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烟草叶片膜保护酶系统对土壤Hg,Cd,Pb胁迫的响应*

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摘要 采用盆栽实验,就烟草膜保护酶系统对土壤Hg,Cd,Pb胁迫的响应进行研究。结果表明:随着Hg,Cd,Pb浓度的增加,POD活性逐渐增加,CAT活性逐渐减小,SOD活性在三种元素共同作用时逐渐下降,在元素单一或两两作用时,SOD活性呈单峰曲线,但总体水平仍较低。土壤Hg,Cd,Pb的这种影响表现出三元素共同作用>两两元素作用>单一元素作用。影响的结果造成活性氧产生与清除之间的不平衡,致使相关生理生化过程紊乱。三种重金属对烟草活性氧清除系统的影响表现出明显地协同作用。

关键词: Hg,Cd,Pb胁迫 烟草 膜保护酶系统

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RESPONSES OF MEMBRANE PROTECTION ENZYME SYSTEM OF TOBACCO LEAVES ON Hg, Cd AND Pb STRESSES IN SOIL*

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ABSTRACT Pot experiment was used to study the responses of membrane protection enzyme system of tobacco leaves on Hg, Cd and Pb stresses in soil. The results showed that POD activity gradually increased with increasing concetrations of Hg, Cd and Pb. CAT and SOD activity gradually decreased under three heavy metals common existing and SOD variation curve showed unimodal curve under single or two elements existing with increase of concentration of Hg, Cd and Pb. The effects of Hg, Cd and Pb in soil: three elemets together > two elements together > single element only. The effects resulted in an imbalance — activated oxygen produce and scavenge and physiological biochemical process disorder. There was a synergistic action for the effect of Hg, Cd and Pb in soil on membrane protection enzyme system in tobacco leaves.

Key words: Hg, Cd and Pb stress. Tobacco leaves. Membrane protection enzyme system

Hg, Cd and Pb are several of the most serious pollutants due to their toxicity to known to affect agricultural biota and will affect human health by entering into Vegetative Chain. Recent years, people are concerned heavy metal pollution of the soil-plant system[1-3], many researchers studied the effect of heavy metals especially individual heavy metal on photosynthesis, respiration, biomass production and quality of plants, but no systematic studies of effects of heavy metals on membrane protection enzyme system of plant. We systematically studied the responses of membrane protection enzyme system of tobacco leaves on Hg, Cd and Pb stresses in soil.

1 MATERIAL AND METHODS

1.1 Material

Tobacco (*Nicotiana tabacum* L) leaves.

1.2 Methods

1.2.1 Cultivating method

Primary compound fertilizer was applied at one time to the selected typical yellow soil (pH 5.2) in Guizhou Province. Each seedling of tobacco was transplanted to a pot containing the soil and treated by sewage irrigation at one time with $HgCl_2$, $CdCl_2 \cdot 2.5\ H_2O$, $Pb(Ac)_2$ at given concentrations. As shown in the Table. 1, ten testing groups of Hg, Cd and Pb were set with the concentrations for each group being calculated in terms of pure Hg, Cd and Pb. at the same time, another CK group was set. (without treating with Hg, Cd and Pb).

Five tobacco seedlings for each group grew by pot cultivation for 25 days. The middle sections of leaves at the same leaf location were collected for measurement after washing by clean water and distilled water, and dried by absorbent paper.

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Table 1 Ion concentration of Hg, Cd and Pb Unit·mg·kg^{-1}

Hg	Cd	Pb	Hg+Cd	Cd+Hg	Pb+Cd	Cd+Pb	Pb+Hg	Hg+Pb	Hg+Cd+Pb
10	10	100	30+10	30+10	300+10	30+100	300+10	30+100	10+10+10
30	30	300	30+30	30+30	300+30	30+300	300+30	30+300	30+30+300
50	50	500	30+50	30+50	300+50	30+500	300+50	30+500	50+50+500
100	100	1000	30+100	30+100	300+100	30+1000	300+100	30+1000	100+100+1000

1.2.2 Measuring method

For measuring the activities of CAT, SOD and POD see[1]. Units are μmol H_2O_2·(min·mg)$^{-1}$, U·mg^{-1} and U·mg^{-1}.

2 RESULTS AND DISCUSSION

2.1 Effects of Hg, Cd and Pb in soil on the activity of CAT

Results (Fig. 1) indicated that the activity of CAT was gradually decreased with increasing concentrations of Hg, Cd and Pb. Particularly in Hg + Cd + Pb group the activity of CAT would drop sharply. Fig. 1 indicated that the synergistic effect of three heavy metal elements of Hg, Cd and Pb on activity of CAT was obviously greater than that of single or two elements. The effect of Pb on the activity of CAT was most obvious among three heavy metals.

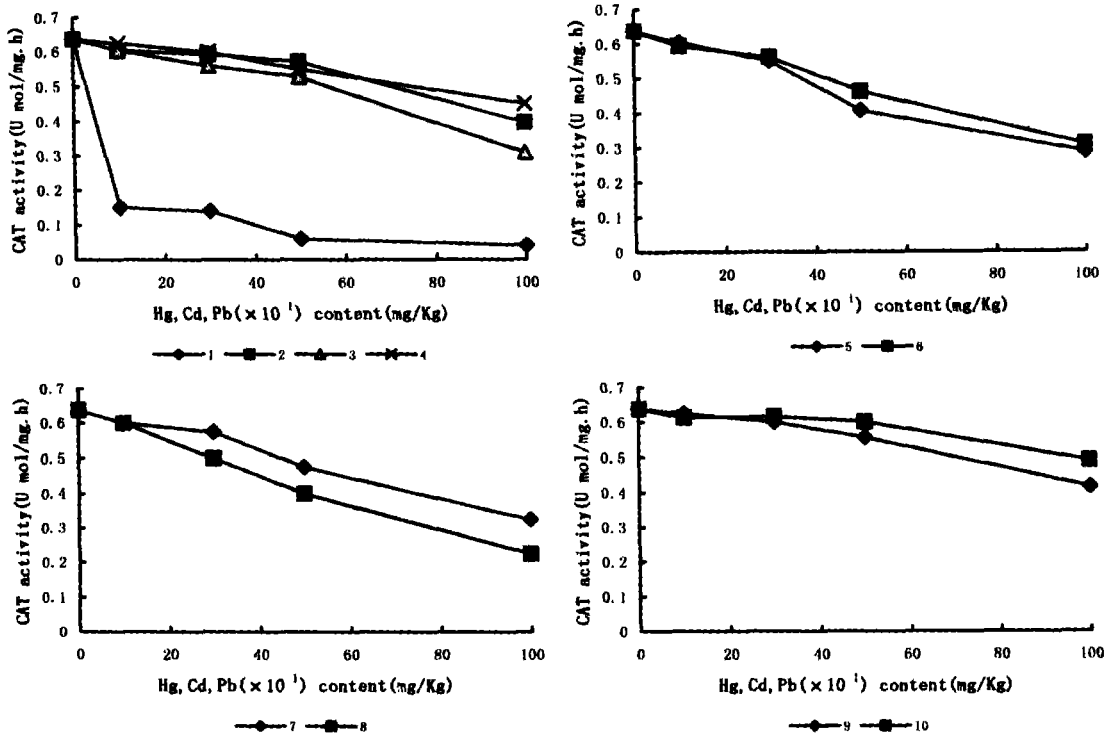

Fig. 1 Effects of Hg, Cd and Pb in soil on CAT activity in tobacco leaves

1. Hg+Cd+Pb; 2. Single Cd; 3. Single Pb; 4. Single Hg; 5. Cd(30mg)+Pb; 6. Pb(300mg)+Cd; 7. Pb(300mg)+Hg; 8. Hg(30mg)+Pb; 9. Hg(30mg)+Cd; 10. Cd(30mg)+Hg.

CAT is able to scavenge superfluous H_2O_2 in cells and to maintain H_2O_2 at a normal level. Therefore it effectively prevents the producing of ·OH and then the potential damage caused by ·OH. The decrease of activity of CAT in this study will be likely to produce large amounts of H_2O_2 and then cause cell membrane lesion or damage.

2.2 Effects of Hg, Cd and Pb in soil on the activity of SOD

Results (Fig. 2) indicated that the activity of SOD was gradually increased with increasing concentrations of Hg, Cd and Pb and greater than that of CK group under single and two elements. However, with continuously increasing concentrations of three heavy metals the activity of SOD would then drop sharply or slowly and even lower than that of CK group. The activity of SOD showed single peak curve and general activity level was decreased. But under common stress of three heavy metals, the activity of SOD was sharply decreased.

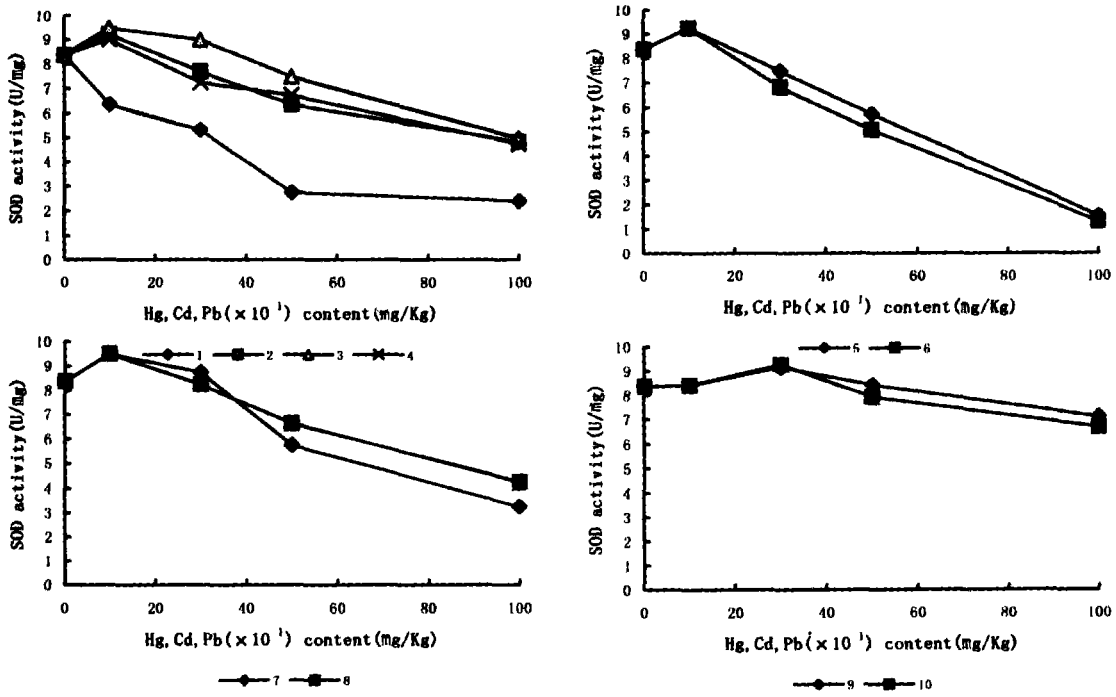

Fig. 2　Effects of Hg, Cd and Pb in soil on SOD activity in tobacco leaves

1. Hg + Cd + Pb; 2. Single Cd; 3. Single Pb; 4. Single Hg; 5. Cd(30mg) + Pb; 6. Pb(300mg) + Cd; 7. Pb(300mg) + Hg; 8. Hg (30mg) + Pb; 9. Hg(30mg) + Cd; 10. Cd(30mg) + Hg.

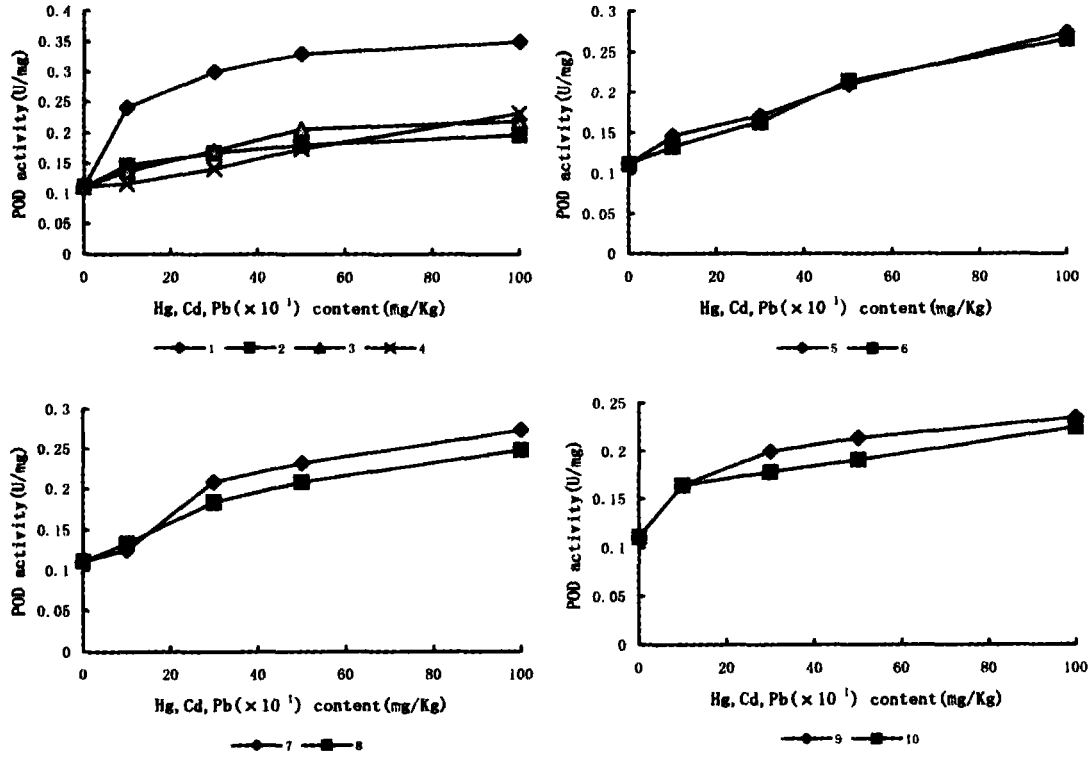

Fig. 3 Effects of Hg, Cd and Pb in soil on POD activity in tobacco leaves

1. Hg + Cd + Pb; 2. Single Cd; 3. Single Pb; 4. Single Hg; 5. Cd(30mg) + Pb; 6. Pb(300mg) + Cd; 7. Pb(300mg) + Hg; 8. Hg(30mg) + Pb; 9. Hg(30mg) + Cd; 10. Cd(30mg) + Hg.

The variation curve of SOD activity of tobacco showed single peak curve under stress of single or two heavy metal elements with increasing of Hg, Cd and Pb concentrations, indicating that tobacco could induce the adversity resistance system under stress of Hg, Cd. and Pb. Increase of SOD activity could hold back the increase of $\bar{O}_2$ in the plant body caused by Hg, Cd and Pb stress. However with further increasing of Hg, Cd and Pb concentrations and surpassing the limitation of dismutation ability it would hence cause decreasing of the activity of SOD and even was lower than that of CK group. This protective reaction led to show single peak curve for variation of SOD activity. The activity of SOD was sharply decreased under common stress of three heavy metals. For effects of Hg, Cd and Pb in soil on the activity of SOD in tobacco leaves, common stress of three heavy metals was further greater than that of single or two heavy metals.

2.3 Effects of Hg, Cd and Pb in soil on the activity of POD

Results (Fig. 3) of this experiment showed that the activity of POD was gradually increased with increasing concentrations of Hg, Cd and Pb. Increasing of POD activity was greater in three heavy metal treatment group than single heavy metal treatment groups and two heavy metal treatment group lay in between single and three heavy metal treatment groups.

Increasing of POD activity with increasing concentrations of Hg, Cd and Pb, indicating that the content of peroxides was increased under heavy metal stress, and the POD activity was induced increasing to oxidize and decompose peroxides which would cause injury to plant body, by catalytic action of H_2O_2.

Effect of POD was similar with action of oxidase of indole acetic acid which could oxidize IAA. So Hg, Cd and Pb in soil could change content of IAA in plant body through improving POD activity to destroy and reduce IAA content or change it into in no-activated substances.

In addition, POD-H_2O_2 decomposition system had participated degradations of chlorophyll and showed highly negative correlation with chlorophyll content[4-6] and further effected growth of plant and accumulation of biomass.

3 SUMMARY

The membrane protection enzyme system is an enzyme reaction system which effectively prevents unsaturated fatty acid from being oxidized by free oxide radicals. CAT, SOD and POD are three important protective enzymes in plant body which are able to remove activated oxygen. $\bar{O}_2$ is removed by SOD to form H_2O_2 which is then catalyzed by POD and CAT to form H_2O. Therefore it effectively prevents the $\bar{O}_2$ and H_2O_2 from being accumulated and simultaneously limits the inducement of lipid per-oxidation by free oxide radials.

Under Hg, Cd and Pb stress, CAT and SOD activity level is generally decreased and POD activity is obviously increased indicating that the effect of three heavy metals (Hg, Cd and Pb) is greater than that of single or two heavy metals. The effects will result in imbalance between produce and remove of activated oxygen and accumulation of activated oxygen. At the same time, POD-H_2O_2 decomposition system can participate degradation of chlorophyll, POD characteristics IAA oxidizing enzyme and so on. The results would lead to cell membranes functional disorder growth of plant inhibition, biomass decrease. Hg, Cd and Pb have an obvious synergistical action on membrane protection enzyme system in tobacco leaves.

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