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Effect of Mercury to Seed Germination, Coleoptile Growth and Root Elongation of Four Vegetables

[1,2]T. Ling, [1,2]Y. Fangke and [1,2]R. Jun
[1]School of Environmental and Municipal Engineering,
Institute of Environmental Ecology, Lanzhou Jiaotong University,
Lanzhou 730070, Peoples' Republic of China
[2]Demonstration Center for Experimental,
Teaching of Environmental Science and Technology in Colleges of Gansu Province,
Lanzhou 730070, Peoples' Republic of China

Abstract: The present investigation reports the results of the effects of mercury on seed germination, coleoptile growth and root elongation of four vegetables. The effects of seven serial doses of mercury (0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mM) on four vegetable crops were studies. Seeds were surface sterilized and washed with distilled water. The seeds were germinated in Petri dishes with double layer of filter paper soaked in distilled water (control) and 0.1-3.2 mM $HgCl_2$ solutions. The seeds were set under a photoperiod of 12 h and 32±1/25±1°C day/night temperature. The root and shoot length of seedling were measured and the germination percentage was recorded after 96 h. The different concentration of Hg^{2+} showed reduction in coleoptile growth and root elongation with increase in concentration of Hg^{2+}. All the treated species were significantly inhibited when the Hg^{2+} concentration up to 0.8 mM. *Brassica oleracea* was the most sensitive species to mercury among the four test species, *B. campestris* was the most resist species to mercury pollution. These four vegetables were more sensitive to mercury stress in coleoptile growth and root elongation than seed germination.

Key words: Coleoptile, germination, mercury, root, vegetable

INTRODUCTION

Following the industrialization, the anthropogenic factor has become the most important source of heavy metals in the environment. Heavy metal contamination of soil, water and air has caused serious environmental hazard in the biosphere due to rapid industrialization and urbanization. Heavy metals might accumulate in the food chains, with risks for the health of animals and humans, which are less sensitive to metal toxicity than plants, but they are capable of concentrating heavy metals in certain tissues and organs (Xiong, 1998; Peralta *et al*., 2001; Parmar *et al*., 2002; Liu *et al*., 2006; Street *et al*., 2007; Emese *et al*., 2009). The influence of metals on development and reproduction of plants can be firstly quantified by determining the germination traits of seeds and growth performance of seedling. In the presence of high concentrations of some heavy metals, most of plant species performed

Corresponding Author: Ren Jun, School of Environmental and Municipal Engineering,
Institute of Environmental Ecology, Lanzhou Jiaotong University,
Lanzhou 730070, Peoples' Republic of China
Tel: 86 0931 4938177 Fax: 86 0931 4838177

the reduction of seed germination and seedling growth (Patra and Sharma, 2000; Peralta *et al*., 2001; Abedin and Meharg, 2002; Jeliazkova *et al*., 2003; Shafiq and Iqbal, 2005; Marchiol *et al*., 2006; Marques *et al*., 2007; Ashraf and Ali, 2007; Hussain *et al*., 2007; Mahmood *et al*., 2007; Marques *et al*., 2007; Singh Sengar *et al*., 2008; Jun *et al*., 2009).

Mercury (Hg) is a toxic heavy metal that is of significant concern as an environmental pollutant. Since mercury is not very phytotoxic in normally occurring concentrations. In polluted regions, mercury is a non-degradable toxic heavy metal pollutant when it is accumulated by plants. The information is scare about its uptake mechanism and growth inhibition. There are a wide range of sources that emit mercury to the atmosphere. Approximately half of the atmospheric budget of vapor-phase mercury is attributed to anthropogenic sources and half to natural sources (Nriagu, 1989). Mercury is readily transported in the atmosphere (Carpi *et al*., 1997) and has an atmospheric half-life of approximately one year (Lindqvist and Pehkonen, 1999).

Mercury is not essential to living cells and performs no known biological function. Mercury has a strong affinity for sulfur and mercury's primary mode of toxic action in living organisms is thought to be the interference of enzyme function and protein synthesis by binding to sulfhydryl groups (Sharma, 1985; Garcia and Reyes, 2001; Patra *et al*., 2004). Maximum work has been carried out on seed germination and seedling growth of different plant species in field exposed to mercurials (Sharma, 1985; Fargasova, 1994; Helal, 1995; Mishra and Choudhuri, 1997a, b, 1998, 1999; Bonifacio and Montano, 1998; Jain *et al*., 1998; Al-Yemeni, 2001; Munzuroglu and Gechil, 2002; Neelima and Reddy, 2003; Li *et al*., 2005; Bhanumathi and Jayabalan, 2007; Devi *et al*., 2007; Bandana *et al*., 2008; Umadevi *et al*., 2009). The present study was undertaken with a view to find out the toxic effect of mercury to seed germination, root elongation and coleoptile growth of four vegetables. With the specific aim of determining: 1) what degree mercury inhibits the four vegetables' seed germination and seedling growth? 2) Which vegetables are more sensitive to mercury?

MATERIALS AND METHODS

The effects of seven serial doses of mercury (0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mM) on four vegetable crops were studies. Seeds of Cabbage (*Brassica rapa*), Cole (*B. napus*), Head Cabbage (*B. oleracea*) and Spinach (*Spinacia oleracea*) were offered by Gansu Academy of Agricultural Sciences of China. The experiment was conducted in Institute of Environmental Ecology of Lanzhou Jiaotong university from Gansu for 4 months. 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 is 150 mm) with double layer of filter paper soaked in distilled water (control) and 0.1-3.2 mM $HgCl_2$ solutions. The seeds were set under a photoperiod of 12 h and 32±1/25±1°C day/night temperature. The root and shoot length of seedling were measured and the germination percentage was recorded after 96 h. A 1-mm radical emergence from seeds was considered seed germination. The root and coleoptile length of 10 germinated seeds which selected stochastically were measured at 96 h under each treatment.

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

RESULTS AND DISCUSSION

Germination under Mercury Stress

The germination percentage of vegetable seeds occurred at all mercury treatments from 0.0 mM to 3.2 mM for each species (Table 2). A two-way ANOVA showed that seed germination was significantly affected by different species (F_3 =7.33, p<0.001), mercury treatment (F_5 =11.03, p<0.001) and interaction between species and mercury treatment (F_{36}=2.62, p<0.001) (Table 1). The germination percentage was significantly different among mercury treatments in four vegetable species (one-way ANOVA: $F_{6,36}$ = 13.26, p<0.001 for *Brassica oleracea*; $F_{6,36}$ = 14.29, p<0.001 for *B. campestris*; $F_{6,36}$ = 56.62, p<0.001 for *B. rapa*; $F_{6,36}$ = 18.69, p<0.001 for *Spinacia oleracea*). For all species, the lowest germination percent always occurred in highest mercury concentration treatments. Compared with the control, higher concentration mercury treatments (0.8, 1.6, 3.2 mM) significantly inhibited germination for *Brassica oleracea*, *B. rapa* and *Spinacia oleracea*. The germination of all four species

Table 1: The analysis of variance for the effects of different species, mercury treatments and their interaction on seed germination (%), coleoptile growth and root elongation for four vegetable species

Traits	Source of variation	df	F-value	p-value
Germination	Mercury treatment	6	7.33***	<0.001
	Species	3	11.03***	<0.001
	Mercury treatment×species	36	2.62***	<0.001
Coleoptile growth	Mercury treatment	6	5.01***	<0.001
	Species	3	4.24***	<0.001
	Mercury treatment×species	36	1.79*	0.027
Root elongation	Mercury treatment	6	2.79*	0.018
	Species	3	1.58	0.126
	Mercury treatment×species	36	0.94	0.565

*Means significant correlation at 0.05 level. **Means significant correlation at 0.01 level. ***Means significant correlation at 0.001 level

Table 2: Influence of mercury on seed germination, coleoptile growth and root elongation of four vegetables

Attribute	Treatment	*Brassica oleracea*	*B. campestris*	*B. rapa*	*Spinacia oleracea*	F-value
Germination (%)	0.0 mM	72.33±39.51Aa	100.00±0.00Ab	42.00±4.90Ac	79.67±11.06Aa	93.39***
	0.1 mM	72.00±5.93Aa	100.00±0.00Ab	39.33±1.97Ac	88.33±4.46Ad	282.88***
	0.2 mM	57.33±12.18Ba	100.00±0.00Ab	36.67±2.94ABc	73.33±10.78Ad	62.74***
	0.4 mM	56.33±10.69Ba	100.00±0.00Ab	34.50±3.45Bc	78.00±8.00Ad	100.12***
	0.8 mM	55.33±13.19Ba	98.67±1.03Ab	26.83±3.76Cc	70.67±8.82Bd	80.84***
	1.6 mM	52.67±11.15Ba	94.00±1.79Ab	28.33±2.58Cc	64.00±12.46Bd	61.61***
	3.2 mM	26.00±12.65Ca	55.67±28.21Bb	11.17±3.37Dc	38.00±5.51Cb	8.53**
	F-value	13.26***	14.19***	56.62***	18.69***	
Coleoptile length (mm)	0.0 mM	25.52±1.00Aa	24.57±0.50Aa	13.00±0.42Ab	24.12±1.14Aa	41.41***
	0.1 mM	26.95±0.26Ba	20.47±0.39Bb	11.03±0.23Bc	22.40±0.41Ab	30.10***
	0.2 mM	22.80±0.40Ca	16.58±0.35Cb	10.30±0.67Cc	20.05±4.29Ba	47.89***
	0.4 mM	19.92±0.61Da	15.02±0.35Db	8.28±0.17Dc	18.79±0.46Ba	116.97***
	0.8 mM	14.28±0.72Ea	13.52±0.27Eb	5.22±0.18Ec	12.62±0.41Cd	63.93***
	1.6 mM	11.33±1.63Fa	7.82±0.19Fb	4.07±0.14Fc	9.52±0.61Dd	92.55***
	3.2 mM	3.03±0.24Ga	4.52±0.42Gb	2.55±0.19Gc	6.42±0.34Ed	32.55***
	F-value	63.44***	214.95***	80.26***	13.17***	
Root length (mm)	0.0 mM	27.87±0.59Aa	25.12±0.47Ab	13.20±0.35Ac	27.55±0.68Aa	10.16**
	0.1 mM	30.52±0.40Ba	22.05±0.52Bb	12.45±0.19Bc	34.17±0.44Bd	340.60***
	0.2 mM	19.43±0.38Ca	18.47±0.35Cb	11.03±0.23Cc	30.28±0.93Cd	126.23***
	0.4 mM	18.45±0.23Da	17.75±0.21Db	9.72±0.25Dc	24.75±0.26Dd	39.64***
	0.8 mM	15.20±0.23Ea	14.77±0.55Ea	6.40±0.13Eb	24.50±0.45Dc	231.94***
	1.6 mM	12.35±0.38Fa	8.53±0.35Fa	5.70±0.14Fb	21.52±0.45Ec	233.53***
	3.2 mM	5.82±0.28Ga	5.83±0.52Ga	4.28±0.21Gb	18.92±0.58Fc	155.30***
	F-value	317.86***	150.49***	145.26***	48.41***	

Results are Means±SD. For each attribute the mean values with the same lowercase letters among species in same column are not significantly different at 5% level of probability (Duncan's multiple comparisons test) and with the same upper-case letters among treatments in same row are not significantly different. *Means significant correlation at 0.05 level. **Means significant correlation at 0.01 level. ***Means significant correlation at 0.001 level

Table 3: Correlations of mercury concentration (Y) to germination percentage (X), root and shoot length (X)

Trait	Species	Linear model	R^2	F-value	p-value
Germination	*Brassica oleracea*	Y=723.3333-6.4167X	0.5574	50.39	<0.001
	Brassica campestris	Y=636.1429-5.2262X	0.3341	20.07	<0.001
	Brassica rapa	Y=493.0714-4.4405X	0.7674	132.03	<0.001
	Spinacia oleracea	Y=725.2381-6.2976X	0.5409	47.14	<0.001
Coleoptile growth	*Brassica oleracea*	Y=415.8619-3.8286X	0.9140	425.60	<0.001
	Brassica campestris	Y=343.4167-3.1613X	0.9733	1461.57	<0.001
	Brassica rapa	Y=194.8548-1.7988X	0.9760	1633.60	<0.001
	Spinacia oleracea	Y=400.1905-3.6619X	0.9225	476.42	<0.001
Root elongation	*Brassica oleracea*	Y=414.8952-3.8113X	0.9173	443.69	<0.001
	Brassica campestris	Y=345.0976-3.1637X	0.9644	1085.19	<0.001
	Brassica pekinensis	Y=175.6786-1.603x	0.9673	1183.64	<0.001
	Spinacia oleracea	Y=237.6071-2.0351X	0.7094	97.67	<0.001

was not affected by lower mercury concentration treatments (0.1 mM) and was significantly inhabited by the highest mercury treatment (3.2 mM) comparing control and other treatments. *B. rapa* performed poorer germination at all mercury treatments and control (Table 2).

The germination was significantly different among species at six kinds of mercury treatments and one control. At control, the germination percentages of *Brassica oleracea* and *Spinacia oleracea* did not perform significant difference, but significantly lower than *B. campestris* and higher than *B. rapa*. At 0.1, 0.2, 0.4, 0.8 and 1.6 mM mercury treatments, there was significant difference of the germination percentages among four species and *B. campestris* always performed the highest germination percentage, *B. rapa* performed the lowest germination percentage. At the highest concentration mercury treatments (3.2 mM), *Brassica oleracea* and *B. rapa* did not performed significant difference and were significantly lower than other 2 species (Table 2). There was a significant negative correlation between the mean percent germination and mercury concentration for all four vegetable species (Table 3).

Coleoptile Growth under Mercury Stress

A two-way ANOVA showed that coleoptile growth was significantly affected by different mercury treatment (F_6 =5.01, $p<0.001$) and species (F_3 =4.25, $p<0.001$), but not by the interaction between species and mercury treatment (Table 1). The coleoptile length was significantly different among mercury treatments in each vegetable species (one-way ANOVA: $F_{6,36}$ = 63.44, $p<0.001$ for *Brassica oleracea*; $F_{6,36}$ = 214.95, $p<0.001$ for *B. campestris*; $F_{6,36}$ = 80.26, $p<0.001$ for B. rapa; $F_{6,36}$ = 13.17, $p<0.001$ for *Spinacia oleracea*). The coleoptile growth of all 4 vegetables dramatically and significantly decreased with the addition of mercury concentration. Compared with the control, lower concentration mercury treatments (0.1 mM) significantly stimulated the coleoptile growth of *Brassica oleracea*, but other 3 species did not perform same responses. For *Brassica oleracea*, there was significant difference of coleoptile growth among 6 treatments and the control, but the highest coleoptile length appeared at 0.1 mM mercury treatment and the lowest coleoptile length appeared at the highest concentration treatment (3.2 mM). For *B. campestris* and *B. rapa*, the highest coleoptile length appeared at control and the lowest coleoptile growth was always caused by the highest concentration treatment (3.2 mM). The lowest concentration mercury treatment (0.1 mM) did not significantly effect the coleoptile growth of *Spinacia oleracea*. Comparing the lower concentration treatments and control, three higher concentration treatments (0.8, 1.6 and 3.2 nM) performed siginificantly lower coleoptile growth and significant difference each other (Table 2).

The coleoptile growth was significantly different among species at six kinds of mercury treatments and one control. At control, *Brassica oleracea*, *B. campestris* and *Spinacia oleracea* did not performed significant difference in the coleoptile growth and their coleoptile lengths were significantly higher than the coleoptile length of *B. rapa*. At 0.1 mM treatment, *B. campestris* and *Spinacia oleracea* did not performed significant difference in the coleoptile growth and their coleoptile lengths were significantly higher than *Brassica oleracea* and *B. rapa* and there was significant difference between *Brassica oleracea* and *B. rapa*. At 0.2 and 0.4 mM treatments, the coleoptile lengths of *Brassica oleracea* and *Spinacia oleracea* were significantly higher than the coleoptile lengths of *B. campestris* and *B. rapa*. At higher concentration mercury treatments (0.8, 1.6 and 3.2 mM), there was significant difference of the coleoptile growth among four species and At 0.8, 1.6 and 3.2 mM treatments, *Brassica oleracea* always performed the biggest coleoptile growth, *B. rapa* performed the smallest coleoptile growth (Table 2). There was a significant negative correlation between the mean coleoptile length and mercury concentration for all four vegetable species (Table 3).

Root Elongation under Mercury Stress

A two-way ANOVA showed that root elongation was significantly affected by different mercury treatment (F_6 =2.80, P=0.018), but not by the species and the interaction between species and mercury treatment (Table 1). The root elongation was significantly different among mercury treatments in each vegetable species (one-way ANOVA: $F_{6,36}$ = 317.86, $p<0.001$ for *Brassica oleracea*; $F_{6,36}$ = 150.49, $p<0.001$ for *B. campestris*; $F_{6,36}$ = 145.26, $p<0.001$ for *B. rapa*; $F_{6,36}$ = 48.41, $p<0.001$ for *Spinacia oleracea*). The root elongation of all 4 vegetables dramatically and significantly decreased with the addition of mercury concentration. Compared with the control, lower concentration mercury treatments (0.1 mM) significantly stimulated the root elongation of *Brassica oleracea* and *Spinacia oleracea*, but other 2 species did not perform same responses. For *Brassica oleracea*, there was significant difference of root elongation among 6 treatments and the control, but the highest root elongation appeared at 0.1 mM mercury treatment and the lowest root elongation appeared at the highest concentration treatment (3.2 mM). For *B. campestris* and *B. rapa*, the highest root length appeared at control and the lowest root length was always caused by the highest concentration treatment (3.2 mM). Comparing the lower concentration treatments and control, three higher concentration treatments (0.8, 1.6 and 3.2 nM) performed significantly lower root elongation and significant difference each other (Table 2).

The root elongation was significantly different among species at six kinds of mercury treatments and one control. At control, *Brassica oleracea* and *Spinacia oleracea* did not performed significant difference in the root elongation and were significantly higher than *B. campestris* and *B. rapa*. At lower concentration mercury treatments (0.1, 0.2, 0.4 mM), there was significant difference of the root elongation among four species, *Brassica oleracea* always performed the biggest root elongation, *B. rapa* performed the smallest root elongation. At higher concentration mercury treatments (0.8, 1.6 and 3.2 mM), *B. campestris* and *Spinacia oleracea* did not performed significant difference in the root elongation and their root lengths were significantly higher than *B. rapa* and lower than *Spinacia oleracea* (Table 2). There was a significant negative correlation between the mean root length and mercury concentration for all four vegetable species (Table 3).

In this study, we have examined the toxicity of mercury on seed germination, coleoptile growth and root elongation in four vegetable species. Depending on the Hg^{2+} concentration and the plant species, the inhibition effects of mercury on vegetable were different. Seed

germination, root and coleoptile growth of vegetable are susceptible to Hg^{2+} pollution. The inhibitory effect on root and coleoptile growth of vegetables was more pronounced at higher concentrations of Hg^{2+}. Root and coleoptile length of seedlings decreased with increasing concentrations of Hg^{2+}. Mercury toxicity for shoot and root cell may result from the displacement of other cations from binding sites in the plasma membranes and cell wall (Sharma, 1985).

In this study, we have examined the toxicity of mercury on seed germination, coleoptile growth and root elongation in four vegetable species. Depending on the Hg^{2+} concentration and the plant species, the inhibition effects of mercury on vegetable were different. Seed germination, root and coleoptile growth of vegetable are susceptible to Hg^{2+} pollution. The inhibitory effect on root and coleoptile growth of vegetables was more pronounced at higher concentrations of Hg^{2+}. Root and coleoptile length of seedlings decreased with increasing concentrations of Hg^{2+}. Mercury toxicity for shoot and root cell may result from the displacement of other cations from binding sites in the plasma membranes and cell wall (Sharma, 1985).

Seed germination had been no significantly inhibited by the lower levels (= 0.4 mM) of Hg^{2+}, but at the same Hg^{2+} concentration, the root and shoot growth was significantly decreased comparing to the control. In general, our results were consistent with previous reports regarding the effect of heavy metals (Fargasova, 1994; Helal, 1995; Mishra and Choudhuri, 1997a, b, 1999; Bonifacio and Montano, 1998; Yemeni, 2001; Jeliazkova and Craker, 2002; Munzuroglu and Gechil, 2002; Li *et al*., 2005). Among the different vegetable seedling, the shoot appeared to suffer the most severe growth inhibition. To this concern, it had been suggested that the reduction in coleoptile growth could be due to inhibition of mercury on coleoptile cell division or elongation, or on the extension of cell cycle. Our results indicated that mercury affected root and coleoptile growth more than seed germination was not a reliable indicator for metal tolerance in early root development of plants. Radicle and plumule growth, the primary plant organs that sense, contact and accumulate heavy metal(s) from the substrate, are thought to be a reliable indicator of metals tolerance in plants (Patra *et al*., 2004; Ozdener and Kutbay, 2009), may be it's because of the seed coat is able to reduce the amount of mercury entering the seed, but after the seed germination, radicle and plumule have no barrier to protect. Some other studies have the same conclusion (Leon *et al*., 2005).

Mercury has inhibitory effect on the germination and seedling growth of the test species. Germination inhibition due to heavy metals has been reported by many workers (Fargasova, 1994; Helal, 1995; Mishra and Choudhuri, 1997a, b, 1999; Bonifacio and Montano, 1998; Al-Yemeni, 2001; Jeliazkova and Craker, 2002; Munzuroglu and Gechil, 2002; Li *et al*., 2005). Ionic toxicity might be the cause of drastic effects of salts on seed germination (Mishra and Choudhuri, 1997a), or it might be due to osmotic effect (Shaukat *et al*., 1999). Mercury also causes alterations in protein structure, alterations in calcium transport, along with the inhibition of glucose transport and enzyme function. The direct mechanism involving mercury's inhibition of cellular enzymatic processes by binding with the hydroxyl radical in amino acids appears to be a major part of the connection to allergic/immune reactive conditions (Sharma, 1985; Garcia and Reyes, 2001; Patra *et al*., 2004).

From this study it is concluded that seed germination, root and coleoptile growth of the vegetable species were significantly reduced by mercury and different species show different levels of tolerance to mercury, *Brassica oleracea* was the most sensitive species to mercury among the four test species, *B. campestris* was the most resist plants to mercury pollution and mercury stress is more sensitive to the coleoptile growth and root elongation.

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