixing bacteria and mycorrhizal fungi as well as fauna in the process of decontamination, thus decreasing their biodiversity (Lombi et al., 2001). Phytoremediation is a potential solution to the problem of heavy metal pollution using plants, and is considered as a cost-effective and environmentally-friendly green technique for heavy metal polluted soil, attracting interest and attention world widely (Mahar et al., 2016). This technology employs plants with ecophysiological adaptations to metalliferous soils to filter (rhizofiltration) or absorb (phytoextraction/phytoaccumulation) heavy metal(s) followed by their harvest. The major limitations to this powerful technology include small biomass of plants adapted to grow on nutrient-poor contaminated soils and disposal of the heavy metal-enriched biomass. The type of plants can also make a significant difference in pollutant removal (Khan, 2001).

The early phytoremediation studies focused on the hyperaccumulator species, which are plants able to accumulate unusually high levels of metals in their tissues (Shrivastava et al., 2020). The hyper accumulators plants such as trees and grasses are now being actively evaluated (Pasricha et al., 2021). In extremely contaminated areas such as disposal fields of tannery wastes, there generally exists only pioneer plant species which could be adaptive to the heavy metal contaminated soil and could be potential candidates for elementary phytoremediation of the contaminated soil, though the metal accumulation capability of pioneer plants might not be able to meet the standard of that of hyper accumulator plants (Navarro-Noya et al., 2010; Sun et al., 2016). It is therefore necessary for the phytoremediation of heavy metal contaminated soil to evaluate the metal accumulation capability of the adaptive pioneer plants.

In this study, soils and herbaceous plants in a decommissioned tannery waste disposal area polluted by tannery waste in Jingzhou, Hubei Province, China, were collected and analyzed. The contamination level of Cr, Cu, Zn and Pb of soil and pioneer plants were evaluated. Besides, the Cr accumulators and species with relatively high Cr tolerant capacity and large biomass were confirmed and identified. Furthermore, contamination levels of Cr, Cu, Zn and Pb of soil and pioneer plants were evaluated.

Materials and methods

Field survey and sampling

The survey was conducted in a decommissioned tannery waste disposal area located in Jingzhou, Hubei Province, China, which belongs to a subtropical continental zone with a warm, wet climate and annual average temperatures of 15.9–16.6 °C and average precipitations of 1100–1300 mm.

The tannery waste disposal area (*Fig. 1*) covers an area of 1.4 × 10^6 m^2, holding a center point GPS information about 30.008° North latitude and 111.607° East longitude.

The tannery waste disposal area was constructed in 1970 s and decommissioned 1990 s. During the operation of the tannery waste disposal area, numerous hazardous tannery wastewater effluent and solid waste was introduced into natural ecosystems. Furthermore, because of the seasonal floodwater overflow in the area, chromium-contained hazardous waste was dispersed along the river coast wise, resulting widespread pollution. In the most seriously polluted areas, there exist hardly wood plants but some pioneer herbaceous plant species adapted to the chromium-contaminated soil. Therefore, a very reduced plant cover on the tannery waste-contaminated site was noted, suggesting a strong selection pressure.

In September 2019, a detailed investigation on the soils and pioneer herbaceous plant species was conducted. Four sampling sites were selected: wastewater treatment plant and nearby, wastewater outfall and riverside, deserted tannery and nearby, and the uncontaminated area as comparison. At each site, almost all species of herbaceous plants were sampled, and all the plant samples included roots and shoots (defined as the above-ground parts in this paper). The roots sampling were carried out according to the procedure of excavation method (Bertin et al., 2003). At least 15 single individual plants of each species were randomly collected within the sampling area and mixed in 3 independent samples. Plant samples were placed loosely in a labeled polythene bag, and were transported to the lab as quickly as possible. Soil samples (0–15 cm) were collected from each site, with benchmark soil samples taken from uncontaminated forest sites around the tanning area (*Fig. 2*).

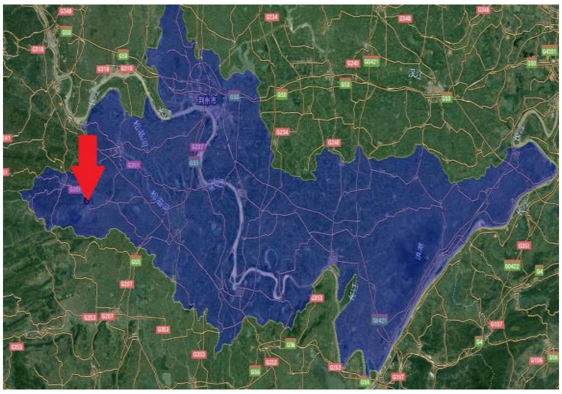

Figure 1. *Map showing the study area*

Figure 2. *Two grasses sampling. (a) Cynodon dactylon L., (b) Eleusine indica L. Gaertn*

Heavy metal analysis

Soil samples were air-dried at room temperature and milled to pass through a 100-mesh nylon sieve. Whole plants were washed thoroughly with running tap water to remove adhering substrate materials, rinsed twice for 30 s with distilled water (Liu et al., 2006), air-dried for 5 h, then separated into shoot and root parts. The samples were first oven-dried at 105 °C for 30 min, then at 70 °C for 48 h to constant weight, milled in a metal-free mill, and passed through a 100-mesh nylon sieve.

The samples (soil: ~0.40 g, shoot: ~0.40 g, root: ~0.30 g) were digested in a microwave digestion reactor (HP1510, Shanghai HengPing Instrument & Meter Co., Ltd. China) equipped with a temperature control microwave power system of differential procedures, holding a mixture of 8 ml HNO_3 (G.R. 65%) + 2 ml H_2O_2 (G.R. 30%), 7 ml HNO_3 + 2 ml H_2O_2 and 7 ml HNO_3 + 1 ml H_2O_2, respectively. The digests were brought to 50 ml with deionized distilled water after cooling to room temperature. Then the aqueous solutions were filtered through filter paper, serially diluted and analyzed on a Hitachi Z-2000 flame atomic absorption spectrometer (Hitachi, Japan) for metals (Cr, Cu, Zn and Pb). Standard materials were included for assurance control.

The dry weight of the digested soil or plant sample was presented in the milligram per kilogram dry weight (mg/kg DW). The mean value of three samples of each plant species were taken as the heavy metal concentration of the corresponding species.

Soil pH and organic matter analysis

The slurry soil samples (5 g dry matter/25 ml water) were prepared for pH measurement using a pH meter (model pHS-2C, Beijing shanghai Instrument Co., Ltd., Beijing, China). Organic matter measurements were performed according to the literature (Liu et al., 2006).

Data analysis

Bioaccumulation coefficient (BC) and translocation factor (TF) were employed to describe the heavy metal accumulation capacity of plants, which were defined as the ratio of heavy metal concentration of plant shoot/root to that in soil, and the ratio of the metal concentration of the shoots to the metal concentration of the roots of dry biomass bases, respectively (Baker et al., 1994). Pearson’s correlation analysis was undertaken to assess the relationship of concentrations of heavy metals between shoots and roots (Zhou et al., 2015). The data in this paper were analyzed by MATLAB 9.0 (MathWorks. Inc, USA).

Results

Metal concentration in soil

The heavy metal content, pH and organic matter of the soil samples in the investigated areas are listed in *Table 1*. It is described that pH of three sites of polluted zone were higher than that of the uncontaminated area, and the contaminated soil contained significantly more organic matter than that of the uncontaminated soil, due to the emission of tannery waste with high organic matter content.

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Table 1. *Heavy metal content, pH and organic matter of samples (mg/kg)*

Site	pH	Organic matter (%)	Cu	Pb	Zn	Cr
Sewage treatment plant and nearby	7.24	8.73	71.45	50.72	97.78	3109.98
Sewage outfall and riverside	6.64	5.68	60.76	43.81	118.97	1477.89
Deserted tannery and nearby	7.02	7.04	68.20	41.91	43.74	1361.39
Uncontamined area	5.74	2.83	48.16	34.70	23.05	104.69
Standard for soils*	—	—	150–200^a 50–100^b	100–140^a 90–120^b	200–250	250–300^a 150–200^b

*Soil environmental quality—risk control standard for soil contamination of agricultural (GB 15618-2018) of the People's Republic of China
^{a}Standards for paddy field
^{b}Standards for other agricultural field except paddy field

Cu, Pb and Cr contents in the investigated area were greater than soil environmental background values in China. Compared with the unpolluted soils, the tanning zone cannot be considered contaminated with Cu, Pb and Zn. However, extremely high concentrations of Cr (the maximum value was 3109.98 mg/kg DW in the wastewater treatment plant and nearby) were found in the soil of the tanning area, with 13–30 times higher than that of unpolluted soil, indicating that the tanning zone has been severely contaminated by Cr.

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Heavy metals in the plants

During the investigation, 18 herbaceous plant species belonging to 11 families were found in deserted tannery and nearby. All species and their total Cu, Pb, Zn, Cr concentrations for shoots and roots are showed in *Table 2*. It can be seen that different plants had different metal-enrichment capabilities, and metal concentrations in different parts of the plants were different. Generally, most of the above heavy metals in the aerial part and root of plants were of significant difference. Cu and Zn concentrations in all shoots and roots from four sites were within the scope of normal contents in plants. Pb concentrations of some plants were slightly higher than that of the normal content. However, Cr concentrations in shoots and roots of most plants were extremely greater than the normal contents. The Cr concentration maximum values (774.05 mg/kg DW and 2334.56 mg/kg DW in shoot and root, respectively) were found in *Cynodon dactylon* L., which holds an extremely wide distribution of all warm countries. Besides, no species investigated in this paper could be qualified as chromium hyper-accumulator, according to the criterion of Cr hyper-accumulator suggested by Baker and Brooks holding the Cr concentration > 1000 mg/kg dry leaf tissue (Baker and Brooks, 1989).

As shown in *Table 2*, there were 11 herbaceous species whose Cr concentrations of shoots were higher than 200 mg/kg. However, the field investigation showed that there existed only very small number of individuals and low biomass for three herbaceous species, i.e., *Humulus scandens* (Lour.) Merr, *Rumex acetosa* L., and *Fimbristylis aestivalis* (Retz.) Vahl. Thus 8 herbaceous species (*Cynodon dactylon* L., *Chenopodium glaucum* L., *Rorippa montana* (Wall.) Small, *Malachium aquaticum* (L.) Fries., *Oenanthe javanica* (Blume) DC., *Agerarum houstonianum* Mill., *Eleusine indica* (L). *Gaertn*, *Saccharum officenarum* L.) were identified as the dominant adaptive species or hyper-tolerant plants in the area. The averages concentrations of heavy metals of all the pioneer species were 10 times higher than the plants from non-polluted environments.

Table 2. *Heavy metal concentrations in different plants of deserted tannery and nearby (mg/kg)*

Plant species	**Cu**		**Pb**		**Zn**		**Cr**	
	Aerial part	**Root**	**Aerial part**	**Root**	**Aerial part**	**Root**	**Aerial part**	**Root**
Cynodon dactylon L.	31.59	13.26	54.75	56.24	143.51	135.24	774.05	2334.56
Humulus scandens (*Lour.*) *Merr.*	28.05	33.99	23.60	15.24	65.37	51.04	216.90	1235.46
Solidago decurens Lour.	12.85	38.56	62.01	50.13	73.50	115.26	75.90	1265.35
Chenopodium glaucum Linn.	29.43	35.26	21.27	30.22	104.48	75.21	552.95	1925.34
Rorippa montana (*Wall.*) *Small*	24.47	15.24	9.70	7.56	65.20	75.20	294.07	1156.35
Arrhenatherum elatius (*Linn.*) *Pressl*	13.82	42.44	58.89	47.62	110.55	136.52	196.85	1392.62
Malachium aquaticum (*L.*) *Fries.*	21.39	20.75	67.96	26.70	113.43	79.51	219.50	983.40
Oenanthe javanica (*Blume*) *DC.*	19.78	16.25	61.77	41.86	67.98	60.26	242.40	1005.22
Boehmeria nivea (*Linn.*) *Gaudich*	21.82	15.42	59.57	36.59	57.52	63.21	78.04	1123.21
Eragrostis pilosa (*L.*) *Beauv.*	21.83	9.62	2.91	15.83	31.30	86.00	55.66	784.84
Rumex acetosa Linn.	30.44	39.58	3.97	18.59	93.11	55.94	218.27	799.08
Amaranthus retroflexus L.	16.52	2.01	53.30	29.44	74.67	36.44	181.70	932.67
Agerarum houstonianum Mill.	18.21	3.57	30.70	18.76	50.25	64.44	242.44	1035.08
Fimbristylis aestivalis Retz.	10.53	8.97	29.60	20.87	64.32	43.56	234.60	1008.23
Eleusine indica L. Gaertn	19.08	13.25	30.90	24.48	79.97	56.78	241.48	1178.34
Zizania aquatica L.	9.99	3.01	4.88	19.35	23.78	21.38	10.62	936.49
Saccharum officenarum L.	21.20	1.56	6.11	25.01	68.38	73.22	364.18	1251.06
Aster ageratoides Turcz.	7.95	3.15	24.96	23.20	192.49	30.71	110.26	1017.41
Plants grow in uncontaminated area	3.67-24.4	3.21-18.32	0.88-40.66	18.37-43.4	14.69-156.87	13.46-123.87	1.98-75.11	70.51-118.76

Bioaccumulation coefficient and translocation factor

Bioaccumulation coefficient (BC) is generally employed to depict the enrichment capacity of plants to heavy metal (Hu et al., 2020). The greater the coefficient, the higher the capacity. The chromium BCs of 8 dominant pioneer plants are shown in *Figure 3*. All the BCs values of plants for Cu were no more than 1. The BCs for Pb and Zn of some plants were higher than 1. There existed only one species, *Chenopodium glaucum* L., whose BCs for Cr in roots were higher than 1. All the BCs for Cr in shoots were much lower than 1. The Cr bioaccumulation capacities of roots were much higher than those of homologous shoots. *Chenopodium glaucum* L. held the greatest BC for Cu and Cr. *Oenanthe javanica* (Blume) DC. showed the greatest BC for Pb. As shown in *Figure 4*, the TFs of most plants were higher than 1, indicating that these plants favored translocation of Cu, Pb and Zn to the shoots of their own. However, the TFs for Cr of all the 8 species were lower than 0.4, implying that the plants did not prefer their translocation of Cr an extreme Cr pollution pressure.

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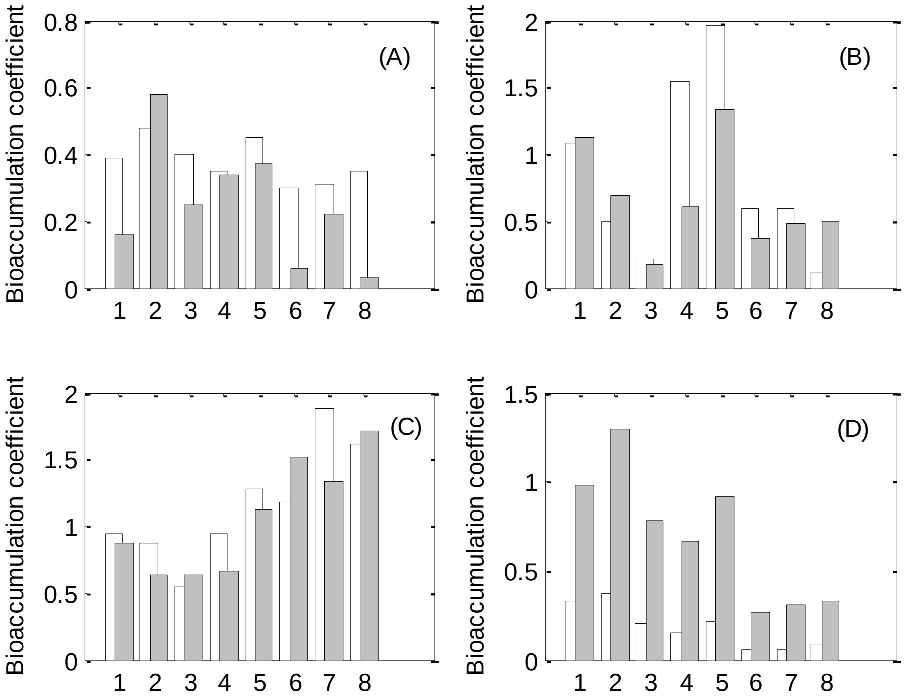

Figure 3. *BCs of heavy metals. Opened bar: shoots; filled bar: roots. (A) Cu, (B) Pb, (C) Zn, (D) Cr. 1. Cynodon dactylon L.; 2. Chenopodium glaucum Linn.; 3. Rorippa montana (Wall.) Small; 4. Malachium aquaticum (L.) Fries.; 5. Oenanthe javanica (Blume) DC.; 6. Agerarum houstonianum Mill.; 7. Eleusine indica L. Gaertn; 8. Saccharum officenarum L.*

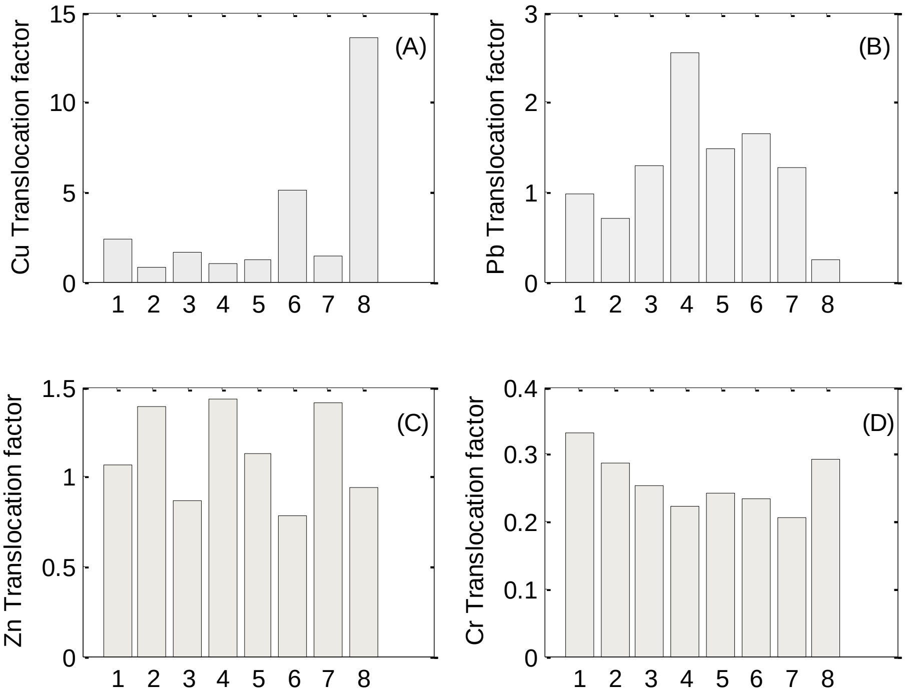

***Figure 4**. TFs of heavy metals. (A) Cu; (B) Pb; (C) Zn; (D) Cr. 1. Cynodon dactylon L.; 2. Chenopodium glaucum Linn.; 3. Rorippa montana (Wall.) Small; 4. Malachium aquaticum (L.) Fries.; 5. Oenanthe javanica (Blume) DC.; 6. Agerarum houstonianum Mill.; 7. Eleusine indica L. Gaertn; 8. Saccharum officenarum L.*

Correlation of heavy metal bioaccumulation

The correlation coefficient matrix of heavy metal concentrations of plant shoots and roots is shown in *Table 3*. The Cr concentrations of shoots were significantly correlated with that in roots ($r = 0.99$) in these highly polluted sites, and the scatter plot together with ordinary least square regression further depicted the significant correlation relationship (*Fig. 5A*). The Cr BC held almost the same way (*Fig. 5B*) as Cr concentration, indicating that the Cr bioaccumulation in shoots was greatly depended on that in roots. It is worth noting from *Table 3* that the Cr accumulation was positively correlated with Cu and Zn both in shoots and in roots, implying probably the synergistic absorption of these heavy metals.

Table 3. *Correlation coefficient matrix of heavy metal concentrations between shoots and roots of 8 dominant pioneer species (Cynodon dactylon L., Chenopodium glaucum Linn., Rorippa montana (Wall.) Small, Malachium aquaticum (L.) Fries., Oenanthe javanica (Blume) DC., Agerarum houstonianum Mill., Eleusine indica L. Gaertn, Saccharum officenarum L.)*

	Cu		**Pb**		**Zn**		**Cr**	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Cu-shoot	1.00							
Root	0.52	1.00						
Pb-shoot	-0.00	0.20	1.00					
Root	0.48	0.14	0.62	1.00				
Zn-shoot	0.76*	0.46	0.49	0.69	1.00			
Root	0.80*	0.04	0.27	0.64	0.81*	1.00		
Cr-shoot	0.92**	0.26	0.01	0.66	0.74*	0.86**	1.00*	
Root	0.92**	0.35	0.00	0.63	0.75*	0.81*	0.99*	1.00*

*$p < 0.05$; **$p < 0.01$

Discussion

The plant response to heavy metals in soil depends on the plant species, total soil metal concentration, bioavailability of the metal itself of soils, etc. Accumulation and exclusion were suggested as the two basic strategies by which plants respond to elevated concentration of heavy metals (Vogel-Mikuš et al., 2005), and the corresponding categories of plants were considered as accumulators and excluders (Sun et al., 2016) respectively. In metal accumulator species, TF greater than 1 was common, indicating a very efficient capability to transport metal from roots to shoots, most likely due to efficient metal transporter systems (Zhang et al., 2007). The TFs of heavy metal excluder species were typically lower than 1 (Baker and Brooks, 1989), showing an exclusive strategy of heavy metal accumulation.

Chromium is an essential trace element in metabolism of human beings and animals. However, excess Cr is highly toxic to animals and plants and may induce cancer and separatism. Hyperaccumulator plants can accumulate extremely high levels of metals in their tissues compared to accumulators (Baker and Brooks, 1989). Three chromium hyperaccumulators, i.e., *Dicoma niccolifera* Wild (Wang et al., 2012), *Brassica campestris* L. ssp. *Pekinensis* (Zhao et al., 2019) and *Leersia hexandra* Swartz (Zhang et al., 2007) in China, were reported. With the maximum Cr concentration 2978 mg/kg in the dry leaf matter, *Leersia hexandra* Swartz, a hydrophilous plant, mostly grows in the swamp, paddy field and riverside and is widely distributed over China. However, none individual of *Leersia hexandra* Swartz was found in the investigated area, and neither reported on other tanning areas in China, perhaps due to inadaptable to the soil polluted by tannery waste or the earth environment.

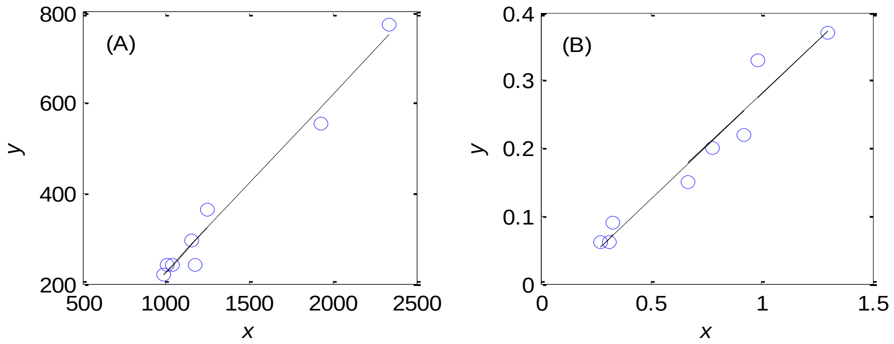

Figure 5. *Scatter plot of Cr concentration/bioaccumulation coefficient in shoots and roots of 8 dominant pioneer species (Cynodon dactylon L., Chenopodium glaucum Linn., Rorippa montana (Wall.) Small, Malachium aquaticum (L.) Fries., Oenanthe javanica (Blume) DC., Agerarum houstonianum Mill., Eleusine indica L. Gaertn, Saccharum officenarum L.), together with ordinary least square regression. (A) Cr concentration, the solid line stands y = 0.39x-167.98, r = 0.99. (B) Bioaccumulation coefficient, the solid line stands y = 0.30x-0.03, r = 0.97*

A comparison of our results with the criterion used to classify the hyperaccumulator plants indicates that plants collected from tanning area under study were not Cr hyperaccumulators but hyper-tolerant (Boularbah et al., 2006). This was confirmed by bioaccumulation coefficients and translocation factors generally lower than 1 (*Figs. 2* and *3*).

However, it is found in the present study that the Cr concentration of *Cynodon dactylon* L. reached 774 mg/kg DW in shoots, indicating that it had a stronger Cr-extraction ability than other plant species in the area. *Cynodon dactylon* L. was a representative plant blooming on the tanning zone. In fact, this grass has an extremely wide distribution, being found in all warm countries and even persisting in colder climates. It is the most widely used lawn grass and an important pasture grass in warm parts of the world. It can grow in very diverse conditions of soil and moisture, withstanding drought well and also tending to eliminate other plants and forming dense cover. Moreover, it is reported that *Cynodon dactylon* L. may be a potentially source of bio-based energy due to its vast acreage (Cantrell et al., 2009). It can be cut for hay when in full bloom. Normally 4 cuttings per year are possible. These characteristics thus make it possible for *Cynodon dactylon* L. to be considered as the potential suitable candidate for bioremediation of Cr-contaminated soils in the area. However, the exploration on the biochemical mechanisms of chromium hyper-tolerance and detoxification of such species should be taken into further research.

Conclusions

The phytoextraction of heavy metals by pioneer herbaceous plants from a decommissioned tannery waste disposal area in south central China was researched. The Cr concentrations were ranged 1300–3100 mg/kg in the surveyed soils, 13–30 times higher than the unpolluted control soil, indicating the area was severely contaminated by Cr from tannery waste. In this area, 18 species of plants were identified as the dominant plants with the ability to tolerate high concentrations of Cr, implying that they can be considered as the adaptive pioneer species for ecological restoration of Cr contaminated soils, although none of them were found to be the hyperaccumulators of Cr. With the advantages of high Cr tolerance, universality, value, etc., *Cynodon dactylon* L. is more suitable for Cr contaminated soil remediation than other herbaceous plants. Additionally, the microbial dynamics of heavy metal contaminated soils along with herbaceous plants should be considered for further studies.

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