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EFFECT OF Cd^{2+} STRESS ON SEED GERMINATION CHARACTERISTICS OF RYEGRASS, INDIAN MUSTARD AND GRAIN AMARANTH

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Abstract

In order to select a better plant to extract cadmium in heavy metal contaminated soil, a hydroponic germination experiment was designed to evaluate the effects of cadmium on seed germination characteristics of Grain amaranth (*Amaranthus hypochondriacus L.*), Indian mustard (*Brassica juncea*) and Ryegrass (*Lolium perenne L.*). The results showed that fresh weight, dry weight and germination index of Ryegrass were promoted at the low cadmium concentrations (≤ 25 mg/L), while that of 3 plants was inhibited at the high cadmium concentrations (≥ 50 mg/L). The inhibition rates of root and bud length of three plants were correlated significantly with cadmium concentrations ($P < 0.05$). The bud sensitivity of three plants to cadmium was in order of Grain amaranth, Ryegrass and Indian mustard, and the sensitivity of root length of three plants to cadmium was in order of Grain amaranth, Indian mustard and Ryegrass, based on the half inhibitory concentration (IC_{50}). The tolerance of three plants to cadmium was in order of Ryegrass, Indian mustard and Grain amaranth based on the value of membership function. Our findings suggest that the root length inhibition rate can probably be used as an important indicator to assess ability of heavy metal (Cd^{2+}) stress. Ryegrass is speculated to have better resistance to Cd^{2+} stress and may be further applied to heavy metal Cd^{2+} ecological restoration.

Key words: cadmium, germination, hyperaccumulator, seed

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1. Introduction

Heavy metal has caused major environmental risk for soil and groundwater due to improper human activity discharges (Mahar et al., 2016). Moreover, heavy metals enter in food chain and endanger human health (Sarwar et al., 2017). Cadmium is considered the most harmful in soil pollution (Irfan et al., 2013; Huang et al., 2018). It is mainly derived from atmospheric deposition, fertilizer use, municipal sludge and sewage irrigation (Monu et al., 2008). Cadmium is a nonessential metal for plant growth (Akhter et al., 2012; Meng et al., 2009; Wang et al., 2009), and can affect biochemical and physiological behavior of plants such as photosynthesis (Baszyński, 1986; Cocarta et al., 2017) and nutrient uptake (Gill et al., 2011; Radojčić et al., 2017). Biological toxicity of cadmium is mainly manifested in inhibiting the germination of plant seeds and root growth, destroying plant cytoplasm and chlorophyll (Koolivand et al., 2017; Padmaja et al., 1990; Shahabivand et al., 2017).

The seed germination characteristic is closely related to the growth of the plants, and can be evaluated with some indexes, such as germination rate, germination potential, germination index, vigor index, root length, bud length etc. The seed germination is dramatically sensitive (Liu et al., 2011) to heavy metal stress (Gu et al., 2018; He et al., 2017;

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Moya et al., 1993). These indexes represent the plant's ability to grow and they will change because of the effects of heavy metal concentrations. In this paper, the indexes were used to evaluate the effect of heavy metal (Cd^{2+}) in plants.

Heavy metal contaminated soil can be cleaned by phytoremediation, physical and chemical remediation technologies (Ehsan et al., 2014). Among them, phytoremediation is more successful because of its low cost, environmental friendliness, and less interference with the types of land applications (Hu et al., 2017; Raziuddin et al., 2011). It is important to choose the appropriate accumulator species for in situ remediation of heavy metal contaminated soil by phytoremediation method (Rojjanateeranaj et al., 2017). Ryegrass (*Lolium perenne L.*), Grain amaranth (*Amaranthus hypochondriacus L.*) and Indian mustard (*Brassica juncea*) are often utilized in phytoremediation studies for heavy metal pollution soil, because of the fast growth of these plants and a multiple-cuts arrangement in a short time (about 4–6 months), providing the opportunity to remove heavy metals from contaminated soil (Ding et al., 2013; Yuan et al., 2014). Indian mustard is the dicotyledon of the angiosperm, and prefers dry and sunny conditions. Ryegrass is angiosperm. It is a perennial plant and suitable for cool and humid climates. Amaranth is an annual herb of the genus Aphid. It is a thermophilic crop with a growth period of more than 4 months, but it also grows well under temperate and cold temperate climates. Furthermore, Indian mustard, Ryegrass and Grain amaranth showed a good accumulation potential for heavy metal ions, especially cadmium (Ali et al., 2017; Hu et al., 2013).

Accumulation potential of a heavy metal is controlled by a number of factors including the plant species and plant age present in the soil (Tang et al., 2016). Therefore, a series of experiments on seeding inhibition and root length characteristics have been conducted to determine the biological toxicity and bioavailability of heavy metals at different concentrations (Pereira et al., 2017; Sharma and Archana, 2016). It is important to study dose-effect relationship of heavy metal in phytoremediation, in order to screen the proper plant seeds (Éric and Erick, 2017). In addition, most studies have focused on crops phytoremediation (Arena et al., 2017, Chen et al., 2017). Grain amaranth, Indian mustard, Ryegrass are all considered to be potential hyperaccumulators for remediation of heavy metals in soil (Acosta et al., 2017; Mahmud et al., 2018). But there is still no comparative study on among three plants to remedy heavy metal Cd pollution soil, and do not quantitatively describe the concentrations of heavy metals that could be remedied based on different plants.

In this paper, the seed germination test of 3 plants, Grain amaranth, Indian mustard and Ryegrass were conducted under cadmium stress, by determining the germination characteristics of three plants using different concentrations of cadmium ion (Cd^{2+}). Bud length inhibition rate, root length inhibition rate and other indicators were introduced into the study, and a comparative analysis was performed on the potential of the three plants for cleaning Cd^{2+} contamination soil, based on dose-toxicity analysis method. Finally, a suitable plant was selected for remediation of soil contaminated with Cd^{2+}. Our study will provide a possible theoretical and experimental basis for the selection of plants for remediation of heavy metals (in particular, Cd^{2+}) contaminated soil.

2. Material and methods

2.1. Experimental method

The germination experiments were performed in biochemical incubators (PGX-450L, SaiFu Experimental Instrument Co., Ltd., Ningbo, China). As hydroponics test water we used deionized water. Cadmium treatment was prepared by using $CdCl_2 \cdot 2.5H_2O$ reagent (analytically pure). Cadmium treatment concentrations were 0, 1, 10, 25, 50 and 100 mg/L respectively. Meanwhile, cadmium treatment concentration of 0 mg/L was set as a control group (CK).

The seeds of Grain Amaranth, Indian mustard and Ryegrass were purchased from Xian Jinrui Seed Co., Ltd., Shaanxi, China. The seeds of full particles, uniform size and no damage were picked out. The seeds were surface sterilized with 70% alcohol for 2 min, rinsed with deionized water. Then they were arranged in a sequence in a petri dish with a filter paper. Each dish contained 20 seeds and the space between each seed was maintained, ensuring a good contact with the cadmium solution ($CdCl_2$ solution) and sufficient growth space. Three replications were designed in the test.

Seeds germinated under dark conditions in the incubator at (25 ± 1)°C. The paper bed in the petri dish was kept moist to ensure the normal development of the seeds (Zhang et al., 2012). Seed samples were collected on the 3rd and 7th days after germination. Five samples of germination seeds were randomly selected in each petri dish to measure the root length (RL), bud length (BL), fresh weight (FW) and dry weight (DW) of 7-day-old seedlings. The root and shoot lengths of plants are measured with a ruler, and the dry and fresh weight are measured with analytical balances. Then, the germination potential (GP) of the samples was calculated in the third day after germination. Besides, the germination rate (GR), germination index (GI), vigor index (VI), bud length inhibition rate (BLR) and root length inhibition rate (RLR) (Salvatore et al., 2008) were determined after 7th day treatment. The following indexes were calculated (Eqs. 1-6):

$$GR=N_7/N_t \times 100\% \quad (1)$$

where, N_7 is the number of seed germination in the 7th days, N_t is the number of seeds tested.

$$GP=N_3/N_t \times 100\% \quad (2)$$

where, N_3 is the number of seed germination in the 3th days.

$$GI=\sum G_t/C_t \quad (3)$$

where, G_t is the number of germination of seeds, C_t is germination days.

$$VI=GI\times BL \quad (4)$$

$$BLR=(1- BL/ BL_{blank})\times 100\% \quad (5)$$

where, BL_{blank} is plant bud length in blank solution.

$$RLR=(1- RL/ RL_{blank})\times 100\% \quad (6)$$

where, RL_{blank} is plant root length in blank solution.

2.2. Comprehensive tolerance evaluation method

The seed germination tolerance indexes of three plants under Cd^{2+} stress were determined by membership function method based on fuzzy mathematical membership function (Canavese et al., 2014). Firstly, the membership function values of GR, GP, FW, DW, BL, RL, GI and VI of each plant seed at different Cd^{2+} concentrations were calculated using Eq. (7). Secondly, the mean value of each index membership value was calculated at different Cd^{2+} concentrations. Finally, the average values of the membership function values of all indexes of each plant (*TM*) were calculated at all Cd^{2+} concentrations to evaluate the plant's tolerance to Cd^{2+}. The greater *TM*, the stronger the tolerance of the plant seeds to Cd^{2+}, and vice versa.

$$M(\rho)=(M-M_{min})/(M_{max}-M_{min}) \quad (7)$$

where M= the index value of a hyperaccumulator in the same Cd^{2+} concentration, M_{max} and M_{min} are their maximum and minimum value, respectively.

No control values are included.

2.3. Data processing and analysis

Variance analysis was performed by Duncan multiple comparison of one-way (ANOVA) using the SPSS 21.0 software. In addition, the means of three replicate data were analyzed. All data reported in this work were revealed as means ± standard deviation.

3. Results and discussion

3.1. Response of GR and GP of three plants to cadmium concentrations

The GRs of three plant seeds at different Cd^{2+} concentration were shown in Fig. 1. The GR of Ryegrass, Grain amaranth and Indian mustard were decreased with the increase of Cd^{2+} concentration. At different Cd^{2+} concentrations of 1, 10, 25, 50 and 100 mg/L, the GRs of the Grain amaranth seed were insignificant compared with the control. The GRs of Ryegrass and Indian mustard were not significantly different from control, at 1, 10, 25 mg/L Cd^{2+} concentrations. But at higher Cd^{2+} concentrations (50 and 100 mg/L), GRs of two plant seeds were significantly smaller than the control ($P < 0.05$).

At all Cd^{2+} concentrations shown in Fig. 2, the GPs of Grain Amaranth were not significant compared to control. This means that Cd^{2+} concentration had a slight effect on the GP of Grain Amaranth. The GP of Indian mustard was much lower compared to the control ($P < 0.05$), at only 100 mg/L Cd^{2+} concentration. At 10 and 100 mg/L Cd^{2+} concentrations, the GRs of Ryegrass were remarkably different compared with the control ($P < 0.05$), and it was notably higher compared to the control at 10 mg/L Cd^{2+} concentration. However, it was remarkably lower than the control at 100 mg/L Cd^{2+} treatment.

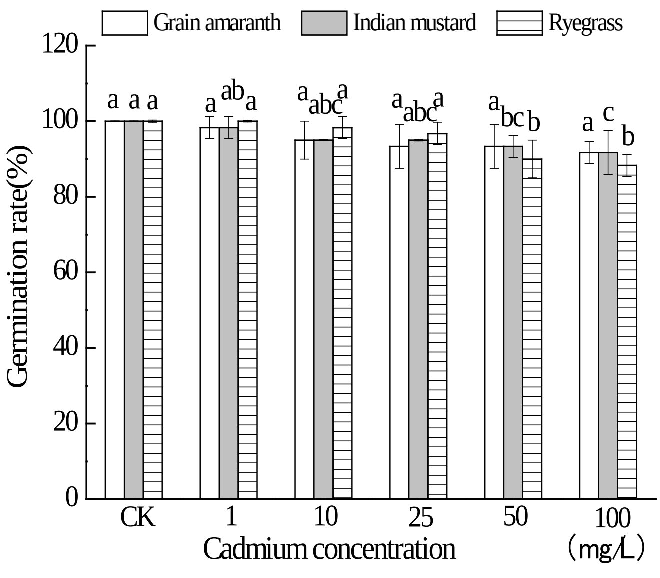

Fig. 1. Response of three plant seed GRs to Cd^{2+} stress. Different letters show a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

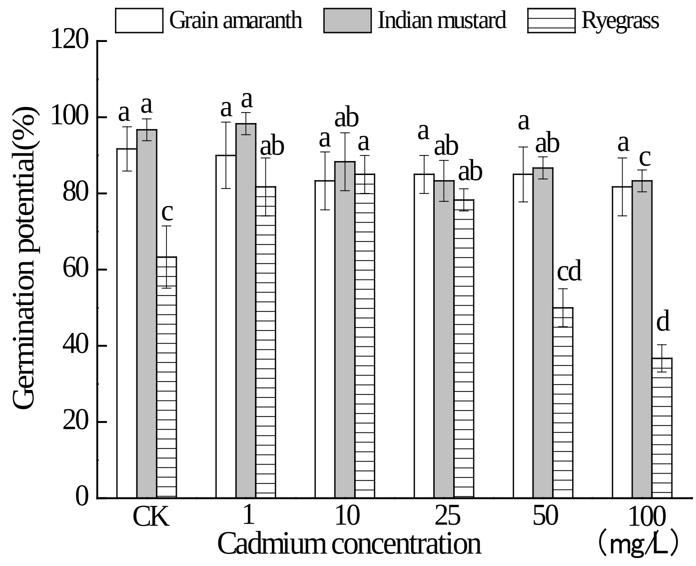

Fig. 2. Response of three plants with seed GP to Cd^{2+} stress. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

The above analysis shows that the GP of Ryegrass can be promoted (low Cd^{2+} concentration) and be suppressed (high Cd^{2+} concentration) probably. This result are in agreement with the findings of other studies (Liu et al., 2011).

3.2. Effects of cadmium on FW and DW of three plants

The FW and DW of seedling of three plant seeds at different Cd^{2+} concentrations were recorded as shown in Figs. 3-4. In all the range of heavy metal Cd^{2+} concentration (1-100 mg/L) set by the experiment, with the increase of Cd^{2+} concentration, the FWs of Grain amaranth and Indian mustard seedling were declined except the Ryegrass. There were a remarkable difference between Grain amaranth and the control ($P < 0.05$) at all Cadmium concentrations, except for 1 mg/L Cd^{2+} concentration. The FWs of Indian mustard at the 25, 50, 100 mg/L Cd^{2+} treatments were decreased strikingly, compared to the control ($P < 0.05$), and there were not remarkably different at the 1 and 10 mg/L Cd^{2+} concentrations. The FW of Ryegrass was increased at low Cd^{2+} concentrations, and the FW of Ryegrass of 10 mg/L Cd^{2+} concentration was significantly promoted ($P < 0.05$), up to the highest value, and 33.3% higher compared to the control. The FW of Ryegrass at 50 and 100 mg/L Cd^{2+} treatments were significantly inhibited by Cd^{2+} concentration ($P < 0.05$). With increasing concentration of Cd^{2+}, the DW of Grain amaranth and Indian mustard were decreased, except for Ryegrass. At high Cd^{2+} concentration (50 and 100 mg/L), the DWs of Grain amaranth were much lower than the control ($P < 0.05$).

Further, the DWs of Indian mustard were decreased significantly than control, at 10, 25, 50 and 100 mg/L Cd^{2+} (P < 0.0 5). The DW of Ryegrass of 25 mg/L Cd^{2+} reached the highest value, and 10.4% higher than the control. The DW of Ryegrass of 100 mg/L Cd^{2+} was significantly inhibited compared to the control ($P < 0.05$).

The FW and DW of Ryegrass were increased at lower concentrations compared to the control, while the higher concentration was declined. This result is the same as the change in GP. It shows that Cd^{2+} concentration in the lower range (≤ 25 mg/L) has facilitation to the FW and DW of Ryegrass.

3.3 Effects of Cd^{2+} on BL and RL of three plants

Effects of different Cd^{2+} concentration on BL and RL of three plant seedlings were also measured (Figs. 5-6).

There was a significant difference between Grain amaranth of all Cd^{2+} treatments and the control ($P < 0.05$) for the BL. At 100 mg/L Cd^{2+} concentration, the BL of Grain amaranth was dramatically decreased compared to the control ($P < 0.05$), and the BL was only 10% of the control. There is no significant difference Indian mustard of 1, 10 and 25 mg/L Cd^{2+} concentrations compared with the control. The BLs of Indian mustard 50,100 mg/L Cd^{2+} concentrations were decreased significantly compared with that of the control ($P < 0.05$). Indicating that Indian mustard had been significantly inhibited at higher Cd^{2+} concentrations (≥50 mg/L).

The BLs of Ryegrass of all Cd^{2+} concentrations were remarkably different than control ($P < 0.05$). But the BLs of Ryegrass at 25-100 mg/L Cd^{2+} were significantly inhibited by Cd^{2+} concentration, which was markedly smaller compared to the control ($P < 0.05$), revealing that the BL of Ryegrass was significantly inhibited at higher Cd^{2+} concentration. In all range of heavy metal Cd^{2+} concentrations, the RLs of Grain amaranth, Indian mustard and Ryegrass were decreased significantly with the increase of Cd^{2+} concentration, and roots of the three plants were more sensitive to Cd^{2+} stress.

The RL of Grain amaranth of 100 mg/L Cd^{2+} concentration was only 8.4% of the control. Indian mustard RL of 100 mg/L Cd^{2+} concentration was only 5.4% of the control. At 100 mg/L Cd^{2+} concentration, Ryegrass RL was only 7.5% of control. The roots of the plants are markedly inhibited and almost stop growing. Based on above analysis, the change of the Cd^{2+}concentration was demonstrated to impact on plant BL and RL more significance than GP and GR of plants, because bud and root of the plants after germination completely were exposed to the environment was directly affected by Cd^{2+} stress in a petri dish (Salvatore et al., 2008). However, the seed was protected by the shell (Kuriakose and Prasad, 2008). Therefore, the changes of Cd^{2+} concentrations to the influence of bud and root growth were greater than its impact on plant GR and GP for the three plants.

3.4. Effects of cadmium on GI and VI of three plant seeds

As Fig. 7 shows, the GIs of Grain amaranth and Indian mustard were declined with the exception of Ryegrass, as the Cd^{2+} concentration increased. The GI of Grain amaranth of 100 mg/L Cd^{2+} treatment was decreased significantly compared to the control. Indian mustard of 50 and 100 mg/L Cd^{2+} treatments, GIs were decreased sharply compared to the control ($P < 0.05$).

The GIs of 1, 10 and 25 mg/L Cd^{2+} concentrations were rapidly increased compared to the control ($P < 0.05$) for Ryegrass. 50 and 100 mg/L Cd^{2+} treatments decreased gradually compared with the control, and 100 mg/L Cd^{2+} was markedly change ($P < 0.05$).

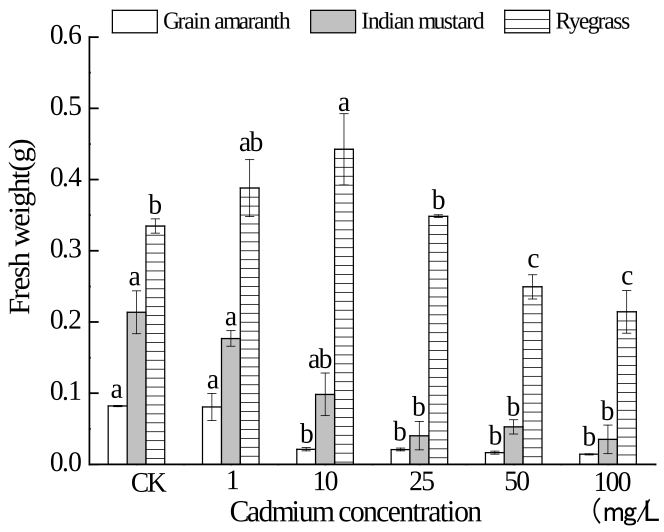

Fig. 3. The fresh weight of the three plants at different Cd^{2+} concentrations. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

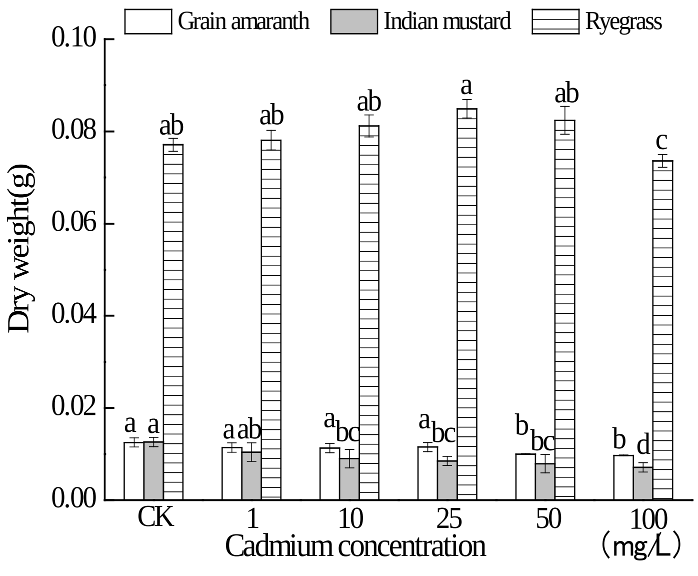

Fig. 4. The dry weight of the three plants at different Cd^{2+} concentrations. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

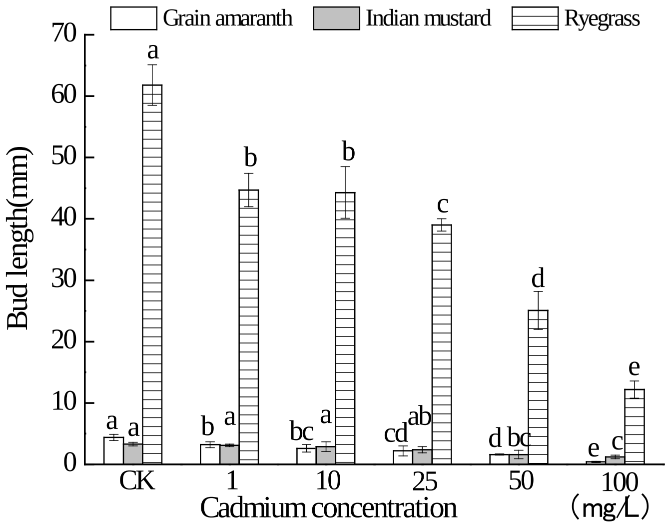

Fig. 5. Bud length of three plants at different Cd^{2+} concentrations. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

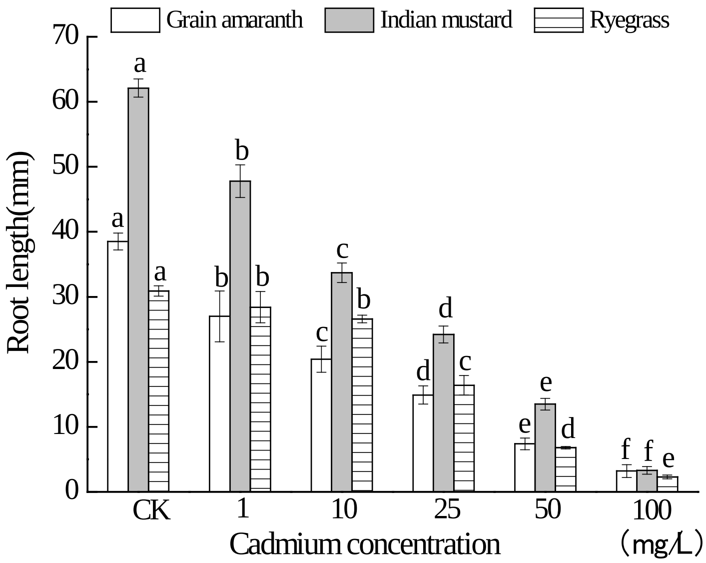

Fig. 6. Root length of three plants with different Cd^{2+} concentrations. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

VI is a more comprehensive evaluation indicator for the seed vigor, based on the seed germination and seedling growth capacities. The higher the VI value is, the greater the vigor of the seed. VI of the seed was cut down with the higher Cd^{2+}, as shown in Fig. 8. At 100 mg/L Cd^{2+} concentration, the VIs of three kinds of seeds were reached the lowest with decreases of 80.9%, 71.5%, 84.4% compared with the control, respectively.

The VIs of Grain amaranth of 25, 50, 100 mg/L Cd^{2+} concentrations decreased significantly ($P < 0.05$) compared to the control, while other Cd^{2+} concentrations were insignificant. At 1, 10 and 25 mg/L Cd^{2+} concentrations, VIs of the Indian mustard compared with the control did not significantly change. The 50 and 100 mg/L Cd^{2+} treatments decreased rapidly, which was significantly differed from the control ($P < 0.05$). The VIs of Ryegrass of all other Cd^{2+} concentrations decreased significantly compared with that of the control except 10 mg/L Cd^{2+} treatment ($P < 0.05$). At high Cd^{2+}concentrations (25, 50, 100 mg/L), the Ryegrass VIs decreased markedly, compared with the control ($P < 0.05$).

3.5. Comprehensive evaluation of cadmium tolerance in seed germination of three plants

Different plants presented a different behavior of resistance and assimilate mechanism to heavy metal cadmium. It was more concerned by researchers to select plants which presented tolerance and remediation abilities for heavy metal in soil. It was significant to control and decontaminate soil of heavy metal pollution. Comprehensive index was general used to determine the heavy metal resistance of plants (Liu et al., 2013a; Shi et al., 2009).

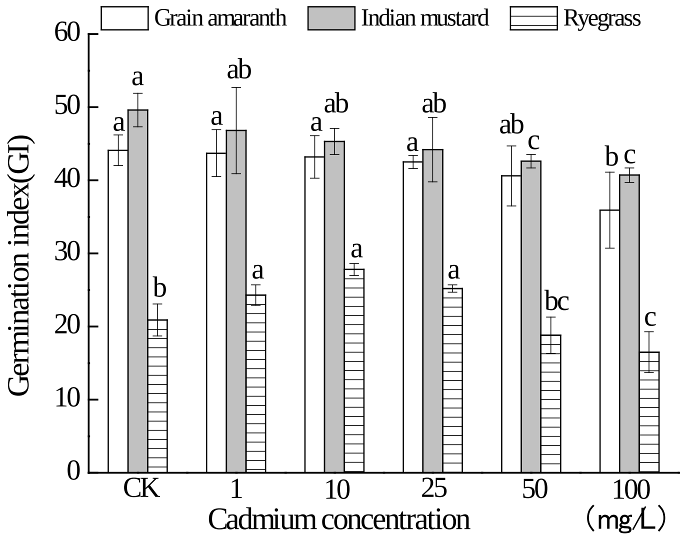

Fig. 7. GI of three plant seeds at different Cd^{2+} concentrations. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

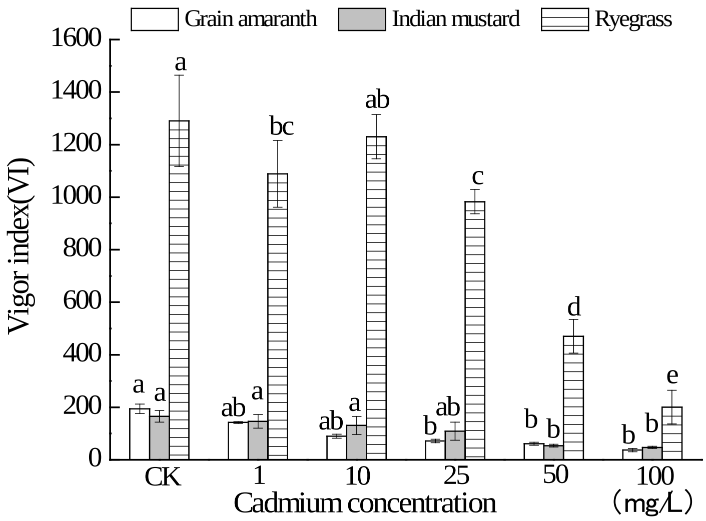

Fig. 8. VI of three plant seeds at different Cd^{2+} concentrations. Different letter shows a remarkable change ($P < 0.05$) for all treatments based on Duncan's multiple comparison of one-way. CK is the control (0 mg/L Cd^{2+} concentration)

The fuzzy mathematics membership function method was used to calculate the value of the integrated membership function of three plants. The membership function value was calculated by eight indexes, GR, GP, FW, DW, GI, VI, RL and BL of three plants (Table 1). The values of the integrated membership functions for Grain amaranth, Indian mustard and Ryegrass were 0.402, 0.434 and 0.556 respectively. Therefore, the order of cadmium resistance of 3 plants was: Ryegrass > Indian mustard > Grain amaranth.

3.6. The effect of cadmium on BLR and RLR of three plants

The BLR and RLR of plants were important indicators for studying the bioavailability and ecological toxicity of heavy metals in soil (Lin et al., 2016). The sensitivity for different plants in different Cd^{2+}concentration can be determined by the inhibition degree of three plant bud and root length. Thus it was helpful to determine suitable plant species to clean heavy metal Cd^{2+} pollution soil. Each Cd^{2+}concentrations presented different inhibitory effect on seed bud and root growth of 3 plants (Table 2), and the inhibitory effect was increased significantly with Cd^{2+} concentration improving. Cd^{2+} concentration was significantly correlated with the BLR and RLR of 3 plant seeds ($P < 0.05$). The results of the regression analysis of BLR and RLR of 3 plant seeds were calculated in all Cd^{2+}concentrations (Table 2).

The value of the half inhibitory concentration (IC_{50}) (that is, cadmium concentration at 50% inhibition rate) was calculated by the regression equation (Zhang et al., 2007). The IC_{50}s of bud length of Grain amaranth, Indian mustard and Ryegrass were 29.2, 65.6 and 43.4mg/L respectively.

Table 1. The membership function value of cadmium tolerance of three plant seeds during germination

Plants	***The membership function value***								***TM***	***Order***
	GR	GP	FW	DW	GI	VI	BL	RL		
Grain amaranth	0.397	0.398	0.286	0.400	0.677	0.413	0.226	0.420	0.402	3
Indian mustard	0.448	0.312	0.333	0.467	0.528	0.509	0.353	0.521	0.434	2
Ryegrass	0.544	0.614	0.513	0.567	0.533	0.577	0.457	0.642	0.556	1

Table 2. Regression analysis of BLR and RLR, *X* is the Cd^{2+} concentration

Plants	***Inhibition rate Y (%)***	***Regression equation***	R^2	IC_{50} ***(mg/L)***
Grain amaranth	BLR	Y=0.5978X+32.3	0.9702	29.2
Indian mustard	BLR	Y=0.6106X+10.0	0.9278	65.6
Ryegrass	BLR	Y=0.5671X+25.4	0.9744	43.4
Grain amaranth	RLR	Y=0.5836X+40.4	0.8633	16.4
Indian mustard	RLR	Y=0.6551X+36.2	0.8692	21.1
Ryegrass	RLR	Y=0.8824X+15.1	0.8699	39.6

Thus, the sensitivity order of the bud length of the three plant seeds to Cd^{2+} concentrations was: Grain amaranth > Ryegrass > Indian mustard. The IC_{50}s of the root length of Grain amaranth, Indian mustard and Ryegrass were 16.4, 21.1 and 39.6 mg/L respectively. The sensitivity order of root length of three plant seeds to Cd^{2+} concentrations was: Grain amaranth > Indian mustard > Ryegrass. This also indicated that the ryegrass presented a better cadmium tolerance, which was consistent with the previous comprehensive evaluation results.

In summary, seed germination was an important stage to evaluate the tolerance of plant seeds to cadmium stress. The current study showed that, at the light Cd^{2+} concentrations (1, 10 and 25 mg/L), the FW, DW and GI of Ryegrass were promoted. Grain amaranth and Indian mustard growth indexes were insignificantly inhibited.

At the high Cd^{2+} concentrations (50 and 100 mg/L), the growth indexes of 3 plants were significantly inhibited. This may be related to the different mechanisms of cadmium tolerance in different plant seeds. Studies have shown that the low concentration of Cd^{2+} can promote some plant seeds higher reactive oxygen free radicals in cells, to stimulate the activity of protease and to promote the cells divide rapidly and value-added, so as to promote the growth of the seed (Liu et al., 2012, 2013b). At high Cd^{2+} concentrations, Cd^{2+} ion penetrated seeds skin into inside, interfered with nucleic acid in plant cells, induced high reactive oxygen free radicals, caused the phenomenon of peroxide, inhibited the germination of the seeds (Talanova et al., 2001). This was consistent with some scholar research results on wheat, cabbage, rice and white trifolium and solanum nigrum respectively (Sun et al., 2014; Yun et al., 2014).

The method of membership function was used to calculate the cadmium resistance of three plant seeds, and the order of resistance to Cd^{2+} stress was: Ryegrass > Indian mustard > Grain amaranth. This indicated that the Ryegrass presented possibly higher tolerance for cadmium pollution soil.

At present, seed germination, bud and root length test are used to detect the toxicity of heavy metal to seed. It was a common method to quickly discover the physiological toxicity of plants at heavy metal stress (Tang et al., 2016). That can draw the inhibition of Cd^{2+} on the root length was greater than on bud length for 3 plant seeds, which was attributed to bud growth and root growth process are different, the necessary nutrients for bud growth is supplied mainly by the embryos, less affected by the external environment. Yet the root is easier to be affected by the toxicity of cadmium, it penetrates seed skin directly to grow (Rojjanateeranaj et al., 2017; Sfaxi-Bousbih et al., 2010). In addition, when the seeds are sprouting after imbibition, radicle first break through seed coat, which causes the root to accumulate the Cd^{2+} time more than the bud, and there are many exchange sites in the root cell wall to immobilize heavy metal ions.

At the same time, Cd^{2+} can induce the root system to produce stress ethylene, resulting in damage to the root cells and inhibiting root growth and development (Deng et al., 2010, Irfan et al., 2013). The RLR of three plants is a more sensitive index under heavy metal cadmium stress compared with the BLR, which can be better used to indicate the poisoning of plant seeds. This is consistent with the results of the root and bud length study of crops such as wild stork, acidophilus, wheat, tomato and so on under Cd^{2+} stress, indicating that Cd^{2+} toxicity has the same effect on bud and root growth of crops (Yun et al., 2014). The BL and RL of Ryegrass are insensitive to cadmium stress, revealing that it presented better resistance to cadmium concentration and may be suitable as a potential remediation species for cadmium pollution.

4. Conclusions

Seed germination of Grain amaranth, Indian mustard and Ryegrass were all affected by cadmium. As less than Cd^{2+}concentration of 25 mg/L, the FW, DW and GI of Ryegrass were promoted, while Seed germination of 3 plants were inhibited significantly (> 50 mg/L) at high Cd^{2+}concentrations. The root length of plants was more sensitive than shoot length to cadmium stress. The shoot and root length of Ryegrass were not sensitive to cadmium stress. Therefore, Ryegrass was speculated to present better resistance to Cd^{2+} stress and can be further applied to heavy metal Cd^{2+} ecological restoration. The RLR can probably be used as an important index to estimate heavy metal stress.

Considering that different plant seeds may have different mechanisms of cadmium stress, it is necessary to further carry out experimental research on the physiological and biochemical and genetic structure of seeds. In addition, this paper was only seed selection for a hydroponic experiment of Cd^{2+} single pollution, hence the ecological remediation of combined pollution of heavy metals needs further study.

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References

Acosta S.G., Cameselle C., Bustos E., (2017), Electrokinetic - enhanced ryegrass cultures in soils polluted with organic and inorganic compounds, *Environmental Research*, **158**, 118-125.

Akhter F., Mcgarvey B., Macfie S.M., (2012), Reduced translocation of cadmium from roots is associated with increased production of phytochelatins and their precursors, *Journal of Plant Physiology*, **169**, 1821-1829.

Ali A., Guo D., Mahar A., Wang Z., Muhammad D., Li R., Wang P., Shen F., Xue Q., Zhang Z., (2017), Role of *Streptomyces pactum* in phytoremediation of trace elements by *Brassica juncea* in mine polluted soils, *Ecotoxicology and Environmental Safety*, **144**, 387-395.

Arena C., Figlioli F., Sorrentino M.C., Izzo L.G., Capozzi F., Giordano S., Spagnuolo V., (2017), Ultrastructural, protein and photosynthetic alterations induced by Pb and Cd in *Cynara Cardunculus* L., and its potential for phytoremediation, *Ecotoxicology & Environmental Safety*, **145**, 83-89.

Baszyński T., (1986), Interference of Cd^{2+} in functioning of the photosynthetic apparatus of higher plants, *Acta Societatis Botanicorum Poloniae*, **55**, 291-304.

Canavese D., Ortega N.R.S., Queirós M., (2014), The assessment of local sustainability using fuzzy logic: An expert opinion system to evaluate environmental sanitation in the algarve region, Portugal, *Ecological Indicators*, **36**, 711-718.

Chen Z., Zheng Y., Ding C., Ren X., Yuan J., Sun F., Li Y., (2017), Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops, *Ecotoxicology & Environmental Safety*, **145**, 111-118.

Cocarta D.M., Subtirelu V.R., Badea A., (2017), Effect of sewage sludge application on wheat crop productivity and heavy metal accumulation in soil and wheat grain, *Environmental Engineering and Management Journal*, **16**, 1093-1100.

Deng X.P., Xia Y., Hu W., Zhang H.X., Shen Z.G., (2010), Cadmium-induced oxidative damage and protective effects of N-acetyl-L-cysteine against cadmium toxicity in *Solanum nigrum* L, *Journal of Hazardous Materials*, **180**, 722-729.

Ding P., Zhuang P., Li Z., Xia H., Lu H., (2013), Accumulation and detoxification of cadmium by larvae of Prodenia litura (Lepidoptera: Noctuidae) feeding on Cd-enriched amaranth leaves, *Chemosphere*, **91**, 28-34.

Ehsan S., Ali S., Noureen S., Mahmood K., Farid M., Ishaque W., Shakoor M.B., Rizwan M., (2014), Citric acid assisted phytoremediation of cadmium by *Brassica napus* L., *Ecotoxicology & Environmental Safety*, **106**, 164-172.

Éric M., Erick L., (2017), New environmental technology uptake and bias toward the status quo: The case of phytoremediation, *Environmental Technology & Innovation*, **7**, 102-109.

Gill S.S., Khan N.A., Tuteja N., (2011), Differential cadmium stress tolerance in five indian mustard (*Brassica juncea* L.) cultivars: An evaluation of the role of antioxidant machinery, *Plant Signaling & Behavior*, **6**, 293-300.

Gu Q., Chen Z.P., Cui W.T., Zhang Y.H., Hu H.L., Yu X.L., Wang Q.Y., Shen W.B., (2018), Methane alleviates alfalfa cadmium toxicity via decreasing cadmium accumulation and reestablishing glutathione homeostasis, *Ecotoxicology and Environmental Safety*, **147**, 861-871.

He S.Y., Yang X.E., He Z.L., Baligar V.C., (2017), Morphological and physiological responses of plants to cadmium toxicity: A review, *Pedosphere*, **27**, 421-438.

Hu J., Wu S., Wu F., Leung H.M., Lin X., Wong M.H., (2013), Arbuscular mycorrhizal fungi enhance both absorption and stabilization of Cd by Alfred stonecrop (Sedum alfredii Hance) and perennial ryegrass (*Lolium perenne* L.) in a Cd-contaminated acidic soil, *Chemosphere*, **93**, 1359-1365.

Hu X., Liu X., Zhang X., Cao L., Chen J., Yu H., (2017), Increased accumulation of Pb and Cd from contaminated soil with scirpus triqueter by the combined application of NTA and APG, *Chemosphere*, **188**, 397-402.

Huang D.L., Zeng G.M., Xu P., Zhao M.H., Lai C., Li N.J., Huang C., Zhang C., Cheng M., (2018), Biosorption behavior of immobilized *Phanerochaete chrysosporium* for heavy metals removal, *Environmental Engineering and Management Journal*, **17**, 2789-2794.

Irfan M., Hayat S., Ahmad A., Alyemeni M.N., (2013), Soil cadmium enrichment: Allocation and plant physiological manifestations, *Saudi Journal of Biological Sciences*, **20**, 1-10.

Kuriakose S.V., Prasad M.N.V., (2008), Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes, *Plant Growth Regulation*, **54**, 143-156.

Koolivand A., Mahvi A.H., Jahed G.R., Yari A.R., (2017), Concentrations of chromium, cadmium and nickel in two consumed fish species of Persian gulf, Iran, *Environmental Engineering and Management Journal*, **16**, 1637-1642.

Lin H., Zhang H.L., Dong Y.B., Tian Y., Chen S., Liu L.L., (2016), Enrichment characteristics of various heavy metals by four herbaceous plants in pair combination under hydroponic culture, *Research of Environmental Sciences*, 1154-1162.

Liu T.T., Wu P., Wang L.H., Zhou Q., (2011), Response of soybean seed germination to cadmium and acid rain, *Biological Trace Element Research*, **144**, 1186-1196.

Liu Y., Zhang W., Liu M.D., (2012), Effect of cadmium-benzo (a) pyrene single and combined pollution on wheat seed germination, *Journal of Agro-Environment Science*, **31**, 265-269.

Liu Z.L., He X.Y., Chen W., Zhao M.Z., (2013), Ecotoxicological responses of three ornamental herb species to cadmium, *Environmental Toxicology and Chemistry*, **32**, 1-6.

Liu J.G., Ma X., Wang M., Sun X., (2013), Genotypic differences among rice cultivars in lead accumulation and translocation and the relation with grain Pb levels, *Ecotoxicology & Environmental Safety*, **90**, 35-40.

Mahar A., Wang P., Ali A., Awasthi M.K., Lahori A.H., Wang Q., Li R.H., Zhang Z.Q., (2016), Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review, *Ecotoxicology & Environmental Safety*, **126**, 111-121.

Mahmud J.A., Hasanuzzaman M., Nahar K., Rahman A., Fujita M., (2018), Insights into citric acid-induced cadmium tolerance and phytoremediation in *Brassica Juncea* L.: Coordinated functions of metal chelation, antioxidant defense and glyoxalase systems, *Ecotoxicology & Environmental Safety*, **147**, 990-1001.

Meng H.B., Hua S.J., Shamsi I.H., Jilani G., Li Y.L., Jiang L.X., (2009), Cadmium-induced stress on the seed germination and seedling growth of *Brassica Napus* L., and its alleviation through exogenous plant growth regulators, *Plant Growth Regulation*, **58**, 47-59.

Monu A., Bala K., Shweta R., Anchal R., Barinder K., Neeraj M., (2008), Heavy metal accumulation in vegetables irrigated with water from different sources, *Food Chemistry*, **111**, 811-815.

Moya J.L., Ros R., Picazo I., (1993), Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants, *Photosynthesis Research*, **36**, 75-80.

Padmaja K., Prasad D.D.K., Prasad A.R.K., (1990), Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* L. seedlings by cadmium acetate, *Photosynthetica*, **24**, 399-405.

Pereira d. A.R., Furtado D.A.A.A., Silva P.L., Mangabeira P.A.O., Olimpio S.J., Pirovani C.P., Ahnert D., Baligar V.C., (2017), Photosynthetic, antioxidative, molecular and ultrastructural responses of young cacao plants to Cd toxicity in the soil, *Ecotoxicology & Environmental Safety*, **144**, 148-157.

Radojčić R.I., De M.A., Proietti C., Hanousek K., Sedak M., Bilandžić N., Jakovljević T., (2017), Poplar response to cadmium and lead soil contamination, *Ecotoxicology & Environmental Safety*, **144**, 482-489.

Raziuddin, Farhatullah, Hassan G., Akmal M., Shah S.S., Mohammad F., Shafi M., Bakht J., Zhou W., (2011), Effects of cadmium and salinity on growth and photosynthesis parameters of brassica species, *Pakistan Journal of Botany*, **43**, 333-340.

Rojjanateeranaj P., Sangthong C., Prapagdee B., (2017), Enhanced cadmium phytoremediation of *Glycine max* ` L. through bioaugmentation of cadmium-resistant bacteria assisted by biostimulation, *Chemosphere*, **185**, 764-771.

Salvatore M.D., Carafa A.M., Carratù G., (2008), Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates, *Chemosphere*, **73**, 1461-1464.

Sarwar N., Imran M., Shaheen M.R., Ishaque W., Kamran M.A., Matloob A., Rehim A., Hussain S., (2017), Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives, *Chemosphere*, **171**, 710-721.

Sfaxi-Bousbih A., Chaoui A., El Ferjani E., (2010), Cadmium impairs mineral and carbohydrate mobilization during the germination of bean seeds, *Ecotoxicology and Environmental Safety*, **73**, 1123-1129.

Shahabivand S., Parvaneh A., Aliloo A.A., (2017), Root endophytic fungus piriformospora indica affected growth, cadmium partitioning and chlorophyll fluorescence of sunflower under cadmium toxicity, *Ecotoxicology & Environmental Safety*, **145**, 496-502.

Sharma R.K., Archana G., (2016), Cadmium minimization in food crops by cadmium resistant plant growth promoting rhizobacteria, *Applied Soil Ecology*, **107**, 66-78.

Shi J., Li L., Pan G., (2009), Variation of grain Cd and Zn concentrations of 110 hybrid rice cultivars grown in a low-Cd paddy soil, *Journal of Environmental Sciences*, **21**, 168.

Sun X.M., Zhao M.Z., Liu Z.L., (2014), Effects of Cd stress on the physiological characteristics of *Trifolium repens* L. seed germination, *Environmental Science & Technology*, **37**, 52-56.

Talanova V.V., Titov A.F., Boeva N.P., (2001), Effect of increasing concentrations of heavy metals on the growth of barley and wheat seedlings, *Russian Journal of Plant Physiology*, **48**, 100-103.

Tang X., Li Q., Wu M., Lin L., Scholz M., (2016), Review of remediation practices regarding cadmium-enriched farmland soil with particular reference to China, *Journal of Environmental Management*, **181**, 646-662.

Wang C., Sun Q., Wang L., (2009), Cadmium toxicity and phytochelatin production in a rooted-submerged macrophyte vallisneria spiralis exposed to low concentrations of cadmium, *Environmental Toxicology*, **24**, 271-278.

Yuan M., He H., Xiao L., Zhong T., Liu H., Li S., Den P., Ye Z., Jing Y., (2014), Enhancement of Cd phytoextraction by two Amaranthus species with endophytic Rahnella sp. JN27, *Chemosphere*, **103**, 99-104.

Yun Y., Li W., Zhang Y.L., Qian J., Yue W., (2014), Ecotoxicity of single and combined contamination of 5-fluorouracil and Cd on seed germination of wheat, chinese cabbage and rice, *Journal of Agro-Environment Science*, **33**, 1075-1081.

Zhang J.X., Na M.L., Xu M.G., (2007), Inhibition and toxicity of Cu, Zn, Pb on root elongation of vegetable in contaminated soil, *Journal of Agro-Environment Science*, **26**, 945-949.

Zhang X.X., Li C.J., Nan Z.B., (2012), Effects of cadmium stress on seed germination and seedling growth of elymus dahuricus infected with the neotyphodium endophyte, *Science China Life Sciences*, **55**, 793-799.