Intraspecific differences in effects of co-contamination of cadmium and arsenate on early seedling growth and metal uptake by wheat

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Abstract

A hydroponic experiment was carried out to study intraspecific differences in the effects of different concentrations of cadmium (Cd) (0–10 mg/L) and arsenate (As(V)) (0–8 mg/L) on the growth parameters and accumulation of Cd and As in six wheat varieties Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8. The endpoints of wheat seedlings, including seed germination, biomass, root length and shoot height, decreased with increasing the Cd and As concentrations. Significant differences in seed germination, biomass, root length, shoot height and the accumulation of Cd and As were observed between the treatments and among the varieties ($p < 0.05$). The lethal dosage 50% were about 20, 80, 60, 60, 80 and 20 mg As/L for Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8, respectively, and the corresponding values for Cd were about 30, 80, 20, 40, 60 and 10 mg Cd/L, respectively. Among the six varieties, Duokang-1 was found to be the most resistant to Cd and As toxicity, and Zhongmai-8 was the most sensitive to Cd and As co-contamination. The resistance of the six varieties was found dependant on the seedling uptake of Cd and As. Duokang-1 was the most suitable for cultivation in Cd and As co-contaminated soils.

Key words: Cd; As; varieties; co-contamination; wheat seedling

Introduction

Cadmium (Cd) is a trace element which is toxic to plants, animals and as well as humans. It has been revealed that the strong toxicity of Cd can cause plant growth inhibition (Avery and Sakar, 1991) and even death due to its interaction with photosynthesis, respiration and nitrogen assimilation in plants (Sanit di Toppi and Gabbriellini, 1999). Arsenate (As(V)) is an unessential plant nutrient element and toxic to plants (Lepp, 1981). It can hamper plant growth due to its inhibiting leaf uptake of other chemicals (Carbonell *et al.*, 1995).

Contamination of Cd and As limits wheat growth, even decreases the production seriously under the condition of high concentration stress and also deteriorates crop quality. With the quick increase in population and the environment pollution being more serious, the problems of food quality and quantity become more and more concerned. Approaches have been sought to prevent the accumulation of Cd and As in crops in order to reduce their contents in human diets as well as to alleviate the health risks associated with exposure to Cd and As. One of the best cost-effective and efficient approaches is to develop the Cd and As-exclusive varieties. To breed Cd

and As-exclusive variety, it is important to find out the potential accumulation of Cd and As in existing varieties and their physiological responses to Cd and As. The great differences in Cd and As tolerance have been found among varieties within a species (Grant *et al.*, 1998; Jalil *et al.*, 1994). Wheat, the main food in North China, is an important crop in China and its cultivating covers about 22% of the total arable lands. However, little investigation on intraspecific variation in Cd and As toxicity of wheat has been reported.

Researches have shown that Cd or As could affect the uptake of each other as well as the uptake of other elements by plants (Das *et al.*, 1997; Carbonell *et al.*, 1995). Therefore, interaction between them for their plant uptake and toxicity might exist. Numerous researches have investigated the single toxicity of Cd or As on plant growth. Coexistence of Cd and As was found in some polluted soils, however, little information is available on the effects of their co-contamination on plant growth and uptake. Assessment of their joint toxicity is more significant because it is more close to the natural environment pollution situations. Due to the complexity for joint metal uptake by plant, such assessments are less reported. It is unknown whether and how Cd and As interfere each other on plant growth and metal uptake.

In this study, the influence of Cd and As on seed germination, biomass, root length, shoot height, LD₅₀, and

the accumulation of Cd and As in the six wheat varieties were investigated by a hydroponic experiment to determine the toxicity of Cd and As co-contamination to wheat and find out the varieties which have more resistance to Cd and As co-contamination.

1 Materials and methods

Six wheat varieties (*Triticum aestivum* L., obtained from Chinese Academy of Agricultural Sciences, Beijing, China), DuoKang-1, Jing-9428, Jing-411, Jingdong-8, Zhongmai-8 and Jingdong-11, were chosen for the test. The six varieties are widely planted in China and were found to be differently sensitive to arsenic stress (Liu *et al.*, 2005). Among them, Duokang-1 was the most resistant variety to arsenic treatment.

1.1 Germination, biomass and seedling toxicity assay

Effects of different concentrations of Cd and As(V) on seed germination were evaluated. The concentrations of Cd and As were 0, 2, 4, 6, 8, 10 mg Cd/L and 0, 1, 2, 4, 8 mg As/L in the culture solutions, which were prepared freshly by dissolving $\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$ and $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ with deionized water, respectively. The pH was adjusted to 5.8 by using 2 mmol/L Mes-Tris. LD_{50} (lethal dosage 50%) of Cd and As were determined according to the wheat germination inhibition rates with the treatments of 0 to 100 mg/L Cd or As.

Prior to germination, all the seeds were surface-sterilized with 3% H_2O_2 and then rinsed with deionized water. Seed germination was tested on moist filter paper. A piece of filter paper was placed on a Petri dish and moistened with 5.0 ml aqueous mixture of Cd and As. Controls were maintained by moistening the filter paper with 5 ml deionized water. Thirty seeds of each variety were placed on a dish, covered by lid, and incubated in dark at $23 \pm 2^\circ\text{C}$. Germinated seeds were counted 4 d after initiation. Seeds were considered germinated when both the plumule and radicle were extended to more than 2 mm from their junctions. The biomass was weighed at the same time. Each treatment was replicated three times.

Seeds germinated for 4 d were transferred to vitreous pots containing 50 ml test solutions of 0, 1, 2, 4 and 8 mg As/L and 0, 2, 4, 6, 8 and 10 mg Cd/L. Each treatment was replicated three times. The pots were then placed randomly in a growth cabinet to ensure normal growth conditions. Temperature was maintained at $23 \pm 2^\circ\text{C}$. The test solutions were renewed every day to avoid the change in concentration and speciation. After 2-d growth, shoot height was then measured from the culm base to the tip of the longest leaf and root length was measured from the root-shoot junction to the tip of the longest root. Root length and shoot height of the wheat grown in the test solution containing Cd and As were expressed as inhibition rate (%) of root length and inhibition rate (%) of shoot height compared with the controls.

1.2 Determination of Cd and As in seedlings

For the determination of Cd and As, three uniform seedlings 6 d after initiation were rinsed with iced CaCl_2

solution (Hart *et al.*, 1998), and then with iced deionized water four times and blotted with tissue paper thoroughly. The fresh weights were recorded and digested with 5 ml ultra-pure concentrated HNO_3 and 3 ml 30% w/v H_2O_2 using a closed-vessel microwave digestion protocol (CEM Mars V, Matthews, NC, USA). Arsenic was determined by hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-610A model atomic fluorescence spectrophotometer, Beijing BRAIC Instrumental Company, Beijing, China) and Cd by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Optima 2000 DV, Perkin Elmer Co., Wellesley, MA, USA). All measurements were performed in triplicates.

1.3 Statistical analysis

Statistical analysis was performed by using Microsoft-Excel, SPSS 11.5 and the geochemical speciation model MINTEQAII (Allison *et al.*, 1991). The data represent means calculated from three replicates. A least significant difference test (LSD-test) was employed for comparison of the changes at $p < 0.05$.

2 Results

2.1 Toxicity of Cd and As co-contamination on seed germination

The mean (average of all 6 varieties) germination inhibition rates (GIRs) over control increased significantly ($p < 0.05$) with increasing the concentrations of Cd and As. The GIRs were 0–28.5%, 4.4%–34.6%, 16.4%–40.4%, 20.7%–43.4% and 31.2%–51.1% at 0, 2, 4, 6, 8, 10 mg/L Cd and 0, 1, 2, 4, 8 mg/L As, respectively. When Cd concentration was fixed, the GIRs increased with the increase in As concentration for all the varieties and vice versa. As we know if Cd and As have interactive effects each other, either synergistic or antagonistic, the germination inhibition rates for the Cd and As co-contamination would be different from the additive inhibition rates of the separate treatments with Cd and As. If the germination inhibition rates are the same for the two situations, only additive effect can be interpreted. In this study, the additive germination inhibition rates of Cd and As obtained individually were higher than those of Cd and As co-contamination, however the differences were insignificant ($p > 0.05$). Therefore, coexistence of Cd and As only exerted additive effect on seed germination.

There were differences in germination for different wheat varieties ($p < 0.05$) with the treatments of Cd and As. GIRs for all the varieties decreased with increasing Cd and As concentrations except Duokang-1. The GIRs were 64.6%, 8.8%, 61.3%, 54.1%, 49.5% and 67.8% for Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8 for the treatment with Cd at 10 mg/L and As at 8 mg/L (Fig.1), respectively. Duokang-1 was the only variety, whose germination showed a considerable resistance to the high concentration of Cd (10 mg/L) and As (8 mg/L).

In this study, germination inhibition rate at 50% was

Fig. 1 Seed germination inhibition rates (%) compared with the control at different concentrations Cd and As ($n=3$).

considered as the lethal dosage 50% (LD_{50}). The LD_{50} of Cd and As for the six wheat varieties are presented in Fig.2, which were about 20, 80, 60, 60, 80 and 20 mg As/L and 30, 80, 30, 40, 60 and 10 mg Cd/L for Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8, respectively. These data indicated that Duokang-1 had the most and Zhongmai-8 had the least resistance to the toxicity of Cd and As.

2.2 Toxicity of Cd and As co-contamination on biomass

The mean biomass inhibition rates (BIRs) (average of 6 varieties) increased significantly ($p < 0.05$) with increasing the concentration of Cd or As. The BIRs were 0–31.3%, 6.0%–36.5%, 22.8%–49.3%, 29.1%–54.7%, and 46.4%–67.8% at 0, 2, 4, 6, 8, 10 mg/L Cd and 0, 1, 2, 4, 8 mg/L As, respectively. The additive inhibition rates of the separate treatments with Cd and As were significantly higher than the inhibition rates of the coexistence of Cd and As ($p <$

0.05), indicating the antagonistic effects of Cd and As on biomass.

Although no variety had a particular resistance to Cd and As in terms of biomass (Fig.3), differential responses were observed among the varieties ($p < 0.05$). The BIRs for Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8 were 70.9%, 58.3%, 69.0%, 77.9%, 61.5% and 79.1%, respectively, when Cd (10 mg/L) and As (8 mg/L) co-existed. Duokang-1 had the most and Zhongmai-8 had the least resistance to Cd and As co-contamination.

2.3 Toxicity of Cd and As co-contamination on wheat early development

Effects of Cd and As on root inhibition rates (RIRs) are presented in Fig.4. The RIRs were 0–64.8%, 4.3%–65.4%, 14.7%–72.8%, 27.8%–80.3%, and 48.5%–87.2% at the Cd concentrations of 0, 2, 4, 6, 8, 10 mg/L and As

Fig. 2 Lethal dosage 50% (LD_{50}) of As(V) (a) and Cd (b) ($n=3$).

Fig. 3 Biomass inhibition rates (%) compared with the control at different concentrations Cd and As ($n=3$).

Fig. 4 Root length inhibition rates (%) of the wheat at different concentrations Cd and As ($n=3$).

concentrations of 0, 1, 2, 4, 8 mg/L, respectively. Analysis of variance showed that Cd and As concentrations and wheat varieties had significant effects ($p < 0.05$) on the RIRs.

The RIRs for all varieties increased significantly with the increase of Cd and As concentrations. Differential responses in root elongation were noted for different Cd and As concentrations and among the varieties (Fig.4) ($p < 0.05$). The RIRs for Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8 were 92.6%, 76.7%, 88.3%, 86.8%, 84.1% and 94.8%, respectively, for the treatment with 10 mg/L Cd and 8 mg/L As (Fig.4).

The difference in RIRs indicated that the resistance of wheat varieties to Cd and As co-contamination was in the order: Duokang-1 > Jingdong-8, Jing-411, Jingdong-11, Jing-9428 > Zhongmai-8.

Effects of Cd and As on shoot inhibition rates (SIRs) are presented in Fig.5. Analysis of variance indicated that Cd and As concentrations and different wheat varieties also had significant effects on the SIRs data ($p < 0.05$). Significant reduction in shoot height was observed with the increase of Cd and As concentrations. The SIRs were 0–44.1%, 7.0%–44.8%, 9.5%–45.8%, 29.3%–57.7% and 36.0%–63.5% for the treatments with 0, 2, 4, 6, 8, 10 mg/L

Fig. 5 Shoot height inhibition rates (%) of the wheat with different concentrations Cd and As ($n=3$).

Table 1 Concentrations of As (mg/kg) in seedlings under different concentrations of Cd and As (based on fresh weight and represented as mean $\pm$ SE, $n=3$)

Cd addition (mg/L)	As(V) addition (mg/L)	Variety						Mean
		Jing-9428	Duokang-1	Jingdong-11	Jing-411	Jingdong-8	Zhongmai-9	
0	0	/	/	/	/	/	/	/
	1	9.0 $\pm$ 0.7	6.4 $\pm$ 0.6	8.8 $\pm$ 0.5	7.7 $\pm$ 0.7	7.0 $\pm$ 0.4	12.6 $\pm$ 1.2	8.6 $\pm$ 0.9
	2	12.9 $\pm$ 1.2	7.2 $\pm$ 0.4	10.2 $\pm$ 0.8	8.6 $\pm$ 0.7	8.5 $\pm$ 0.7	13.1 $\pm$ 1.2	10.1 $\pm$ 1.1
	4	15.6 $\pm$ 1.4	9.6 $\pm$ 0.8	14.0 $\pm$ 1.3	12.1 $\pm$ 1.1	11.3 $\pm$ 1.1	20.0 $\pm$ 1.8	13.8 $\pm$ 1.3
	8	21.2 $\pm$ 1.7	13.9 $\pm$ 1.2	18.0 $\pm$ 1.5	15.8 $\pm$ 1.3	14.6 $\pm$ 1.4	24.7 $\pm$ 2.2	18.0 $\pm$ 1.6
2	0	/	/	/	/	/	/	/
	1	8.8 $\pm$ 0.6	6.2 $\pm$ 0.4	8.4 $\pm$ 0.5	7.2 $\pm$ 0.4	6.73 $\pm$ 0.6	10.8 $\pm$ 0.8	8.0 $\pm$ 0.9
	2	11.4 $\pm$ 1.1	7.2 $\pm$ 0.6	10.0 $\pm$ 0.8	8.2 $\pm$ 0.7	7.34 $\pm$ 0.7	12.9 $\pm$ 1.3	9.5 $\pm$ 0.9
	4	13.3 $\pm$ 1.2	9.1 $\pm$ 0.7	13.2 $\pm$ 1.2	11.9 $\pm$ 1.1	10.2 $\pm$ 0.9	13.5 $\pm$ 1.2	11.9 $\pm$ 1.2
	8	19.8 $\pm$ 1.9	13.1 $\pm$ 1.2	17.3 $\pm$ 1.5	15.4 $\pm$ 1.5	14.4 $\pm$ 1.3	21.7 $\pm$ 2.1	17.0 $\pm$ 1.5
4	0	/	/	/	/	/	/	/
	1	8.6 $\pm$ 0.7	6.1 $\pm$ 0.5	8.3 $\pm$ 0.8	7.0 $\pm$ 0.6	6.5 $\pm$ 0.6	11.3 $\pm$ 1.1	7.9 $\pm$ 0.7
	2	11.3 $\pm$ 1.0	7.0 $\pm$ 0.5	10.0 $\pm$ 1.0	7.9 $\pm$ 0.7	7.1 $\pm$ 0.5	13.0 $\pm$ 1.2	9.4 $\pm$ 0.9
	4	13.1 $\pm$ 1.2	9.0 $\pm$ 0.7	12.0 $\pm$ 1.1	11.2 $\pm$ 1.0	9.7 $\pm$ 0.9	13.3 $\pm$ 1.3	11.4 $\pm$ 1.1
	8	19.2 $\pm$ 1.7	12.8 $\pm$ 0.9	17.0 $\pm$ 1.6	15.0 $\pm$ 1.3	14.1 $\pm$ 1.4	21.9 $\pm$ 1.8	16.7 $\pm$ 1.4
6	0	/	/	/	/	/	/	/
	1	7.5 $\pm$ 0.6	5.0 $\pm$ 0.5	7.1 $\pm$ 0.6	6.6 $\pm$ 0.5	6.2 $\pm$ 0.6	7.5 $\pm$ 0.7	6.6 $\pm$ 0.6
	2	11.0 $\pm$ 1.1	6.8 $\pm$ 0.7	9.4 $\pm$ 0.8	7.4 $\pm$ 0.5	7.0 $\pm$ 0.7	14.1 $\pm$ 1.2	9.3 $\pm$ 0.8
	4	12.9 $\pm$ 1.3	8.5 $\pm$ 0.6	12.9 $\pm$ 1.3	10.7 $\pm$ 0.9	9.3 $\pm$ 0.8	14.4 $\pm$ 1.3	11.4 $\pm$ 1.1
	8	17.3 $\pm$ 1.5	12.1 $\pm$ 1.1	16.5 $\pm$ 1.4	14.2 $\pm$ 1.3	13.9 $\pm$ 1.4	19.6 $\pm$ 1.7	15.6 $\pm$ 1.6
8	0	/	/	/	/	/	/	/
	1	7.2 $\pm$ 0.3	4.7 $\pm$ 0.4	6.8 $\pm$ 0.6	6.3 $\pm$ 0.6	5.0 $\pm$ 0.4	9.0 $\pm$ 0.7	6.5 $\pm$ 0.5
	2	10.6 $\pm$ 0.9	6.5 $\pm$ 0.5	9.2 $\pm$ 0.8	7.0 $\pm$ 0.6	6.6 $\pm$ 0.5	12.7 $\pm$ 1.2	8.7 $\pm$ 0.8
	4	12.7 $\pm$ 1.2	8.4 $\pm$ 0.6	11.4 $\pm$ 1.1	10.1 $\pm$ 0.9	8.7 $\pm$ 0.8	13.0 $\pm$ 1.2	10.7 $\pm$ 1.0
	8	16.8 $\pm$ 1.4	12.0 $\pm$ 1.1	15.6 $\pm$ 1.4	13.6 $\pm$ 1.2	12.9 $\pm$ 0.9	19.1 $\pm$ 1.8	15.0 $\pm$ 1.5
10	0	/	/	/	/	/	/	/
	1	7.2 $\pm$ 0.6	4.6 $\pm$ 0.4	6.7 $\pm$ 0.5	6.1 $\pm$ 0.4	4.9 $\pm$ 0.5	7.7 $\pm$ 0.7	6.2 $\pm$ 0.6
	2	10.0 $\pm$ 0.8	6.2 $\pm$ 0.3	8.9 $\pm$ 0.8	7.0 $\pm$ 0.6	6.4 $\pm$ 0.5	12.3 $\pm$ 1.2	8.5 $\pm$ 0.7
	4	11.0 $\pm$ 0.9	7.1 $\pm$ 0.6	10.1 $\pm$ 0.7	8.9 $\pm$ 0.9	8.6 $\pm$ 0.7	13.1 $\pm$ 1.1	9.8 $\pm$ 1.0
	8	16.3 $\pm$ 1.5	11.4 $\pm$ 1.0	15.0 $\pm$ 1.5	13.2 $\pm$ 1.1	12.5 $\pm$ 1.2	19.3 $\pm$ 2.0	14.6 $\pm$ 1.5
Significance ^a among Cd treatments		**	*	*	*	*	**	*
Significance ^a among varieties		**	**	**	**	**	**	**

^a By analysis of variance; * $p < 0.05$; ** $p < 0.01$; /: no As detected.

Cd and 0, 1, 2, 4, 8 mg/L As, respectively. More reduction in shoot height was caused by the coexistence of Cd and As than by the separate treatment with Cd and As. The SIRs for Jing-9428, Duokang-1, Jingdong-11, Jing-411, Jingdong-8 and Zhongmai-8 were 66.8%, 55.3%, 62.0%, 61.7%, 60.9% and 74.5%, respectively, for the treatment with 10 mg/L Cd and 8 mg/L As. Shoot height had different responses to Cd and As at all the concentrations for all the varieties ($p < 0.05$). Duokang-1 was the most resistant, and Zhongmai-8 was the least resistant to Cd and As co-contamination.

Calculated from Figs.4 and 5, the additive inhibition rates of Cd and As individual effects on root and shoot elongations were higher than those of the coexistence of Cd and As; co-contamination caused more root and shoot reduction than the Cd or As separate treatment, therefore, Cd and As had antagonistic effects on root and shoot elongations.

2.4 Accumulation of Cd and As in wheat seedlings

The concentrations of Cd and As in wheat seedlings increased with the increase in Cd and As concentrations (Tables 1 and 2). Differential As ($p < 0.01$) and Cd uptakes by seedlings were observed among the varieties, but the differences of Cd uptakes were not significant ($p > 0.05$) among the varieties. Duokang-1 accumulated the least, while Zhongmai-8 accumulated the most Cd and As. Data

in Tables 1 and 2 also showed that Cd supply decreased As uptake ($p < 0.05$) and As supply decreased Cd uptake by wheat ($p < 0.01$), indicating that Cd and As had an antagonistic effect on their uptake by wheat seedlings.

3 Discussion

The results of this study showed that wheat germination was considerably affected by both Cd and As except Duokang-1, who showed a high tolerance in germination even when Cd and As co-existed at the concentration of 10 mg/L Cd and 8 mg/L As. The LD₅₀ of Cd and As was different for the varieties and Duokang-1 had the most resistance to Cd and As co-contamination. High resistance to Cd and As could be achieved by (1) complexation of Cd and As by such as peptides with SH-groups (Maitani *et al.*, 1996; Sneller *et al.*, 1999; Schmäger *et al.*, 2000); (2) reduction of As influx by suppressing phosphate/arsenate uptake systems (Meharg and Macnair, 1992; Meharg, 1994); (3) enhanced production of antioxidants that detoxify free reactive oxygen species (ROS) produced in response to Cd and As (Hartley-Whitaker *et al.*, 2001; Mylona *et al.*, 1998); and (4) depressed the total ion activity in the solution caused by the Cd and As coexistence (the data calculated by MINTEQAII assisted the result).

The effects of co-contamination of Cd and As on seed

Table 2 Concentrations of Cd (mg/kg) in seedlings under different concentrations of Cd and As (based on fresh weight and represented as mean $\pm$ SE, $n=3$)

As(V) addition (mg/L)	Cd addition (mg/L)	Variety						Mean
		Jing-9428	Duokang-1	Jingdong-11	Jing-411	Jingdong-8	Zhongmai-9	
0	0	/	/	/	/	/	/	/
	2	185.6 $\pm$ 12.3	158.4 $\pm$ 14.4	181.5 $\pm$ 16.8	170.2 $\pm$ 15.7	162.4 $\pm$ 14.6	190.7 $\pm$ 18.8	174.8 $\pm$ 10.9
	4	306.5 $\pm$ 27.5	276.3 $\pm$ 24.8	297.8 $\pm$ 25.6	292.3 $\pm$ 26.4	285.8 $\pm$ 23.3	309.5 $\pm$ 30.1	294.7 $\pm$ 15.4
	6	394.6 $\pm$ 33.6	352.8 $\pm$ 32.7	385.5 $\pm$ 20.8	375.6 $\pm$ 35.2	362.4 $\pm$ 33.1	410.3 $\pm$ 40.5	380.2 $\pm$ 31.2
	8	414.8 $\pm$ 28.9	387.6 $\pm$ 35.4	408.3 $\pm$ 34.2	404.8 $\pm$ 35.7	391.2 $\pm$ 28.8	416.1 $\pm$ 31.2	403.8 $\pm$ 35.3
	10	468.6 $\pm$ 43.8	412.5 $\pm$ 21.7	462.1 $\pm$ 22.5	451.2 $\pm$ 45.0	425.1 $\pm$ 40.7	477.5 $\pm$ 43.2	449.5 $\pm$ 42.5
1	0	/	/	/	/	/	/	/
	2	166.7 $\pm$ 11.4	141.2 $\pm$ 12.3	164.7 $\pm$ 15.7	156.4 $\pm$ 13.8	147.7 $\pm$ 12.6	175.5 $\pm$ 15.5	158.7 $\pm$ 13.1
	4	253.1 $\pm$ 21.2	217.1 $\pm$ 15.7	241.1 $\pm$ 23.8	237.6 $\pm$ 20.6	225.8 $\pm$ 18.8	262.9 $\pm$ 23.5	239.6 $\pm$ 22.4
	6	347.5 $\pm$ 31.2	312.5 $\pm$ 27.6	342.8 $\pm$ 30.7	335.8 $\pm$ 26.3	319.5 $\pm$ 25.5	362.1 $\pm$ 35.5	336.7 $\pm$ 24.7
	8	407.2 $\pm$ 30.8	367.9 $\pm$ 35.3	398.6 $\pm$ 32.1	389.1 $\pm$ 28.5	372.8 $\pm$ 23.5	415.8 $\pm$ 30.6	391.9 $\pm$ 37.2
	10	438.9 $\pm$ 41.5	398.2 $\pm$ 37.6	435.2 $\pm$ 23.8	424.6 $\pm$ 41.5	412.5 $\pm$ 26.9	448.4 $\pm$ 43.6	426.3 $\pm$ 42.8
2	0	/	/	/	/	/	/	/
	2	133.3 $\pm$ 11.5	118.6 $\pm$ 10.7	131.5 $\pm$ 12.2	128.2 $\pm$ 11.6	121.3 $\pm$ 12.1	134.5 $\pm$ 13.3	127.9 $\pm$ 14.8
	4	230.2 $\pm$ 20.5	201.7 $\pm$ 15.3	228.8 $\pm$ 21.4	224.6 $\pm$ 19.6	210.7 $\pm$ 20.7	234.8 $\pm$ 22.6	221.8 $\pm$ 21.7
	6	311.7 $\pm$ 30.8	245.8 $\pm$ 21.5	292.6 $\pm$ 24.3	283.2 $\pm$ 27.4	258.3 $\pm$ 24.5	332.2 $\pm$ 30.1	287.3 $\pm$ 28.5
	8	351.4 $\pm$ 23.6	312.4 $\pm$ 30.7	342.9 $\pm$ 32.2	331.9 $\pm$ 22.8	321.7 $\pm$ 25.6	355.7 $\pm$ 34.3	336.0 $\pm$ 27.8
	10	416.3 $\pm$ 40.5	387.7 $\pm$ 36.7	405.2 $\pm$ 37.4	399.7 $\pm$ 39.1	391.6 $\pm$ 27.5	428.9 $\pm$ 42.7	404.9 $\pm$ 39.8
4	0	/	/	/	/	/	/	/
	2	115.5 $\pm$ 10.2	109.6 $\pm$ 5.8	114.6 $\pm$ 10.7	106.9 $\pm$ 9.9	92.1 $\pm$ 6.8	133.5 $\pm$ 12.6	108.7 $\pm$ 12.8
	4	222.9 $\pm$ 21.1	176.5 $\pm$ 16.5	218.7 $\pm$ 20.3	203.9 $\pm$ 18.8	183.6 $\pm$ 16.4	228.6 $\pm$ 21.1	205.7 $\pm$ 19.5
	6	277.4 $\pm$ 22.7	212.3 $\pm$ 20.6	269.4 $\pm$ 19.8	255.6 $\pm$ 24.1	225.7 $\pm$ 22.5	313.0 $\pm$ 30.1	258.9 $\pm$ 24.8
	8	335.8 $\pm$ 29.8	265.8 $\pm$ 25.4	315.9 $\pm$ 30.2	305.4 $\pm$ 18.9	278.3 $\pm$ 13.2	340.8 $\pm$ 30.6	307.0 $\pm$ 30.1
	10	392.1 $\pm$ 33.4	312.5 $\pm$ 27.5	375.4 $\pm$ 35.2	367.4 $\pm$ 33.8	328.4 $\pm$ 30.5	432.8 $\pm$ 42.4	368.1 $\pm$ 35.5
8	0	/	/	/	/	/	/	/
	2	107.1 $\pm$ 10.5	65.4 $\pm$ 4.6	98.6 $\pm$ 8.9	85.6 $\pm$ 7.3	71.2 $\pm$ 4.6	112.7 $\pm$ 11.1	90.1 $\pm$ 8.9
	4	210.7 $\pm$ 19.5	164.7 $\pm$ 14.6	201.8 $\pm$ 17.8	191.7 $\pm$ 18.3	175.8 $\pm$ 17.1	213.9 $\pm$ 21.5	193.1 $\pm$ 14.6
	6	225.4 $\pm$ 21.3	176.3 $\pm$ 16.5	212.3 $\pm$ 21.1	196.8 $\pm$ 17.8	182.5 $\pm$ 17.9	237.9 $\pm$ 21.3	205.2 $\pm$ 19.8
	8	302.8 $\pm$ 27.2	233.4 $\pm$ 21.5	297.6 $\pm$ 26.3	281.1 $\pm$ 25.4	245.1 $\pm$ 22.1	311.6 $\pm$ 30.7	278.6 $\pm$ 25.2
	10	353.5 $\pm$ 28.1	295.7 $\pm$ 25.6	347.7 $\pm$ 33.2	335.2 $\pm$ 30.1	310.1 $\pm$ 27.6	367.2 $\pm$ 35.2	334.9 $\pm$ 31.3
Significance ^a among As treatments		**	**	**	**	**	**	**
Significance ^a among varieties		NS						

^a By analysis of variance; ** $p < 0.01$; NS: not significant; /: no Cd detected.

germination, biomass, root and shoot elongation were observed. Their coexistence had an additive effect on seed germination and antagonistic effects on biomass, root and shoot elongation. The antagonistic effect may be caused by the reductions of the ion activity of Cd and As in solution and the reductions of Cd and As uptake by wheat when Cd and As coexisted. The results calculated by MINTEQAII also indicated that the coexistence of Cd and As decreased the ion activity of Cd and As in the cultural solution and the highest reduction rate of total ion activity reached 22% with Cd and As coexistence, which supported the above conclusion.

With the increases in the concentrations of Cd and As in the growth media, root length reduced more significantly than the other endpoints and the reduction followed the order: root length > shoot height, biomass > germination percent. More sensitivity of root length to Cd and As could be ascribed by the fact that plant root was the first point to contact with the toxicants in the growth media. In the previous study for the toxicity of As to wheat (Liu *et al.*, 2005), we observed that both of the root length and shoot height of wheat were sensitive to As. The differences between these two observations could be attributed to the existence of Cd. Cadmium can easily penetrate the root through the cortical tissue (Yang *et al.*, 1998) and enter the roots; consequently roots are likely to experience Cd damage first (Sanità di Toppi and Gabrielli, 1999). The previous work by Blum (1997) also showed that root was the most sensitive to Cd treatment.

The data of inhibition rates of germination, biomass, root length and shoot height showed that Duokang-1 was the most and Zhongmai-8 was the least tolerant to Cd and As co-contamination. Different wheat varieties had different sensitivity to Cd and As toxicity. We also observed the differences of Cd and As accumulation in seedlings among the varieties and Duokang-1 had the least and Zhongmai-8 had the most Cd and As accumulations, which confirmed that the different resistances to Cd and As among the varieties could depend on the metal uptake by seedlings. Differences in metal accumulations among varieties of the same species have also been observed in different plants, including barley, soybean, corn, lettuce, wheat and cucumber (Grant *et al.*, 1999).

4 Conclusions

Differences in seed germination, biomass, root length, shoot height, Cd and As accumulations were noted between the treatments and among the varieties ($p < 0.05$). Among the six varieties, Duokang-1 was found to be the most resistant, and Zhongmai-8 was the most sensitive to Cd and As co-contamination. The different resistances among the six varieties could be ascribed to the uptake of

Cd and As by seedlings. We might get a conclusion from this study that Duokang-1 was suitable for cultivation in soils with Cd and As contaminations.

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