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Effect of cadmium and arsenic contamination on element content and effectiveness in rhizosphere soils of different forages

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Abstract. To explore the effects of Cd and As composite contaminated soils on the element contents and effectiveness in rhizosphere soils of different forages, the root morphology, rhizosphere soil elements and their availability of 21 forages were studied and analyzed. The results showed: The 21 cultivars showed significant differences in root morphological characteristics. Root length is an index of root morphology that has the highest correlation with other elements and availability. And the correlation between the availability of each element and root morphology was higher than that of element content. Among them, Fe availability was significantly correlated with root morphological characteristics (P<0.05). The contents of different elements and availability varied greatly among the different cultivars. Among the six rhizosphere soil elements, the difference of toxic metal Cd was the largest, while the difference of Al availability was the largest. According to the correlation analysis between the elements and availability of each cultivar, the interaction of the availability of each element is higher than that of the soil element under the composite contaminated of Cd and As. Toxic metals Cd and As affected each other, and there was a very significant positive correlation between the content and availability. Fe, Mn and P had higher correlation with Cd and As.

1 Introduction

Toxic metal pollution is one of the important research problems in soil ecological remediation, which is shown not only by single element pollution, but also by two or even multi-element compound pollution[1]. Among them, the problem of cultivated land caused by Cd and As combined pollution is causing more and more economic losses to China. These two elements have a wide range of pollution and great toxicity. However, there are complex additions, synergism and antagonism between soil and organisms, which makes the impact of their combined pollution on the environment more complex than that of single pollution. The interaction between different metal elements also provides a new idea for biological detoxification and metal pollution remediation[2-3]. The interaction between metal and other elements is very complex under the stress of metal compound pollution, which will show different forms of action due to the existence of different elements. At present, the research on Cd and As compound polluted soil mainly focuses on the different absorption of toxic elements by cultivated varieties[4-5]. There are relatively few studies on the effects of metal pollution elements in soil on root morphology and the content of other elements in soil, which need to be studied.

Gejiu City, Yunnan Province is an industrial city rich in mineral resources. The problems left by the early mining and metallurgy are the main sources of Cd and As pollution in the local soil. According to the investigation, the local characteristic dairy industry has developed into a regular model, forages is the main forage crop. Therefore, 21 forages cultivars planted locally were used as test materials on the farmland soil polluted by Cd and As. The root morphological characteristics, different element contents and effective statuses of the forages cultivars were investigated to explore the interaction between each element under Cd and As composite contaminated soils. It is hoped to provide a basis for the future regulation of toxic metal elements in contaminated soils and the safe prevention and control during forages production in agricultural fields.

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2 Materials and methods

2.1 Experimental site location and forage plants

The experimental area was located Zhadian District, Jijie Town, Gejiu City, Yunnan Province, with an altitude of 1428 m, 103 ° 15 ′ E and 23 ° 46 ′ N. Soil physical and chemical properties: The pH between 7.64-8.19, 40.26 g/kg of organic content, 2.54 g/kg of total nitrogen content, 1.36 g/kg of total phosphorus content, 2.26 g/kg of total potassium content, 142.23 mg/kg of alkali-hydrolyzable nitrogen content, 179.5 mg/kg of available phosphorus content, 178.91 mg/kg of available potassium, 5.09 mg/kg Cd and 226.13 mg/kg As, respectively. The contents of Cd and As in the experimental area exceeded the control values of soil pollution risk of agricultural land in *"Soil environmental quality risk control standard for soil contamination of agricultural land"* (GB 15618-2018) by 1.27 times and 2.26 times, which belonged to severe pollution.

The tested materials were 21 cultivars of forages mainly comprising 3 families (*Gramineae*, *Leguminosae*, and *Compositae*), including 18 species and 21 cultivars (Table 1.).

Table 1. Species, families and genera of forages materials.

Number	Cultivar	Species	Family
1	Farn	*Festuca arundinacea* Schreb.	*Gramineae*
2	Denata	*Dactylis glomerata* L.	*Gramineae*
3	Reyan 4	*Pennisetum purpureum* Schumach. × *Pennisetum glaucum* (L.) R. Br.	*Gramineae*
4	Pearl millet	*Pennisetum alopecuroides* (L.) Spreng.	*Gramineae*
5	Ye Mingzhu	*Phleum pratense* L.	*Gramineae*
6	Dongmu-70	*Secale cereale* L.	*Gramineae*
7	Munzhou I	*Pennisetum giganteum* z.x.lin	*Gramineae*
8	VNS	*Coronilla varia* L.	*Leguminosae*
9	Bondi	*Lolium Multiflorum* L.	*Gramineae*
10	Maddy	*Lolium perenne* L.	*Gramineae*
11	Super King Tang	*Sorghum bicolor* × S. *Sudanense*	*Gramineae*
12	12SU9001	*Sorghum bicolor* × S. *Sudanense*	*Gramineae*
13	12SU9003	*Sorghum sudanense* (Piper) Stapf.	*Gramineae*
14	12SU9004	*Sorghum sudanense* (Piper) Stapf.	*Gramineae*
15	13FB7001	*Sorghum bicolor* (L.) Moench	*Gramineae*
16	12FS9003	*Sorghum bicolor* (L.) Moench	*Gramineae*
17	WL525HQ	*Medicago Sativa* L.	*Leguminosae*
18	Grand Slam	*Cichorium pumilum* Jacq.	*Compositae*
19	You-12	*Zea mays* ssp. *mexicana*	*Gramineae*
20	Rainbow	*Pennisetum purpureum* Schum cv.Guiminyin	*Gramineae*
21	Chaosheng	*Lactuca indica* L.	*Compositae*

2.2 Experimental design

The test materials were sown in October 2019. In the in-situ field test, each material was set up with three replicates. The plot area was 3m × 5m, and the materials were randomly distributed. In the in-situ field test, each material was set up with three groups of repetition, the plot area was 3m × 5m, and the materials were randomly distributed. During the growth period, insecticidal, weeding, watering and topdressing were carried out according to the growth status. Samples were collected in June 2020. Five sampling sites were sampled evenly and randomly by "X" method, and the plant root and rhizosphere soil samples were collected. The aboveground and underground parts of the plant were opened with scissors and put into polyethylene bags.

2.3 Determination of root morphology

The plant root scanner EPSON perfection V700 photo was used for scanning and analysis.

2.4 Determination of element content and available state in rhizosphere soil

The content of cadmium was determined by flame atomic absorption spectrometry (Thermo ICE 3000 SERIES) and recorded it in mg/kg[6-7]. The content of arsenic was determined by atomic fluorescence spectrometer (AFS-9710) and recorded it in mg/kg[8-9]. Determination of total phosphorus and available phosphorus in soil by molybdenum antimony anti colorimetric Spectrophoto-metry (Metash UV-5800)[6]. The content and effective state of Fe, Mn and Al were determined by ICP-OES (ThermoScientific iCAP6300) [6、10].

2.5 Statistical analyses

The statistical significance analyzed used one-way analysis of variance (ANOVA) and Duncan test the difference of the average value of different treatments at the 0.05 level by SPSS 20.0 (n=3). And the correlation was analyzed by Spearman method.

3 Results

3.1 Differences in rhizosphere soil pH and root morphology

The rhizosphere soil pH of the 21 cultivars ranged from 7.33 to 8.70, and the differences were not significant (P > 0.05), whereas obvious differences were observed in root morphology among the different cultivars (Table 2.). The maximum difference of total root length was 13.41 times, the maximum value was 'Denata' of *Gramineae*, and the minimum value was 'Grand slam' of *Compositae*, and the maximum differences of root length, root surface area, root volume and root diameter among cultivars were 1.97, 6.26, 30.82 and 179.3 times, respectively. Except for root length, the maximum value is *Compositae*.

Table 2. Soil pH and root morphology in the Rhizosphere of 21 cultivars.

Cultivar	pH	total root length (cm)	root length （cm）	root surface area （cm^2 ）	root diameter （mm）	root volume （cm^3）
Farn	7.73±0.07a	210.94±52.21efg	8.68±2.09d	28.96±8.83h	0.43±0.04d	0.3±0.13c
Denata	7.71±0.04a	681.71±160.46a	11.63±2.52cd	125.67±28.13abcde	0.48±0.02d	1.49±0.3c
Reyan 4	8.27±0.02a	619.69±112.16ab	17.85±0.88a	113.94±27.1abcdef	0.58±0.04cd	1.68±0.51c
Pearl millet	8.02±0.09a	397.92±36.45bcde	11.89±2.11bcd	74.03±10.86defgh	0.59±0.04cd	1.11±0.23c
Ye Mingzhu	8.00±0.12a	370.36±78.34de	11.79±1.02bcd	41.66±5.32fgh	0.38±0.06d	0.39±0.09c
Dongmu-70	7.46±0.10a	301.13±21.73def	13.5±0.6abcd	52.4±14.97efgh	0.55±0.16cd	0.84±0.45c
Munzhou I	8.13±0.03a	611.69±170.39abc	15.31±1.61abc	151.24±13.62abc	0.62±0.11cd	1.9±0.6c
VNS	8.07±0.05a	411.66±66.17bcde	15.14±1.28abc	107.97±9.56abcdefg	0.89±0.17cd	2.41±0.51c
Bondi	8.00±0.00a	273.13±9.89defg	12.48±1.09bcd	33.22±3.67gh	0.39±0.05d	0.33±0.08c
Maddy	8.11±0.03a	431.44±99.5bcde	8.87±0.32d	53.42±12.3efgh	0.39±0.01d	0.53±0.12c
Super King Tang	8.01±0.01a	350.36±6.32de	14.29±1.49abc	114.47±1.2abcdef	1.04±0.01cd	2.98±0.05c
12SU9001	7.90±0.03a	279.56±28.65defg	14.29±1.49abc	78.79±6.54cdefgh	0.90±0.05cd	1.78±0.16c
12SU9003	7.62±0.05a	200.82±37.54efg	12.38±1.57bcd	51.44±8.64efgh	0.84±0.09cd	1.07±0.2c
12SU9004	8.13±0.04a	279.49±14.29defg	12.4±2.22bcd	95.41±8.74bcdefgh	1.09±0.1cd	2.64±0.45c
13FB7001	7.82±0.19a	467.84±38.72abcd	14.41±0.39abc	134.12±18.27abcd	0.91±0.08cd	3.11±0.65c
12FS9003	7.48±0.03a	384.67±53.46cde	16.31±0.52abc	153.14±29.23abc	1.25±0.1cd	4.9±1.19c
WL525HQ	7.68±0.05a	281.75±39.49defg	17.14±0.89ab	148.21±53.75abcd	1.60±0.43c	3.73±1.03c
Grand Slam	8.03±0.03a	50.84±11.02g	11.51±3.28cd	158.32±55.72ab	11.71±0.6a	46.98±17.68b
You-12	7.58±0.02a	246.25±26.03defg	15.05±1.57abc	117.32±10abcdef	1.10±0.21cd	2.62±0.97c
Rainbow	7.87±0.12a	466.04±71.62abcd	16.09±1abc	144.36±15.32abcd	0.53±0.04cd	1.48±0.42c
Chaosheng	7.73±0.05a	72.24±1.1fg	14.12±0.84abc	181.37±23.41abcd	9.08±1.16b	53.79±15.24a

Note: Data are the mean ± standard errors of three replicates. Different lowercase letters represent significant differences between data in the same column ($P < 0.05$).

3.2 Difference of element content in rhizosphere soil

There were differences in the contents of different elements in the rhizosphere soil of different forage grasses (Figure 1.). The Cd and P contents in soil of ‘Denata’ was the highest. The highest contents of As, Fe, Mn and Al were ‘You-12’, ‘Reyan 4’, ‘Faren’ and ‘Grand Slam’, respectively, with significant difference ($P < 0.05$). After planting ‘Reyan 4’, the contents of Cd, As and Mn in rhizosphere soil were the lowest, the lowest contents of Fe were ‘denata’ and ‘Dongmu-70’, the lowest contents of Al were made in China, and the lowest contents of P were ‘Munzhou I’. Among the six rhizosphere soil elements, the biggest difference was the Cd content, and the difference reached 4.76 times.

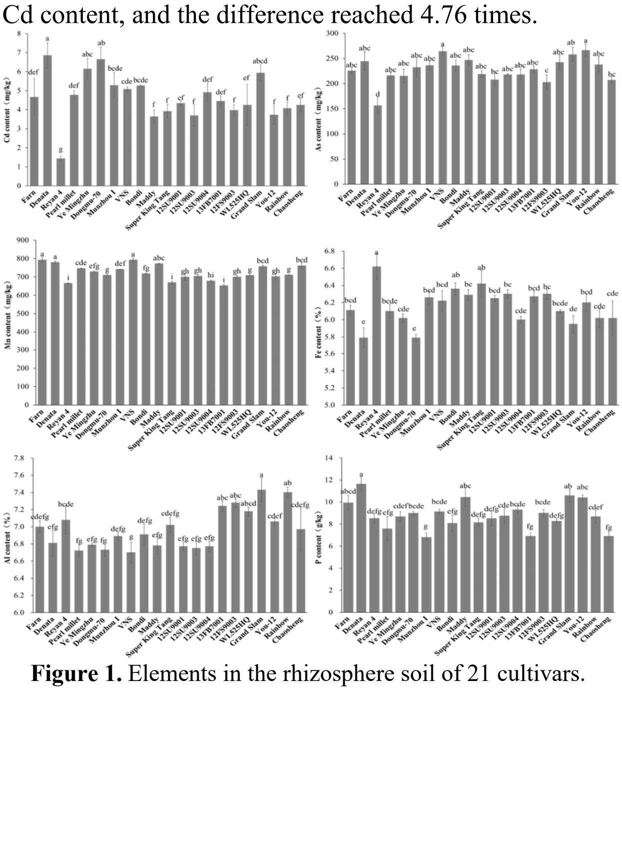

Figure 1. Elements in the rhizosphere soil of 21 cultivars.

3.3 Difference of effective content of elements in rhizosphere soils

The highest availability of As and P was found in ‘Denata’ (Figure 2.), the lowest in ‘Super King Tang’ and ‘12SU9004’, respectively. And ‘Yemingzhu’, ‘Dongmu 70’ and ‘Super King Tang’ had the highest availability of Cd, Mn and Al, while ‘You-12’, ‘Reyan 4’ and ‘VNS’ had the lowest. Moreover, The highest availability of Fe was ‘Reyan 4’, the lowest was ‘denata’ and ‘Dongmu-70’. There were significant differences in rhizosphere soil availability among different cultivars, and the biggest difference among these available states was Al availa- bility, which was 4.22 times higher than that in rhizosphere soil of different cultivars.

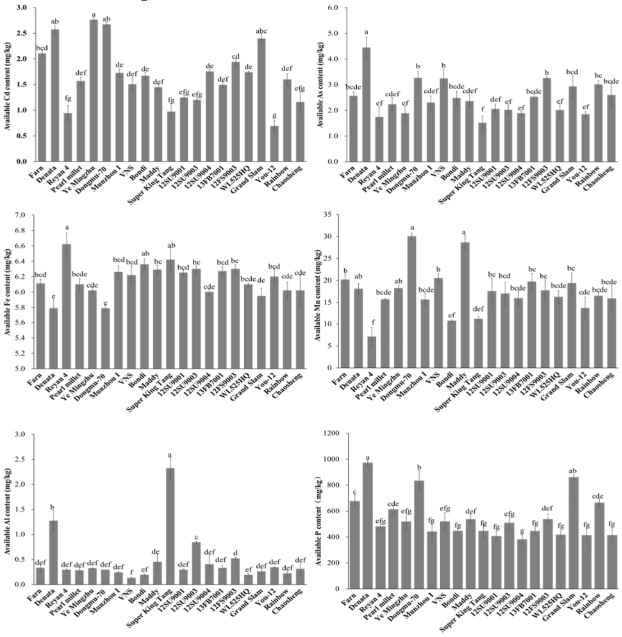

Figure 2. Availability of elements in the rhizosphere soil of 21 cultivars.

3.4 Correlation analysis

The root morphology of forages was not significantly correlated with the content of Cd and As, the same as the soil pH (Table 3-5.). But the pH was significantly correlated with the As availability, and the root length was negatively correlated with the available state of Cd, Fe, Mn and P ($P < 0.05$). The contents of Cd and As were significantly positively correlated with the available state of Cd and As, and both of them were significantly correlated with Fe, Mn content and availability. ($P < 0.05$). There was extremely significant positive correl-ation between Cd, As availability and P availability (P＜0.01). The correlation of the available state of each element to the root morphology in the soil polluted by Cd and As was higher than that of the element content, and the interaction of the available state of each element was higher than that of the soil element.

Table 3. Correlation analysis between root morphology and soil elements among 21 cultivars.

	pH	Total root length	Root length	Root surface area	Root diameter	Root volume
Cd content	-0.171	-0.051	-0.222	-0.002	0.090	0.096
As content	-0.157	-0.104	-0.004	0.120	0.081	0.085
Fe content	0.305*	0.090	0.154	-0.098	-0.276*	-0.297*
Mn content	0.012	-0.065	-0.359**	-0.095	0.203	0.189
Al content	-0.052	-0.137	0.151	0.373**	0.303*	0.175
P content	-0.088	-0.015	-.0278*	-0.111	0.036	0.013
Cd availability	-0.231	-0.019	-0.294*	-0.092	0.084	0.047
As availability	-0.303*	0.153	-0.134	0.130	0.127	0.134
Fe availability	-0.090	0.071	-0.264*	-0.291*	-0.288*	-0.258*
Mn availability	-0.233	-0.088	-0.298*	-0.172	0.028	0.072
Al availability	-0.047	0.086	-0.123	0.010	-0.100	-0.085
P availability	-0.237	0.066	-0.291*	-0.033	0.177	0.120

Note: *At the 0.05 level, correlations were significant.
** At the 0.01 level the correlation was extremely significant.
The same below.

Table 4. Correlation analysis of the content of elements in rhizosphere soil of 21 cultivars.

	Cd	As	Fe	Mn	Al	P
Cd	1					
As	0.358**	1				
Fe	-0.632**	-0.257*	1			
Mn	0.375**	0.351**	-0.320*	1		
Al	-0.207	0.039	0.284*	-0.212	1	
P	0.102	0.205	-0.264*	0.297*	-0.002	1

Table 5. Correlation analysis of availability in rhizosphere soil of 21 cultivars.

	Cd	As	Fe	Mn	Al	P
Cd	1					
As	0.469**	1				
Fe	0.463**	0.306*	1			
Mn	0.415**	0.380**	0.521**	1		
Al	-0.085	-0.040	-0.145	-0.159	1	
P	0.628**	0.593**	0.325**	0.409**	0.115	1

4 Discussions

Roots showed a high degree of plasticity to changes of soil environmental factors during the growth process. Difference in genotype and growing environment can lead to changes in morphological characteristics of part or the whole root system[11]. Metal ions on the root surface can be absorbed actively and passively through cation exchange, diffusion and metabolic energy, and show a high selectivity to some metal elements[12]. Larger root morphological characteristics, such as root length, root surface area, root volume and root tip number, not only help to increase the contact area between plant roots and soil, and promote roots to absorb trace elements in soil. It may also increase the absorption and accumulation of metal elements[13]. In this experiment, the effect of pH on the rhizosphere soil of all forage cultivars before and after planting was small. There were significant differences in total root length, root surface area, root diameter and root volume among different forages, and the degree of difference varied with cultivars, which was consistent with the results of Gao et al.[14]. In root morphology, the total root length and root length of *Gramineae* were generally higher than those of *Leguminosae* and *Compositae*, while the root surface area, root diameter and root volume of *Compositae* were higher than those of *Gramineae* and *Leguminosae*, which was mainly related to the growth characters and interspecific differences of each material. Li et al.[15] also shows that the change in root form structure is an important embodiment of plant adaptation to stress. In addition, correlation analysis showed that there was a significant correlation between Fe availability and root morphology, and it was found that root length had more correlation with the content of Mn, P and the available state of Cd, Fe, Mn, which fully indicated that root length was a very important index in Cd and As contaminated soil, which may be more affected by some trace elements and Cd.

There are complex additive, synergistic and antagonistic effects between soil and organisms, which makes the impact of metal compound pollution on the environment more complex than that of single pollution[16]. Cd and As exist in different forms in soil with different migration characteristics and bioavailability. After Cd and As enter the soil environment, the interaction between soil pH, and hazardous metals is an important influencing factor[17]. Studies have shown that toxic elements tend to affect mineral elements in soils[18-19]. However, the contents of both toxic metals including Cd and As showed extremely significant positive correlation in this experiment ($P < 0.01$), and it indicated that the interaction had a large effect and showed a synergistic relationship. Guo et al.[20] reported that the coexistence of Cd and As in soil could additive to alfalfa, and Cd promote the accumulation of As. At the same time, the toxic elements were positively correlated with Fe content and negatively correlated with Mn content, but the effectiveness was positively correlated with the available state of Fe, Mn and P, which had obvious synergistic effect. This result suggests that increased or accumulated nutrients such as Fe and P in soils may exacerbate the risk of Cd or As contamination in cultivated forages.The interactive interaction between Cd, As and each element is an important factor affecting the absorption and accumulation of the pollutants toxic metals in soils by forages. It is similar to that of Cheng et al.[21].

5 Conclusions

There were significant differences in root morphology among different cultivars, and the root length was more affected by some trace elements and Cd in the composite contaminated soils. The correlation between the available state of each element and root morphology was higher than that of element content, and the available state of rhizosphere soil elements had a greater effect on plant root morphology. There was a significant positive correlation between Cd and As, and the interaction was synergistic. At the same time, in the element content of rhizosphere soil, the difference of Cd content was the biggest, while in the available state, the difference of Al content was the biggest. The elements with high correlation with metal Cd and As are Fe, Mn and P. The results can provide a certain scientific basis for the selection of agricultural cultivars of farmland polluted by hazardous metals in mining area and the appropriate measures to control the pollution of Cd and As in farmland soil, and realize the effective control and safe utilization.

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