Bull. Environ. Contam. Toxicol. (2003) 71:998–1004

DOI: 10.1007/s00128-003-0225-8

Environmental Contamination and Toxicology

Combined Toxic Effects of Cadmium and Acid Rain on *Vicia faba* L.

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Received: 31 August 2002/Accepted: 24 June 2003

Cadmium is an environmental toxic element and widely exists in different soils (Fan 1991). Many studies demonstrate high contents of Cd^{2+} in soil inhibiting plant growth (Aery and Jagetiya 1997; Bhupal Raj and Patnaik 1999; Belimov and Dietz 2000). The main reasons are probably injury to plant organs (Lagriffoul et al. 1998; Reed et al. 1999), and changes of plant protective enzymes (Chen et al. 1998; Jain et al. 1999; Chien et al. 2001). Some authors discussed the relations of soil bio-available Cd and other elements, such as P (Jentschke et al. 1999), Ca (Skorzynska Polit et al. 1998), Ca, Mg, K, Zn, Cu, Pb, Ni (Obata and Umebayashi 1997; Keltjens et al. 1998), because toxic effects of Cd^{2+} on plants depend on various factors in the environment. However, few studies emphasized effects of acid rain on Cd^{2+} toxicity to plants. Hunan, a province in southern China, is located in the center area of acid rain; meanwhile, Hunan is rich in heavy metal minerals. Pollution of both heavy metals (including Cd^{2+}) and acid rain is increasingly severe in this area. It is of importance to consider combined influences of Cd^{2+} and acid rain on plant growth. *Vicia faba* L. was selected as the tested plant because it is easily found in this area and commonly used in toxicological experiments. The primary objective of this study was to investigate combined toxic effects of Cd^{2+} and acid rain on *Vicia faba* L. through a pot experiment.

MATERIALS AND METHODS

Soil sample for this pot experiment was collected from Changsha County, an unpolluted area, and contained low heavy metal contents. The soil sample was air-dried, passed through a 2 mm sieve, and weighed 5.0 kg to each of 15 plastic tubs. These 15 tubs were divided into three groups. $Cd(CH_2COO)_2$ (pure) was added to each group to make external Cd^{2+} contents be 0.0, 4.0, 6.0, 8.0, 10.0 mg/kg, respectively. Two weeks later 10 *Vicia faba* L. seedlings (Qidou 2) were planted in each tub. The first group was watered with distilled water (dw), the second and third groups were watered with simulated acid rain (pH 4.5 and 3.5, respectively). Heights of the *Vicia faba* L. plants were measured 90 days later. Before blossoming, injured symptoms were recorded (photos were taken), protective enzymes (surperoxide dismutase SOD and peroxidase POD) in the leaf and root tissues were determined, and the ultra-structures of leaf cells were observed on transmission electronic microscope H600. Determination of SOD and POD was followed the

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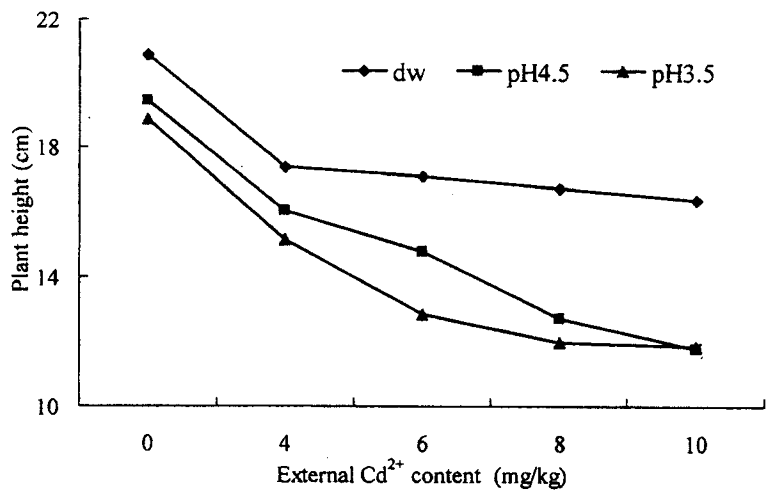

Figure 1. Height of *Vicia faba* L. plants treated with different Cd^{2+} concentrations and different simulated acid rain.

procedures in Methods of Plant Bio-Chemistry Analysis (Jin and Ding 1981), measurement of chlorophyll a contents was described in Analytical Technology of Plant Physiological and Biochemistry (Bai et al. 1995), and observation of ultra-structures of leaf cells was the same as in the study of Liu et al. (2001).

RESULTS AND DISCUSSION

Heights of *Vicia faba* L. decreased with increasing soil external Cd^{2+} contents and with decreasing pH values of acid rain (Figure 1), which shows that soil Cd^{2+} and/or acid rain could inhibit growth of *Vicia faba* L. seedlings, especially for the condition with higher Cd^{2+} contents and higher acidity of rain water. The plants grew in soils without external Cd^{2+} had higher heights than those grew in soils containing external Cd^{2+}. Watered with distilled water, the plants had almost same heights although they grew in soils containing various external Cd^{2+} contents. For the plants watered with acid rain (pH 4.5 and 3.5, respectively), however, the heights decreased continuously with increasing Cd^{2+} contents in soils, and were much shorter than those watered with distilled water, meaning acid rain (pH 4.5 and 3.5) accelerating toxic effects of Cd^{2+} on growth of *Vicia faba* L. plants. During the processes of cultivation, we found that the survival ratios of *Vicia faba* L. seedlings were closely related to soil Cd^{2+} contents and pH values of rain water. Generally, higher survival ratios were found in the soils with lower Cd^{2+} contents and watered with high pH solutions.

Injury symptoms of *Vicia faba* L. plants treated with external Cd^{2+} and acid rain were observed on the leaves and roots by comparing with the control condition (Figure 3). The normal root system was very flourishing with many nodules, and the root color was very fresh (Figure 3a). Treated with moderate Cd^{2+} (6.0 mg/kg)

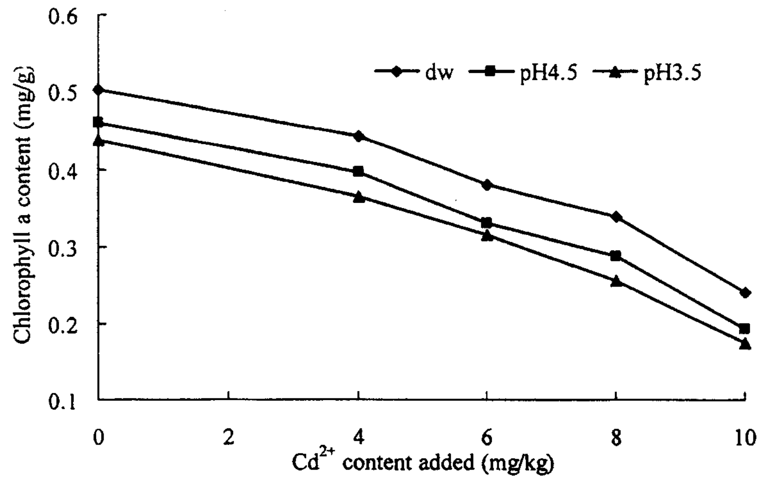

Figure 2. Chlorophyll a content in the leaf tissues of *Vicia faba* L. treated with different Cd^{2+} concentrations and different simulated acid rain.

and moderate simulated acid rain (pH 4.5), the plant height was much shorter. In this case, the old leaves were much easier to die, the number of lateral roots was much fewer, there were no nodules on the root system, and the root color was brown (Figure 3b). In the experiment, the root color became dark brown with increasing external Cd^{2+} contents and decreasing pH values of acid rain. There were some death cells on the root outer layer, which apparently indicates that combined toxic effects of Cd^{2+} and acid rain on growth of *Visia faba* L. plants appeared first from the root system.

Ultra-structures of *Vicia faba* L. leaf cells observed on electronic microscope were given in Figure 4. In the control condition, the membrane of chloroplasts was smooth and there were many thylakoids inside (Figure 4a). Under complex pollution of Cd^{2+} and acid rain, however, the chloroplasts deformed, thylakoids decomposed, and many black small balls scattered among the chloroplast matrix (Figure 4b). This phenomenon had also been observed in the chloroplasts of *Trapa bicornis* Osbeck and in *Hydrilla verticillata* leaf cells due to Cd^{2+} pollution (Li and Shi 1999; Shi et al. 2000).

When plants live in a polluted environment, a large number of free radicals will be produced. The protective enzymes, such as SOD, POD, and CAT, could clean some of these free radicals, and the others could cause damage to the plant cells. Therefore, the activities of SOD and POD in plant cells are useful indicators to show the injury symptoms of plants.

Generally, SOD activities in both leaf and root tissues of *Vicia faba* L. plants were declined with increasing external Cd^{2+} contents and decreasing pH values of acid

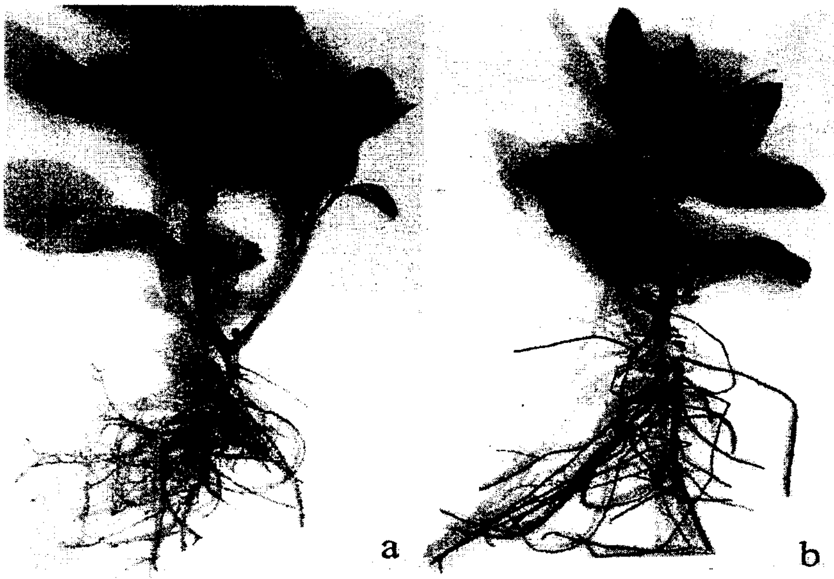

Figure 3. *Vicia faba* L. plants treated with external Cd^{2+} and simulated acid rain (a: treated with 0.0 mg/kg of Cd^{2+} and distilled water, as the control; b: treated with 6.0 mg/kg of Cd^{2+} and simulated acid rain of pH 4.5).

Table 1. SOD activities in the tissues of *Visia faba* L. (NU/g)

External Cd^{2+} (mg/kg)	Leaf tissue			Root tissue		
	distilled water	pH 4.5	pH 3.5	distilled water	pH 4.5	pH 3.5
0.0	57.3	62.0	52.2	74.8	60.4	50.8
4.0	55.5	53.9	46.8	66.7	48.2	45.7
6.0	55.4	52.6	46.1	63.8	47.5	42.2
8.0	51.7	50.9	45.0	58.9	44.4	40.3
10.0	47.9	48.4	43.6	51.2	41.6	39. 6

rain (Table 1). This demonstrates that the *Vicia faba* L. plants produced many free radicals and consumed a great amount of SOD to resist complex pollution of Cd^{2+} and acid rain. Cd^{2+} existing in soils resulted in decreasing SOD activities, which agreed with the study of Chen et al. (1998); meanwhile acid rain, especially for serious acid rain with pH 3.5, also led to decreasing SOD activities. SOD activities in the *Vicia faba* L. root tissues were lower than those in the leaf tissues, which shows that the root tissues of this plant are more sensitive to combined pollution of Cd^{2+} and acid rain than the leaf tissues.

POD is an induced enzyme, although it is also involved in the processes of cleaning free radicals of plants. More serious polluted environment could induce plants to

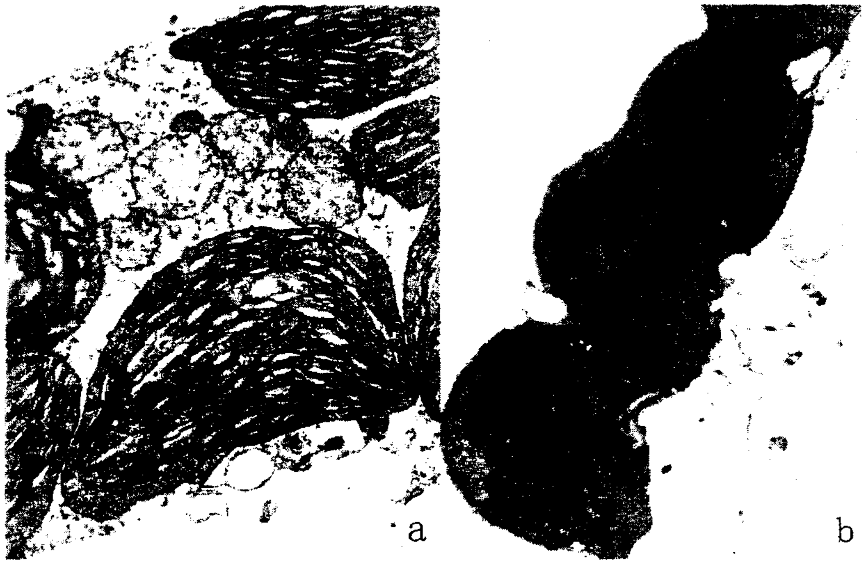

Figure 4. Chloroplasts in the *Vicia faba* L. leaf cells (a: treated with 0.0 mg/kg of Cd^{2+} and distilled water, as the control; b: treated with 6.0 mg/kg of Cd^{2+} and simulated acid rain of pH 4.5).

Table 2. POD activities in the tissues of *Visia faba* L. ($\times 10^2$ NU/g)

External Cd^{2+} (mg/kg)	Leaf tissue			Root tissue		
	distilled water	pH 4.5	pH 3.5	distilled water	pH 4.5	pH 3.5
0.0	3.91	4.19	5.54	12.1	15.0	17.3
4.0	3.80	4.90	5.77	14.2	18.0	23.2
6.0	3.75	4.01	4.82	15.6	20.2	22.0
8.0	3.64	3.79	3.96	16.1	17.9	19.1
10.0	3.30	3.41	3.80	20.0	15.7	13.7

produce more peroxides and enhance POD activities. Therefore higher POD activities reflect more serious damages happened on plant organs. In our experiment, POD activities in the *Vicia faba* L. root tissues were much higher than those in the leaf tissues (more than 3 times, see Table 2). Because of serious complex pollution of Cd^{2+} and acid rain, some of *Vicia faba* L. roots and leaves were dying, so that POD activities declined rapidly after reached maximum levels. For the plants treated with different Cd^{2+} levels but watered with distilled water, POD activities increased rapidly in the root tissues and decreased slowly in the leaf tissues with increasing external Cd^{2+} contents. This was probably due to intense reaction of free radicals in the root tissues mitigating the pressure in the leaf tissues. POD could clean part of free radicals to protect plant tissues from damages.

Meanwhile, it could induce to synthesize some carbon materials those are waterproof and protect the tissues in deep layer from damages. Hence we observed dark color (brown) on *Visia faba* L. roots in the polluted environment of Cd^{2+} and acid rain (see Figure 3b). This color became darker with increasing external Cd^{2+} contents and decreasing pH values of acid rain.

Acknowledgments. This work is part of the key project of the Chinese Ministry of Education ([2000] 156-00209) and the cooperation project of the Chinese Ministry of Science and Technology and the French Ministry of Research (PRA E 00-04), partly financially supported from the Education Commission of Hunan Province. Presented at the First International Conference on Pollution Eco-Chemistry and Ecological Processes, Shenyang, China, August 26-31, 2002.

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