Complex toxic effects of Cd^{2+}, Zn^{2+}, and acid rain on growth of kidney bean (*Phaseolus vulgaris* L)

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Abstract

Complex toxic effects of Cd^{2+}, Zn^{2+}, and acid rain on growth of kidney bean (*Phaseolus vulgaris* L) were studied in a pot experiment by measurement of fresh weights of the plants, determination of surperoxide dismutase (SOD), peroxidase (POD), and lipid peroxidation (MDA) in the plant organs, and observation of injury symptoms. The experimental results demonstrated that all treatments of Cd^{2+}, Zn^{2+}, and/or acid rain significantly decreased fresh weights of kidney bean and caused toxic effects on growth of the plants, especially higher amounts of Cd^{2+} and Zn^{2+} and higher acidity of acid rain. Combination of these three pollutant factors resulted in more serious toxic effects than any single pollutant and than combinations of any two pollutants. SOD, POD, and MDA in the plant organs changed with different pollution levels, but MDA content in the leaves showed the best relationship between the pollution levels and toxic effects.

Keywords: Complex toxic effect; Cd^{2+}; Zn^{2+}; Acid rain; Kidney bean (*Phaseolus vulgaris* L)

1. Introduction

Ecological effects of heavy metals, especially Cd and Zn, have been widely studied in soil–plant systems (Zheng et al., 1996; Lorenz et al., 1997; Wu et al., 1997a; Murray et al., 2000). The results indicate that plant yields decrease due to a toxic effect of heavy metals on the ecosystem (Scherer et al., 1997; Wu et al., 1997a). Cd^{2+} can accumulate and redistribute in some plant organs, resulting in plant injuries (Lagriffoul et al., 1998; Reed et al., 1999), changes in plant protective enzymes (Chen et al., 1998; Chien et al., 2001), or changes in photosynthetic sensitivity of plants at different growth stages (Skorzynska Polit and Baszynski, 1997). Compared to the control, Cd treatments increase lipid peroxidation and autoxidation rates of plant leaves and roots estimated from rate of MDA formation (Zhang et al., 2000), excess $CdCl_2$ reduces SOD activity (Chien et al., 2001), and there is a relationship between the POD activity and the Cd content (Lagriffoul et al., 1998), so that some authors use enzyme activities of plants to reflect soil pollution levels. Field experiments show that addition of Zn and other heavy metals can cause interactions with soil Cd, leading to increases in plant absorption coefficients, which are even more serious in acid and neutral soils (Wu et al., 1997b). Hunan, a province in southern China, is called "the town of non-ferrous metals in China", because mining of heavy metals (Pb, Zn, Cu, Cd, As, etc.) has been conducted for about 500 years. Field investigations show that outputs and quality of crops in mining areas are much lower than those in the areas with no mining activities, due to serious heavy metal pollution in the local soils and waters (Liu et al., 2005). Meanwhile, Hunan is in the center of the area of acid rain in southern China. Recent research implies that acid rain is an important factor in increasing the activity and mobility of heavy metals in soils (Liao et al., 2005). A

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laboratory study indicates that simulated acid rain influences leaching of Cd and Cu in soils, and the released amounts increase with increasing acidity of simulated acid rain. Cd is more sensitive to acid rain than Cu (Xie et al., 1991). Therefore, it is very important to study complex effects of heavy metals (Cd^{2+} and/or Zn^{2+}) and acid rain on plants growing in polluted soil–plant systems such as in Hunan. The objective of this work was to study complex toxic effects of Cd^{2+}, Zn^{2+}, and acid rain on growth of kidney bean (*Phaseolus vulgaris* L) by measuring the fresh weights of the plants and by determining activities of SOD, POD, and MDA in the various plant organs.

2. Materials and methods

A pot experiment with kidney bean (*Phaseolus vulgaris* L) was conducted. Treatments of heavy metals and simulated acid rain were designed as follows: Cd^{2+} (0~10 mg/kg) and acid rain solutions (pH values of 5.6, 4.5, 3.5, and 3.0), Zn^{2+} (0~300 mg/kg) and acid rain solutions (pH 5.6~3.0), complex treatments (CK: no Cd^{2+} or Zn^{2+}, but treated with acid rain; CX1: 0.5 mg/kg Cd^{2+}, 20 mg/kg Zn^{2+}, and acid rain; CX2: 5 mg/kg Cd^{2+}, 150 mg/kg Zn^{2+}, and acid rain). A vegetable garden soil (no heavy metal pollution reported) was collected from the suburb of Changsha, the capital city of Hunan Province, then air-dried, passed through a 5-mm sieve, and weighed 5.0 kg to each pot. Solutions of pure $Cd(CH_2COO)_2$ and/or $Zn(CH_2COO)_2$ were added to each pot as sources of heavy metals so that concentrations of Cd^{2+} and/or Zn^{2+} in the pot soil reached the levels described above, then 20 g of rape cake was added as base manure. After 2 weeks, six 7-day-old kidney bean seedlings were transplanted in each pot. Five days later, simulated acid rains (0.242 L, calculated from the yearly precipitation 1500 mm and evapotranspiration 40% in Changsha, and the pot area 3.27×10^{-2} m^2) were watered evenly from the plant tops into the pots. This watering process was repeated 10 times (once every 3 days), and solution pH values of acid rain ranged from 5.6 to 3.0. A replicate experiment was carried out followed the same processes above. Injury symptoms of the plants were observed and recorded during the whole growth period. Fresh weights of the plants, activities of SOD and POD in the different organs, and MDA contents in the leaves were determined after 35 days, and this analytical process was repeated twice for each replicate by using new fresh plant organs. Determination of SOD, POD, and MDA was followed the standard procedures described by Jin and Ding (1981).

Table 2
Fresh weights (g/plant) of kidney bean plants treated with acid rain and Cd^{2+} or Zn^{2+}

Cd^{2+} added (mg/kg)	pH 5.6	pH 4.5	pH 3.5	pH 3.0
0	13.2±0.3	12.0±0.2	9.7±0.3	7.7±0.1
0.5	11.7±0.1	16.5±0.3	15.5±0.3	10.0±0.4
2	12.0±0.4	14.5±0.1	11.0±0.7	9.5±0.2
5	11.2±0.1	14.0±0.2	5.8±0.3	5.0±0.1
10	7.5±0.2	6.2±0.2	5.2±0.5	4.3±0.1

Zn^{2+} added (mg/kg)	pH 5.6	pH 4.5	pH 3.5	pH 3.0
0	13.2±0.3	12.0±0.2	9.7±0.3	7.7±0.1
20	11.7±0.2	16.0±0.6	15.3±0.7	14.0±0.4
60	9.7±0.1	12.7±0.3	10.2±0.3	11.3±0.1
150	9.0±0.4	6.3±0.1	7.0±0.2	6.0±0.1
300	5.2±0.5	5.3±0.4	4.5±0.4	3.5±0.1

Significant differences

Cd^{2+} added (mg/kg)	$p<0.01$	Zn^{2+} added (mg/kg)	$p<0.01$	pH	$p<0.01$
0	C	0	C	5.6	B
0.5	A	20	A	4.5	A
2	B	60	B	3.5	C
5	D	150	D	3.0	D
10	E	300	E		

3. Results

3.1. Effects of single Cd^{2+}, Zn^{2+}, or acid rain on growth of kidney bean

Average fresh weights of kidney bean plants were used as a growth index. Treatments of single Cd^{2+}, Zn^{2+}, or acid rain severely inhibited growth of the plants (Table 1). It was obvious that with increasing soil Zn^{2+} concentration or increasing acidity of acid rain, the fresh weights of kidney bean plants decreased significantly. Although there were no significant differences observed when Cd^{2+} concentration was in a range of 0.5~5 mg/kg, the fresh weights were significantly different among three levels of no Cd^{2+}, lower Cd^{2+} concentration (0.5~5 mg/kg), and higher Cd^{2+} concentration (10 mg/kg) in the soil. Compared to the case without Cd^{2+}, Zn^{2+}, or acid rain, the fresh weights decreased

Table 1
Fresh weights of kidney bean plants treated with single Cd^{2+}, Zn^{2+}, or acid rain

Cd^{2+} added (mg/kg)	0.0	0.5	2	5	10
Weight (g/plant)	13.2±0.3	11.7±0.1	12.0±0.4	11.2±0.1	7.5±0.2
Significant difference ($p<0.01$)	A	B	B	B	C
Zn^{2+} added (mg/kg)	0.0	20	60	150	300
Weight (g/plant)	13.2±0.3	11.7±0.2	9.7±0.1	9.0±0.4	5.2±0.5
Significant difference ($p<0.01$)	A	B	C	D	E
Acid rain (pH)	5.6 (distilled water)	4.5	3.5	3.0	
Weight (g/plant)	13.2±0.3	12.0±0.2	9.7±0.3	7.7±0.1	
Significant difference ($p<0.01$)	A	B	C	D	

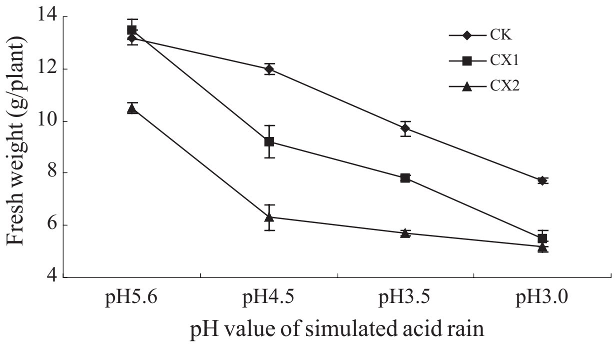

Fig. 1. Fresh weights of kidney bean plants treated with Cd^{2+}, Zn^{2+}, and acid rain (CK: no Cd^{2+} or Zn^{2+} added to the soil, and plants watered with simulated acid rain; CX1: 0.5 mg/kg Cd^{2+} and 20 mg/kg Zn^{2+} added, and plants watered with acid rain; CX2: 5 mg/kg Cd^{2+} and 150 mg/kg Zn^{2+} added to the soil, and plants watered with acid rain).

by 43%, 61%, or 42% for the soil containing 10 mg/kg of Cd^{2+}, 300 mg/kg of Zn^{2+}, or watered with simulated acid rain of pH 3.0, respectively.

3.2. Complex effects of Cd^{2+}, Zn^{2+}, and acid rain on growth of kidney bean

Table 2 gave fresh weights of kidney bean plants treated with acid rain and Cd^{2+}, acid rain and Zn^{2+}, and significant differences at $p<0.01$ level. The results indicated that there were significant differences on kidney bean growth among various pollution treatments although the greatest fresh weights appeared at pH 4.5 and 0.5 mg/kg of soil Cd^{2+} or pH 4.5 and 20 mg/kg of Zn^{2+}. Under the effects of simulated acid rains (pH 4.5∼3.0), plant fresh weights increased generally at lower Cd^{2+} or Zn^{2+} levels, and then decreased apparently at higher Cd^{2+} or Zn^{2+} levels, which was different from the situations of single Cd^{2+}, Zn^{2+}, or acid rain pollution. Higher fresh weights appearing at pH 4.5 and lower levels of Cd^{2+} or Zn^{2+} was probably because acid rain caused a great release of soil base cations (such as Ca^{2+} and Mg^{2+}) from the soil and these cations compensated toxic effects of Cd^{2+} or Zn^{2+} in the soils. On the other hand, in the case of high acidity of acid rain (pH 3.0) and high Cd^{2+} (10 mg/kg) or Zn^{2+} (300 mg/kg), the fresh weights declined considerably compared to those with either high acidity of acid rain or high Cd^{2+} (or Zn^{2+}) levels, implying that complex effects of Cd^{2+} (or Zn^{2+}) and acid rain became more detrimental, to some extent, to plant growth.

The experimental results indicated that fresh weights of kidney bean plants decreased significantly with increasing both acidity of acid rain and total contents of soil Cd^{2+} and Zn^{2+} (Fig. 1). Compared to the treatments of only acid rain (CK), Cd^{2+} and Zn^{2+} increased toxic effects on growth of kidney bean, resulting in a significant decrease of fresh weights. Meanwhile, when the soil contained certain contents of Cd^{2+} and Zn^{2+}, acid rain (pH 4.5∼3.0) had the same trend to increase these toxic effects on the plants. A comparison of Fig. 1 with Tables 1 and 2 demonstrated that complex effects of Cd^{2+}, Zn^{2+}, and acid rain were more harmful to the plant growth than complex effects of Cd^{2+} (or Zn^{2+}) and acid rain, and much more harmful than those of either Cd^{2+}, or Zn^{2+}, or acid rain.

3.3. SOD/POD activities and MDA contents of kidney bean

SOD and POD are two major protective enzymes in plants. When plants grow in the polluted environment, changes of these two enzymes in plant organs can eliminate free radicals caused from pollution, and prevent the plants from injury. All pollution treatments (addition of Cd^{2+}, Zn^{2+}, and/or acid rain to the soils) caused activity changes of SOD and POD in the plant organs (Table 3), showing that SOD and POD were stimulated to inhibit the free radicals. In our experiment, there was no a certain pattern for changes of SOD activity according to changes of pollution level; however, POD activity was higher in the cases treated with Cd^{2+} (or Zn^{2+}) and acid rain than in the cases treated with single acid rain or Cd^{2+}, Zn^{2+}, and acid rain together. The highest activities of SOD were found in the leaves, but those of POD in the roots. This was the same as in our previous experiment for *Vicia faba* L treated with Cd^{2+} and acid rain (Liao et al., 2003) and

Table 3
Activities of SOD and POD (U/g)[a] in various organs of kidney bean plants treated with Cd^{2+}, Zn^{2+}, and acid rain

Treatment	pH value of acid rain	SOD			POD		
		Root	Stem	Leaf	Root	Stem	Leaf
No heavy metals	5.6	21.3±2.4	24.0±2.5	36.6±3.2	108.7±9.6	60.0±4.0	75.0±6.3
No heavy metals	4.5	30.8±2. 7	41.7±2.9	54.8±3.3	487.3±22.1	150.0±12.2	206.2±18.1
No heavy metals	3.5	31.5±1.6	37.2±3.5	39.0±4.3	532.3±21.1	318.6±33.8	359.9±26.1
No heavy metals	3.0	27.0±3.1	49.4±4.1	43.3±6.6	438.6±38.2	112.5±10.0	153.7±16.4
5 mg/kg Cd^{2+}	5.6	23.4±4.6	18.4±1.9	45.3±2.5	352.4±31.6	326.1±20.5	464.9±36.5
5 mg/kg Cd^{2+}	4.5	18.3±1.8	19.2±3.7	20.9±1.6	299.9±25.3	247.4±16.1	299.9±27.4
5 mg/kg Cd^{2+}	3.5	22.4±2.7	18.6±3.2	34.0±5.1	363.6±26.3	239.9±18.1	337.4±27.2
5 mg/kg Cd^{2+}	3.0	17.0±1.6	16.2±2.9	32.4±5.1	517.3±30.2	221.2±14.5	277.4±15.4
150 mg/kg Zn^{2+}	5.6	32.6±2.4	26.8±2.1	54.9±3.2	637.3±26.1	273.7±26.0	453.6±21.7
150 mg/kg Zn^{2+}	4.5	34.1±3.7	40.9±2.2	50.5±2.6	667.3±32.1	254.9±19.1	401.1±25.3
150 mg/kg Zn^{2+}	3.5	23.9±2.1	31.4±1.8	50.1±4.5	277.4±26.3	251.2±13.6	224.9±20.2
150 mg/kg Zn^{2+}	3.0	22.7±1.1	23.2±1.6	44.0±3.9	434.9±27.1	153.7±11.8	273.7±27.2
5 mg/kg Cd^{2+}+150 mg/kg Zn^{2+}	5.6	29.9±3.2	24.6±2.4	44.8±4.9	138.7±13.6	93.7±5.4	86.2±6.2
5 mg/kg Cd^{2+}+150 mg/kg Zn^{2+}	4.5	48.4±3.3	43.7±2.4	45.1±4.1	674.8±32.6	198.7±14.1	172.4±13.3
5 mg/kg Cd^{2+}+150 mg/kg Zn^{2+}	3.5	31.1±2.1	18.2±3.5	40.3±2.9	554.8±33.5	251.2±8.1	389.9±22.7
5 mg/kg Cd^{2+}+150 mg/kg Zn^{2+}	3.0	41.1±5.6	39.7±4.2	43.0±2.8	423.6±25.0	97.5±10.2	153.7±13.1

[a] One activity unit of SOD (U/g) was defined as the amount of SOD enzyme which caused 50% inhibition of the initial reaction rate in the absence of the enzyme at 550 nm. One activity unit of POD (U/g) was defined as the amount of POD enzyme which caused a 0.001 absorbance increase per min at 470 nm.

indicated that plant leaves and roots were more sensitive to soil pollution than plant stems.

Rates of MDA formation indicated autoxidation rates in plant leaves and roots, and Cd treatments increased MDA contents compared to the control (Zhang et al., 2000). In our experiment, MDA contents in the leaves of kidney bean increased significantly ($p<0.01$) with the amounts of Cd^{2+} or Zn^{2+} added to the soils, or with the acidity of acid rain (Fig. 2), showing that more serious pollution caused higher autoxidation rates in the plant leaves. Therefore, for kidney bean plants and the corresponding soils, MDA contents in the leaves seemed to reflect pollution levels better and more clearly than activities of SOD and POD in the roots, stems, or leaves.

3.4. Injury symptoms of kidney bean plants

All kidney bean plants in polluted soils had injury symptoms to some extent. These symptoms included changing color of the leaves from green to yellow, increasing areas of yellow spots on the leaves, and increasing height differences of polluted plants and unpolluted plants. Generally, significant symptoms appeared after the 4th addition of acid rain, especially pH 3.5 and 3.0, and for addition of the highest amount of Cd^{2+} (10 mg/kg) or Zn^{2+} (300 mg/kg). With increasing additions of acid rain, increasing acidity of acid rain, and increasing amounts of Cd^{2+} or Zn^{2+} added to soils, these injury symptoms became more and more serious. In the soil containing 10 mg/kg Cd^{2+} and watered with acid rain of pH 3.0, the plants appeared the shortest, and about one-third of their leaves were covered with yellow spots. In the soil containing 300 mg/kg Zn^{2+}, the symptoms were even more significant, compared to the soil containing 10 mg/kg Cd^{2+}. When watered with acid rain of pH 3.0, some leaves started dying. The injury symptoms in soils polluted with Zn^{2+} were more obvious than those with Cd^{2+}, probably due to the much higher level of Zn^{2+} added to the soils. These symptoms mostly corresponded to changes of fresh weights and MDA in leaves.

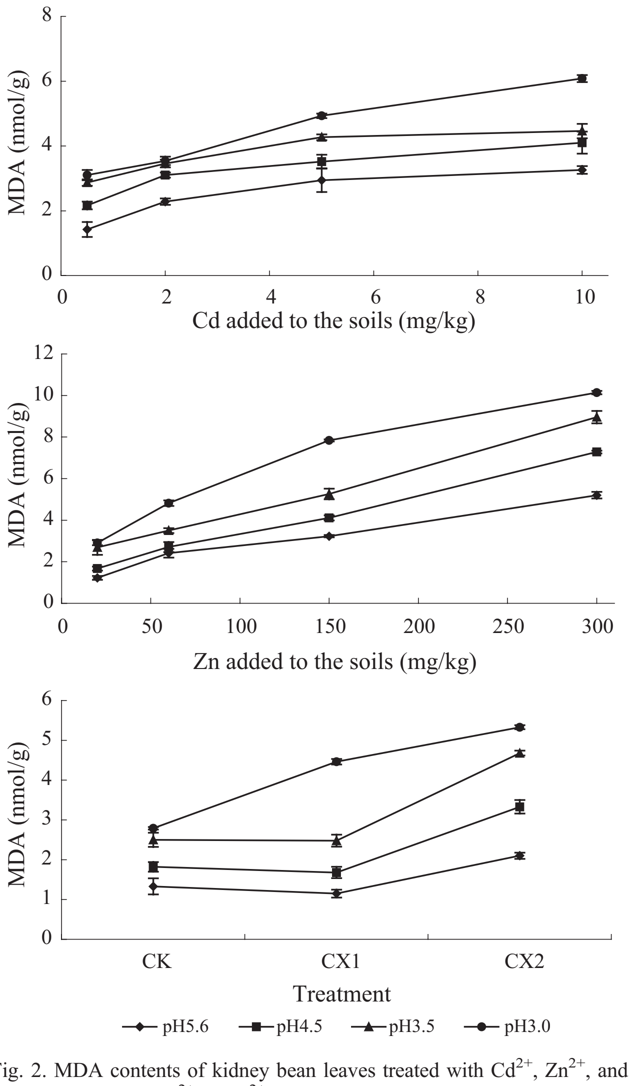

Fig. 2. MDA contents of kidney bean leaves treated with Cd^{2+}, Zn^{2+}, and acid rain. (CK: no Cd^{2+} or Zn^{2+} added to the soil, but plants watered with simulated acid rain; CX1: 0.5 mg/kg Cd^{2+} and 20 mg/kg Zn^{2+} added to the soil, and plants watered with acid rain; CX2: 5 mg/kg Cd^{2+} and 150 mg/kg Zn^{2+} added to the soil, and plants watered with acid rain).

4. Discussion

Many studies have demonstrated toxic effects of Cd^{2+} on growth of plants, in both terrestrial and aquatic ecosystems. These toxic effects are probably caused by accumulation and redistribution of Cd^{2+} in some plant organs, and by changes in plant protective enzymes (Chien et al., 2001), resulting in direct plant organ injuries (Reed et al., 1999). Therefore growth of plants (estimated from the yields, fresh weights, or heights) is inhibited. Our present experiment for kidney bean (*Phaseolus vulgaris* L) and the previous experiment for broad bean (*Vicia faba* L) (Liao et al., 2003) have verified this (Table 1). Zn is also a toxic element in soil–plant systems, although some authors consider toxicity of Zn lower than that of Cd (Aery and Jagetiya, 1997). Addition of Zn^{2+} to the Cd^{2+}-polluted soils enhanced the injury to growth of kidney bean plants (Fig. 1). Zn^{2+} itself can cause toxic effects on plants (Table 1); on the other hand, it can also compete with Cd^{2+} on soil complex sites, leading to more Cd^{2+} entering the soil solution and relatively enhancing available Cd^{2+} contents.

Our previous batch experiment indicated that acid rain not only led to soil acidification but also to release of heavy metals from the soils, mostly due to exchange processes of external cations with soil Ca^{2+}, Mg^{2+}, Al^{3+} and heavy metals. Desorption ratios of soil heavy metals were directly related to acidity of acid rain and contents of base cations. More Ca^{2+}, Mg^{2+} and less Al^{3+} would be released from the soils experimentally polluted by heavy metals and acid rain than from the unpolluted soils (Liao et al., 2005). Thus application of acid rain to the soil polluted by Cd^{2+} and/or Zn^{2+} would lead to three results: (1) direct injury of acid rain to plants, (2) Ca^{2+} deficit, caused by leaching of acid rain, enhancing the effect of Cd^{2+} toxicity (Skorzynska Polit et al., 1998), and (3) more soil available Cd^{2+} and Zn^{2+} to plants. Therefore, the serious complex toxic effects of Cd^{2+}, Zn^{2+}, and acid rain on growth of kidney bean plants in our experiment were reasonable (Fig. 1).

When plants live in a polluted environment, a large number of free radicals will be produced, causing damage to the plant cells. Under this condition, activities of SOD and POD in plants will change automatically to remove these free radicals. Therefore, activities of SOD and POD in plant cells are often used to show injury degrees of plants due to pollution. However, activities of SOD and POD generally increase at the early stage of plants suffering pollution. Once the pollution becomes more severe (for example, Cd^{2+} concentration higher than 5 mg/kg, or Zn^{2+} higher than 60 mg/kg, or pH of acid rain lower than 3.5 in our experiment) and injury symptoms of plants become more apparent, activities of SOD and POD in these plants will decrease. This makes judgment on toxic effects of pollutants confused, as some cases in our experiment (Table 3).

A study on effects of surfactants on aquatic plants indicated that MDA contents in the plant tissues were positively related to surfactant concentrations in the solutions, and MDA reflected environmental pollution levels (Liu, 2001). Two similar positive relations were also obtained from our experiment: (1) between the MDA contents in the kidney bean leaves and the contents of Cd^{2+} or Zn^{2+} added to the soils, and (2) between the MDA contents and the acidity of acid rain (Fig. 2). This means that MDA content in plant tissues is a useful index to evaluate pollution levels and to judge toxic effects of pollutants.

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