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Response of Soybean Seed Germination to Cadmium and Acid Rain

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Abstract Cadmium (Cd) pollution and acid rain are the main environmental issues, and they often occur in the same agricultural region. Nevertheless, up to now, little information on the combined pollution of Cd^{2+} and acid rain action on crops were presented. Here, we investigated the combined effect of Cd^{2+} and acid rain on the seed germination of soybean. The results indicated that the single treatment with the low level of Cd^{2+} (0.18, 1.0, 3.0 mg L^{-1}) or acid rain (pH ≥3.0) could not affect the seed germination of soybean, which was resulted in the increased activities of peroxidase and catalase. The single treatment with the high concentration of Cd^{2+} (>6 mg L^{-1}) or acid rain at pH 2.5 decreased the activities of peroxidase and catalase, damaged the cell membrane and then decreased the seed germination of soybean. Meanwhile, the same toxic effect was observed in the combined treatment with Cd^{2+} and acid rain, and the combined treatment had more toxic effect than the single treatment with Cd^{2+} or acid rain. Thus, the combined pollution of Cd^{2+} and acid rain had more potential threat to the seed germination of soybean than the single pollution of Cd^{2+} or acid rain.

Keywords Combined pollution · Cadmium · Acid rain · Soybean seed · Germination

Introduction

Cadmium (Cd) is widely used in many fields, including smelting, plating, plastic and dye manufacturing [1–4]. The use has led to the accumulation of Cd in soil [5, 6]. Excess concentration of Cd^{2+} in soil has caused the decrease in the growth, development and yield

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of crop [7]. Some toxic symptoms, such as poor growth, stunted growth and chlorosis, have been observed in plants exposed to the excess concentration of Cd^{2+} [8].

Acid rain has been a well-known environmental problem for decades in the world [9–11]. Acid rain can cause soil acidification [10, 12]. Thus, acid rain directly and indirectly inhibits the growth of crops [9, 13–15]. Many groups have reported the effect of acid rain on the growth of crop and ascertained the maximum pH value of acid rain in which the yield of crop is decreased [9, 13–15]. Acid rain and Cd^{2+} pollution often occur in the same agricultural region. Meanwhile, acid rain can increase the content of Cd^{2+} in soil, and the increased Cd^{2+} can be utilized by crop [16]. Recently, some researchers have studied the combined effect of acid rain and Cd^{2+} on the growth of crops and have found that acid rain increases the toxic effect of Cd^{2+} on *Vicia faba* L. and *Phaseolus vulgaris* L. [17, 18]. These studies have been conducted using crops at the seedling stage. Seed germination is the beginning of crop life, and it directly affects the growth and biomass of crop [19]. Therefore, it is important to investigate the combined effect of Cd^{2+} and acid rain on the seed germination of crop.

In the present work, the combined effects of Cd^{2+} and acid rain on the germination percentage, germination energy, germination index, vigour index, activities of catalase (CAT) and peroxidase (POD), content of malonydiadehyde (MDA) and membrane permeability of soybean during seed germination were investigated. The objective was to reveal the combined effect of Cd^{2+} and acid rain on the seed germination of soybean. The results can provide theoretical basis for the early monitoring and risk assessment of Cd^{2+} pollution and acid rain.

Materials and Methods

Plant Material and Treatments

It was reported that the average pH value and concentration of Cd^{2+} in soil (Jiangsu province, China) was 5.93 and 0.18 mg mg^{-1}, respectively [20]. Thus, six pH values of acid rain (pH 2.5, 3.0, 3.5, 4.0, 5.0, 5.9) and seven concentrations of Cd^{2+} (0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1}) were selected in the present work. Three solutions were prepared as follow: (1) the stock solution of the simulated acid rain at pH 1.0 was prepared with the solution of H_2SO_4 and HNO_3 in the ratio of 4.7 (*v*/*v*) by chemical equivalents, according to the general anion composition of rainfall in Southern China [21] and then diluted to pH 2.5, 3.0, 3.5, 4.0, 5.0 and 5.9. (2) The 1 gL^{-1} cadmium nitrate ($Cd(NO_3)_2$) stock solution was freshly prepared by dissolving appropriate quantities of cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$) in deionized water. The solutions of Cd^{2+} (0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1}) were prepared from the stock solution by serial dilution with deionized water. (3) The complex solution of Cd^{2+} and acid rain was prepared by dissolving appropriate quantities of $Cd(NO_3)_2 \cdot 4H_2O$ in the simulated acid rain.

Soybean seeds (Su Zao No.1) were obtained from Wuxi Seed CO., LTD, China. Soybean seeds were sterilized with $HgCl_2$ (0.1%) solution for 8 min, rinsed with deionized water for several times. The seeds were divided into four groups and treated with Cd^{2+} and acid rain: (1) the control group: soybean seeds were soaked in deionized water (pH 7.0); (2) single treatment with acid rain: soybean seeds were soaked in the simulated acid rain at pH 2.5, 3.0, 3.5, 4.0, 5.0 and 5.9; (3) single treatment with Cd^{2+}: soybean seeds were soaked in the solution of Cd^{2+} (0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1}); (4) combined treatment with Cd^{2+} and acid rain: soybean seeds were soaked in the complex solution of

Cd^{2+} and acid rain. Seeds with the four treatments mentioned above were placed in the culture dishes (pasteurized in oven at 100 °C for 1 h) with filter sheets (θ=15 cm) to germinate in the culture container kept at a constant temperature of 25 °C. Each dish received 50 healthy seeds. Each treatment was performed in triplicate. During germination, about 30 mL treatment solution (deionized water with pH 7.0, acid rain, Cd^{2+} solution, the complex solution of Cd^{2+} and acid rain) was added to each dish, so that about half the volume of each seed was immersed. The treatment solution was replaced every day. Germinated seeds were counted, and germination percentage, germination energy, germination index and vigour index were then calculated after seeds were germinated for a week. Seeds of each treatment were sampled for the determination of the activities of POD and CAT, content of MDA and membrane permeability.

Determination

Germination percentage was: GP=germinated seeds/total seeds×100.

Germination energy was the germination percentage of seeds on the third day of germination.

Germination index was expressed as Eq. 1:

$$\mathrm{GI} = \sum (Gt/Dt) \tag{1}$$

where GI is the germination index, *Gt* and *Dt* are the amount of the germinated seeds and the germination time, respectively.

Vigour index was expressed as Eq. 2:

$$\mathrm{VI} = S \times \sum (Gt/Dt) \tag{2}$$

where VI is the vigour index of seed, *S* is the growth vigour of seedling in the certain time (expressed with the average fresh weight of a seedling), *Gt* and *Dt* are the amount of the germinated seeds and the germination time, respectively.

The level of lipid peroxidation was expressed as the content of MDA [22]. Samples were repeatedly and ultrasonically extracted with ethanol/water (80/20, *v/v*) containing 1 mg L^{-1} butylated hydroxytoluene (BHT). After centrifugation, supernatants were pooled, and an aliquot of appropriately diluted sample was added to a test tube with an equal volume of either (1) −TBA solution containing 20% (*w/v*) trichloroacetic acid (TBA) and 0.01% (*w/v*) BHT, or (2) +TBA solution containing the above plus 0.65% TBA. Samples were heated at 95°C for 25 min, and after cooling, the absorbance was read at 440, 532 and 600 nm. The content of MDA is expressed as Eq. 3:

$$\mathrm{MDA}\left(\mathrm{nmol\,ml^{-1}}\right) = 10^6 \times \frac{A - B}{157000} \tag{3}$$

where $A = \frac{\mathrm{Abs}_{532+\mathrm{TBA}} - \mathrm{Abs}_{600+\mathrm{TBA}}}{\mathrm{Abs}_{532-\mathrm{TBA}} - \mathrm{Abs}_{600-\mathrm{TBA}}}$
$B = (\mathrm{Abs}_{440+\mathrm{TBA}} - \mathrm{Abs}_{600+\mathrm{TBA}}) \times 0.0571$

Here, 157000 was the molar extinction coefficient for MDA. The molar absorbance of 1–10 mM sucrose at 532 and 440 nm was 8.4 and 147, respectively, giving a ratio of 0.0571.

Frozen fresh tissue were ground to fine powder with a mortar and pestle under liquid nitrogen. The proteins were then extracted at 4 °C by grinding with a cold 50 mM potassium phosphate (pH 7.0). The homogenate was centrifuged at 4 °C for 20 min at 12,000×*g*. The supernatant was used for the measurement of enzymatic activity. The activity of POD was measured by spectrophotometric method [23]. The reaction mixture contained phosphate buffer (pH 7.0, 25 mM), guaiacol (0.05%), 10 mM H_2O_2, and the activity of POD was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E=26.6 mM^{-1} cm^{-1}). The activity of CAT was measured by titration method [24]. In the measurement, H_2O_2 was used as reaction substrate. Residual H_2O_2 was oxidized by $KMnO_4$ (0.1 M) standard solution. The activity of CAT was expressed by the amount of enzyme that decomposes H_2O_2 per gramme (fresh weight) within 10 min.

Membrane permeability was measured by relative conductivity method described by the previous report [25]. Seeds were rinsed, placed into 40 mL deionized water and gently tumbled at ambient room temperature. Conductance of deionized water was measured after 15 min (C_1), 2 h (C_2), and 2 h after a freeze–thaw treatment (C_{total}). The rate of electrolyte leakage was expressed as $\%/h=100\times(C_2-C_1)/(1.75\ C_{total})$.

Statistical Analysis

Each treatment was performed in triplicate. The data were analyzed through ANOVA using SPSS 11.5 and origin 8.0. Student's t test was applied to determine the significance between different treatments. Statistical significance was set at the $p\leq0.05$ confidence level.

Results

Germination Indexes of Soybean Seeds

The germination indexes (germination percentage, germination energy, germination index and vigour index) of soybean seeds treated with Cd^{2+} and acid rain were listed in Table 1. As shown in Table 1, the single treatment with the low concentration of Cd^{2+} (0.18, 1.0, 3.0 mg L^{-1}) could not change the germination percentage and germination energy of soybean. The single treatment with 6 mg L^{-1} Cd^{2+} decreased the germination percentage and germination energy of soybean. The decrease was more obvious with increasing the concentration of Cd^{2+}. The single treatment with acid rain at pH 2.5 severely decreased the germination percentage and germination energy of soybean. The single treatment with acid rain at the other test pH value could not affect the germination percentage and germination energy of soybean. When the soybean seeds were treated with 0.18, 1.0 or 3.0 mg L^{-1} Cd^{2+} and acid rain at pH ≤4.0, the germination percentage and germination energy of soybean were decreased compared with those of the control soybean seeds. The results indicated that the combined treatment with the low concentration of Cd^{2+} and the high pH value of acid rain showed the toxic effect on the seed germination of soybean. The combined treatment with the high concentration of Cd^{2+} (6.0, 10.0, 20.0, 50.0 mg L^{-1}) and acid rain at all test pH value decreased the germination percentage and germination energy of soybean. The decrease was higher than that of soybean treated with the single Cd^{2+} or acid rain. The results indicated that the combined treatment with Cd^{2+} and acid rain increased the potential toxic effect of the single Cd^{2+} or acid rain on the seed germination of soybean.

Germination Index and Vigour Index of Soybean Seeds

It was also observed from Table 1 that the single treatment with the low concentration of Cd^{2+} (0.18, 1.0, 3.0, 6.0 mg L^{-1}) could not affect the germination index and vigour index of

Table 1 Germination index of soybean treated with Cd^{2+} and acid rain

Cd^{2+} concentration (mg L^{-1})	AR (pH)	Germination percentage (%)	Germination energy (%)	Germination index (%)	Vigor index (%)
0.0	7.0	92.00aAB	85.00aA	43.89aA	30.83aAB
	2.5	86.00bA	80.00bA	40.31cA	27.68bA
	3.0	90.00abA	83.00abA	42.78bcA	30.45aA
	3.5	92.00aA	85.00aA	44.57abA	31.61aA
	4.0	94.00aA	86.33aA	45.09aA	31.58aA
	5.0	93.00aA	85.33aA	44.17aA	31.39aA
	5.9	92.33aAB	85.00aA	43.89aA	31.25aA
0.18	7.0	93.00aA	84.00aA	43.59aA	31.23aAB
	2.5	82.00cAB	76.00dB	40.09cA	27.59cA
	3.0	85.00cB	77.33dB	41.20bcA	28.95bcA
	3.5	86.00bBC	78.67cdB	41.33bcBC	29.74abB
	4.0	88.67bBC	80.00cB	42.22abB	30.30abA
	5.0	91.00abAB	81.00bcB	42.44abB	30.34abAB
	5.9	92.00aAB	83.00abA	43.50aA	31.25aA
1.0	7.0	94.00aA	83.00aA	43.54aA	31.41aB
	2.5	85.33eA	76.67bB	40.93cA	28.29bA
	3.0	86.00deAB	78.33abAB	41.57bcA	29.24bA
	3.5	88.67cdeB	81.00abB	42.31abcBC	30.60aAB
	4.0	90.00bcdAB	82.00aB	42.69abB	30.68aA
	5.0	92.00abcA	82.00aAB	43.50aAB	31.04aAB
	5.9	93.00abA	82.33aA	43.31aA	31.12aA
3.0	7.0	90.00aBC	75.67aB	43.20aA	30.05aAB
	2.5	81.00cB	66.67eC	39.56dA	25.93cB
	3.0	83.33cBC	67.00eC	39.69cB	26.69bB
	3.5	84.00bcBC	68.00deC	40.46bcCD	27.42bC
	4.0	85.00abC	71.00cdC	40.80bC	28.87abB
	5.0	86.67abB	72.00bcC	40.98bC	28.91abBC
	5.9	88.00abBC	74.00abB	43.06aA	29.97aB
6.0	7.0	86.67aCD	68.00aC	42.76aA	29.30aA
	2.5	77.00cC	55.00cD	36.91eB	24.29cB
	3.0	81.00bcC	58.00cD	38.39deC	25.64bcB
	3.5	82.00abcCD	63.33bD	39.28cdD	27.18abC
	4.0	83.33abcCD	65.00abD	39.61cdC	27.22abB
	5.0	85.00aBC	66.67abD	40.50bcC	27.74abC
	5.9	86.00abCD	67.00aC	41.65abB	28.59aC
10.0	7.0	83.33aD	64.00aD	40.57aB	27.21aC
	2.5	70.00cD	51.00dE	29.24eC	19.24eC
	3.0	75.00bD	54.00cdE	32.06dD	21.46dC
	3.5	78.00abD	56.67bcE	34.83cE	23.35cdD
	4.0	81.00aD	57.00bE	35.61cD	24.75bcC
	5.0	82.00aC	58.00bE	37.98bD	25.92abD
	5.9	82.33aD	63.33aD	40.02abC	26.75aD
20.0	7.0	75.00aE	67.00aCD	38.80aC	25.62aD

Table 1 (continued)

Cd^{2+} concentration (mg L^{-1})	AR (pH)	Germination percentage (%)	Germination energy (%)	Germination index (%)	Vigor index (%)
	2.5	53.33dE	48.00eF	27.22eD	16.50dD
	3.0	60.00cE	54.00dF	31.02dE	19.80cD
	3.5	65.00bcE	56.67cdF	33.33cdF	21.86bD
	4.0	70.00abE	61.00bcF	35.93bcD	24.17aC
	5.0	73.33aD	64.00abF	37.04abD	24.44aD
	5.9	74.00aE	66.67aD	37.78abD	24.79aE
50.0	7.0	25.00aF	22.00aE	15.09aD	9.99aE
	2.5	8.00eF	8.00eG	5.56eE	3.42eE
	3.0	14.00dF	12.00deG	8.33dF	4.92deE
	3.5	18.00cF	15.00cdG	10.83cdG	6.97cdE
	4.0	21.00bcF	17.00bcG	12.41bcE	7.91bcD
	5.0	22.00abcE	21.00abG	13.80abE	9.05aE
	5.9	24.00abF	21.33abE	14.54abE	9.40abF

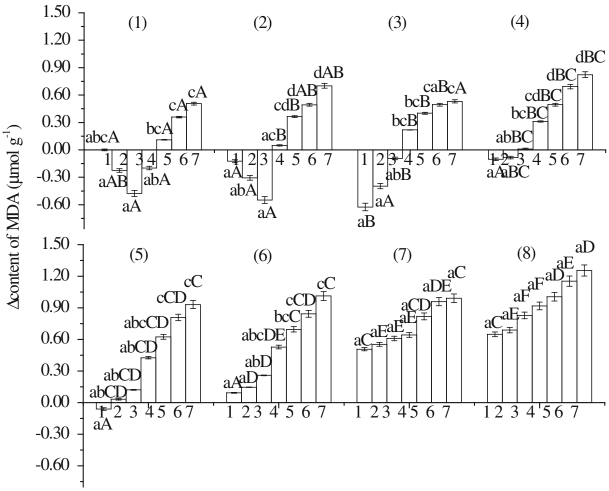
Values represent the average of three samples. Significant differences at $p<0.05$ were showed with different letter in the same line. Lowercase letters shows the difference between the treatments with the same concentration and the different pH value. Uppercase letters shows the difference between the treatments with the same concentration and the different pH value

soybean. When the concentration of Cd^{2+} was increased to 10 mg L^{-1}, the germination index and vigour index of soybean were decreased compared with those of the control soybean. The decrease depended on the increase in the concentration of Cd^{2+}. The change in the germination index and vigour index of soybean treated with the single acid rain was the same as that of the germination percentage and germination energy of soybean. When soybean seeds were treated with Cd^{2+} and acid rain, germination index and vigour index were decreased compared with those of the control soybean, and the decrease was higher than that of the soybean treated with the single Cd^{2+} or acid rain.

MDA Content of Soybean Seeds

Figure 1 showed the content of MDA in soybean seeds treated with Cd^{2+} and acid rain. It was found from Fig. 1 that the treatment with the single Cd^{2+} firstly decreased and then increased the content of MDA in soybean seeds with increasing the concentration of Cd^{2+}. When the concentration of Cd^{2+} was 1 mg L^{-1}, the content of MDA in soybean seeds was minimum. When the pH of acid rain was lower than 4.0, the treatment with the single acid rain increased the content of MDA in soybean seeds. When the pH of acid rain was equal to or higher than 4.0, the treatment with the single acid rain could not affect the content of MDA in soybean seeds. When soybean seeds were treated with 1.0 mg L^{-1} Cd^{2+} and acid rain at pH 4.0, the content of MDA in soybean seeds was increased compared with that of the control soybean seeds. The combined treatment with 10 mg L^{-1} Cd^{2+} and acid rain at all test pH value increased the content of MDA in soybean seeds, and the increase was higher than that of soybean treated with the single Cd^{2+} or acid rain. The results indicated that the combined treatment with Cd^{2+} and acid rain increased the potential toxic effect of the single Cd^{2+} or acid rain on the cell membrane.

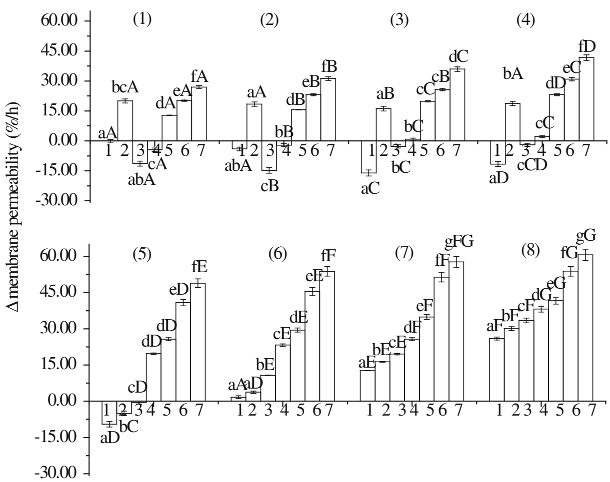
Fig. 1 Effect of Cd^{2+} and acid rain on the content of MDA during the seed germination of soybean. Δ content of MDA shows the difference in the content of MDA between the treatment group and the control group. At the same concentration of Cd^{2+}, values followed by the same *lowercase letters* are not significantly different at $p<0.05$. At the same pH, values followed by the *uppercase letters* are not significantly different at $p<0.05$. *1–7* represent pH 7.0, 5.9, 5.0, 4.0, 3.5, 3.0 and 2.5 acid rain, respectively; *(1)–(8)* represent 0, 0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1} Cd^{2+}, respectively



Membrane Permeability of Soybean Seeds

The membrane permeability in soybean seeds treated with Cd^{2+} and acid rain was shown in Fig. 2. It was observed from Fig. 2 that when the concentration of Cd^{2+} was higher than 1 mg L^{-1}, the membrane permeability of soybean seeds treated with the single Cd^{2+} was increased with increasing the concentration of Cd^{2+}. When the concentration of Cd^{2+} was lower than 10 mg L^{-1}, the membrane permeability was decreased compared with that of the control soybean. The single treatment with acid rain at the high pH value (pH >3.5, except pH 5.9) decreased the membrane permeability of soybean. The opposite phenomenon was observed in soybean treated with the single acid rain at the low pH value (pH ≤3.5). For the combined treatment with Cd^{2+} and acid rain, the membrane permeability in soybean was higher than that of the control soybean, and the increase was higher than that of the single treatment with Cd^{2+} or acid rain. The results indicated that acid rain aggravated the damage to the cell membrane of soybean exposed to Cd^{2+}; in turn, Cd^{2+} also aggravated the damage to the cell membrane of soybean under acid rain.

Fig. 2 Effect of Cd^{2+} and acid rain on the membrane permeability during the seed germination of soybean. Δ membrane permeability shows the difference in the membrane permeability between the treatment group and the control group. At the same concentration of Cd^{2+}, values followed by the same *lowercase letters* are not significantly different at $p<0.05$. At the same pH, values followed by the *uppercase letters* are not significantly different at $p<0.05$. *1–7* represent pH 7.0, 5.9, 5.0, 4.0, 3.5, 3.0 and 2.5 acid rain, respectively; *(1)–(8)* represent 0, 0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1} Cd^{2+}, respectively



Antioxidant Enzyme Activities of Soybean Seeds

The activities of CAT and POD in soybean treated with Cd^{2+} and acid rain were given in Figs. 3 and 4, respectively. It was shown in Figs. 3 and 4 that the single treatment with the low concentration of Cd^{2+} (0.18, 1, 3, 6 mg L^{-1}) increased the activities of CAT and POD in soybean during seed germination, indicating that the single treatment with the low concentration of Cd^{2+} increased the ability of CAT and POD to remove free radicals. The opposite effect was observed in the activities of CAT and POD in the single treatment with the high concentration of Cd^{2+} ($\geq$10 mg L^{-1}), indicating that the single treatment with the high concentration of Cd^{2+} decreased the ability of CAT and POD to remove the free radicals. It was also observed from Figs. 3 and 4 that compared with those of the control soybean, the activities of CAT and POD in soybean treated with the single acid rain at pH 4.0–5.9 were increased, while the activities of CAT and POD in soybean treated with the single acid rain at pH 2.5, 3.0 and 3.5 were decreased. For the combined treatment with Cd^{2+} and acid rain, the minimum concentration of Cd^{2+} in which the activities of CAT and POD were obviously lower than that of the control soybean was smaller than that of soybean treated with the single Cd^{2+}. Meanwhile, the pH of acid rain at which the activities of CAT and POD was obviously lower than that of the control soybean was higher than that of soybean treated with the single acid rain. Obviously, the combined effect of Cd^{2+} and acid rain on the activities of CAT and POD was more obvious than that of the single Cd^{2+} or acid rain.

Discussions

It is well known that germination percentage is an important indicator used to evaluate seed quality. Germination energy represents the seed vigour. Germination index and vigour index can reflect the germination status. Here, germination percentage, germination energy, germination index and germination vigour were selected to evaluate the combined effect of Cd^{2+} and acid rain on the seed germination of soybean.

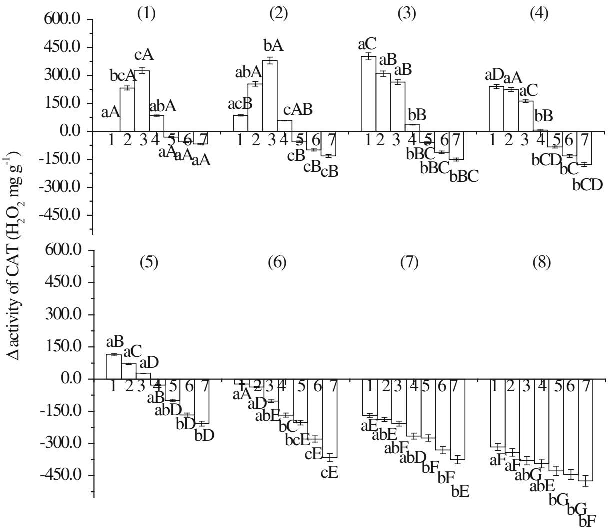
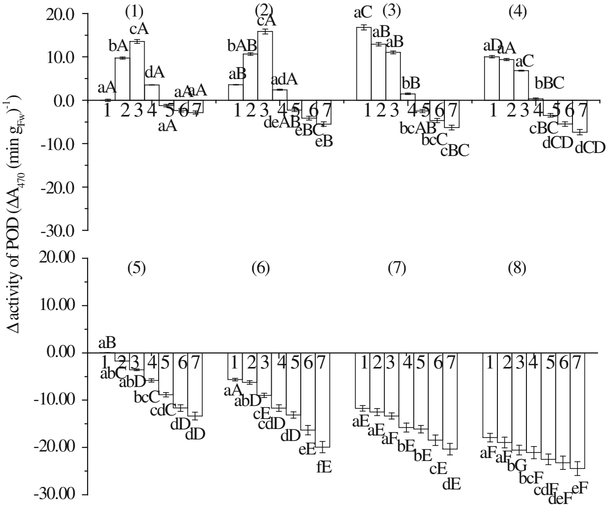


Fig. 3 Effect of Cd^{2+} and acid rain on the activity of CAT during the seed germination of soybean. Δ activity of CAT shows the difference in the activity of CAT between the treatment group and the control group. At the same concentration of Cd^{2+}, values followed by the same *lowercase letters* are not significantly different at $p<0.05$. At the same pH, values followed by the *uppercase letters* are not significantly different at $p<0.05$. *1–7* represent pH 7.0, 5.9, 5.0, 4.0, 3.5, 3.0 and 2.5 acid rain, respectively; *(1)–(8)* represent 0, 0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1} Cd^{2+}, respectively

Fig. 4 Effect of Cd^{2+} and acid rain on the activity of POD during activities of the seed germination of soybean. Δ activity of POD shows the difference in the activity of POD between the treatment group and the control group. At the same concentration of Cd^{2+}, values followed by the same *lowercase letters* are not significantly different at $p<0.05$. At the same pH, values followed by the *uppercase letters* are not significantly different at $p<0.05$. *1–7* represent pH 7.0, 5.9, 5.0, 4.0, 3.5, 3.0 and 2.5 acid rain, respectively; (*1*)–(*8*) represent 0, 0.18, 1.0, 3.0, 6.0, 10.0, 20.0, 50.0 mg L^{-1} Cd^{2+}, respectively



It was found that acid rain at pH ≥3.0 or the low concentration of Cd^{2+} (0.18 and 1 mg L^{-1}) could not change the germination percentage, germination energy, germination index and vigour index of soybean seeds; thus, these treatments could not affect the seed germination of soybean (Table 1). These concentrations of Cd^{2+} and the pH values of acid rain were safe to the seed germination of soybean. But the combined treatment with acid rain at pH 3.5, 4.0 and the low concentration of Cd^{2+} (0.18 and 1 mg L^{-1}) decreased the germination percentage, germination energy, germination index and vigour index of soybean (Table 1). The results indicated that the combined treatment with the safe concentration of Cd^{2+} and acid rain (H^+) has potential threat to the seed germination of soybean. Moreover, the combined treatment with the safe pH value of acid rain (such as pH 5.0 or 4.0) and the high concentration of Cd^{2+} (≥3 mg L^{-1}) decreased the seed germination of soybean. The same effect was observed in the combined treatment with the safe concentration of Cd^{2+} (0.18, 1 mg L^{-1}) and the low pH value of acid rain (pH 3.0, 2.5). The results indicated that when the soil is with the safe pH value and the high concentration of Cd^{2+}, the soil could threaten the seed germination of soybean. When the concentration of Cd^{2+} in the soil is safe and the pH value in the soil is low, the soil could also threaten the seed germination of soybean. Furthermore, the combined treatment with the high concentration of Cd^{2+} (≥3 mg L^{-1}) and the low pH value of acid rain (pH 3.0, 2.5) decreased the seed germination of soybean, and the decrease was more obvious than that of the single treatment with the high concentration of Cd^{2+} or the low pH value of acid rain. These results indicated that the combined pollution of Cd^{2+} (≥3 mg L^{-1}) and acid rain (pH 3.0, 2.5) has more threat to the seed germination of soybean than that of the single pollution of Cd^{2+} or acid rain.

The cell membrane is a selective permeable boundary around a cell or an organelle [26]. It controls the substances in and out cell and organelle, transfers signals from the outside to inside of cell, participates in the synthesis and assembly of substance in the cell and provides physical connections for cell construction and substances in the outside of cells [26]. Thus, the seed germination of plant depends on the integrity of structure and stability of function in the cell membrane. It has been reported that the abiotic stress can cause the excess accumulation of free radicals in plants [27, 28]. The excess free radicals oxidize the unsaturated fatty acids in the membrane lipid of cell [29], leading to the peroxidation of cell membrane lipid [30, 31]. Subsequently, the cell membrane is damaged, the selectivity of the cell membrane is destroyed and then the electrolyte leakage from the cytoplasm (the membrane permeability) is increased. Hence, the content of MDA and membrane permeability can reflect the injury degree of the cell membrane [30, 31]. CAT and POD are two major antioxidant enzymes, and they are relative to the seed germination of plant [19]. CAT and POD can effectively remove the free radicals (including O^{2-}, OH^{-}, H_2O_2, etc.) induced by the abiotic stress in order to protect the plant cell from the damage of abiotic stress [28]. It was found from our experimental results that the treatment with the single acid rain (pH ≥3.5) or Cd^{2+} (<6 mg L^{-1}) increased the activity of CAT and POD in soybean (Figs. 3 and 4). The increase could lead to the increase in the ability of CAT and POD to remove free radicals and then could make the soybean seeds maintain a balance in the production and scavenging of free radicals. Consequently, the content of MDA and cell membrane permeability was not obviously changed (Figs. 1 and 2), indicating the cell membrane was not damaged. Thus, the single treatment with the low concentration of Cd^{2+} and the high pH value of acid rain could not affect the seed germination of soybean (Table 1). The decrease in the activities of CAT and POD was observed in the single treatment with the low pH value of acid rain (such as pH 2.5) and the high concentration of Cd^{2+} (≥10 mg L^{-1}). The decrease in the activities of CAT and POD led to the increase in the content of MDA and membrane permeability and then the decrease in the seed germination of soybean. The combined treatment with Cd^{2+} and acid rain (including the safe concentration of Cd^{2+} and the safe pH value of acid rain) decreased the activities of CAT and POD, and then increased the content of MDA and the cell membrane permeability. The toxic effect was more obvious than that of the treatment with the single Cd^{2+} or acid rain. Thus, the decrease in the seed germination of soybean in the combined treatment with Cd^{2+} pollution and acid rain was resulted in the oxidative stress induced by the decrease in the activities of CAT and POD of soybean.

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