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Original Research

Effects of Cadmium on Seed Germination, Coleoptile Growth, and Root Elongation of Six Pulses

Ling Tao[1, 2], Meiying Guo[1, 2], Jun Ren[1, 2]*

[1]School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou 730070, Lanzhou, 730070, P. R. China
[2]Engineering Research Center for Cold and Arid Regions Water Resource Comprehensive Utilization, Ministry of Education, Lanzhou, 730070, P. R. China

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Abstract

The ecotoxicological effects of Cd^{2+} on germination and early seedling growth of six pulses were investigated. Seeds of these plants were exposed to seven different concentrations of Cd (0, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM). The results indicated that root and coleoptile growth of six pulse plants were more sensitive than seed germination for measurement of toxic Cd^{2+} pollution. Different species show different levels of tolerance to Cd^{2+} pollution. *V. angularis* and *Dumasia villosa* are the most sensitive to Cd^{2+}, and their germination percentage, and root and coleoptile growth were significantly lower than other tested species. By contrast, *Vigna radiata* and *Lablab purpureus* are the most resistant species, their germination and seedling growth almost were not influenced by Cd^{2+} pollution significantly compared to control. There were significantly negative correlations between seedling growth and increasing concentrations of Cd^{2+} for *V. angularis*. *V. angularis* and *Dumasia villosa* are the most sensitive to Cd^{2+}. By contrast, *Vigna radiata* and *Lablab purpureus* are the most resistant species.

Keywords: cadmium, coleoptiles, germination, pulse, root

Introduction

Heavy metal pollution was of considerable importance and relevant to the present scenario due to the increasing levels of pollution and its obvious impact on human health through the food chain [1]. It is a known fact that the widespread accumulation of metals in the environment is increasingly becoming a problem for organisms of every kind. The main sources of elevated heavy metals concentration in soils were agricultural, manufacturing, mining, waste disposal practices, and the use of sewage sludge as fertilizer in agricultural fields. Metal contamination of agricultural soil has increased parallel to industrialization. Heavy metals concentration was introduced due to the application of metals containing agrochemicals such as pesticides and fertilizers. Previous studies had showed that Hg, Zn, Cd, Co, Pb, Cu, As, Al, Cr, and Ni reduce the germination of many kinds of seeds [2-16].

Cadmium pollution was increasing in the environment due to mining, industrial usage, and many anthropogenic activities. Irrigation with polluted water had induced heavy metal accumulation not only in the soil but also in plant parts, including seeds and tissues [17]. Excess Cd caused a number of toxic symptoms in plant growth retardation and inhibition of photosynthesis. Cadmium could also enter the soil or water from spills or leaks at hazardous waste sites if

*e-mail: renjun@mail.lzjtu.cn

large amounts of dissolved cadmium were present at the site where it was potentially available to rooted plants. The available Cd thereby entered biogeochemical cycles, becoming bioconcentrated [18-21]. There were some studies about cadmium toxicity for seed germination [22, 23], but they were not well described processes and not for seeds of pulse plants. Pulses are widely grown around the world; the aim of our study was to compare the effects of Cd^{2+} on seed germination, root, and shoot growth of six pulse crops. Through the study, we want to know:

1) To what degree cadmium inhibits the six pulse crops' seed germination and seedling growth
2) Which pulses are most sensitive to cadmium

This study was carried out in June 2008 in the Institute of Environmental Ecology, Lanzhou Jiaotong University.

Experimental Procedures

The effects of several doses of cadmium (0, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM) on six pulse crops were investigated. Seeds of soybean (*Glycine max* (Linn.)), mung bean (*Vigna radiata* (Linn.)Wilczek.), Lentil (*Lablab purpureus* (Linn.)Sweet), sweet pea (*Lathyrus odoratus* Linn.), adzuki bean (*Vigna angularis* (Willd.) Ohwietohashi.), and black bean (*Dumasia villosa* DC.) were offered by Gansu Academy of Agricultural Sciences of China. Seeds were surface sterilized in 0.5% sodium hypochlorite solution for 20 min and washed thoroughly with distilled water. The seeds were germinated in Petri dishes (diameter=150mm) with a double layer of filter paper soaked in distilled water (control) and 0.05-1.6 mM $CdCl_2$ solutions. The seeds were set under a photoperiod of 12 hr, and 30±1/25±1ºC day/night temperature. The seeding was harvested after 96 hr and the germination rate was recorded for every 24 hr. A 1-mm radical emergence from seeds was considered seed germination. The root and shoot length of germinated seeds, however, were measured only at 96 hr of incubation.

Data are the results from six separate analyses with 50 seeds in each Petri. Statistical analysis was performed based on STATISTICA [24]. The data were analyzed through two-way and one-way analysis of variance (ANOVA) to determine the effect of treatments, and Duncan's multiple comparison tests were performed to determine the statistical significance of the differences between means of treatments.

Results

Germination under Cadmium Stress

The germination of pulse seeds occurred at all cadmium treatments from 0.0 mM to 1.6 mM in all species. A two-way ANOVA showed that seed germination was significantly affected by different species ($F_{30} = 4.13$, $p < 0.001$), and the absence of a significant cadmium treatment and interaction demonstrates that the response of all species to cadmium concentration was similar, although the percentage of germination varies between the species (Table 1).

Table 1. The analysis of variance for the effects of different species, cadmium treatments, and their interaction on seed germination (%) and shoot and root elongation for six pulse species.

Traits	Source of variation	df	*F*	*P*
Germination	Cadmium treatment	6	3.33**	0.01504
	species	5	4.13***	<0.0001
	Cadmium treatment × species	30	1.38	0.1178
Shoot growth	Cadmium treatment	6	1.89	0.1216
	species	5	1.18	0.2635
	Cadmium treatment × species	30	0.92	0.5945
Root elongation	Cadmium treatment	6	1.77	0.1468
	species	5	0.89	0.6278
	Cadmium treatment × species	30	0.53	0.9751

*significant difference at 0.05 level
**significant difference at 0.01 level
***significant difference at 0.001 level

The germination was significantly different among cadmium treatment and control in 5 pulse species. Only in the case of *Vigna radiata* was its germination not significantly effected by cadmium treatment ($F_{6,35} = 1.714$, $p = 0.1468$), and it performed a higher germination percentage and stronger tolerance to cadmium. Compared with the control and lower concentration cadmium treatments (0.1 and 0.2 mM), higher concentration cadmium treatments (1.6 and 3.2 mM) significantly ($p < 0.001$) inhabited germination for *V. angularis*, *Lathyrus odoratus*, and *Dumasia villosa* (Table 2).

The germination was significantly different ($p < 0.001$) among species at six kinds of cadmium treatments and one control (Table 1). At all cadmium treatments and control, the germination percentages of *V. angularis* and *Dumasia villosa* was significantly lower than the other five species (Table 2).

Coleoptile Growth under Cadmium Stress

A two-way ANOVA showed that coleoptile growth was not significantly affected by different species, cadmium treatment and the interaction between species and cadmium treatment (Table 1). The shoot length was significantly different among cadmium treatments in five pulse species. Only in the case of *Dumasia villosa* was shoot length not significantly effected by cadmium treatment. Compared with the control and shorter-concentration cadmium treatments (0.1 and 0.2 mM), higher-concentration cadmium treatments (1.6 and 3.2 mM) significantly inhibited shoot length for *Glycine max*, *Vigna radiate*, *Dumasia villosa*, and *Lathyrus odoratus*. *Lathyrus odoratus* performed shorter shoot length at all cadmium treatments ($p<0.001$). Compared with control, for *Vigna radiate*, 0.1 mM cadmium treatment signifi-

Table 2. Influence of Cd on seed germination and root and shoot growth of six pulses. Results are means±sd.

Attribute	Treatment	*Glycine max*	*Vigna radiate*	*V. angularis*	*Lablab purpureus*	*Lathyrus odoratus*	*Dumasia villosa*	F-value
Germination (%)	0.0 mM	92±5.99[a]	91±2.42[a]	83±3.72[b]	93±2.6[a]	94±2.61[a]	79±3.52[b]	16.80***
	0.1 mM	84±4.63[a]	91±1.63[b]	82±7.42[a]	94±3.79[b]	94±3.41[b]	76±4.8[c]	14.26***
	0.2 mM	65±28.47[a]	92±1.51[b]	82±7.69[bc]	86±3.67[b]	93±1.47[b]	71±7.46[ac]	4.93***
	0.4 mM	86±8.2[ab]	92±1.51[b]	81±10.78[ac]	85±7.24[ab]	93±2.66[b]	71±6.03[d]	8.00***
	0.8 mM	93±3.93[ab]	90±1.97[ab]	83±8.92[ac]	77±15.11[c]	94±2.95[b]	66±8.98[d]	9.74***
	1.6 mM	82±4.27[ae]	91±1.1[b]	72±6.62[c]	77±12.88[ac]	89±1.17[eb]	63±6.42[d]	14.73***
	3.2 mM	87±11.5[a]	92±0.82[a]	61±3.72[b]	78±11.48[c]	87±3.74[a]	56±6.98[b]	24.26***
	F-value	8.987**	49.09***	13.32**	6.361**	3.787*	8.897**	
Root length (mm)	0.0 mM	41.7±3.79[a]	22.5±2.37[b]	23.5±3.17[b]	31.9±3.35[c]	12.5±0.57[d]	22.0±4.37[be]	59.10***
	0.1 mM	36.0±3.04[a]	22.1±3.92[be]	18.4±5.36[b]	31.0±1.67[c]	13.7±2.75[d]	23.6±3.66[e]	31.01***
	0.2 mM	24.7±3.6[a]	17.1±2.82[b]	16.8±4.39[b]	27.6±3.51[c]	10.1±1.99[a]	20.2±2.90[b]	21.71***
	0.4 mM	20.2±3.0[ae]	10.4±1.22[b]	13.9±4.39[c]	23.1±2.01[d]	6.9±1.01[a]	18.5±2.56[e]	33.32***
	0.8 mM	14.8±2.18[a]	7.2±1.57[bc]	9.5±2.18[b]	20.4±2.00[c]	5.3±0.98[d]	13.7±2.65[a]	46.64***
	1.6 mM	12.3±1.59[a]	5.3±0.78[b]	8.0±1.46[c]	17.1±1.44[b]	4.2±0.84[d]	10.1±0.74[e]	94.78***
	3.2 mM	10.5±1.24[a]	4.6±1.11[b]	6.4±1.56[c]	12.3±0.32[d]	2.9±1.19[e]	7.3±1.44[c]	51.90***
	F-value	8.993**	7.801**	6.365**	6.368**	8.996**	1.714	
Shoot length (mm)	0.0 mM	27.8±4.96[a]	46.0±5.35[b]	26.1±2.91[a]	20.8±3.90[c]	8.7±0.73[d]	21.4±1.68[d]	67.06***
	0.1 mM	23.4±1.97[a]	35.7±7.76[b]	22.3±3.60[a]	20.5±3.45[c]	10.0±0.92[a]	21.1±1.95[a]	25.80
	0.2 mM	20.9±1.34[a]	31.6±5.89[b]	8.6±1.74[c]	17.1±2.95[c]	10.5±1.36[d]	21.5±3.16[a]	41.88***
	0.4 mM	18.7±3.98[ae]	23.3±7.30[b]	12.1±1.82[c]	15.3±1.09[d]	7.1±2.10[ac]	21.3±2.52[eb]	15.61***
	0.8 mM	18.3±4.08[a]	17.6±3.14[ac]	18.0±0.77[ac]	15.5±1.22[b]	5.3±1.25[c]	20.1±1.76[a]	31.04***
	1.6 mM	14.1±1.18[a]	11.8±0.22[b]	19.7±2.03[c]	14.3±3.41[d]	4.2±0.74[a]	16.6±1.48[e]	49.90***
	3.2 mM	11.9±2.48[ae]	9.1±1.06[b]	21.1±2.40[c]	12.3±0.86[d]	2.0±0.63[a]	10.1±0.93[be]	90.45***
	F-value	29.474***	12.815***	19.786***	36.514***	5.69*	13.78**	

For each attribute the mean values with the same superscript letters among species are not significantly different at 5% level of probability (Duncan's multiple comparisons test).
*significant difference at 0.05 level, **significant difference at 0.01 level, ***significant difference at 0.001 level

cantly inhibited the shoot length ($p<0.001$), 0.2 mM and 0.4 mM cadmium treatments had no significant influence on shoot growth for *Glycine max*, *Lathyrus odoratus*, and *Dumasia villosa*. Lower cadmium treatments (0.1 and 0.2 mM) promoted the shoot length for *Vigna Lathyrus odoratus* (Table 2).

The shoot length was significantly different among species at six kinds of cadmium treatments and one control ($p<0.001$) (Table 1). The shoot length of *Glycine max* and *Vigna radiata* decreased with the addition of cadmium concentration. *Vigna radiata* shoot length was significantly higher than the other five species (Table 2). There was a significant negative correlation between the mean shoot length and cadmium concentration for three species:

Glycine max: $y = 112.744–0.8973x$, $r^2=0.12$, $F_{1.5}=5.5$, $p=0.024$

Vigna ratiata: $y=254.1873–2.2007x$, $r^2=0.14$, $F_{1.5}=6.32$, $p=0.016$

Lablab purpureus: $y=44.7416–0.3642x$, $r^2=0.068$, $F_{1.5}=2.9$, $p=0.096$

Dumasia villosa: $y=96.0798–0.7415x$, $r^2=0.146$, $F_{1.5}= 6.82$, $p=0.013$.

Root Elongation under Cadium Stress

A two-way ANOVA showed that root elongation was significantly affected by different species, cadmium treatment, and the interaction between species and cadmium treatment (Table 1). Root elongation was significantly different among cadmium treatments in 6 pulse species. Compared with the control and shorter concentration treatments (0.1, 0.2 and 0.4 mM), the highest concentration cadmium treatments (0.8 and 1.6 mM) significantly inhibited root elongation for *Glycine max* ($p < 0.001$). For *Lathyrus odoratus*, root elongation dramatically decreased at 1.6 mM cadmium treatment and was significantly shorter than other species ($p<0.001$). The root elongation of all species performed a decline with the increase of cadmium concentration (Table 2).

There was a significant difference of root elongation among species ($p<0.001$) at six kinds of cadmium treatments and one control (Table 1). At control treatment and lower cadmium concentration treatments (0.1 and 0.2 mM), the root length of *Glycine max* and *Lablab purpureus* was significantly longer than the other four species. At higher cadmium concentration treatments (1.6 and 3.2 mM), the root length of *Lathyrus odoratus* was significantly shorter than the other five species (Table 2). There was a negative correlation between the mean root elongation and cadmium concentration, and there was a significant negative correlation for *V. angularis* ($y=124.8185-1.0662x$, $r^2=0.13$, $F_{1,5}=6.02$, $p=0.0186$).

Discussion

Of all the non-essential heavy metals, cadmium is perhaps the metal that has attracted the most attention in soil science and plant nutrition due to its potential toxicity to humans, and also its relative mobility in the soil-plant system [18]. The Cd^{2+} showed a strong inhibitory effect on germination; root elongation and coleoptile growth of the test species, especially at high cadmium concentrations, and root elongation and coleoptile growth were much more sensitive than the germination by this study. High levels of Cd^{2+} supply can inhibit seed germination, and subsequent seedling growth demonstrate that inhibition of root elongation is considered to be the first evident effect metal toxicity in plants [2, 3, 6, 17, 19, 22], maybe because of the seed coat it is able to reduce the amount of Cd entering the seed, but after seed germination cadmium is considered strongly toxic because cadmium compounds in the soil are more or less insoluble as the metal ions are tightly bound to humus and clay particles. Shoot and root after germination has no barrier to protect. Some other studies have the same conclusion [18, 19].

Conclusions

This study concluded that seed germination of all the test species were not so sensitive like shoot and root elongation to Cd^{2+}. Coleoptile growth and root elongation of the test species were more accurate for measuring the toxicity of Cd^{2+} pollution, and different species show different levels of tolerance to Cd^{2+}. *V. angularis* and *Dumasia villosa* are the most sensitive to Cd^{2+}. By contrast, *Vigna radiata* and *Lablab purpureus* are the most resistant species.

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