

**Separate and combined effects of Cu and Cd on seedling growth and active oxygen metabolism system of *Trifolium repens* L.**

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**1. ABSTRACT**

Pot-culture experiments were used to examine the individual and combined effects of Cu and Cd pollutants on *Trifolium repens* L. seedlings, both on their growth and their active oxygen metabolism system, mainly superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities. The results showed that the negative action took place at low concentrations of Cu (less than 500 ppm) and Cd (less than 0.5 ppm), which had no obvious effects on the seedlings' growth. However, as the concentrations of Cu and Cd increased (500-3000 ppm and 0.5-50 ppm respectively), synergistic activities was observed, showing obvious negative effects ( $P$  less than 0.05). Compared with the control samples, the seedlings affected by Cu and Cd pollutants were shorter and smaller, their fresh/dry weight and content of soluble protein decreased drastically, their leaf electric conductivity increased, and the contents of their leaf pigments decreased. Chlorophyll *a* was more sensitive than chlorophyll *b* to Cu and Cd pollutants, and chlorophyll *b* was more sensitive than carotenoid. It was also shown that the active oxygen metabolism of *T. repens* seedlings was destroyed by high amounts of Cu and Cd, the balance of the anti-oxidase system was broken, and the CAT and SOD activities noticeably decreased while POD activity evidently increased. Cd had a more noticeable effect on seedling growth than Cu.

**2. INTRODUCTION**

With increased knowledge and growing concern for the environment, the problem of soil pollution attracts more and more attention (1-6). Heavy metals, such as Cu and Cd, are toxic substances that pollute the environment. With the development of exploitative mining and metallurgy, many heavy metals, such as Cu and Cd, enter the environment, causing serious soil pollution (7). Wu et al. (8) and Hu et al. (9) studied the removal trends and transformation of heavy metal pollutants (Cu, Pb, Zn, etc.) in farmland ecosystems. Liu et al. (10), Song et al. (11), and Zhou et al. (12) reported the effects of heavy metal pollutants (Cd, Cu, As, etc.) on seed germination and seedling growth of crops. It was also well documented by Zheng and Chen (13), Yu et al. (14), and Wang et al. (15) that heavy metal pollutants (Cu, Cd, Pb, Zn, etc.) injured soil-crop systems. Furthermore, Abuzid (16), et al. thoroughly studied the effects of Cu on plants and other heavy metals on maize seedling growth. However, the research plants mentioned above are all agricultural crops such as rice, wheat, maize, etc., and there have been few reports regarding heavy metals' harm on reclamation plants in industrial and mining wastelands. In recent decades, with the development of exploitative mining, the wastelands of industry and mining are quickly expanding, and account for a large percentage of polluted soil.

**Table 1.** Experimental design of Cu and Cd treatments (ppm)

Cu (ppm)	Cd (ppm)				
	0	0.5	2.5	10	50
0	0	0 + 0.5	0 + 2.5	0 + 10	0 + 50
500	500 + 0	500 + 0.5	500 + 2.5	500 + 10	500 + 50
1000	1000 + 0	1000 + 0.5	1000 + 2.5	1000 + 10	1000 + 50
2000	2000 + 0	2000 + 0.5	2000 + 2.5	2000 + 10	2000 + 50
3000	3000 + 0	3000 + 0.5	3000 + 2.5	3000 + 10	3000 + 50

The zero in table represents no Cu or Cd was added to the soil.

Research on the effects of heavy metal pollutants on reclamation plants in the mineral wastelands has attracted many scientists (17,18).

The objective of this study is to uncover the physiological/ecological toxic effects of Cu and Cd pollution on *T. repens* and to collect basic data for selecting the reclamation plant species for industrial and mining wastelands.

### 3. MATERIAL AND METHODS

#### 3.1. Experiment materials

*T. repens* is a clonal herb (19). *T. repens* seedlings from the same clone were taken from the Wugongli copper tail mining wasteland in Tongling, Anhui, then cultivated for 2 weeks in the greenhouse of Anhui Normal University. The seedlings, with approximately equal height and biomass, were selected and rinsed with tap water followed by rinsing with distilled water. The stolon cuttings, 5 cm in length and each with 6 nutritious leaves, were taken from the rinsed seedlings.

#### 3.2. Experiment design

For the control experiment, the stolon cuttings were planted in plastic pots (20cm in diameter, 25cm in depth) and filled with garden soil (pH value 7.12, organic matter content 13.6 ppm, with concentrations of Cu and Cd at 28.35 ppm and 0.25 ppm respectively). To study the effects of the heavy metals (Cu, Cd) on the plants, the garden soil was further treated as follows:  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  or  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$  was added by means of a hydro solvent equivalent to the content of pure Cu in soil at 500, 1000, 2000, 3000 ppm, respectively, and the content of pure Cd at 0.5, 2.5, 10, 50 ppm respectively. Combined treatments of Cu and Cd were introduced according to table 1. Ten cuttings were planted in each pot (2 kg soil each). Three replicates were set for each treatment. The pot plants were cultivated for 60 days in the greenhouse before being used for measurement and analysis.

#### 3.3. Methods of analysis

##### 3.3.1. Measurement of seedlings' growth

The above-ground height and fresh/dry biomass of each treatment were measured.

##### 3.3.2. Measurement of soluble protein content

Using Coomassie brilliant blue G-250 method (20), the photo densities under a 595 nm wavelength were measured with a 721A spectrophotometer.

##### 3.3.3. Measurement of cell membrane osmolarity

Leaf samples (0.2g) were taken from each pot respectively, then cut into 1 cm long fragments and put into an Erlenmeyer flask filled with 20 mL double distilled water. This was shaken on an electric oscillator at a velocity of 400  $\text{r} \cdot \text{min}^{-1}$  for one hour. The electric conductivities were measured with a DDS-12 conductometer. ( $\mu\text{S} \cdot \text{cm}^{-1}$ )

##### 3.3.5. Measurement of chlorophyll content

Using the spectrophotometric method (21), 0.25 g of leaf samples were obtained, ground and extracted with 80% acetone. Then the photo densities were measured under the 663 nm, 645 nm, and 440 nm wavelengths.(ppt·FW)

##### 3.3.6. Measurement of protective enzyme activity

Peroxidase (POD) activity: Using the spectrophotometric method (21), enzymatic activity was indicated by variations of absorbance per minute, meaning  $\Delta\text{OD}_{470} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \cdot \text{FW}$ .

Catalase (CAT) activity: This was measured by the decomposed quantity of hydrogen peroxide (22). ( $\text{U} \cdot \text{g}^{-1} \cdot \text{FW} \cdot \text{min}^{-1}$ )

Superoxide dismutase (SOD) activity: Using Chen's method (21), the absorbance under a 560 nm wavelength was measured after the enzyme was centrifuged in a frozen state at a velocity of 12000  $\text{r} \cdot \text{min}^{-1}$  for 20 minutes, according to the photo inhibition of SOD on nitroblue tetrazolium (NBT). ( $\Delta\text{OD}_{560} \cdot \text{g}^{-1} \cdot \text{FW} \cdot \text{h}^{-1}$ )

#### 3.4. Statistical analysis

Statistical analysis was performed using the SPSS statistical package (version 11.5). The data was tested for significance ( $P < 0.05$  and  $P < 0.01$ ) by one-way ANOVA.

### 4. RESULTS

#### 4.1. Effects on the seedling growth

The *T. repens* seedlings polluted by Cu and Cd were mostly shorter and smaller. The average height, fresh/dry weight, and soluble protein content differed significantly between the polluted plants and the control samples ( $P < 0.01$ ) (table 2). Under exclusive Cu pollution, as the Cu gradient concentration increased, the average height, fresh/dry weight and soluble protein content all decreased gradually, except for a slight increase in very low Cu concentrations ( $\leq 500$  ppm). There was an apparent negative correlation between Cu concentration and the seedlings growth, with correlation coefficients of –

**Table 2.** Effects of Cu and Cd in separate and combined treatments on the seedling growth of *Trifolium repens*

Cu + Cd (ppm)	Average height (cm)	Fresh weight (mg)	Dry weight (mg)	Protein content (mg)
0 (CK)	10.73±0.23	1189.54±37.14	548.41±16.64	56.43±2.13
0 + 0.5	10.62±0.45	1178.46±34.34	550.47±29.00	55.82±1.97
0 + 2.5	10.35±0.42	1123.37±34.22	517.92±38.32	54.11±1.58
0 + 10	8.64±0.29	851.13±35.63	336.70±8.59	44.61±3.21
0 + 50	6.51±0.32	489.02±27.01	187.50±10.93	26.25±1.82
500 + 0	10.84±0.37	1195.38±62.97	589.26±25.27	58.03±1.69
500 + 0.5	11.15±0.17	1243.41±34.66	593.63±22.00	61.34±2.14
500 + 2.5	10.40±0.09	1157.22±35.63	567.54±35.60	55.15±1.69
500 + 10	8.81±0.61	896.76±43.24	370.18±9.25	45.82±0.34
500 + 50	6.83±0.21	532.71±24.42	200.20±14.87	27.15±0.37
1000 + 0	9.33±0.39	905.50±22.62	353.93±10.89	49.50±1.40
1000 + 0.5	9.64±0.27	1018.82±38.46	450.93±16.31	50.43±0.51
1000 + 2.5	9.01±0.26	868.05±60.14	398.34±13.32	44.41±0.91
1000 + 10	8.67±0.26	833.03±40.17	297.19±8.28	41.36±1.65
1000 + 50	6.65±0.41	503.54±23.61	186.75±16.20	27.17±0.84
2000 + 0	8.45±0.39	642.08±5.56	248.73±6.21	34.06±2.01
2000 + 0.5	8.54±0.37	673.85±46.70	256.82±14.03	34.38±1.12
2000 + 2.5	7.06±0.18	420.26±40.53	168.05±16.21	20.35±0.96
2000 + 10	6.51±0.20	324.51±24.50	135.52±10.23	18.75±1.32
2000 + 50	6.39±0.48	217.07±6.05	82.34±3.89	12.91±0.47
3000 + 0	6.26±0.15	228.46±9.80	103.34±4.67	12.64±0.27
3000 + 0.5	6.31±0.11	304.05±7.30	127.83±2.82	16.11±0.51
3000 + 2.5	6.25±0.10	292.83±28.24	100.42±4.40	14.65±0.44
3000 + 10	5.50±0.38	257.72±13.58	84.05±5.74	12.34±2.38
3000 + 50	5.11±0.31	134.93±12.65	60.75±4.51	10.72±0.97

Data represent means ±SD (standard deviation,  $n=3$ ). The zero in table represents no Cu or Cd was added to the soil.

.9765\*\* (average height), -0.9856\*\* (fresh weight), -0.9588\*\* (dry weight), and -0.9750\*\* (protein content) respectively (\*  $P<0.05$ , \*\*  $P<0.01$ , hereafter). The same trends appeared under exclusive Cd pollution, with correlation coefficients of -0.9545\*\*, -0.9590\*\*, -0.9235\*\*, -0.9813\*\*, respectively.

The combined effects of Cu and Cd pollution mainly corresponded with the effects of separate Cu or Cd pollution on average height, fresh/dry weight and soluble protein content of the *T. repens* seedlings, i.e. showing negative correlation between the concentration of Cu and Cd and the growth indices above, except that at low concentrations of Cu (<500 ppm), the indices increased identically. Statistical analysis showed that the combined correlation coefficients were 0.924, 0.924, 0.911 and 0.937, respectively (>0.7, the same is below), and dual and linear regression equations were  $Y=10.465-0.00126X_{Cu}-0.0542X_{Cd}$ ,  $Y=1167.507-0.272 X_{Cu}-9.148 X_{Cd}$ ,  $Y=524.361-0.128X_{Cu}-4.549X_{Cd}$  and  $Y=57.245-0.0129X_{Cu}-0.411X_{Cd}$ , respectively.

#### 4.2. Effects on leaf electric conductivity

Cell membranes are a kind of selectively permeable membrane, which can regulate and control the transport of material as well as intracellular and extracellular interchange. Osmolarity of cell membranes is one of the indices indicating a plant's response to polluting substances. The leaf electric conductivity of *T. repens* decreased slightly at low concentration, the lowest being 500ppm of Cu and 0.5 ppm of Cd. However, at higher concentrations (>500 ppm of Cu and 0.5 ppm of Cd), as the

concentration of Cu and Cd increased, the leaf electric conductivity increased rapidly (figure 1). Under separate Cu or Cd pollution, electric conductivities showed positive and extremely significant correlations, with a correlation coefficient of  $r_{Cu}=0.9564^{**}$ , and a regression equation of  $Y=45.9827+0.0246X_{Cu}$ ; while  $r_{Cd}=0.9661^{**}$  and  $Y=60.1175+0.9365X_{Cd}$ . Under combined Cu and Cd pollution, electric conductivities increased with the increase in concentrations of Cu and Cd, and the joint correlation coefficient was 0.945, with a dual and linear regression equation of  $Y=53.415+0.01969X_{Cu}+0.763X_{Cd}$ . Under combined Cu and Cd pollution, electric conductivities at the Cu concentration of 500 ppm were lower than corresponding data under isolated Cd pollution. Similarly, at the Cd concentration of 0.5 ppm, the electric conductivities were also lower than their corresponding data under isolated Cd pollution.

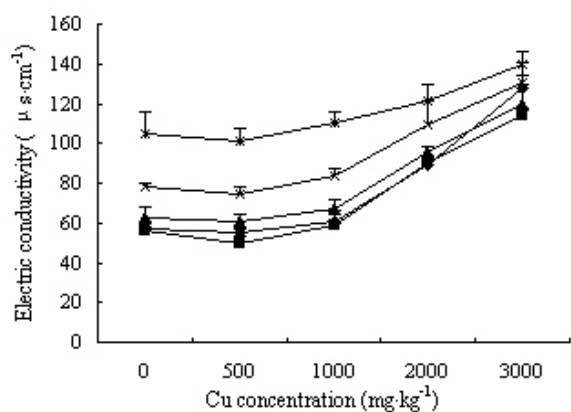
#### 4.3. Effects on leaf pigment content

Chlorophyll is a photosynthetic pigment in plants, and its content indicates, to some extent, the level of photosynthetic activity. Low chlorophyll content leads to a low level of photosynthesis, and in turn leads to low biomass and poor plant growth (23). It was shown that chlorophyll content reached its peak under the combined pollution of 500 ppm Cu and 0.5 ppm Cd. However, as concentrations of Cu and Cd continued to rise, the chlorophyll content decreased proportionately (table 3). During separate tests of pollution by Cu or Cd, content of chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoid of the seedlings' leaves all showed negative correlations, with the correlation coefficients for Cu at –

**Table 3.** Effects of Cu and Cd in separate and combined treatments on leaf pigments contents of *Trifolium repens* (ppt-FW)

Cu+Cd (ppm)	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoid
0 (CK)	1.062±0.022	0.490±0.021	1.554±0.042	0.502±0.023
0 + 0.5	1.003±0.030	0.395±0.010	1.398±0.023	0.473±0.023
0 + 2.5	1.005±0.048	0.342±0.021	1.347±0.063	0.322±0.021
0 + 10	0.898±0.025	0.302±0.154	1.200±0.032	0.336±0.013
0 + 50	0.737±0.028	0.304±0.019	1.041±0.043	0.317±0.012
500 + 0	1.007±0.024	0.482±0.008	1.489±0.031	0.495±0.017
500 + 0.5	1.072±0.055	0.490±0.010	1.562±0.011	0.503±0.007
500 + 2.5	0.972±0.016	0.489±0.006	1.461±0.022	0.347±0.007
500 + 10	0.876±0.051	0.421±0.004	1.297±0.048	0.341±0.005
500 + 50	0.595±0.054	0.350±0.021	0.945±0.074	0.299±0.004
1000 + 0	0.960±0.027	0.479±0.011	1.439±0.037	0.453±0.012
1000 + 0.5	0.971±0.041	0.475±0.021	1.446±0.061	0.411±0.010
1000 + 2.5	0.862±0.032	0.320±0.012	1.182±0.043	0.399±0.019
1000 + 10	0.757±0.046	0.286±0.005	1.043±0.048	0.348±0.007
1000 + 50	0.505±0.037	0.224±0.003	0.729±0.039	0.291±0.008
2000 + 0	0.843±0.041	0.418±0.011	1.261±0.048	0.452±0.008
2000 + 0.5	0.806±0.016	0.399±0.012	1.205±0.008	0.421±0.011
2000 + 2.5	0.732±0.010	0.305±0.006	1.037±0.005	0.398±0.014
2000 + 10	0.523±0.015	0.287±0.005	0.810±0.144	0.334±0.005
2000 + 50	0.408±0.036	0.246±0.009	0.654±0.045	0.288±0.009
3000 + 0	0.433±0.012	0.263±0.011	0.696±0.016	0.407±0.009
3000 + 0.5	0.394±0.017	0.212±0.011	0.606±0.007	0.389±0.005
3000 + 2.5	0.248±0.029	0.160±0.011	0.408±0.037	0.361±0.014
3000 + 10	0.259±0.012	0.136±0.008	0.395±0.020	0.312±0.010
3000 + 50	0.232±0.016	0.133±0.007	0.365±0.023	0.280±0.014

Data represent means  $\pm$ SD (standard deviation,  $n=3$ ). The zero in table represents no Cu or Cd was added to the soil.



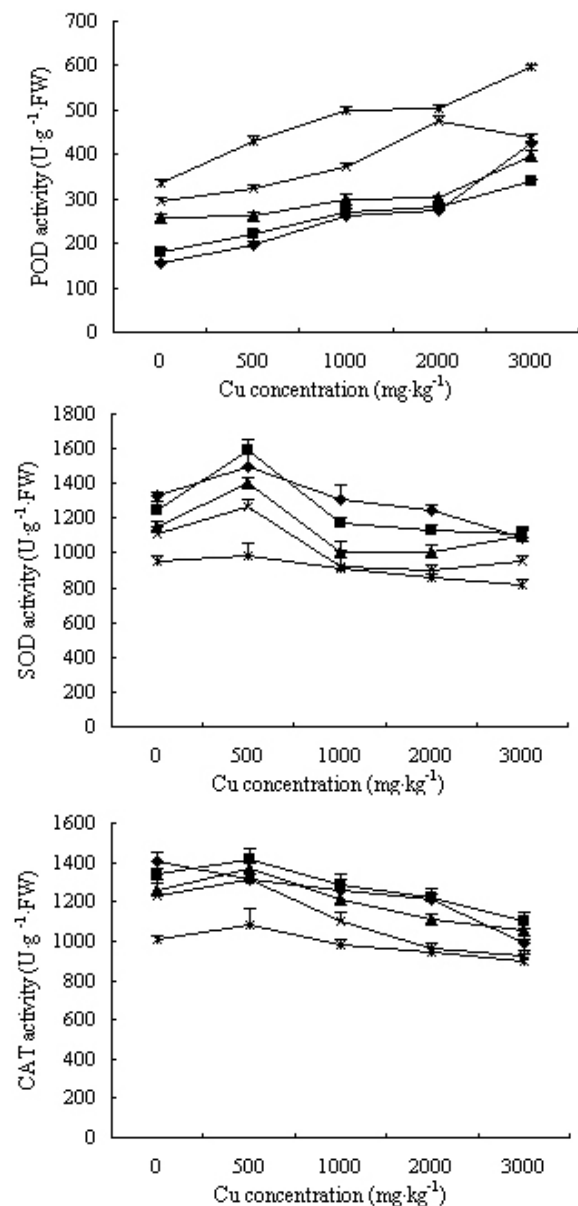
**Figure 1.** Effects of Cu and Cd in separate and combined treatments on leaf electric conductivity of *Trifolium repens* ( $\mu\text{s}\cdot\text{cm}^{-1}$ ). Each point represents means  $\pm$ SD (standard deviation,  $n=3$ ). Cd:  $\blacklozenge$  0 ppm  $\blacksquare$  0.5 ppm  $\blacktriangle$  2.5 ppm  $*$  50 ppm.

.9439\*\*, -0.9250\*\*, -0.9402\*\*, -0.9529\*\*, respectively; while those for Cd were -0.9541\*\*, -0.5710\*\*, -0.8577\*\*, -0.5644, respectively. The combined effects of Cu and Cd pollution showed the same trend, and the leaf pigment content with correlation coefficients of -0.944, -0.799, -0.919 and -0.739 respectively, and dual and linear regression equations  $Y=1.078-0.000206X_{\text{Cu}}-0.00664X_{\text{Cd}}$ ,  $Y=0.459-0.0000693X_{\text{Cu}}-0.00262X_{\text{Cd}}$ ,  $Y=1.537-0.000275X_{\text{Cu}}-0.00926X_{\text{Cd}}$ , and  $Y=0.429-0.0000136X_{\text{Cu}}-0.00257X_{\text{Cd}}$  respectively. It was also shown that

chlorophyll a was the most sensitive to Cu and Cd pollution, followed by chlorophyll b then carotenoid. Compared to the controls, the content of chlorophyll a, chlorophyll b, and carotenoid of the seedlings' leaves decreased an average of 32.95%, 32.67% and 25.49% respectively.

#### 4.4. Effects on SOD, POD, CAT activity in active oxygen metabolism system

It was shown that under the separate and combined pollution experiments using Cu and Cd, SOD and CAT activity both decreased, while POD activity gradually increased with the increase of concentrations of Cu and Cd (figure 2). Under exclusive Cu pollution, SOD and CAT activity increased slightly at the beginning, then decreased rapidly with greater Cu concentration, with correlation coefficients of -0.8336\* and -0.9695\*\*, respectively. The same trends took place with the increase of Cd concentration, with correlation coefficients of -0.8808\* and -0.9434\*\*, respectively. However, POD activity increased with greater concentrations of Cu or Cd separately, showing positive correlation (with correlation coefficients of  $r_{\text{Cu}}=0.9596$ \*\*, and  $r_{\text{Cd}}=0.7780$ , respectively). Under the combined pollution of Cu and Cd, SOD and CAT activity decreased with the increased concentrations of Cu and Cd, with correlation coefficients of -0.758 and -0.905, respectively. The Dual and linear regression equations were  $Y=1305.812-0.0797X_{\text{Cu}}-6.434X_{\text{Cd}}$  and  $Y=1348.138-0.0964X_{\text{Cu}}-5.053X_{\text{Cd}}$ , respectively. Also during the combined Cu and Cd pollution conditions, POD activity proportionately increased with rising concentrations of Cu and Cd (with correlation coefficient of 0.921, regression



**Figure 2.** Effects of Cu and Cd in separate and combined treatments on of SOD, POD and CAT activity (U·g<sup>-1</sup>·FW). Each point represents means ±SD (standard deviation, n=3). Cd: ♦ 0 ppm ■ 0.5 ppm ▲ 2.5 ppm × 10 ppm \* 50 ppm.

$$\text{equation } Y = 206.673 + 0.06118X_{\text{Cu}} + 3.941X_{\text{Cd}}$$

## 5. DISCUSSION

### 5.1. Effects on seedling growth

Heavy metal pollutants damage plants in various ways. Heavy metals can affect the physiological metabolism of the plants directly by damaging their plasma membrane system, or indirectly by retarding the growth and development of plants via its effects on the physical and chemical characteristics of the soil and its nutrient

content (7,24).

In our study, with the increase of gradient concentrations of Cu and Cd (polluted separately and conjunctively), the average height, fresh/dry weight, soluble protein content and chlorophyll content of the seedlings all decreased gradually, while electric conductivity increased. Cd is a heavy metal with strong toxicity, and low concentrations may disorder cells' metabolism and damage the cell structure (25). As for plants, the principal affecting mechanism of Cd may be that Cd has a strong attraction to -SH on some important biomacromolecules, such as proteins, nucleic acids, etc.; it also shows strong attraction to other side chains, e.g., phosphoric acid functional masses. Furthermore, Cd moves easily and usually concentrates on vigorously growing roots, which injures root systems and hinders their absorbance of nutrients, stunting plant growth (12,23). Cu's mechanism for harming plants may be through physiological actions stimulated by enzymes because Cu has been found in several oxidases in plants that take part in plant metabolism. High concentrations of Cu restrict growth of the root system, leading to deficiency of macroelements like Mg and Fe, and causes a lack of chlorophyll in the plant. This hinders plant growth and causes it to lose biomass (13,14).

Heavy metal pollutants usually lead to a decline of chlorophyll content (23). Cu and Cd entering a plant break the balance of chlorophyllase activities, inducing a faster rate of chlorophyll decomposition. At the same time, because Cu and Cd reacted with the some of the -SH groups distributed in the peptide chains of several enzymes (protochlorophyllide reductase,  $\delta$ -aminolevulinic synthetase and porphobilinogen deaminase) involved in the biological synthesis of chlorophyll, the normal enzyme structure was changed, restraining enzyme activities and hindering synthesis of chlorophyll (26). The reason for the increase in electric conductivity might be that Cu and Cd reacted with the -SH groups on cellular membrane proteins or phosphatide material in the phospholipid bilayer. This would change phosphatide structure of membrane proteins as well as the cell membrane structure. Thus, the membrane systems were damaged and osmolarity increased, causing several soluble substances to penetrate the cells, thereby increasing electric conductivity (25,27,28).

No obvious effects were found on plant growth under low concentrations of Cu (<500 ppm) or Cd (<0.5 ppm). However, when the concentrations increased to a higher level (>500 ppm for Cu and >0.5 ppm for Cd, respectively), the damage Cu and Cd caused to *T. repens* plants became obvious and more destructive with the rising concentrations. Under conditions of combined pollution by 500 ppm Cu and 0.5 ppm Cd, the average height, fresh/dry weight, soluble protein content and chlorophyll contents of the seedlings reached their highest values, while electric conductivity reached its lowest point. The reason may be that an antagonistic reaction takes place during the combined pollution of low concentrations of Cu and Cd (Cu<500 ppm and Cd<0.5 ppm respectively), so the toxic effects were alleviated to some extent, and that stimulates

plant growth (23,29). However, at higher concentrations of Cu and Cd (Cu: 500~3000 ppm, Cd: 0.5~50 ppm), there existed synergistic activities, so toxicity rose gradually.

### 5.2. Effects on active oxygen metabolism of seedling

Under heavy metal stress, the plant produces a lot of active oxygen radicals, damaging some biomacromolecules (such as proteins and nucleic acids), and thus affecting the normal metabolism (30-32). SOD, POD and CAT together comprise an effective active-oxygen-metabolic system, and their harmonious and identical actions can effectively scrub radicals and peroxides (28,33,34). In a definite range, SOD and CAT react together, which can not only convert  $O_2^-$  and  $H_2O_2$  into  $H_2O$  and  $O_2$ , but can also inhibit the formation of  $\cdot OH$  which has toxicity and high activity; POD and CAT may stimulate  $H_2O_2$  to change into  $H_2O$ , thus effectively restraining accumulation of  $O_2^-$  and  $H_2O_2$  and inhibiting the switch on effect of free radicals on peroxidation of membrane lipids (24,30,35-38). Under the stress of Cu and Cd pollution in separate and combined states, the active-oxygen eliminating system (composed of SOD, POD and CAT) was destroyed, the balance of the protective enzyme system was broken, and the activity of SOD and CAT decreased noticeably while POD activity increased, leading to a series of physiological and biochemical disorders.

Low concentrations of Cu and Cd possibly engendered an antagonistic reaction, which alleviated toxicity to some extent, thus slightly increasing rather than decreasing SOD and CAT activity. However, as the concentrations of Cu and Cd (Cu: 500~3000 ppm, Cd: 0.5~50 ppm) increased, synergistic actions caused rapidly decreasing SOD and CAT activity. SOD is a protective enzyme for the membrane system that protects it from the damage of oxygen free radicals. As an eliminating substance for free radicals, SOD could change  $O_2^-$  into  $H_2O_2$  and  $H_2O$ , which would decrease the free radicals in the plant; and SOD activity was correlated with the counteraction of the plant. The decrease of SOD activity would not only induce the accumulation of free radicals in the plant (causing stress and thereby damaging the plant), but would also affect POD and CAT activity at the same time. POD activity increased gradually, probably due to the fact that the introduction of Cu and Cd into a plant produced several harmful peroxides in a series of physiological and biochemical reactions, therefore inducing the number of POD substrates to increase and in turn causing a rise in POD activity.

### 6. ACKNOWLEDGEMENT

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