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# Single and joint stress of acetochlor and Pb on three agricultural crops in northeast China

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#### Abstract

In order to evaluate ecological risk of agrochemicals in agricultural environment, single and joint toxic effects of an important herbicide and a typical heavy metal on root elongation of crops were investigated. Seeds of the three crops including wheat (*Triticum aestivum*), Chinese cabbage (*Brassica pekimensis*) and soybean (*Glycine max*) as the main crops in northeast China were exposed to acetochlor as a herbicide and lead (Pb) as a heavy metal using the pot-culture method, and meadow brown soil as one of the main soils distributed in northeast China was applied in the investigation. The results indicated that the interactive effects of the two pollutants on root elongation of the three crops were very complicated although they had markedly significant (P<0.01) linear interrelationships based on the regression analyses. When the concentration of added Pb<sup>2+</sup> reached 200 mg/kg, acetochlor and Pb had an antagonistic effect on the inhibition of root elongation of the three crops. However, acetochlor and Pb had significantly (P<0.05) synergic effects on the inhibition of root elongation when concentration of added Pb<sup>2+</sup> was up to 1000 mg/kg. At the low concentration of added Pb, joint toxicity of acetochlor and Pb was more dependent on the concentration of Pb. Among the three crops, wheat was the most sensitive to the toxicity of Pb and Chinese cabbage was the most sensitive to the toxicity of acetochlor.

**Key words**: acetochlor; Pb; ecotoxicology; joint effect; Chinese cabbage (*Brassica pekimensis*); soybean (*Glycine max*); wheat (*Triticum aestivum*)

# Introduction

The northeast China distributed with fertile and productive soils is not only an important commodity grain base, but also a pivotal industrial region with the centuriesold history of industrial development in China. With the further development of industrial and agricultural production, industrial sewage irrigation, antiknocking additives in gasoline, atmospheric sedimentation caused by coal combustion and the use of pesticides bring a large amount of lead (Pb) to soil (Zhou and Song, 2004). As a result the pollution of Pb in soils has extensively occurred in northeast China (Zhou et al., 2004). Meantime, acetochlor has been one of the three herbicides most widely used in northeast China (Xiao et al., 2004) after it was used in controlling field weeds in the world since 1982 (Lolpin et al., 1996; Hu, 1998). According to Zheng and Ye (2001), so far more than  $1.0 \times 10^4$  t of acetochlor in every year has been used in China. In the past several years, acetochlor

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and its metabolites had been widely detected in waters and soils (Boyd, 2000; Dagnac et al., 2002). It is not difficult to understand that there is more frequent coexistence of acetochlor and Pb in soil environment. According to the previous investigation (Zhou et al., 2004), acetochlor and Pb were simultaneously detected in agricultural soils of northeast China. Moreover, the toxicity of acetochlor, such as inducing sister chromatid exchanges in cultured human lymphocytes (Hill et al., 1997), mutagenizing germ cells of male rats (Ashby et al., 1997), and altering thyroid hormone-dependent gene expression in Xenopus laevis (Crump et al., 2002), has been reported. Thus, increasing attention has been paid to the pollution problems associated with acetochlor in recent years, and its decomposition in natural or artificial environment has been also a focus (Liu et al., 2005). However, it is regretful that there is litter literature about joint toxic effects of acetochlor and Pb.

In view of the fact that the simultaneous occurrence of acetochlor and Pb is becoming more frequent in northeast China, single and joint toxicity of acetochlor and Pb acting on wheat, soybean and Chinese cabbage as the main crops in northeast China was investigated. Of particular interest was the inhibition rate of root elongation that can really reflect the toxicity of hazardous chemicals in soils.

## 1 Materials and methods

#### 1.1 Materials

Meadow brown soil was one of the main soils distributed in northeast China. The unpolluted surface (0–20 cm) soil was sampled from the Shilihe Ecological Experimental Station (41°31′N and 123°41′E), Shenyang, Liaoning Province, China. The fresh soil samples were air-dried and ground to pass a sieve of 1.0 mm before use. Soil pH was measured at 1:2.5 ratio of soil solution 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 (Li, 1983). Organic matter was determined by the dry combustion method. Selected properties of the soil are shown in Table 1. All reagents used in the study were of analytical grade. The tested form of Pb was Pb(NO<sub>3</sub>)<sub>2</sub>.

To evaluate the toxicity of the herbicide in farmlands effectively, the commercial product (50% of oil soluble concentration of acetochlor) from the Rize Pesticide Company in Dalian, China was used in this study. Pure acetochlor was a colorless liquid. The molecular formula of acetochlor was  $C_{14}H_{20}ClNO_2$ .

The variety of tested wheat (*Triticum aestivum*) was Liaoning Spring No.10, the Chinese cabbage (*Brassica pekimensis*) was Liaobai No.7 and soybean (*Glycine max*) was Liaodou No.16. The crop seeds were surface-sterilized in the clorox solution for 5 min, washed several times with sterilized deionized water.

### 1.2 Plant culture

Acetochlor, Pb and the mixture of acetochlor and Pb were churned up with sieved soils, respectively (Song et al., 2002; Xiao et al., 2004). The synthetic polluted soil was put in the culturing box (LRH-250-A; made in Guangdong) with a temperature of 25±1°C for 24 h. Fifteen sterilized seeds (nine seeds for the soybean) were scattered in each synthetic polluted soil in a culture dish and covered. Then the culture dishes were put into the culturing box (LRH-250-A, made in Guangdong, China) with a temperature of 25±1°C. All treatments were replicated three times to minimize experimental errors. The exposed experiment was finished after 96 h.

## 1.3 Single-factor exposure experiments

According to 0–60% of the inhibitory rate of root elongation by the pollutants, the tested concentration of Pb<sup>2+</sup> was set to be 0, 200, 500, 1000, 1500 and 2000 mg/kg, and the tested concentration (formulated as pure acetochlor) of the herbicide was 0, 2, 5, 10, 15 and 20 mg/kg for wheat and Chinese cabbage, and 0, 5, 10, 20, 30 and 40 mg/kg for soybean.

#### 1.4 Joint exposure experiments

According to the results from the single-factor exposure experiments, the tested concentration of Pb<sup>2+</sup> was set to be 0, 200 and 1000 mg/kg, and the tested concentration (formulated as pure acetochlor) of acetochlor was 0, 2, 5, 10, 15 and 20 mg/kg for wheat and Chinese cabbage and 0, 5, 10, 20, 30 and 40 mg/kg for soybean under the joint exposure experimental conditions.

## 2 Results and discussion

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

# 2.1 Effects of Pb or acetochlor on the three crops

Pb had a poisonous effect on root elongation of the three crops and the differences between the six concentrations were significant (P<0.05). As shown in Fig.1a, there was a significant linear correlation between the inhibitory rate of root elongation and the tested concentration of Pb (P<0.01). The corresponding regression equations are listed in Table 2. According to the regression equations based on the inhibition of root elongation, ID<sub>50</sub> of Pb was calculated. The calculation showed that ID<sub>50</sub> of Pb based on the inhibition of root elongation of wheat, Chinese cabbage and soybean was 1823, 1991 and 3644 mg/kg, respectively, which means that the toxic effect of Pb on wheat was stronger than that on the other two crops.

Similar to the effects of Pb, root elongation was correlated with the concentration of acetochlor (P<0.05). As shown in Fig.1b, there was a significant linear correlation between the inhibitory rate of root elongation and the

Table 1 Basic physical and chemical properties of meadow brown soil

Soil pH	Organic matter (%)	CEC (cmol/kg)	Sand (%, 1–0.05 mm)	Silt (%, 0.05–0.001 mm)	Clay (%, < 0.001 mm)
6.62	2.75	12.3	7.3	75.6	17.1

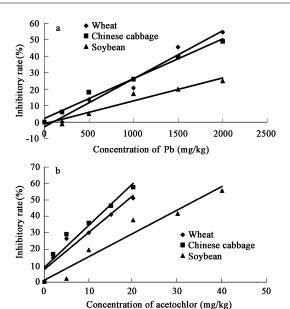


Fig. 1 Toxic effects of Pb (a) and acetochlor (b) on the inhibitory rate of root elongation of three crops under single-factor contamination.

Table 2 Regression equations and  $ID_{50}$  of three crops to Pb

Species	Regression equation	$R^2$	P	ID <sub>50</sub> (mg/kg)
Wheat	$RI_{Pb^{2+}} = 0.029X - 2.861$	0.972	< 0.01	1823
Chinese cabbage	$RI_{Pb^{2+}} = 0.024X + 2.212$	0.987	< 0.01	1991
Soybean	$RI_{Pb^{2+}}^{10} = 0.014X - 1.016$	0.948	< 0.01	3644

 $RI_{Pb^{2+}}$  is the inhibitory rate (%) of root elongation of growing seedlings exposed to Pb, X is the tested concentration of Pb, and  $ID_{50}$  is the half-inhibition dose.

tested concentration of acetochlor (P<0.01). The inhibitory rate of root elongation increased with increasing concentration of acetochlor in the tested soil. The corresponding relationship can be expressed using the regression equations in Table 3. According to Table 3, acetochlor had an obviously poisonous effect on root elongation. Chinese cabbage is the most sensitive plant to the toxicological effect of acetochlor among the three crops, with 50% of the inhibitory rate of root elongation when the concentration of acetochlor was only 16.1 mg/kg. While the inhibitory rate of root elongation of wheat and soybean reached 50%, the concentration of acetochlor was up to 18.8 and 34.3 mg/kg, respectively. According to ID<sub>50</sub> under the single-factor experiment, it can be concluded that the toxicity of

acetochlor to the crops are stronger than that of Pb to the crops.

# 2.2 Joint stress of Pb and acetochlor on the three crops

It was shown by the variance analysis that there were positive linear relationships between the inhibitory rate of root elongation and the concentration of acetochlor when the concentration of Pb remained at 0, 200, or 1000 mg/kg, respectively (Fig.2). These corresponding regression equations are listed in Table 3. As shown in Fig.2 and Table 3, the relationships between the inhibitory rate of root elongation and the concentration of added acetochlor were at the same significance level (P<0.01)

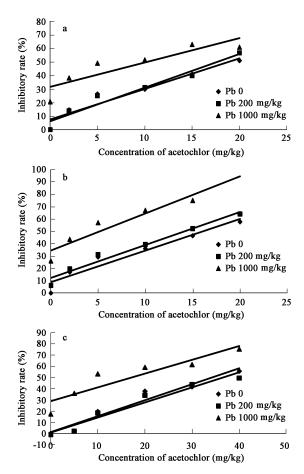


Fig. 2 Joint toxic effects of acetochlor (mg/kg) and Pb (mg/kg) on the inhibitory rate of root elongation of wheat (a), Chinese cabbage (b), and soybean (c).

Table 3 Relationships between the inhibitory rate of root elongation (RI) and the concentration of added acetochlor at the same concentration of added Pb

Crop	Added Pb (mg/kg)	Regression equation	$R^2$	P	$ID_{50} (mg/kg)$
Wheat	0	RI=2.251X+7.673	0.925	< 0.01	18.8
	200	RI=2.479X+6.349	0.948	< 0.01	17.6
	1000	RI=1.793X+31.757	0.784	< 0.05	10.2
Chinese cabbage	0	RI=2.559X+8.865	0.929	< 0.01	16.1
· ·	200	RI=2.669X+12.279	0.966	< 0.01	14.1
	1000	RI=3.003X+34.496	0.891	< 0.05	5.2
Soybean	0	RI=1.427X+1.122	0.948	< 0.01	34.3
•	200	RI=1.347X+0.752	0.946	< 0.01	36.6
	1000	RI=1.233X+28.796	0.843	< 0.05	17.2

RI is the inhibitory rate (%) of root elongation of growing seedlings exposed to the different pollutants, X is the tested concentration of acetochlor and  $ID_{50}$  is the half-inhibition dose.

when the concentration of Pb remained at 0 or 200 mg/kg. However, the significance level was low (P<0.05) when the concentration of Pb reached 1000 mg/kg, which suggests that there are interactive effects between acetochlor and Pb with the increase of Pb concentration. It is also shown in Fig.2 that the inhibitory rate of root elongation remained at the similar levels when the concentration of acetochlor was at the same levels, despite the concentration of added Pb was 0 or 200 mg/kg. According to the regression equations, ID<sub>50</sub> of acetochlor inhibiting root elongation of the three crops was calculated and listed in Table 3. It is showed in Table 3 that the joint toxicity of acetochlor and Pb is the strongest on Chinese cabbage among the three crops.

As shown in Table 4, root elongation of wheat by 200 mg/kg of Pb<sup>2+</sup> was greater than that under the condition of no Pb<sup>2+</sup>, but the differences were not significant. Thus, it can be concluded that acetochlor and Pb2+ had an antagonistic effect on root elongation at that concentration of Pb, although the effect was not significant. It is shown in Table 4 that adding Pb2+ had an inhibitory effect on root elongation, especially when the concentration of Pb<sup>2+</sup> was up to 1000 mg/kg. Acetochlor and Pb<sup>2+</sup> had a markedly synergic effect on root elongation when the concentration of acetochlor was less than 15 mg/kg. However, the synergic effect of Pb<sup>2+</sup> was not obvious when the concentration of acetochlor was higher than 20 mg/kg. According to Table 4, it was also shown that the differences in root elongation between all the tested concentration of acetochlor with Pb addition was not significant (P>0.05), which means that the toxicity of the joint effects of acetochlor and Pb toxic to root elongation of wheat is more dependent on the toxicity of Pb.

It is revealed in Table 5 that the inhibitory rate of root elongation increased with increasing  $Pb^{2+}$  added, but added  $Pb^{2+}$  had a few effect on root elongation of Chinese

cabbage when the concentration of Pb<sup>2+</sup> was 200 mg/kg, which means that acetochlor and Pb had antagonistic effects on root elongation when the concentration of Pb was low. However, Pb had a synergistic effect with acetochlor on root elongation when the concentration of added Pb was up to 1000 mg/kg. When the level of added Pb was 200 mg/kg, the differences between root elongation of Chinese cabbage at all the tested concentration of acetochlor were more significant than those without Pb addition, which also means that the joint toxicity of acetochlor and Pb to Chinese cabbage depends more on the toxicity of Pb at the low concentration of Pb. When the level of added Pb was up to 1000 mg/kg, the differences between root elongation of Chinese cabbage at all the tested concentration of acetochlor were less significant than those without Pb addition, which means that the joint toxicity of acetochlor and Pb to Chinese cabbage is complex at the high concentration of Pb.

As shown in Table 6, root elongation of soybean by 0-20 mg/kg of acetochlor and 200 mg/kg of added Pb<sup>2+</sup> was greater than that under the condition of no Pb2+ added. Thus, it can be concluded that acetochlor and Pb<sup>2+</sup> had an antagonistic effect on root elongation at 200 mg/kg of Pb, although the effect was not distinct. According to Table 6, it is shown that adding Pb<sup>2+</sup> had an inhibitory effect on root elongation, especially when the concentration of Pb<sup>2+</sup> was up to 1000 mg/kg. Acetochlor and Pb<sup>2+</sup> had a markedly synergic effect on root elongation when the concentration of acetochlor was less than 20 mg/kg. However, the synergic effect of Pb2+ was not significant when the concentration of acetochlor was higher than 30 mg/kg. When the concentration of added Pb was up to 1000 mg/kg, the differences between root elongation of soybean at all the tested concentration of acetochlor were less significant than those with no Pb addition or 200

Table 4 Differences in average root elongation (mean±SD, cm) of wheat under joint stress of acetochlor and Pb

Pb <sup>2+</sup> (mg/kg)	Acetochlor (mg/kg)						
	0	2	5	10	15	20	
0	4.21±0.34a	3.59±0.29b	3.10±0.23bc	2.95±0.21c	2.48±0.31d	2.06±0.48d	
200	a 4.21±0.47a	a 3.62±0.38ab	a 3.15±0.51bc	a 2.90±0.48bc	a 2.53±0.52cd	a 1.82±0.57c	
1000	a 3.34±0.42a	a 2.60±0.43b	a 2.14±0.35bc	a 2.03±0.26bc	a 1.56±0.44c	a 1.64±0.43c	
	b	b	b	b	b	a	

<sup>&</sup>lt;sup>a</sup> Means followed by different letters differ at *P*<0.05 (the LSR test). Letters beside means refer to the difference at the same concentration of Pb and under means refer to the difference at the same concentration of acetochlor.

Table 5 Differences in average root elongation (mean±SD, cm) of Chinese cabbage under joint stress of acetochlor and Pb

Pb <sup>2+</sup> (mg/kg)	Acetochlor (mg/kg)						
	0	2	5	10	15	20	
0	4.31±0.47a	3.58±0.28b	3.05±0.28bc	2.76±0.41cd	2.31±0.22de	1.82±0.48e	
	a	a	a	a	a	a	
200	$4.04\pm0.34a$	$3.47 \pm 0.29ab$	$2.97 \pm 0.23$ bc	2.62±0.18cd	$2.06\pm0.31$ de	1.55±0.52e	
	a	a	a	a	ab	a	
1000	$3.18\pm0.22a$	$2.43 \pm 0.19b$	$1.85 \pm 0.25c$	1.42±0.16cd	$1.07 \pm 0.34d$	n	
	b	b	b	b	b		

n: The germination rate  $\leq 50\%$ , root elongation can not be measured.

Table 6 Differences in average root elongation (mean±SD, cm) of soybean under joint stress of acetochlor and Pb

Pb <sup>2+</sup> (mg/kg)	Acetochlor (mg/kg)					
	0	5	10	20	30	40
0	3.98±0.18a	3.89±0.24a	3.20±0.23b	2.48±0.24c	2.32±0.19c	1.77±0.19d
	a	a	a	a	a	a
200	$4.02\pm0.31a$	$3.90\pm0.24a$	$3.26 \pm 0.44b$	2.63±0.19c	$2.25\pm0.48c$	$2.01\pm0.48c$
1000	a 3.29±0.29a b	a 2.55±0.28b b	a 1.86±0.31c b	a 1.63±0.29c b	a 1.53±0.41c a	a 0.98±0.29c a

Table 7 Regression equations between the root elongation and the concentrations of acetochlor and Pb

Species	Regression equation	$R^2$
Wheat	Z=3.927-0.01X-0.92Y	0.906
Chinese cabbage	Z=4.006-0.001X-0.116Y	0.944
Soybean	Z=3.978-0.001X-0.105Y	0.922

Z is the root elongation (cm) of growing seedlings exposed to acetochlor and Pb simultaneously, X is the tested concentration (mg/kg) of Pb, and Y is the tested concentration (mg/kg) of acetochlor.

mg/kg of added Pb, which means that the joint toxicity of acetochlor and Pb depends more on the toxicity of Pb at the high concentration of added Pb.

Although there were significant (P<0.05) linear relationships (Table 7) between root elongation of three crops and the concentrations of the two contaminants, inhibitory effect on root elongation by joint stress of acetochlor and lead was complicated, in particular, greatly differed from the action of single acetochlor or Pb. When the concentrations of acetochlor and Pb were low, there was an antagonism between acetochlor and Pb on root elongation no matter crops were different. When concentration of Pb was high, they were mainly synergistic. The reason may be attributed to fixation of some acetochlor in the outer environment of root systems at the low concentration of acetochlor combined with the low concentration of added Pb. Thus, some of acetochlor and Pb were inhibited to enter into the root systems and to go upwards the shoots. In this way, the toxic effects of acetochlor decreased and the activity of Pb reduced due to the interaction of organic pollutants and heavy metals. Possibly, the combination of Al and/or Fe with N-Gps can vacate some sites for Pb<sup>2+</sup> (Meng et al., 1998) due to the interaction between acetochlor and Pb, thus decreasing the potential toxicity of Pb. Teisseire et al. (1998) identified that herbicide Diuron could reduce the oxidation caused by Cu2+ and increase the activity of glutathione reductase, ascorbic acid, and peroxidase (POD), thus enhancing antioxidation in cells. Nevertheless joint effects of acetochlor and Pb could be determined by the interactive mode and the ration of the two substances (Teisseire et al., 1998).

# **3 Conclusions**

There were significant (P<0.05) linear relationships between root elongation of wheat, Chinese cabbage and soybean and concentrations of acetochlor and Pb. When the concentrations of the two pollutants were low, Pb had antagonistic effects with acetochlor on root elongation of

the three crops. However, Pb had significantly (P<0.05) synergistic effects with acetochlor on root elongation when the concentration of added Pb<sup>2+</sup> was high. Meanwhile, the joint toxicity was more dependent on toxic effects of Pb than on those of acetochlor.

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