

Joint toxicity of methamidophos and cadmium acting on *Abelmoschus manihot*

WANG Xiao-fei^{1,2}, ZHOU Qi-xing^{1,2,*}

(1. Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China. E-mail: zhouqixing2003@yahoo.com; 2. Graduate School of the Chinese Academy of Sciences, Beijing 100039, China)

Abstract: Joint toxicity of methamidophos and cadmium(Cd) on the ornamental *Abelmoschus manihot* was firstly examined and compared with single-factor effects of the two pollutants using ecotoxicological indexes including the inhibitory rate of seed germination, root elongation and inhibitory concentration 50% (IC₅₀). The results indicated that methamidophos and Cd had unobvious ($p > 0.05$) effects on seed germination of the ornamental. There were significant ($p < 0.05$) inhibitory effects of Cd on root elongation of the tested plant. When the concentration of added Cd was low (< 20 mg/L), significant antagonistic effects on root elongation were observed. And synergic effects were observed when Cd was added in high dose (> 20 mg/L). However, the analysis of joint effects indicated that there were antagonistic effects between Cd and methamidophos under all the treatments. At the high concentration of Cd, joint toxicity of methamidophos and Cd was more dependent on concentration of Cd.

Keywords: joint toxicity; cadmium; methamidophos; ornamental

Introduction

Nowadays, more and more ornamentals are being used in families, parks, roads and other public sites with the improvement of people's living levels and changes in the city life style (Wei, 2004; Zhou, 2004). At the same time, increasing chemicals such as pesticides and chemical fertilizers are being applied in horticulture and flower planting (Thapinta, 2000; Zhou, 2001). Methamidophos (C₂H₈NO₂PS, O, S-dimethyl phosphoramidothioate) as one of important pesticides which are most frequently used worldwide for plant protections is a potent acetylcholinesterase inhibitor used to control chewing and sucking insects and spider mites on ornamental plants, vegetables, citrus fruits, stone fruits and other plants (Fuh, 1999; Hung, 2002, Yu, 2003). With the extensive application of methamidophos that may lead to an adverse effect on water-soil-plant systems by the pesticide itself and its metabolites (Singh, 2002), there is more and more obviously potential menace for ornamentals to suffer from methamidophos. Meanwhile, cadmium(Cd) is a very important pollutant in urban soils of China (Wu, 1992; Cheng, 2002; Zhou, 2003). In particular, the application of chemical fertilizers and other pesticides such as calcium super phosphate and Bordeaux mixture can also introduce heavy metals Cd, Cu and Zn to environmental systems, which result in combined pollution of methamidophos and Cd (Chen, 2003; Zhou, 1995; Wang, 2004).

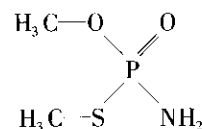
There are more and more reports on combined pollution of agricultural crops (Zhou, 1994; 1995; 2004), but joint toxicity of chemicals on ornamentals is rarely involved. Therefore we firstly examine joint toxicity of methamidophos and Cd acting on *Abelmoschus manihot* as one of the most widely used ornamentals in China. The work may be also useful to virescence and ecological construction of urban environment and remediation of urban soils contaminated by Cd and methamidophos (Song, 2002; Wang, 2003; Zhou, 2004).

1 Materials and methods

1.1 Root-elongation toxicity experiments

The tested form of heavy metal, Cd, used in the experiment, was CdCl₂ · 2.5H₂O (analytical grade), which

was bought from the Second Shenyang Chemical Factory. The pesticide, methamidophos, was obtained from the chemical factory of Zhejiang University of Technology and its type is 40% galactic liquid. The molecular formula of the pesticide is C₂H₈NO₂PS and the structural formula is as follows:



Based on the regression equations from the preparative experimental results that is the range of about 0—50% of the inhibitory rate of root elongation by the pesticide and Cd during the preparative experiment, the tested concentration of single methamidophos and Cd were approximately ascertained to 0, 50, 100, 300, 600, 800 mg/L (formulated as pure methamidophos) and 0, 10, 20, 30, 40, 50 mg/L, respectively. According to the results from single-factor experiments, tested concentrations of methamidophos for joint toxicity were 0, 50, 100, 300, 500 and 700 mg/L that matched the inhibitory rate of root elongation as 0.0%, 19.5%, 21.5%, 29.5%, 37.4% and 45.4% respectively, and the tested concentration of Cd for joint toxicity was 0, 10, 20, 30, 40, 50 mg/L, which inhibited root elongation of the tested plant 0.0%, 6.3%, 18.4%, 30.5%, 42.6% and 54.7% respectively.

1.2 Seed-germination toxicity experiments

Fifteen sterilized seeds of the tested ornamental *Abelmoschus manihot* that were obtained from the Shenyang Agricultural University were put on 2 layers of filter paper in a Petri dish and exposed to single methamidophos and Cd (CdCl₂ · 2.5H₂O) and the admixture of methamidophos and Cd (CdCl₂ · 2.5H₂O) that were dissolved with deionized water under the condition of the darkness with the temperature of 25 ± 1 °C in the culturing box (LRH-250-II, made in Guangdong, China). All the treatments were fourfold to reduce the experimental errors to the maximum. When the length of growing roots in the control solution (no methamidophos and Cd addition) reached 20 mm (NEPA, 1990), the germination experiment was finished, and seed germination and root length of all treatments were measured and calculated.

1.3 Data processing

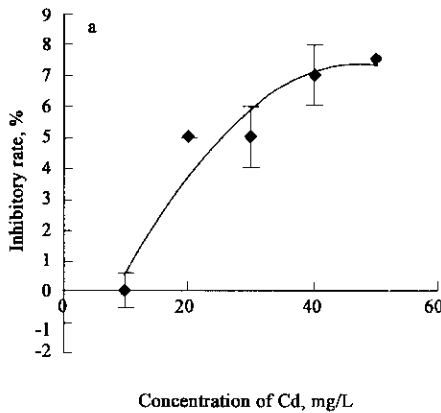
All the data from the experiment were statistically analyzed using the one-way ANOVA procedure of the SPSS statistical package from the SPSS Corp(Wang, 2004). And all statistical significance was set at the level of $p < 0.05$. The data presented are means \pm SD(standard deviation).

2 Results and discussion

2.1 Single-factor effects of Cd or methamidophos

The statistical analysis indicated that there was no significant ($p > 0.05$) difference in the germination rate of *Abelmoschus manihot* when exposed to the tested concentration of single Cd. On the contrary, single Cd had a markedly ($p < 0.05$) poisonous effect on root elongation under the experimental conditions. With increasing concentration of Cd, there was an increase in the inhibitory rate of root elongation. Regression equations to describe the trend can be expressed as follows:

$$RI = 1.21X - 5.76 \quad (R^2 = 0.995, n = 6, p < 0.05). \quad (1)$$



Where RI is the inhibitory rate(%) of root elongation of *Abelmoschus manihot*, and X is the tested concentration (mg/L) of Cd.

According to Fig. 1, it was clearly indicated that seed germination and root elongation had different responses when exposed to single Cd. There was a starting inhibition of root elongation occurred when the tested concentration of Cd reached 4.78 mg/L while inhibition of seed germination occurred when the concentration of Cd exceed 10 mg/L. And when the tested concentration reached 50 mg/L, the inhibitory rate of root elongation reached 54.1%, however, there was only 7.5% inhibition appeared on seed germination. On the one hand, the root may absorb the nutrient from the surroundings during the growing of seedlings, and at the same time embryo can partly provides some necessary nutrients. On the other hand, it is also difficult for Cd to penetrate seeds with hard capsule so that Cd did not have adverse effect on the ornamental, which was similar to the results of Cheng and Zhou(Cheng, 2002), Song et al.(Song, 2002) and Wang and Zhou(Wang, 2005).

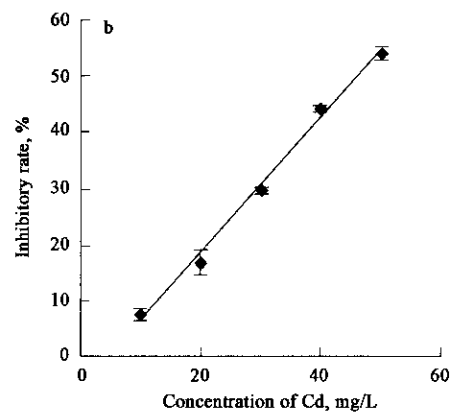


Fig.1 Relationships between Cd(mg/L) and seed germination (a) and root elongation (b)

As described in Fig.2a, there was no regular change of single methamidophos acting on seed germination under the experimental conditions. However, there was a positive linear correlation between added concentration of methamidophos and inhibitory rate of root elongation according to Fig. 2b. The relationship can be expressed using following regression

equation:

$$RI = 0.0398Y + 17.509 \quad (R^2 = 0.9021, n = 6, p < 0.05). \quad (2)$$

Where Y is the tested concentration (mg/L) of methamidophos.

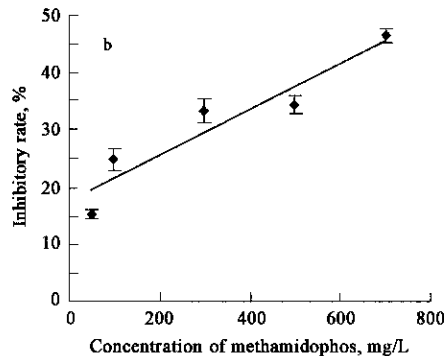
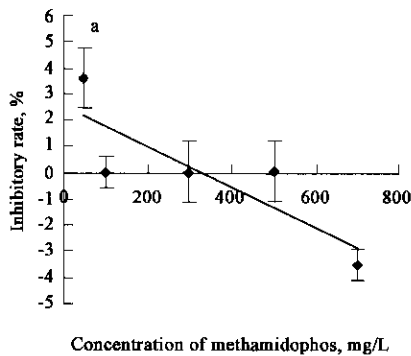


Fig.2 Relationships between methamidophos(mg/L) and seed germination and root elongation

Perhaps during the process of transform, the $-NH_2$ of methamidophos can change into N so that methamidophos cannot have obvious effect on seed germination. However, root, which was dipped into the pesticide directly contact the pollutant. Thus, methamidophos had more adverse effect on

root elongation than on seed germination.

2.2 Joint toxicity of Cd and methamidophos on seed germination

The statistical analysis (Table 1) indicated that there was no linear correlation between the seed germination of *Abelmoschus manihot* and the concentration of Cd and

methamidophos under the tested concentrations. However, it was apparent that seed germination was observably inhibited when the tested seeds were exposed to the high dose of two pollutants (Table 2). When the tested pollutants reached the maximum dose, a relative low value of seed germination occurred, suggesting that seeds of the plant were not sensitive to combine pollution of Cd and methamidophos.

2.3 Joint toxicity of Cd and methamidophos on root elongation

Unlike seed germination, root elongation of *Abelmoschus manihot* could be obviously inhibited by the combined pollution of methamidophos and Cd. There was a markedly positive linear relationship between the inhibitory rate and the concentration of added methamidophos when added Cd remained at the same concentration of 10, 20, 30, 40, and

50 mg/L, respectively. The regression equations are listed in Table 3.

Table 1 Variance analysis of joint effects of methamidophos-Cd on seed germination

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	3944.228 ^a	25	157.769	0.732	0.807
Intercept	587019.975	1	587019.975	2725.048	0.000
Cd	1684.192	4	421.048	1.955	0.110
Methamidophos	407.876	4	101.969	0.473	0.755
Cd × methamidophos	1201.640	16	75.102	0.349	0.989
Error	15940.815	74	215.416		
Total	710999.600	100			
Corrected total	19885.043	99			

Note: a. $R^2 = 0.198$ (Adjusted $R^2 = -0.072$)

Table 2 The percentage of *Abelmoschus manihot* seed germination under the condition of Cd-methamidophos combined pollution

Cd, mg/L	Methamidophos, mg/L					
	0	50	100	300	500	700
0	95.0 ± 5.77	93.33 ± 1.15	93.33 ± 0.58	93.33 ± 1.15	93.33 ± 1.15	96.67 ± 0.58
10	100 ± 0.00	82.5 ± 1.50	87.5 ± 1.26	82.5 ± 1.71	90.0 ± 1.15	80.0 ± 1.15
20	95.0 ± 0.58	75.0 ± 1.29	82.5 ± 1.71	75.0 ± 0.58	75.0 ± 2.38	82.5 ± 1.26
30	100 ± 0.00	84.5 ± 0.58	92.5 ± 0.50	86.7 ± 2.00	86.7 ± 1.00	80.0 ± 1.73
40	92.5 ± 0.96	90.0 ± 0.82	85.0 ± 1.29	87.5 ± 1.26	87.5 ± 0.58	87.5 ± 1.26
50	92.5 ± 0.96	85.0 ± 1.73	82.5 ± 0.96	67.5 ± 0.82	75.0 ± 0.58	77.5 ± 1.5

Notes: * The data presented are mean ± SD (standard deviation)

Table 3 Changes in the inhibitory rate (RI) of root elongation with concentration of added methamidophos at the same concentrations of added Cd (n = 6)

Added Cd, mg/L	Regression equation	R ²	p-value
10	$RI = 0.045Y + 12.9$	0.900	0.018
20	$RI = 0.046Y + 16.6$	0.926	0.025
30	$RI = 0.028Y + 32.0$	0.969	0.003
40	$RI = 0.019Y + 45.6$	0.878	0.138
50	$RI = 0.010Y + 53.4$	0.829	0.704

As shown in Fig. 3, the relationships between RI and the concentration of methamidophos were at high significant level ($p < 0.01$) when the added Cd was 30 mg/L. However, the significant level was low ($p > 0.05$) when the concentration was 10 and 20 mg/L respectively, which suggest that there were interactive effects between Cd and methamidophos when the dose of Cd was low. Perhaps, at the low dose Cd can fix some part of methamidophos in the outer environment of root system. Thus some of the Cd and methamidophos were inhibited to enter the root system and to the other part of the plant. And in that way, the toxic effects of the pesticide decreased. Some researchers pointed out that the combination of organic pollutants and heavy metals will reduce the activity of heavy metals. Wang identified that herbicide chlorimuron-ethyl could reduce the toxicity of Cd when Cd was added in low dose (Wang, 2005). It is also shown in Fig. 3 that when the concentration of methamidophos was 700 mg/L, the inhibitory rate of root elongation remains at similar level (about 50%) in spite of different concentration of Cd.

According to regression equations in Table 3, half effects (IC₅₀) of methamidophos at the same concentration of Cd were calculated (Table 4). It can be seen clearly that IC₅₀ was changed with the concentration of added Cd under the combined concentrations. The value markedly decreased with the increase of Cd and reached the weakest point when the added Cd was 50 mg/L, which means the toxicity of

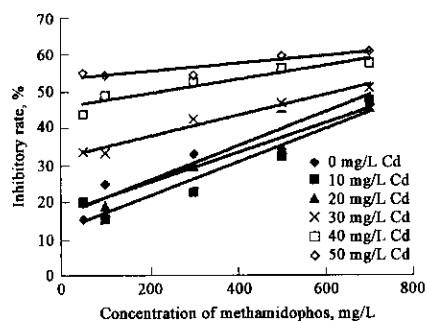


Fig. 3 Relationships between concentrations (mg/L) of methamidophos and Cd and root elongation

methamidophos was enforced with increasing concentration of Cd. That is, Cd had the major effect on the root elongation of the ornamental.

Table 4 Half effects concentration of methamidophos at the different concentration of added Cd

Added Cd, mg/L	10	20	30	40	50
IC ₅₀ , mg/L	833.80	727.28	635.34	234.13	/

Fig. 3 unfolds a comparison between inhibitory rate of root elongation at different concentrations of Cd under the same concentration of methamidophos. The average increase of inhibitory rate had big difference under all Cd concentrations while there was a steady increase in this index. Compared with seeds dipped into high concentrations (above 30 mg/L), those cultured in low concentrations (below 30 mg/L) had a relative high increase, for instance, the average increase was 21.95% and 20.77% under 10 and 20 mg/L Cd and then the value decreased to 2.8% when Cd was 50 mg/L.

When no Cd was added in the cultural solution, the minimum and the maximum value of inhibitory rate were 15.42% and 46.09% respectively. However, the two values

were respectively 15.41% and 47.72% when the adding Cd was at the same concentration of 10 mg/L, then changed to 19.89% and 45.39% when 20 mg/L Cd was added. Thus, it can be deduced that Cd and methamidophos had an antagonistic effect on root elongation when the tested concentration of Cd was lower than 20 mg/L.

Table 5 shows the differences in root elongation under combined pollution of methamidophos and Cd, respectively. It is revealed that when concentration of Cd was below 20

mg/L, there was no significant ($p > 0.05$) difference in root elongation at the same level of methamidophos. Interestingly, when concentration of methamidophos was 100 and 300 mg/L respectively, root elongation was more than that with no Cd addition, which means methamidophos and Cd had a significant antagonistic effect on root elongation when the concentration of Cd was low. However, when the concentration of added Cd was up to 50 mg/L, Cd had a synergic effect with methamidophos on root elongation.

Table 5 Root length of *Abelmoschus manihot* jointly exposed to Cd and methamidophos

Cd, mg/L	Methamidophos, mg/L					
	0	50	100	300	500	700
0	21.50 ± 3.42a	18.24 ± 0.73b	16.32 ± 1.70c	14.60 ± 2.05d	14.36 ± 1.63d	11.84 ± 1.23e
	a	a	a	a	a	a
10	19.54 ± 1.31a	15.58 ± 3.44b	16.50 ± 1.82b	15.12 ± 2.79b	13.23 ± 2.31b	10.20 ± 1.22c
	ab	b	a	a	a	a
20	17.55 ± 1.86a	15.63 ± 1.34a	15.82 ± 2.65a	13.77 ± 2.97ab	10.69 ± 3.21b	10.65 ± 2.43b
	b	b	a	a	ab	a
30	15.10 ± 0.46a	11.77 ± 1.48b	11.82 ± 1.60b	9.64 ± 2.56bc	8.86 ± 0.79c	7.66 ± 1.70c
	bc	c	b	b	b	b
40	12.47 ± 0.38a	9.35 ± 2.59b	8.21 ± 1.27bc	7.43 ± 1.69bc	6.65 ± 1.47c	6.40 ± 0.89c
	cd	cd	c	b	bc	b
50	10.46 ± 1.09a	6.92 ± 1.27b	7.07 ± 2.68b	7.03 ± 2.02b	5.92 ± 1.13b	5.70 ± 1.37b
	d	d	c	b	c	b

Note: * The same alphabet in the same column or row represents unobvious differences

The difference in root elongation between all treatments of methamidophos was less significant than those with low dose of Cd (less than 40 mg/L), which suggests at high concentration of Cd the joint toxicity of methamidophos-Cd more depends on Cd. The results of analysis of variance (Table 6) revealed that root elongation of *Abelmoschus manihot* was significantly ($p < 0.001$) inhibited by single methamidophos and Cd, whereas, the significant value under combined effects of methamidophos-Cd was not significant ($p = 0.601$). This indicates the methamidophos and Cd do not have obvious effect on root elongation. Research about metabolism of methamidophos in animals such as rats, hens and sucking pugs made it known that it can metabolize to methanesulfonic acid that was found incompatible with metals. Methamidophos in plants may follow the route in animals to be changed into methanesulfonic acid, therefore, Cd can react with methanesulfonic acid and the joint toxicity of methamidophos and Cd decreased. Moreover, it was also shown that methamidophos can be rapidly degraded to O,S-dimethyl phosphorothioate and ammonia. Because ammonia released from methamidophos can be absorbed by plants and is beneficial to the growth of plants, the toxic symptom of the ornamental may be alleviated.

Table 6 Variance analysis of joint effects of methamidophos-Cd on root elongation

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1675.064 ^a	25	67.003	15.223	0.000
Intercept	11776.156	1	11776.156	2675.470	0.000
Methamidophos	224.991	4	56.248	12.779	0.000
Cd	906.188	4	226.547	51.470	0.000
Methamidophos × Cd	61.512	16	3.844	0.873	0.601
Error	343.319	78	4.402		
Total	14001.283	104			
Corrected total	2018.383	103			

Note: a. $R^2 = 0.830$ (Adjusted $R^2 = 0.775$)

2.4 Analysis of joint toxicity of two pollutants

According to Xiao (Xiao, 2004), joint toxicity of Cd and methamidophos was analyzed by the following Kungolos-Hadjispyrou model (Kungolos, 1999; Hadjispyrou, 2001):

$$P(T) = 100 - \left[\prod_{i=1}^n (100 - P_i) \right] / 10^{2(n-1)} \quad (3)$$

Where $P(T)$ represents the theory value of inhibitory rate under the combined condition and P_i is the inhibitory rate of pollutant i . $P(E)$ is the observed experimental value of inhibitory rate. When $P(E) > P(T)$, the effect of pollutants is additive effect. When $P(E) = P(T)$, there is cooperative effect among pollutants and $P(E) < P(T)$ means antagonistic effect. The model can be used to analyze acting mechanism of pollutants provided each one of them worked at the same time other than in a certain order.

According to Table 7, $P(E)$ was less than $P(T)$ under all treatments, which suggests that Cd and methamidophos had antagonistic effect on root elongation of the tested plant. It was also clearly that the gap between $P(E)$ and $P(T)$ became large when the pollutants were in high dose, for instance, $P(T)$ was 16.59 more than $P(E)$ when the tested Cd and methamidophos were 50 and 700 mg/L respectively, while the difference was 2.06 when Cd and methamidophos were 10 and 700 mg/L respectively. This indicated that the antagonistic effect was obvious when the concentration of pollutants was high.

3 Conclusions

The effects of methamidophos and Cd were observed on seed germination and root elongation of the ornamental *Abelmoschus manihot*. However, the toxicity of the two pollutants acting on seed germination is less than that on root elongation. There was no markedly difference under the joint concentrations of the two pollutants although there were

Table 7 Analysis of joint toxicity of Cd and methamidophos acting on root elongation

Cd, mg/L	Methamidophos, mg/L	$P(T)$	$P(E)$	$P(E) - P(T)$
10	50	26	20.14	-5.86
	100	27.83	15.41	-12.42
	300	35.2	22.5	-12.7
	500	42.47	32.16	-10.31
	700	49.78	47.72	-2.06
20	50	35.66	19.89	-15.77
	100	37.26	18.92	-18.34
	300	43.66	29.41	-14.25
	500	49.98	45.18	-4.8
	700	56.34	45.39	-10.95
30	50	46.13	33.51	-12.62
	100	47.41	33.28	-14.13
	300	52.82	42.45	-10.37
	500	58.11	46.82	-11.29
	700	63.44	50.8	-12.64
40	50	55.79	43.87	-11.92
	100	56.88	49.01	-7.87
	300	61.28	52.66	-8.62
	500	65.63	56.18	-9.41
	700	70	57.35	-12.65
50	50	66.25	54.98	-11.27
	100	67.09	54.29	-12.8
	300	70.45	54.47	-15.98
	500	73.76	59.53	-14.23
	700	77.09	60.5	-16.59

Notes: * $P(T)$ and $P(E)$ represented the theory value and the effective value of inhibition respectively

significant linear relationships between the concentration of single Cd or methamidophos and the root elongation ($p < 0.05$). According to the root length of the plant, methamidophos and Cd had a significant antagonistic effect on root elongation of the plant when the concentration of Cd was low. However, when Cd was high, there was a synergic effect. Analysis of joint effect indicated that Cd had antagonistic effects with methamidophos on root elongation of the ornamental. Meanwhile, the joint toxicity was more dependent on effects of Cd than those of methamidophos.

References:

- Chen S H, Zhou Q X, Sun T H *et al.*, 2003. Rapid ecotoxicological assessment of heavy metal combined polluted soil using canonical analysis[J]. *Journal of Environmental Sciences*, 15: 854—858.
- Cheng Y, Zhou Q X, 2002. Ecological toxicity of reactive X-3B red dye and Cd acting on wheat(*Triticum aestivum*)[J]. *Journal of Environmental Sciences*,

- 14: 136—140.
- Fuh C B, Wu M L, 1999. Impurity analysis of methamidophos with chromatographic methods[J]. *Analytica Chimica Acta*, 395: 257—263.
- Hadjispyrou S, Kungolos A, Anagnostopoulos A, 2001. Toxicity, bioaccumulation and interactive effects of organotin, cadmium and chromium on *Artemia franciscana* [J]. *Ecotoxicology and Environmental Safety*, 49: 179—186.
- Hung D Q, Wohlers J, Thiemann W, 2002. The mineralisation of methamidophos using ionised AN air water treatment pilot system and ultraviolet irradiation [J]. *Water Research* 36: 2959—2966.
- Kungolos A, Samaras P, Kipopoulou A M *et al.*, 1999. Interactive toxic effects of agrochemicals on aquatic organisms[J]. *Water Science Technology*, 40: 357—364.
- NEPA, 1990. NEPA guidelines for testing of chemicals[M]. Beijing: Chemical Industry Press. 163—225.
- Singh A K, 2002. Acute effects of acephate and methamidophos and interleukin-1 on corticotropin-releasing factor (CRF) synthesis in and release from the hypothalamus in vitro[J]. *Comparative Biochemistry and Physiology Part C*, 132: 9—24.
- Song Y F, Zhou Q X, Xu H X *et al.*, 2002. Ecological toxicity of heavy metals in soils acting on seed germination and root elongation of wheat[J]. *Chinese Journal of Applied Ecology*, 13: 459—462.
- Thapinta A, Hudak P F, 2000. Pesticide use and residual occurrence in Thailand [J]. *Environmental Monitoring and Assessment*, 60: 103—114.
- Wang M E, Zhou Q X, 2005. Single and joint toxicity of chlorimuron-ethyl, Cd and copper acting on wheat *Triticum aestivum* [J]. *Ecotoxicology and Environmental Safety*, 60: 169—175.
- Wang M E, Zhou Q X, Zhang L H, 2003. Chemical behavior and ecological effects of pollutants acting on root-soil interface [J]. *Chinese Journal of Applied Ecology*, 14: 2067—2071.
- Wei S H, Zhou Q X, Wang X *et al.*, 2004. Potential of weed species applied to remediation of soils contaminated with heavy metals [J]. *Journal of Environmental Sciences*, 16: 868—873.
- Wu J C, Xu J X, Yuan S Z *et al.*, 2001. Pesticide-induced susceptibility of rice to brown planthopper *Nilaparvata lugens*[J]. *Entomologia Experimentalis et Applicata*, 100: 119—126.
- Wu Y Y, Tian J L, Zhou Q X, 1992. Study on the proposed environmental guidelines for Cd, Hg, Pb, and As in soil of China [J]. *Journal of Environmental Sciences*, 4: 66—73
- Xiao H, 2004. Ecotoxicological effects and mechanism of excessive acetochlor and urea to ecosystems in phaeozem cropland[D]. Ph. D Thesis. Institute of Applied Ecology, Chinese Academy of Sciences. 69—70.
- Yu Y, Zhou Q X, 2003. Effect of methamidophos on sorption-desorption behavior of copper in soils [J]. *Bulletin of Environmental Contamination and Toxicology*, 71: 979—987.
- Zhou Q X, 1995. Ecology of combined pollution [M]. Beijing: China Environmental Science Press.
- Zhou Q X, Huang G H, 2001. Environmental biogeochemistry and global environmental changes[M]. Beijing: Science Press.
- Zhou Q X, Gao Z M, 1994. Compound contamination and secondary ecological effects of Cd and As in soil-alfalfa ecosystems[J]. *Journal of Environmental Sciences*, 6: 330—336.
- Zhou Q X, Song Y F, 2004. Principles and methods of contaminated soil remediation[M]. Beijing: Science Press.
- Zhou Q X, Wang X, Liang R L *et al.*, 2003. Effects of Cd and mixed heavy metals on rice growth in Liaoning, China [J]. *Soil and Sediment Contamination*, 12: 851—864.

(Received for review August 30, 2004. Accepted October 24, 2004)