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Tissue-specific bioaccumulation of heavy metals in *Ammopiptanthus mongolicus*, the only evergreen shrub in the desert of Northwest China

Yong-Zhi YANG[1,#], Run-Hong GAO[1,#,*], Min-Xin LUO[2], Bing-Hong HUANG[2], Pei-Chun LIAO[2,*]

1. College of Forestry, Inner Mongolia Agricultural University, Hohhot 010019, China.
2. School of Life Science, National Taiwan Normal University, No. 88 Ting-Chow Rd., Sec. 4, Taipei 116, Taiwan.
[#] These authors contributed equally to this work.
[*]Corresponding author's : R.H. Gao: Email: grhzwdm@163.com, tel.: +86-1814-8260396, fax: +86-0471-4301330;
P.C. Liao: Email: pcliao@ntnu.edu.tw, tel.: +886-2-77346330, fax: +886-2-29312904.

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ABSTRACT: The concentration of heavy metals (HMs) in plants is determined by the background values in soil and/or the expression of genes related to HM metabolism. To determine if the bioaccumulation of HMs in *Ammopiptanthus mongolicus*, an evergreen desert shrub commonly used as a traditional medicine, is related to environmental background and/or tissue (organ) specific, the HM concentrations (As, Cd, Cr, Cu, Hg, Pb, Ni, and Zn) in the soil, roots, stems, and leaves of *A. mongolicus* in the desert of northwestern China were measured. Efficiencies of bioconcentration, bioaccumulation, and translocation to different portions of the plant were calculated. A linear mixed-effects model was used to test the effects of environmental background and tissue specificity on bioaccumulation. The results showed that most HMs did not exceed the pollution threshold in the soil samples, and the accumulation in plants did not meet the standard of worldwide averages. The phytoaccumulation of HMs was independent of the background concentration in soil but was related to plant tissues, which reflects the low mobility of HMs in the desert, and suggesting that the phytoaccumulation is related to the metabolic capacity and/or differential expression of HM-related genes in tissues of *A. mongolicus*. The high translocation efficiency of mercury from the underground portion to the leaves makes *A. mongolicus* a potential phytoremediator for mercury contamination. However, the removal efficiency in high-dose contamination soil still needs to be further examined. This study suggests that the desert environment may lack appropriate microorganisms to decompose immobile HMs effectively. In addition, when using *A. mongolicus* as a medicinal plant, special attention should be paid to the accumulation of HMs in different tissues, even if they have not reached the risk threshold.

KEY WORDS: *Ammopiptanthus mongolicus*, bioaccumulation, bioavailability, heavy metals, mobilization, translocation efficiency.

INTRODUCTION

Heavy metals (HMs), which naturally exist in the soil due to weathering of the underlying bedrock, are typically mined from ores during mineral processing (Khalid *et al.*, 2017). Arsenic (As), chromium (Cr), mercury (Hg), lead (Pb), nickel (Ni), and copper (Cu) usually vulcanize (sulfurize) naturally and exist as sulfide ores. Cadmium (Cd) often coexists with zinc sphalerite (ZnS), and the abundance of Cd is controlled by the temperature and fluid conditions of the formation of zinc sphalerite (Khalid *et al.*, 2017). Land use, such as mining, construction, and tillage, may change the HM content in the soil. HM pollution of the environment is increasing mainly due to mining, smelting, diffusion of sewage sludge, and excessive use of phosphate fertilizer (Mejáre and Bülow, 2001). Rock weathering depends on organisms (such as plants and microorganisms) to trigger soil formation, and these mineral-biological interactions are essential for the establishment and growth of plants (Mapelli *et al.*, 2012). However, the composition of edaphic elements, particularly As, Pb, Cr, Hg, Cu, Cd, and uranium (U) (Khalid *et al.*, 2017), depends not only on the classification and weathering degree of *in situ* rocks but also on atmospheric dust from different sources (Suska-Malawska *et al.*, 2019). The sources of these soil elements even affect the accumulation of elements in local plants (Suska-Malawska *et al.*, 2019).

The accumulation of elements in plants is affected not only by the external environment but also by internal regulatory mechanisms. For example, the physiological response of *Conocarpus lancifolius* (Combretaceae) under sewage treatment, e.g., the increase of chlorophyll content, CO_2 assimilation rate, and water usage efficiency, affects the translocation efficiency of Zn, Pb, and Cd (Rasheed *et al.*, 2020). Absorption of HMs from the soil by roots of ground cover plants stabilizes the HMs in the soil and changing their bioavailability and mobility, i.e., phytostabilization (Sylvain *et al.*, 2016). After taking up HMs from the soil, HMs are transported into the root cells via transmembrane carriers and are further translocated to the aerial parts of plants via xylem. In this process, plants might regulate the response to HMs through differential expression of specific genes at different tissues, which affect signaling molecules such as plant hormones, reactive oxygen species (ROS), and NO (Luo *et al.*, 2016). Different plants and even different tissues thus have different efficiencies in the accumulation of various metals (e.g. Sylvain *et al.*, 2016).

Table 1 Sampling site information

Sampling site	Latitude	Longitude	Altitude	Environment
Loc. 1	N40°00'51"	E106°37'50"	1097 m	Bare-sand habitats with few shrubs and herbs
Loc. 2	N37°59'31"	E106°35'08"	1357 m	Coal mine area with abundant shrubs and herbs
Loc. 3	N38°55'21"	E105°46'05"	1590 m	Road traffic island with planted shrubs, suffering severe air pollution
Loc. 4	N41°56'34"	E102°30'57"	1068 m	Sandy land with more gravel in a dried-up riverbed

These discrepancies in HM accumulation among tissues may be due to the differential expression of metal-binding proteins among tissues (Mejáre and Bülow, 2001). HM tolerance and accumulation are associated with several HM chelator-related genes and transporters (ABC transporter, efflux transporters, uptake transporters, etc.) (Chiang *et al.*, 2006). Plants possess homeostatic mechanisms for maintaining a steady state of essential HM ions to survive in variable soil conditions. For example, in *Arabidopsis thaliana*, higher peroxidase activity alleviates damage from reactive oxygen species, thereby improving the plant's tolerance to the HMs Zn and Cd (Chiang *et al.*, 2006).

Nearly 80% of the world's population consumes herbal products as their primary form of health care (Arunkumar and Muthuselvam, 2009, Shaban *et al.*, 2016). Some commonly used herbs accumulate HMs to varying degrees, depending on their growing environment (Haider *et al.*, 2004, Barthwal *et al.*, 2008). *Ammopiptanthus mongolicus* (Maxim. ex Kom.) Cheng f., a traditional herbal medicine and health food in China (Wang *et al.*, 2007), grows in heterogeneous microhabitats in the Northwest desert in China (Jia *et al.*, 2008), e.g. rocky, gravelly, sandy soils of dry valleys, basins, and rocky dunes with a soil depth of less than 30 cm (Liu, 1998). *Ammopiptanthus mongolicus* is the only evergreen broad-leaf shrub in the desert of Northwest China (Liu *et al.*, 2017), where environmental heterogeneity, particularly local precipitation, affects both spatial distribution and population genetic structure (Jiang *et al.*, 2019). Compared with annual herbs, the perennial characteristics of *A. mongolicus* give it a more excellent opportunity to interact with toxicants, including HMs, in the environment.

Because the desert in Northwest China is dry and windy and has undergone weathering for a long time, the mobilization of HM elements in soil may be slower than in rainy climatic zones, which are characterized by higher leaching rates and higher bioactivities (Larocque and Rasmussen, 1998). Understanding the ability of plants to absorb and accumulate HMs in different local environments, particularly under different soil HM content levels, is relevant in selecting candidates for phytoremediator. In addition, the organs and tissues of *A. mongolicus* are used for medicinal or edible purposes. Therefore, investigation of the accumulation of HMs in various tissues or organs of A. mongolicus is also useful for food security and therapeutic application.

In this study, we assessed the ability of *A. mongolicus* to absorb HM elements from the soil, and verify that the HM elements are transferred and accumulated in different parts of the plants. In addition, whether the differences of the degrees of phytoaccumulation are distinct between tissues and are related to the differential environmental background (i.e., the concentration in the soil) were tested. The results of this study will provide a reference for future phytoremediation strategies and serve as a basis for further understanding the elemental accumulation and metabolic specificity of *A. mongolicus* in arid desert.

MATERIALS AND METHODS

Sampling

There were four sampling sites in China: Loc. 1: Dunkou County, Bayannaoer City, Inner Mongolia; Loc. 2: Yangchangwan Coal Mine, Ningdong Town, Lingwu City, Ningxia Province; Loc. 3: the median strip of 314 Provincial Road in Alxa Left Banner**,** Inner Mongolia; and Loc. 4: a dry riverbed 12.6 km south of the frontier station in Yagan of Wentu-Gaole Sumu, Inner Mongolia. The sampling-site information is listed in Table 1. In summary, Loc. 1 is representative of general bare-sand habitats of *A. mongolicus*, with relatively rare accompanying shrubs and herbs; Loc. 2 is in a coal mine area and has a greater abundance of accompanying shrubs and herbs than Loc. 1; Loc. 3, which comprises green plants in a road traffic island (median strip), suffers from severe air pollution; Loc. 4 is in a dried-up riverbed, where there is more gravel than at the other sites in addition to sandy land and only occasional floods form a river channel.

Each soil sample was excavated adjacent to the plants at a depth of 10cm, 20cm, and 30cm from the surface. Two-hundred-gram bulk soils were sampled from each sampling layer, and these soils of the three sampling layers were evenly mixed. For plant-tissue samples, we took leaves (100 leaves each at the top, middle, and bottom of the crown), stems (top, middle, and base of the trunk, 100g dry weight each) and roots (basal area, middle mature area, and an extension area of the tip region, 100g dry weight each). After the samples of each vegetative organ were evenly mixed, 200g each was taken for HM concentration analyses. A total of nine individuals were sampled in each sample plot and divided into three biological replicates. Every biological replicate merged three equal-weight samples of adjacent individuals.

Measuring heavy metal concentrations in soils

Following methods, according to the National Standards in China, were used to measure soil HMs:

GB/T22105.2-2008 for arsenic (As), GB/T17141-1997 for cadmium (Cd), GB/T22105.1-2008 for mercury (Hg), and HJ766-2015 for chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) (Geological and Mineral Industry Standards of the People's Republic of China, 2016). These methods were commonly used for detecting the soil HM pollution in China (Shangguan *et al.*, 2018). Soil Cr, Pb, and Zn were measured by X-ray fluorescence spectroscopy using pressed powder pellets. All soil samples were ground and sifted to obtain a particle size of less than 74 μm and dried for 6–8 hours at 105°C. Samples of 4 g were measured into polyethylene plastic rings and pressed at 10 MPa. The fluorescence intensity of each element was measured by wavelength dispersive X-ray spectroscopy using a GBC Avanta atomic absorption spectrophotometer (AAS) (GBC Scientific Equipment Pty Ltd.).

Soil Hg and As were measured using vapor generation-cold atomic fluorescence spectroscopy (AFS) and hydride generation-cold AFS, respectively. First, soil samples (0.5 g) were decomposed with 10 mL aqua regia at 90~98°C, and then added 22.5M HNO_3 2 mL and kept heating. The decomposed soils were reduced with stannous chloride in 22.5M HNO_3 6 mL and 50 g/L $K_2Cr_2O_7$ 0.5 mL to produce cold vapor mercury. The decomposed soil was also reduced with 50 g/L thiourea-ascorbic acid 10 mL as the reducing agent and reacted with KBH_4 in 3M HCl 2mL solution to produce gaseous hydride. Then, the gasified Hg and As were activated by a high-intensity vacuum cathode lamp with argon as the carrier, and the concentrations were determined in a GBC Avanta AAS (GBC Scientific Equipment Pty Ltd.).

Cu and Ni were measured by inductively coupled plasma (ICP)-atomic emission spectroscopy (AES). Soil samples (0.1 g) were decomposed by heating at 110°C with 32.6M HCl 2 mL and 22.5M HNO_3 2mL, with subsequent heating at 250°C with 56.5M HF 1 mL and 16.6M $HClO_4$ 1 mL, and dissolved in 3M HCl 2 mL after volatilization of $HClO_4$. The concentration of Cu and Ni was determined by a Thermo X-Series 2 ICP mass spectrometer (Thermo Fisher Science).

Soil Cd was measured using graphite furnace atomic absorption spectroscopy (GF-AAS). The soil sample (0.25 g) was first decomposed by heating with 32.6M HCl 5mL and 22.5M HNO_3 2 mL at a lower temperature, and then heating at 250°C with 56.5M HF 10 mL and 16.6M $HClO_4$ 2 mL, and dissolved in 11M HNO_3 1 mL. After adding a 2.5-mL $NH_4H_2PO_4$-CH_4N_2S-EDTA mixture (mixture with a ratio of 10 g: 10 g: 2 g in 100-mL H_2O), the sample was irradiated at the Cd-element wavelength using a vacuum cathode lamp. When the Cd wavelength passed through the graphite furnace sample vapor, it was absorbed by Cd ground-state atoms. The concentration of Cd was then estimated by the degree of weakening of the radiation intensity.

Quantitative analysis was carried out by using an extrapolation method in which the intensity ratio of the mass spectrometric signal of the element to be measured and the mass spectrometric signal of the element in the internal standard was proportional to the concentration of the element to be measured. Concentrations of the soil HMs were calculated with the formula:

$$w_i = (\rho_i - \rho_0) \times \frac{V_1}{V_0} \times \frac{1}{m}$$

, where w_i is the concentration of the i metal (μg/g); ρ_i and ρ_0 is the mass concentration (μg/mL) of the tested samples and blanks acquired from the standard curve, respectively; V_0 and V_1 are the solvent volumes and the solute volume of the dissolved sample, respectively; m is the weight of samples.

Measuring heavy metal concentration in plant tissues

The measurements of HMs in plant tissues followed the National Food Safety Standard in China (GB 5009.11-2014 and GB-5009.268-2016) (China Food and Drug Administration, 2016). All plant samples were washed, dried, and digested by heating in 2% HNO_3 (v/v). After digestion, the Hg was measured with vapor generation-cold AFS, and the other HM elements were determined using ICP-MS as described above. Qualitative analysis was carried out using specific mass-to-charge ratios, and quantitative analysis was carried out by using the extrapolation method. Concentrations of the soil HMs were calculated with the formula:

$$w_i = (\rho_i - \rho_0) \times \frac{V}{m} \times f$$

, where w_i is the concentration of the i metal (μg/g); ρ_i and ρ_0 is the mass concentration (μg/mL) of the tested samples and blanks acquired from the standard curve, respectively; V is the total volume of solutions of the dissolved sample; m is the weight of samples; f is the dilute fraction.

Calculating the bioaccumulation factor (BAF), bioconcentration factor (BCF), and translocation factor (TF)

To evaluate the potential for phytoremediation, the ratios of HM concentrations of root-to-soil, shoot (stem or leaf)-to-soil, and shoot -to-root, i.e., the bioconcentration factor (BCF), bioaccumulation factor (BAF_{shoot}, BAF_{stem}, and BAF_{leaf}), and translocation factor (TF), respectively, were calculated, in which the BAF_{shoot} = (BAF_{stem} + BAF_{leaf})/2, TF = [(C_{stem} + C_{leaf})/2]/C_{root}, where C_{stem}, C_{leaf}, and C_{root} are the concentration of HM in stem, leaf, and root, respectively (Fig. 1).

Testing the effects of tissue specificity and environmental background

To test whether bioaccumulation of HMs was tissue-specific and/or affected by the environmental background (i.e., HM concentrations in soil), linear mixed-effects models (LMMs) were conducted using the R package lme4 (Bates *et al.*, 2015). Plant tissues (three categorical factors: leaf, stem, and root) and HM concentration in soil (the numerical factor) were used as fixed effects to

Table 2 Heavy metal concentrations measured in bulk soils in four sites in Inner Mongolia. The soil environmental quality standards for heavy metals of different countries (Chen *et al.*, 2018) are listed at the bottom of this table.

Site	As (μg/g)	Cd (ng/g)	Cr (μg/g)	Cu (μg/g)	Hg (ng/g)	Ni (μg/g)	Pb (μg/g)	Zn (μg/g)
Loc. 1	9.59±0.03	119.33±0.94	45.57±0.33	16.17±0.09	21.10±0.08	21.33±0.05	19.83±0.13	48.23±0.38
Loc. 2	11.07±0.15	115.00±2.45	**94.67±0.17**	17.43±0.21	11.87±0.76	27.67±0.17	21.37±0.31	53.60±0.29
Loc. 3	11.44±0.10	129.67±1.25	72.03±0.76	25.10±0.08	23.23±0.26	33.80±0.14	20.43±0.09	68.50±0.08
Loc. 4	**17.07±0.03**	158.67±1.70	69.50±1.64	26.83±0.25	16.93±0.26	32.70±0.64	18.93±0.33	64.77±0.95
China (Level1)	15	200	90	35	150	40	35	100
Australia	20	2000	50	100	2000	70	100	200
Canada	12	10000	64	63	6600	50	140	200
France	37	20000	130	190	7000	140	400	9000
Germany	50	20000	400	NA	20000	140	400	NA
Japan	150	150000	250	NA	15000	NA	150	NA
Korea	75	12000	15	450	12000	300	600	900
Norway	2	3000	25	100	1000	50	60	100
Russia	4.5	760	3.8	3.5	1900	2.6	55	16
Sweden	15	400	120	100	1000	35	80	350
UK	20	8000	130	NA	8000	50	450	NA
USA	22	37000	100000	3100	23000	1600	400	23000

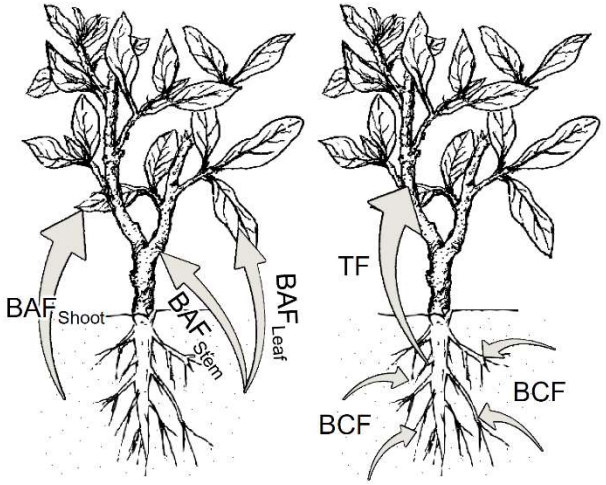

Fig. 1. Schematic diagram of bioaccumulation factor (BAF), bioconcentration factor (BCF), and translocation factor (TF).

predict the average accumulation of HMs in plant tissues, and the sampling sites (Loc. 1~4) were taken as the random factor under the random intercept model. The likelihood and Akaike Information Criterion (AIC) value of every reduced model were calculated. The likelihood-ratio test (LRT) evaluates the likelihood of a change in model fit when each individual factor is added (or dropped) from the model; a smaller AIC value indicates a significant improvement of the model. The significance of the effect of each fixed factor was tested by *t*-tests via Satterthwaite's degrees of freedom method with the R package lmerTest (Kuznetsova *et al.*, 2017).

RESULTS AND DISCUSSION

Heavy metals in the soil

Among the soil samples tested in this study, only the concentrations of As at Loc. 4 (17.067±0.026 μg/g) and Cr at Loc. 2 (94.667±0.170μg/g) exceeded the China's soil environmental quality standards of contaminated soil (Level 1, GB 15618-1995) (Chen *et al.*, 2018) (Table 2).

According to the collation of Chen *et al.* (2018), China's current criteria for HMs can be divided into the standards for the natural environment (GB 15618-1995) and agricultural soil (HJ / T 333-2006), which can also be divided into paddy soil, dry soil, orchard soil, and vegetable soil. Level 1 of GB 15618-1995 is the most stringent (lowest allowable residual dose) standard for the HM concentration of soil. Compared to other countries, China's standards are relatively strict. The environments, even around the coal mine area (Loc. 2) or road traffic island (Loc. 3), are not high in HMs. *Ammopiptanthus mongolicus* is thought to entrap and redeposit of fine sands as well as redistribute HMs in different soil layers (Dai *et al.*, 2017). However, due to the low concentration of HMs around the growing environment, whether *A. mongolicus* can readjust the HMs and soil particle size distributions in the background with high HMs remains to be further explored.

Heavy metals in plant tissue

In plants, most HMs accumulated in the aboveground, especially in leaves, and only Cr was highly accumulated in roots than in leaves or stems (Fig. 2). Compared with the concentration in soil, most HMs were relatively low in plant tissues except Hg (Table S1). For most elements, the level in plant tissues was lower than that in soil (as shown in BAF and BCF), except the accumulation of Hg at Loc. 2 (BAF = 1.349±0.074, Fig. 3, Fig. 4, and Table S2). BAF values of Hg are > 1 indicating the capacity of this species to accumulate Hg in their leaves. We also found that the bioaccumulation of most of the HM elements at roots were mostly less than that of aboveground shoots (i.e., BCF < BAF and TF >1).

The concentrations of As, Cr, and Ni in plant tissues exceeded the estimates of global averages of all tissues from all plants (Table S1) (World Reference Plants (WRP), Dunn, 2007). However, except for the root Cr concentration in Loc. 2 (26.466±0.790 μg/g), which was17-fold higher than the world average (1.5 μg/g), the

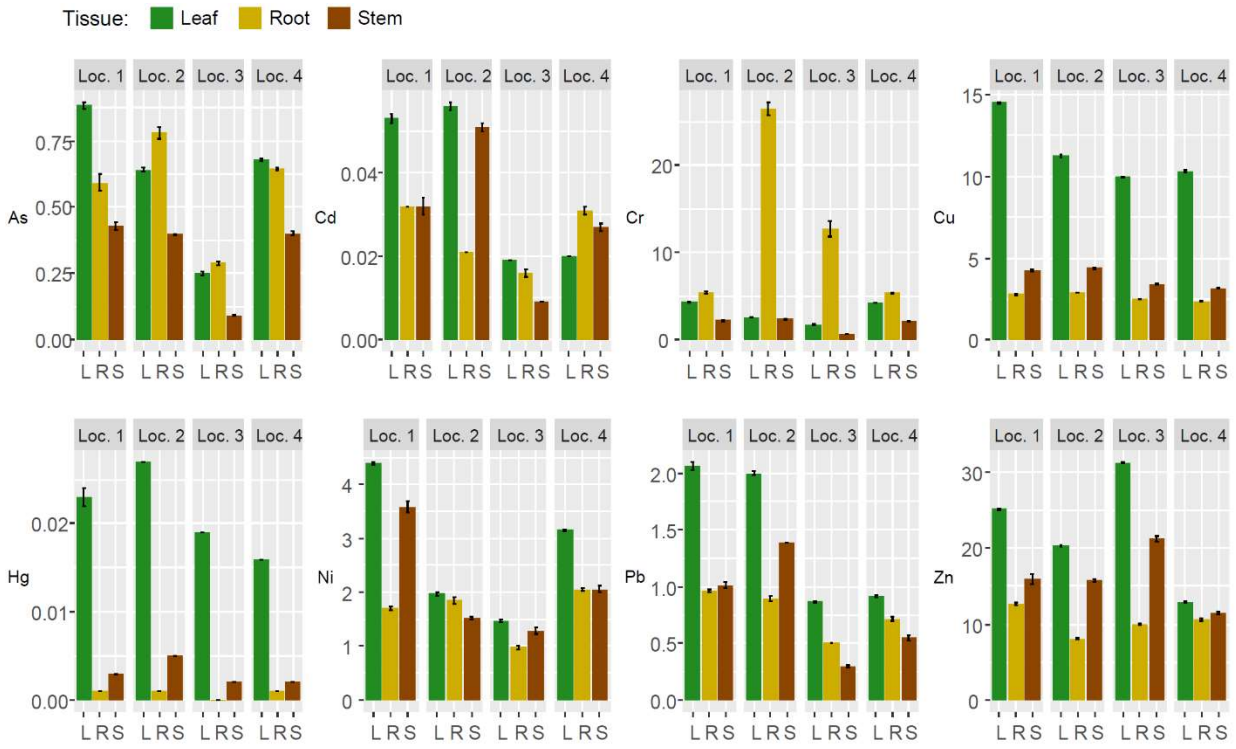

Fig. 2. Heavy metal concentration (μg/g) measured in plant tissues (leaf, root, and stem) from the four sampling sites. The concentration of HMs is relative to the dry weight of plant tissues.

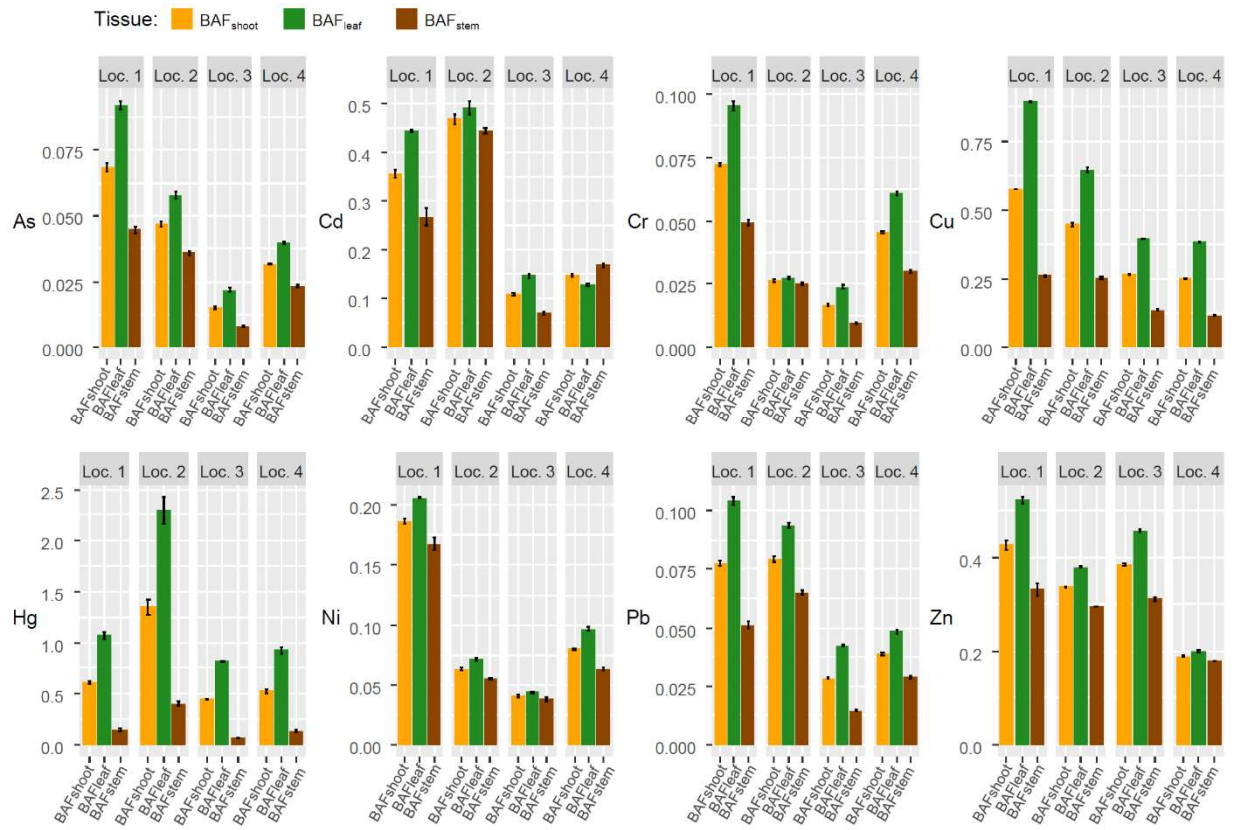

Fig. 3. Bioaccumulation factor (BAF) for the heavy metals in *Ammopiptanthus mongolicus*. BAF calculates a ratio of HMs in the harvestable part of a plant (i.e., the shoots) to that in the soil.

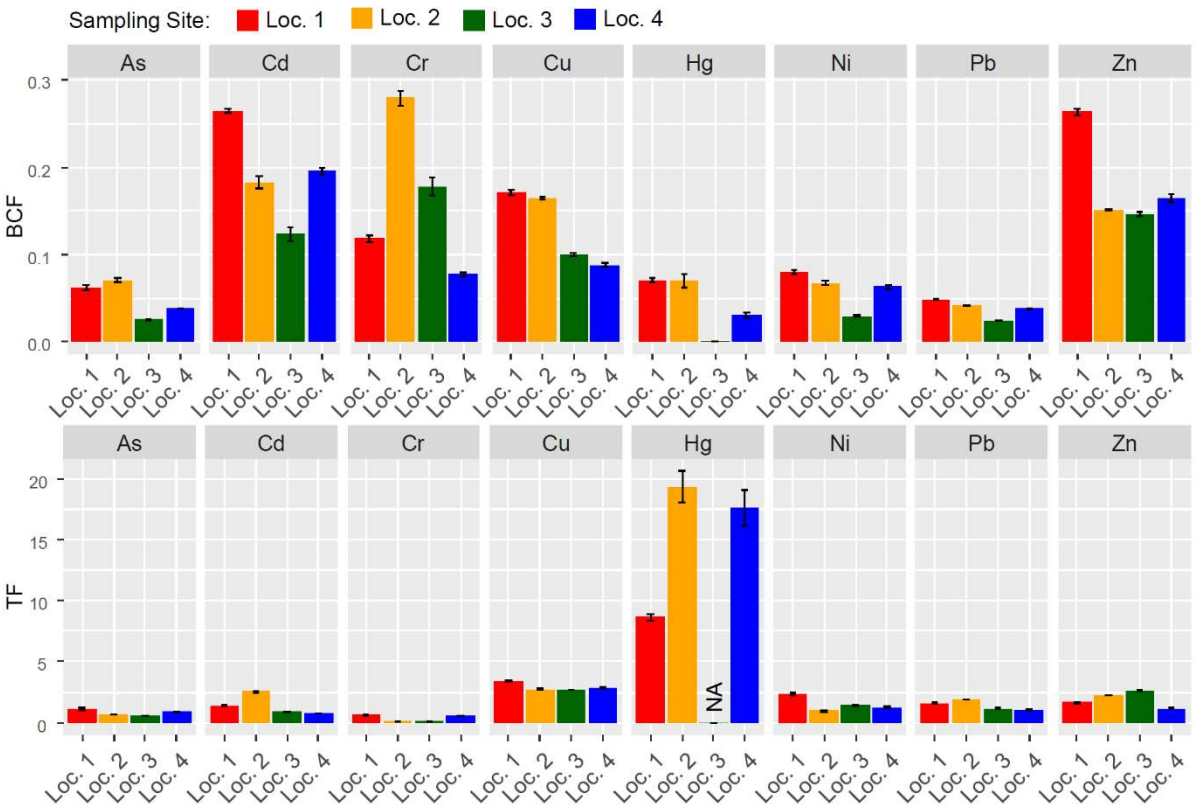

Fig. 4. Bioconcentration factor (BCF) and translocation factor (TF) for the heavy metals (HMs) in *Ammopiptanthus mongolicus*. BCF and TF describes the root-to-soil ratio (HMs from soil to root) and shoot-to-root ratio (from root to shoot) of HMs. BCF and TF of Hg in Loc. 3 are not allowed (NA) because the Hg concentration in the root is too small to be detected.

HM concentration in our samples did not exceed the hyperaccumulator threshold standard of 100–10,000 μg/g or 10-1,000 times the average concentration of WRP (van der Ent *et al.*, 2013, Reeves *et al.*, 2018). In practice, HMs in aboveground tissues (mainly leaves) are the primary removal targets in phytoremediation to avoid expanding the scope of contamination by moving contaminated soils during the removal process (van der Ent *et al.*, 2013).

Most BAF_{Leaf} is larger than BAF_{Stem} except for Cd and Ni (Fig. 3). Leaves may be the central portion to accumulate HMs in *A. mongolicus* (i.e., the sink). Particularly noteworthy is the accumulation of Hg. The high translocation efficiency from root to shoots (especially to leaves) found in *A. mongolicus* (Fig. 4) differs from that in most of other plants, which usually form a barrier at roots preventing the translocating of Hg to the aboveground tissues (Patra and Sharma, 2000, Bontidean *et al.*, 2004, Swapna *et al.*, 2015). Woody stems may mainly transport HMs (especially Hg) rather than store them. This is because sustained absorption of HMs (such as Hg) is metabolically dependent, but woody stems lack related metabolically active cells, while leaf cells do (Yang *et al.*, 2015).

In addition, the BAF_{Leaf} of the four sampling sites is close to or more than one (Fig. 3), indicating that the cumulative amount of Hg may be related to the Hg concentration of the external soil (although the results of LMM show that the soil factor is not a determinant for bioaccumulation, Table 3). Therefore, *A. mongolicus* can be a potential phytoremediator for the removal of Hg from the soil. The high BAF_{Leaf} suggests that to remove Hg only their leaves need to be harvested and no need to cut off the entire aboveground parts. However, the accumulative efficiency of the plant growing at high-Hg soil remains to be evaluated.

Effects of environmental background and tissue specificity on bioaccumulation

Among the LMMs, the null model showed the best fit for predicting Cd and Ni concentrations in plant tissues (Table 3). The failure rejection of the null model indicates that the bioaccumulation of Cd and Ni cannot reflect the environmental differences between the four sampling sites and is not significantly associated with specific tissues. Other environmental factors (e.g., air temperature, soil temperature, precipitation, humidity, etc.) differing among sites might affect the results. In addition, using more specific tissues (such as dermal tissues, cortex, vascular tissues, etc.) may improve the relationship.

The other six HMs in plants exhibited significant effects of tissue specificity; in addition, the Cu concentration in plant tissues was also associated with the background value

Table 3. Summary results of the model selection for the best-fit model for predicting phytoaccumulation by *Ammopiptanthus mongolicus*.

Element	Model	Model selection [a]				
		df	AIC	logLik	χ^2	*P*
As	~ 1 + (1 | Loc.)	3	2.673	1.664		
	~ soil + (1 | Loc.)	4	4.66	1.67	0.013	0.911
	~ tissue + (1 | Loc.)	**5**	**-4.93**	**7.465**	**11.59**	**0.001**
	~ tissue + soil + (1 | Loc.)	6	-2.943	7.471	0.013	0.911
Cd	**~ 1 + (1 | Loc.)**	**3**	**-62.524**	**34.262**		
	~ soil + (1 | Loc.)	4	-61.862	34.931	1.338	0.247
	~ tissue + (1 | Loc.)	5	-60.929	35.464	1.067	0.302
	~ tissue + soil + (1 | Loc.)	6	-60.267	36.133	1.338	0.247
Cr	~ 1 + (1 | Loc.)	3	86.422	-40.211		
	~ soil + (1 | Loc.)	4	87.022	-39.511	1.399	0.237
	~ tissue + (1 | Loc.)	**5**	**82.815**	**-36.408**	**6.207**	**0.013**
	~ tissue + soil + (1 | Loc.)	6	82.025	-35.013	2.79	0.095
Cu	~ 1 + (1 | Loc.)	3	73.832	-33.916		
	~ soil + (1 | Loc.)	4	75.452	-33.726	0.38	0.537
	~ tissue + (1 | Loc.)	5	45.216	-17.608	32.236	1.00E-08
	~ tissue + soil + (1 | Loc.)	**6**	**41.046**	**-14.523**	**6.17**	**0.013**
Hg	~ 1 + (1 | Loc.)	3	-71.66	38.83		
	~ soil + (1 | Loc.)	4	-69.824	38.912	0.164	0.685
	~ tissue + (1 | Loc.)	**5**	**-100.601**	**55.3**	**32.776**	**1.00E-08**
	~ tissue + soil + (1 | Loc.)	6	-100.445	56.222	1.844	0.175
Ni	**~ 1 + (1 | Loc.)**	**3**	**38.059**	**-16.029**		
	~ soil + (1 | Loc.)	4	36.62	-14.31	3.439	0.064
	~ tissue + (1 | Loc.)	5	36.448	-13.224	2.171	0.141
	~ tissue + soil + (1 | Loc.)	6	35.01	-11.505	3.439	0.064
Pb	~ 1 + (1 | Loc.)	3	23.928	-8.964		
	~ soil + (1 | Loc.)	4	24.973	-8.486	0.955	0.328
	~ tissue + (1 | Loc.)	**5**	**17.044**	**-3.522**	**9.929**	**0.002**
	~ tissue + soil + (1 | Loc.)	6	18.089	-3.044	0.955	0.328
Zn	~ 1 + (1 | Loc.)	3	85.531	-39.766		
	~ soil + (1 | Loc.)	4	87.517	-39.759	0.014	0.906
	~ tissue + (1 | Loc.)	**5**	**78.373**	**-34.187**	**11.144**	**0.001**
	~ tissue + soil + (1 | Loc.)	6	80.355	-34.178	0.018	0.894

[a] The best-fit models in model selection are marked in bold.

Table 4. Summary results of the significance test on fixed terms of the best-fit model for the phytoaccumulation in Table 3

Element [a]	Best-fit model	Fixed effects [b]				
		Fixed-term	Estimate	SE	*t*	*P*
As	~ tissue + (1 | Loc.)	Intercept	0.615	0.107	5.718	0.005
		tissue:root	-0.038	0.070	-0.543	0.606
		tissue:stem	**-0.285**	**0.070**	**-4.076**	**0.007**
Cr	~ tissue + (1 | Loc.)	Intercept	3.227	2.903	1.112	0.295
		tissue:root	**9.280**	**4.105**	**2.260**	**0.050**
		tissue:stem	-1.367	4.105	-0.333	0.747
Cu	~ tissue + soil + (1 | Loc.)	Intercept	14.817	1.411	10.499	5.9E-6
		tissue:root	**-8.873**	**0.703**	**-12.623**	**1.5E-6**
		tissue:stem	**-7.716**	**0.703**	**-10.977**	**4.2E-6**
		soil	**-0.155**	**0.062**	**-2.514**	**0.036**
Hg	~ tissue + (1 | Loc.)	Intercept	0.021	0.001	14.670	6.9E-7
		tissue:root	**-0.021**	**0.002**	**-11.920**	**2.1E-5**
		tissue:stem	**-0.018**	**0.002**	**-10.610**	**4.1E-5**
Pb	~ tissue + (1 | Loc.)	Intercept	1.462	0.244	5.995	0.003
		tissue:root	**-0.694**	**0.186**	**-3.725**	**0.010**
		tissue:stem	**-0.649**	**0.186**	**-3.486**	**0.013**
Zn	~ tissue + (1 | Loc.)	Intercept	22.423	2.581	8.689	5.7E-5
		tissue:root	**-12.063**	**2.859**	**-4.220**	**0.006**
		tissue:stem	-6.291	2.859	-2.201	0.070

[a] Significant tests on fixed terms of the best-fit model for Cd and Ni are not shown because the null model (~ 1 + (1 | Loc.)) is inferred as the best-fit model.
[b] The fixed terms with significant effects (P<0.05) are marked in bold.

of Cu in soil (Table 3). However, the association of Cu concentration between plant tissues and soil was negative (t = -2.514, P = 0.036, Table 4), suggesting low copper bioavailability and a higher background concentration of insoluble copper in the soil (Badilla-Ohlbaum *et al.*, 2001, Keiblinger *et al.*, 2018). Copper, which exhibits increased adsorption at pH < 5.0, is less mobile and firmly bound to the soil, and this binding is governed by the concentration of organic matter and oxide (Rieuwerts *et al.*, 1998, García-Carmona *et al.*, 2019). Due to the arid conditions in deserts, the conversion of immobile copper into bioavailable copper ions may be inefficient. Nevertheless, we cannot rule out

the possibility that the decrease of Cu in the soil is due to the uptake by plants because of BAF_{leaf} close to one. However, the BAF of these *A. mongolicus* individuals growing in these habitats varies widely (0.25 ~ 0.58). Whether the differential capacity of bioremediation to copper pollution is related to local adaptation still needs more investigation.

Our results imply that soil HMs could primarily exist in the immobile form in the desert, which is not propitious for bioavailability. The mobilization of edaphic elements usually relies on microorganisms. Compared with forests with more vegetation coverage, the microbial composition in areas with low vegetation cover (such as grassland or desert) is mostly random and uncorrelated, and microbe-microbe interactions (e.g., competition and cooperation) are relatively weak (Wu *et al.*, 2017; Wu *et al.*, 2019). Therefore, soil microorganisms in desert areas have low demineralization activity and low efficiency for HM element mobilization due not only to drought but also to an insufficient complement of functional microbes (Wu *et al.*, 2017).

Our results also reveal tissue specificity of the accumulation of HMs in *A. mongolicus*, which is probably due to the differential expression of metal-binding proteins (Mejáre and Bülow, 2001) and transporters (Chiang *et al.*, 2006, Krämer *et al.*, 2007). This tissue specificity indicates that the active demand of plants may play a more decisive role than the background value of soil in the absorption of HMs. Adaptive change in the composition and/or expression of metal transporter genes is relevant to plants in the face of adversity of excessive soil HM content (Krämer *et al.*, 2007). Mechanisms of physiological (such as HM metabolism) and ecological (such as tolerance, resistance, and adaptability to HM stress in soils) significance for differential accumulation among tissues are worthy of further study. In addition, these HMs in the soil might need to be converted into bioavailable forms for plants' absorption. The root exudates of some plants can alter the surrounding soil microbiome composition by changing soil physicochemical properties; hence improve the bioavailability of HM. *A. mongolicus* might not have the ability thus does not absorb high concentrations of HMs in this way. This might limit the potential of *A. mongolicus* being a phytoremediator.

The potential risk of using *A. mongolicus* as herb medicine

The roots of *A. mongolicus*, which are usually used for medication, accumulate relatively high concentrations of Cr, Cd, and Zn, while the leaves, which are the raw material for health drinks, accumulate many kinds of HMs. Certain HMs, such as Cd, Hg, and Pb, are non-essential metals for living organisms and are toxic even at low-level exposure (El-Kady and Abdel-Wahhab, 2018). Accumulation of HMs may cause a deficiency of some essential nutrients required for immunological defenses and detoxification (Antoniadis *et al.*, 2017). The high BAF_{Leaf} value for Hg indicates that Hg was almost equivalently translocated to leaves from soil. Although the HM accumulation of *A. mongolicus* growing in the sites of this study does not reach a dangerous level, special attention should be paid when manufacturing 'health drink' from leaves of *A. mongolicus* growing in Hg polluted soil.

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