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Ecotoxicity of Chloramphenicol and Hg Acting on the Root Elongation of Crops in North China

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ABSTRACT: Single and joint toxicity of chloramphenicol and Hg acting on wheat (*Triticum aestivum L.*), Chinese cabbage (*Brassica campestris L.*) and corn (*Zea mays L.*) were investigated. The results showed positive correlations between root elongation inhibition of three plants and concentrations of pollutants added to soil (P<0.01) in test concentration range. In terms of root elongation, wheat was the most sensitive to toxicity of chloramphenicol with an IC_{50} (concentration when 50% plants show inhibition) value as high as 26.8 mg/kg and also was the most sensitive one to the toxicity of Hg with the IC_{50} value as high as 300.8 mg/kg. The toxicity of chloramphenicol to the plants is stronger than that of Hg. Chloramphenicol and Hg had an antagonistic effect on the inhibition of root elongation of the three plants when the concentration of added Hg reached 30 mg/kg. Chloramphenicol and Hg had significantly synergistic effects on the inhibition of root elongation when Hg concentration was up to 200 mg/kg (P<0.05).

Key words: chloramphenicol, Hg ecotoxicology, *Triticum aestivum L.*, *Brassica campestris L.*, *Zea mays L.*

INTRODUCTION

Chloramphenicol (CAP) is a broad-spectrum antibiotic active against gram-positive and gram-negative bacteria (Sorensen *et al*., 2003). CAP is one of the most widely used antimicrobials for human and veterinary medication. The administration of CAP to humans in relatively high doses has caused serious toxic effects such as agranulocytosis and aplasticanaemia (Fabiansson *et al*., 1976). With the rapid development of stock breeding, the annual output of veterinary antibiotics in animals' manure is increasing (Jørgensen & Halling-Sørensen, 2000, Paul *et al*., 2005). However, incorrect or excessive usage of veterinary antibiotics in recent decades has caused substantial amounts of antibiotics and their metabolites being released to the environment through manure application onto agricultural land. Veterinary antibiotics enter agricultural soils mainly by fertilizing and grazing (Christian *et al*., 2003, Hamscher *et al*., 2005, Wojciech *et al*., 2006) and transport through surface runoff and plants uptake (Davis *et al*., 2006, Dolliver *et al*., 2007). Previous studies showed that antibiotics could not be eliminated completely at the usual sewage treatment plants, with the highest elimination efficiency reaching to 81%. These drug residues and their metabolites may accumulate in soil and disturb the balance of the soil ecosystem (Ghosal & Kaviraj, 2002, Yoshimur *et al*., 2005, Sarmah *et al*., 2006). The major risk of these antibiotics entering the environment is probably the development and spread of resistant pathogens (Heuer & Smalla, 2007). Additionally, it was reported that some veterinary antibiotics (Jin *et al*., 2009) and personal care products (An *et al*., 2009a, 2009b) resided in soil can effect the growth of plant. Only in recent years, more attention has been paid to the discharge, presence and potential adverse effects of veterinary antibiotics in the environment (De *et al*., 2003, Diao *et al*., 2007, Jensen *et al*., 2007). Since its effective control of diseases, CAP has become one of the veterinary antibiotics with the most widespread use in animal husbandry in China, especially in North China. Although there are some literatures on effects of CAP in aquatic environment (Nicole, 2008, Jjemba, 2006), the knowledge about their toxic effects on terrestrial plants and agricultural crops is still scarce. Thus it is necessary to accumulate information about CAP potential effects on soil and plants.

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In general, many heavy metals including Cd and Hg are nonessential elements with biological toxicity. However, mercury (Hg) pollution is a global environmental problem whose levels in soils have been increased due to human activities like mining and smelting of metals (Steinnes, 1997, Chibunda *et al.*, 2010). Although the availability of soil Hg to plants is low, several studies have shown that Hg could inhibit plant growth and disturb water and nutrient uptake, photosynthesis, oxidative stress and enzymatic activities (Cho & Park, 2000, Patra & Sharma, 2000, Ortega-Villasante *et al.*, 2000, Sun *et al.*, 2010). Some studies about Hg accumulation in several plant species, like pea and spearmint, wheat, rice, oil-seed rape, sugar beet, tomato and so on have been carried out (Beauford *et al.*, 1977, Wang and Greger, 2004, Greger *et al.*, 2005). However, the joint pollution would occur because antibiotics and heavy metals could residue in soil (Liu *et al.*, 2010). Earlier research into ecotoxicology of pollutants mainly focused on pesticides or heavy metals. In recent years, much attention has been devoted to the issues of antibiotic residues in aquatic (Managaki *et al.*, 2007, Hasan *et al.*, 2010). With the rapid development of animal food industry, the consumption of veterinary antibiotics would greatly increase annually, and meanwhile the simultaneous pollution problem would be generated more seriously. Therefore, studies considering the ecotoxicology of veterinary antibiotics and the joint toxicity with heavy metal on plants should be focused.

Wheat (*T. aestivum L*), Chinese cabbage (*B. campestris L.*) and corn (*Zea mays L.*) are the usual crops in North China and were selected as test plants. In this study, we investigated the single and joint toxicity of CAP and Hg exposure in soil by measuring inhibitory rates of root elongation of plants. The study purpose, therefore, is to demonstrate the joint toxicity of antibiotics and heavy metal on plants so as to provide a technical basis for the soil environmental pollution diagnosis.

MATERIAL & METHODS

CAP was purchased from National Institute the Control of Pharmaceutical and Biological Products of China. The molecular formula of CAP is $C_{10}H_{10}N_4O_2S$. The tested form of Hg was $HgCl_2$. Soil samples were taken from the upper 0-20cm layer of an experimental farm field. The fresh soil samples were air-dried and ground to pass a sieve of 2.0 mm before using. Soil pH was measured at 1:2.5 ratio of soil solution in H_2O using a combination glass electrode. The cation exchange capacity (CEC) was determined using the ammonium acetate replacement method, and soil texture was analyzed using the gravitometer method. Organic matter was determined by the dry combustion method (Li, 1983). The main physical and chemical properties of the soil are: dry density 2.03 g /cm^3, pH 6.52, organic matter 2.05%, CEC 11.38 cmol/kg, total N 0.21 g/kg, total P 0.05 g/kg, total K 0.54 g/kg, sand 50.3%, powder 24.2%, clay 25.5%. The variety of tested wheat (*T. aestivum*) was Jing 411, Chinese cabbage (*B. campestris*) was Beijing NO.3 and corn (*Z. mays*) was Nongfeng 13, respectively. Seeds were sterilized with NaOCl solution for 5 minutes and washed several times with sterilized deionized water.

The experimental design was basically performed in accordance with the Organization for Economic Cooperation and Development (OECD) for plant acute toxicity tests (OECD, 1984). 50g air-dried soil was put into the culture dish (diameter: 90mm), and then the pre-confected pharmaceutical solution was added into the dish. The soil moisture was adjusted with deionized water, and then mixed well when the moisture reaches 60% of maximal holding capacity. The dishes were put into the culturing box (JC303A-TS, made in Shanghai, China) for 48 hours under the dark condition with temperature being controlled at 25 !. All treatments were replicated three times. When the length of the growing root cultured in the control solution without contamination reached 20mm, the exposed experiment was finished. And then the root elongation of all the treatments were calculated and measured. The inhibitory rate (IR) of the three plants was calculated as followed:

IR = (a-b)/a (where IR is inhibitory rate; a is the root elongation in control treatment; b is the root elongation in drug treatment)

According to the results of preliminary tests, formal single toxicity test concentrations were determined. Drugs concentrations used in this experiment were based on 10–50% of the inhibitory rates of drugs on plants root elongation, which were calculated and obtained from the preliminary experiment. According to the preliminary test, the tested concentration of the CAP in the formal solution culture was ascertained. They were 0, 1, 5, 10, 20, 25, 30 mg/kg (formulated as pure CAP) for wheat and Chinese cabbage and 0, 1, 5, 10, 20, 30, 50 mg/kg for corn. The tested concentration of Hg was 0, 30, 50, 100, 200, 300, 400 mg/kg for the three plants.

In the combined-pollution experiments, the tested concentrations of CAP were ascertained as the concentration of single-factor experiments. In order to make the combined inhibitory range from 10% to 50%, the concentrations under which the inhibitory rate of root elongation around 5% and 25% were selected. But according to the actual results from single-factor experiments, tested concentrations of Hg was equal to

30, and 200 mg/kg, under which the inhibitory rate of wheat root elongation was 10%, and 33%, the inhibitory rate of Chinese cabbage root elongation was 7%, and 31%, the inhibitory rate of corn root elongation was 12%, and 30%, respectively.

The data of this experiment were statistically processed using SPSS13.0 software. Statistical analysis including calculation of average values, standard deviation, and regression and variance analysis was performed on the data obtained from the experiment. The data are presented by means ± standard deviation (SD) (X±SD). The multiple comparison procedure (LSR test) was used to compare root elongation under the combined pollution of CAP and Hg and statistical significance was set at $P<0.05$.

RESULTS & DISCUSSION

There are three established simple and convenient methods to study the toxicity of pollutants to higher plants, which are the experiment concerning root elongation, the experiment concerning seed germination, and the experiment concerning seedling growth at the early stage (OECD, 1984, Cheng & Zhou, 2002). However, the seed germination is not sensitive when the concentration of a pollutant is low (Gong & Wilke, 2001, Wang & Zhou, 2005a). Shoot elongation is easily affected by the embryo and does not show obvious inhibitory symptom, because a plant embryo can absorb nutrition from the embryo to accomplish its germinating process. And the shoot elongation shows nearly a normal appearance when the concentration of a pollutant is low. Biomass of seedling growth at the early stage is also insensitive when the concentration of a pollutant is high (Wang & Zhou, 2005b). The root of plants, which directly dipped into contaminated soils, is more sensitive to the toxicity of a pollutant. So, in this experiment, only the root elongation under all the treatments was measured and calculated.

As shown in Fig.1, there was a clear dose-response between concentration of CAP and the inhibitory rate of root elongation in the tested soil. There was a significant linear correlation between the inhibitory rate of root elongation and the tested concentration of CAP ($P<0.01$). CAP had a poisonous effect on root elongation of the three plants and its effect on root elongation was significant. The corresponding regression equations were listed in Table 1. According to the regression equations based on the inhibition of root elongation, IC_{50} of CAP was calculated. It was shown that IC_{50} of CAP based on the inhibitory rates of root elongation of wheat, Chinese cabbage and corn were 26.8 mg/kg, 30.0 mg/kg and 44.1 mg/kg, respectively, which meant that the toxic effect of CAP on wheat was stronger than that on the other two plants. As root elongation was concerned, wheat was the most sensitive plant to the toxicity of CAP. Therefore, wheat plants can be used to assess the toxicity of CAP. An et al (An et al., 2009a, 2009b) also found that wheat and rice are commonly used to assess the toxicity of organic and inorganic compounds due to their sensitivity.

As shown in Fig.1, there was a significant linear correlation between the inhibitory rate of root elongation and the tested concentration of Hg ($P<0.01$). Similar to the effects of CAP, inhibitory rate was correlated with the concentration of Hg and the differences among the seven concentrations were significant ($P<0.01$). The positive correlative relationships could be expressed by regression equations listed in Table1. We can see that the correlation coefficient (R^2) ranged from 0.9459 to 0.9885. According to the regression equations based on the inhibition of root elongation, IC_{50} of Hg was calculated. The calculation results showed that IC_{50} of Hg based on the inhibitory rate of root elongation of wheat, Chinese cabbage and corn was 300.8 mg/kg, 396.1 mg/kg and 363.4 mg/kg, respectively, which means that the toxic effect of Hg on wheat was stronger than that on the other two plants. Therefore, wheat was the most sensitive plant to the toxicity of Hg in this study. In terms of IC50 under the single-factor experiment, the results from the present study indicated that the toxicity of CAP to the plants is stronger than that of Hg to the three plants. Perhaps the seeding can absorb the heavy metal, which constitutes a barrier between the embryo and its immediate environment. Thus, Hg could not represent stronger toxicity than CAP. Additionally, wheat was the most sensitive plant to the toxicity of Hg and CAP among the three plants.

Joint effects of pollutants may be similar (additive) to expected effects from separate exposures, or stronger (synergistic, more than additive) or weaker (antagonistic, less than additive) than those from separate exposures (Zhu *et al.*, 2008). The effects of joint pollution depend on the constituents of the mixture and may vary significantly (Jensen & Sverdrup, 2002). According to the experimental data, inhibitory effects of combined CAP and Hg on root elongation were complicated (Table 2, and Fig.2), which greatly differed from action of single CAP or Hg. Table 2 show that there were positive linear relationships between the inhibitory rate of root elongation and the concentration of CAP when the concentration of Hg remained at 0 mg/kg, 30 mg/kg, or 200 mg/kg, respectively. These corresponding regression equations are listed in Table 2, and the correlation coefficient (R^2) ranged from 0.8275 to 0.9851. According

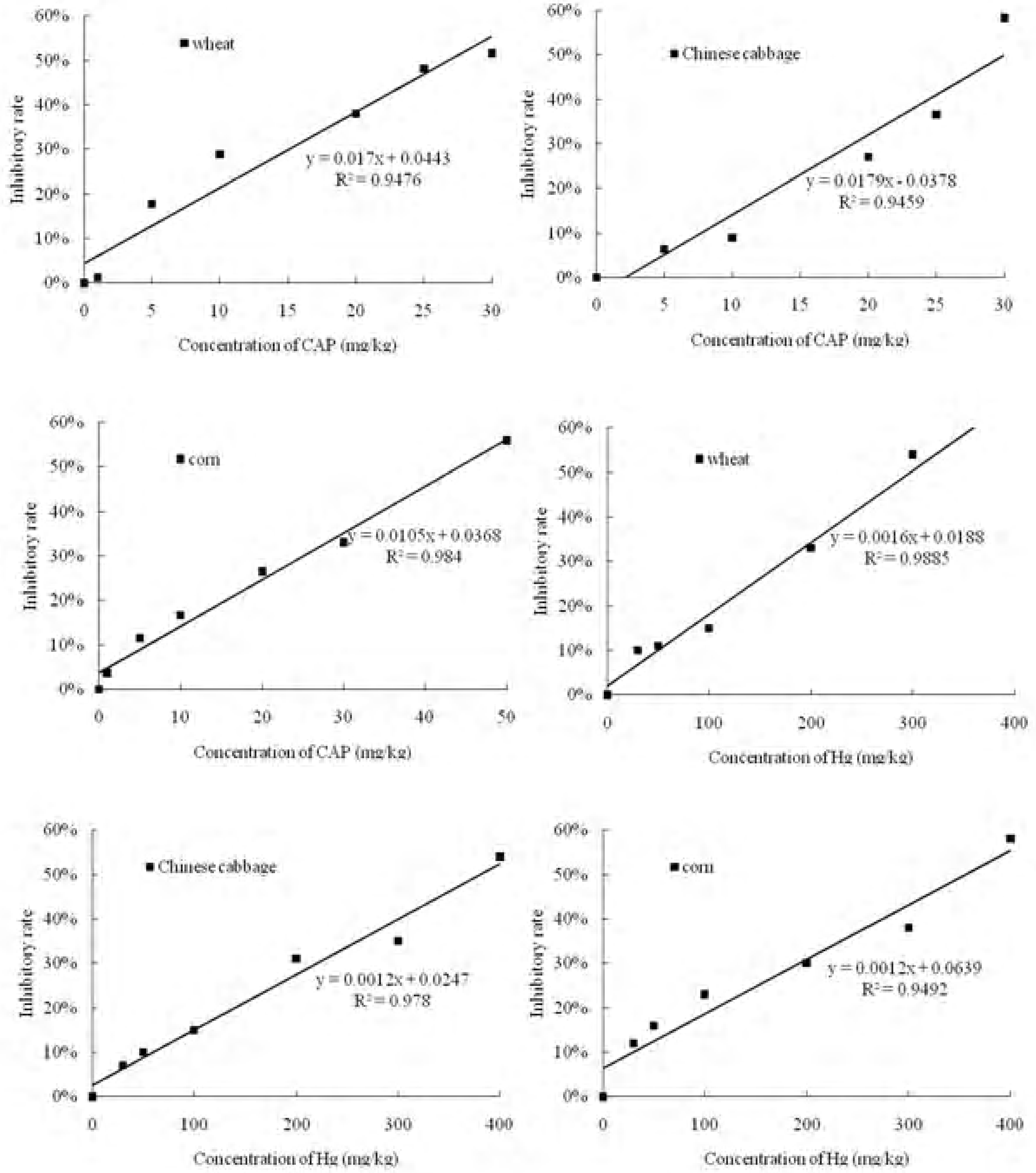
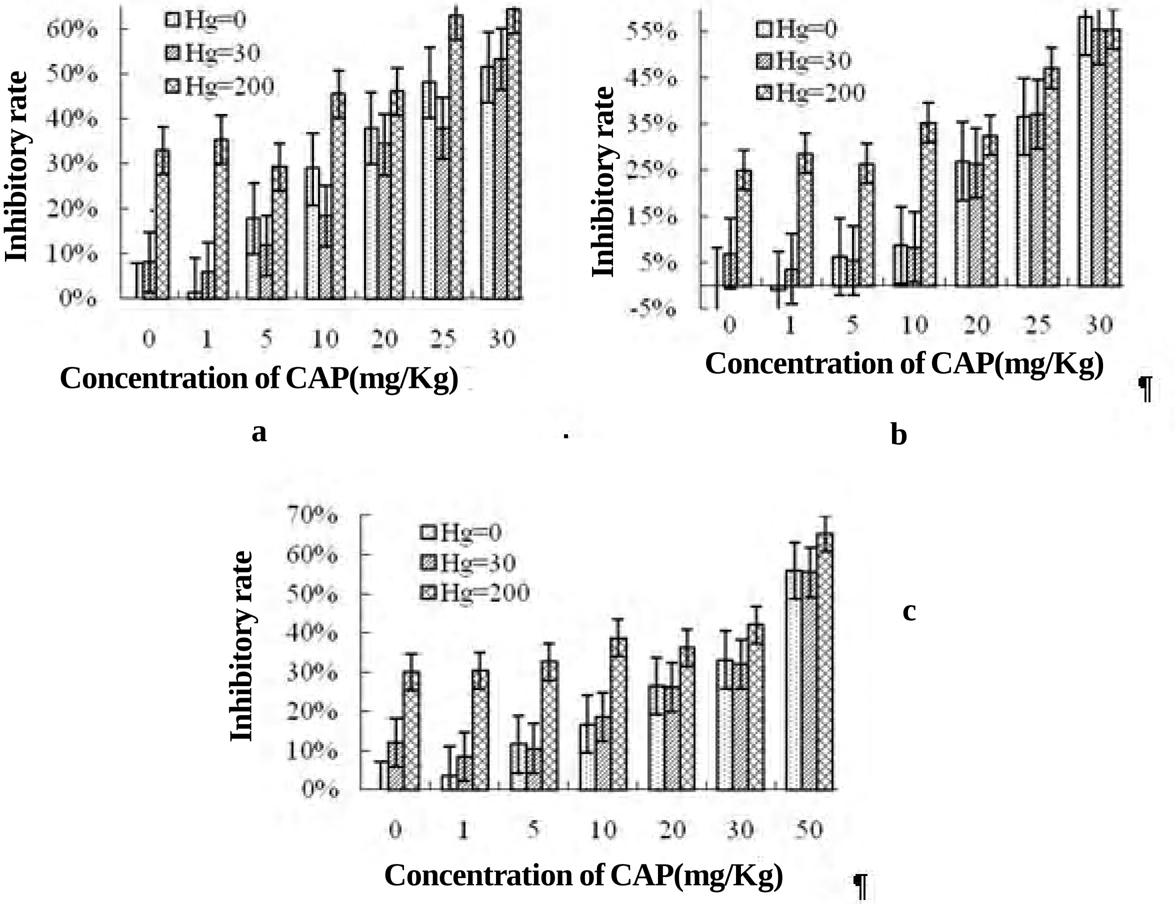


Fig. 1. Toxic effects of CAP and Hg on the inhibitory rate of root elongation of three plants under single-factor contamination

Table 1. Regression equation and IC50 for root elongation of three plants to CAP and Hg

Plant Species	Regression Equation	R^2	*P*	n	IC_{50}/(mg/kg)
Wheat(CAP)	y = 0.017x + 0.0443	0.9476	< 0.01	7	26.8
Wheat(Hg)	y = 0.0016x + 0.0188	0.9885	< 0.01	7	300.8
Chinese cabbage(CAP)	y = 0.0179x – 0.0378	0.9459	< 0.01	7	30.0
Chinese cabbage(Hg)	y = 0.0012x + 0.0247	0.9780	< 0.01	7	396.1
Corn (CAP)	y = 0.0105x + 0.0368	0.9840	< 0.01	7	44.1
Corn(Hg)	y = 0.0012x + 0.0639	0.9492	< 0.01	7	363.4



a

b

c

Fig. 2. Joint toxic effects of CAP (mg/kg) and Hg (mg/kg) on the inhibitory rate of root elongation of wheat (a), Chinese cabbage (b), and corn(c)

Table 2. Relationships between inhibitory rate of root elongation (RI) and concentration of added CAP at the same concentration of added Hg

Crop Species	Added Hg (mg/kg)	Regression Equation	R^2	*P*	n	IC_{50}/(mg/kg)
Wheat	0	y = 0.017x + 0.0442	0.9478	<0.01	7	26.8
	30	y = 0.0147x + 0.0515	0.9770	<0.01	7	30.5
	200	y = 0.0109x + 0.3105	0.8761	<0.05	7	17.4
Chinese cabbage	0	y = 0.0179x - 0.0378	0.9458	<0.01	7	30.0
	30	y = 0.0158x + 0.0001	0.9111	<0.01	7	31.6
	200	y = 0.0086x + 0.246	0.8275	<0.05	7	29.5
Corn	0	y = 0.0105x + 0.0368	0.9851	<0.01	7	44.1
	30	y = 0.009x + 0.0845	0.9779	<0.01	7	46.2
	200	y = 0.0064x + 0.2878	0.8886	<0.05	7	33.2

to Fig.2 and Table 2, the relationships between the inhibitory rate of root elongation and the concentration of added CAP were at the same significant level ($P<0.01$) when the concentration of Hg remained at 0 mg/kg or 30 mg/kg. However, the significant level was lower ($P<0.05$) when the concentration of Hg reached 200 mg/kg, which meant that perhaps there are interactive effects between CAP and Hg with the increase of Hg concentration. Moreover, it was also shown in Fig.2 that the inhibitory rate of root elongation remained at the similar level when the concentration of CAP was at the same levels when the concentration of added Hg was 0 mg/kg or 30 mg/kg. Based on the regression equations, IC_{50} of CAP inhibiting root elongation of the three plants was calculated and listed in Table 2. It was shown in Table 2 that the joint toxicity of CAP and Hg was the strongest on wheat among the three plants. In another word, wheat was the most sensitive plant to the joint pollution in this study, which suggested us that perhaps it can be used as the indicator plant to joint pollution of CAP and Hg.

As shown in Fig.2, adding Hg had an inhibitory effect on root elongation of wheat, especially when the concentration of Hg was up to 200 mg/kg. For example, when CAP concentration was 30mg/kg and Hg was 200mg/kg, the inhibitory rate of Chinese cabbage was 55.0%. But when the concentration of added Hg was 30 mg/kg and the concentration of CAP was 1-20 mg/kg, the inhibitory rates of wheat were lower than that without Hg addition. For example, when CAP concentration was 20mg/kg and Hg was 30mg/kg, the inhibitory rate of wheat was 34.3%, while the inhibitory rate was 38.0% without Hg addition. That indicated that the joint effect of inhibition is weaker than the effect caused by separate exposure on root elongation under that condition. When the concentration of CAP was higher than 25 mg/kg, the antagonistic effect of Hg was not obvious. Perhaps since the total concentration of the pollutants in the soil was higher in the cases of a joint application of pollutants, this might have resulted in greater toxic effects on plants. Additionally, CAP and Hg had a markedly synergistic effect on root elongation of wheat when the concentration of Hg was up to 200 mg/kg ($p<0.05$).

It was shown in Fig.2 that the inhibitory rate increased with increasing Hg added, but adding Hg had less effect on root elongation of Chinese cabbage when the concentration of Hg was 30 mg/kg, which meant that CAP and Hg had antagonistic effects on root elongation when the concentration of Hg was low. However, Hg had a synergistic effect with CAP on root elongation of Chinese cabbage when the concentration of added Hg was up to 200 mg/kg.

The similar as the result of Chinese cabbage, inhibitory rate of corn under 30 mg/kg Hg addition was lower than that under the condition of no Hg addition when the concentration of CAP under 1-20 mg/kg. Therefore, CAP and Hg have an antagonistic effect on root elongation of corn at 30 mg/kg of Hg. According to Fig.2, it was shown that adding Hg had an inhibitory effect on root elongation of corn, especially when the concentration of Hg was up to 200 mg/kg. Obviously, CAP and Hg had a markedly synergistic effect on root elongation of corn when the concentration of Hg was up to 200 mg/kg ($p<0.05$). Wang and Zhou (2005a, 2009) also found that organoic pollution and heavy metal had a synergistic effect when their concentration at a high level. The inhibitory effects of combined CAP and Hg on root elongation can further support Zhou's theory (Zhou *et al.*, 2004) that ecotoxicological effects under the combined pollution were not only related to chemical properties of pollutants but also dependent on the concentration level of pollutants, in particular on the combination of concentrations of pollutants in ecosystems. When the concentrations of CAP and Hg were low, perhaps there was an antagonism between CAP and Hg on root elongation of three plants. How one pollutant (Hg) could help decrease the other pollutant (CAP) acute toxicity may attribute to competition and complex compound formation, inducing metallothionein or changing the enzyme activity (Zhu *et al.*, 2008). In addition, these results meant that at the low concentration of CAP combined with the low concentration of added Hg, Hg can perhaps fix some parts of CAP in the outer environment of the root system. Thus some of the Hg and CAP were inhibited to enter the root system and to go up to the shoot. In that way, the toxic effects of CAP decreased. The combination of organic pollutants and heavy metals will reduce the activity of heavy metals. Further research is still necessary to understand the specific mechanism.

CONCLUSION

According to the experiment, there were significant ($P<0.01$) linear relationships between root elongation inhibition of wheat, Chinese cabbage and corn and concentrations of CAP and Hg in test concentration range. In terms of root elongation, wheat was the most sensitive plant to the toxicity of CAP and Hg. The toxicity of CAP to the three plants was stronger than that of Hg. When the concentrations of the two pollutants were low, Hg had antagonistic effects with CAP on root elongation of the three plants. However, Hg had significantly ($P<0.05$) synergistic effects with CAP on root elongation when the concentration of added Hg was high.

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