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Single and joint effects of pesticides and mercury on soil urease

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Abstract

The influence of two pesticides including chlorimuron-ethyl and furadan and mercury (Hg) on urease activity in 4 soils (meadow burozem and phaeozem) was investigated. The soils were exposed to various concentrations of the two pesticides and Hg individually and simultaneously. Results showed that there was a close relationship between urease activity and organic matter content in soil. Chlorimuron-ethyl and furadan could both activate urease in the 4 soils. The maximum increment of urease activity by chlorimuron-ethyl was up to 14%–18%. There was almost an equal increase (up to 13%–21%) in the urease activity by furadan. On the contrary, Hg markedly inhibited soil urease activity. A logarithmic equation was used to describe the relationship ($P<0.05$) between the concentration of Hg and the activity of soil urease in the 4 tested soils. Semi-effect dose (ED_{50}) values by the stress of Hg based on the inhibition of soil urease in the 4 soils were 88, 5.5, 24 and 20 mg/kg, respectively, according to the calculation of the corresponding equations. The interactive effect of chlorimuron-ethyl or furadan with metal Hg on soil urease was mainly synergic at the highest tested concentrations.

Key words: chlorimuron-ethyl; furadan; mercury; soil urease; combined pollution

Introduction

Pesticides are playing a very important role in the elimination of insect pests, the control of diseases and other aspects of agricultural production. However, they are increasingly polluting agricultural soils because of the wide and indiscriminate use of these artificial chemicals in agriculture. It was reported that the worldwide sale of pesticides was 8.50×10^9 USD in 1960, then quickly increased up to 3.32×10^{11} USD in 2004 (Zhou, 2005). At present, the quantity of pesticides and the land area treated by pesticides are still increasing every year.

Pollution of agricultural soils by heavy metals from sewage sludge or fertilizer application is another environmental problem. According to the searched literature (Chen *et al*., 1999; Wang *et al*., 2001), some agricultural soils in many provinces in China, including Liaoning, Shandong, Hebei, Henan, Jiangsu and Anhui, were badly contaminated by heavy metals. More than 32000 hm^2 of arable land were contaminated by Hg in 1988, and about 1.95×10^8 kg of Hg-contaminated rice were produced each year from 1960–1980. Furthermore, more than 1.20×10^7 t of grain yields had been lost each year due to soil pollution in those areas in China (Chen *et al*., 1999; Wang *et al*., 2001).

Soil enzymes contribute to total biological activity of the soil-plant ecosystems under different states (Dick, 1995, 1997). They are important components for participating in and assuring the correct and integrated sequence of all the biochemical routes in soil biogeochemical cycles (Ladd, 1985). The pesticides and metal Hg, as extraneous components added to soils, will affect behavior of soil enzymes (Guan, 1987; Zhou *et al*., 2004). One of the main emphases in soil enzymatic studies is its relationship (activation, inhibition or irrelevant action) with pollutants extraneous to soil. Although efforts aimed at understanding the possible cause-effect relationships between pesticides or a heavy metal and enzymatic activity have been pursued (Sannino and Gianfreda, 2001; Gianfreda *et al*., 1994; He *et al*., 2000), the joint effects of pesticide-metal interaction on enzymatic activity are less explored. It is possible that the two substances exist in the same arable land very frequently. So it is necessary to investigate the joint effect of pesticides and heavy metals on soil enzymes. For this work soil urease was selected, because of its importance in the nitrogen cycle in soil.

1 Materials and methods

1.1 Chemicals

All reagents used in the work were of analytical grade. Chlorimuron-ethyl (98.8% a.i.) and furadan (99.0% a.i.) were purchased from the Raiser Company in Liaoning Province, China and the Sannoda Company in Hubei Province, China, respectively. The molecular formula of chlorimuron-ethyl and furadan is $C_{15}H_{15}ClN_4O_6S$ and

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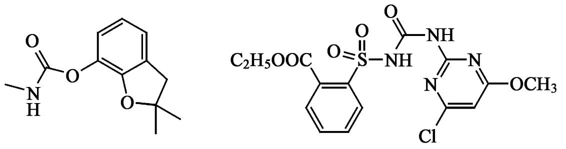
$C_{12}H_{15}NO_3$, respectively. Their structural formulas are described as follows:



Furadan Chlorimuron-ethyl

The chemical form of Hg used in the experiment was $HgCl_2$.

1.2 Soil samples

Two surface (5–20 cm) meadow burozem samples (No. 1 and 2) were collected from 2 fields in the Shenyang Station of Experimental Ecology (41°32′N, 122°23′E). Two surface (5–20 cm) phaeozem samples (No. 3 and 4) were collected from two farmland areas of Heihe City in Heilongjiang Province (49°9′N, 125°12′E and 49°10′N, 125°16′E). The four soil samples were air-dried and passed through a 1 mm-mesh sieve. The basic properties of the soils are listed in Table 1.

Soil pH was determined in a soil suspension (ratio of soil:water=1:2.5). Organic matter, cation exchange capacity (CEC) and texture were determined according to the methods suggested by the Nanjing Agricultural University (1982). Total-C and total-N were determined using the standard elementary analysis procedures (Lu, 2000).

1.3 Experimental methods

Urease activity was determined using the method suggested by Guan (1987). Triplicate samples of air-dried soil (5.00g) were allowed to mix with 1 ml toluene for 15 min. Before adding 5 ml of solution containing the pesticides chlorimuron-ethyl or furadan and $HgCl_2$, respectively or simultaneously, about 30 min later, they were mixed with 10 ml of 10% urea and 20 ml of citrate buffer (pH 6.7), and then placed in the incubator at 37°C for 15 h. After the incubation, the mixtures were immediately filtered. The filtrate was analyzed for urease activity. The concentration of NH_4^+ ions produced from urea hydrolysis was determined using the hypochlorite-alkaline phenol method (Guan, 1987). A unit of urease activity was defined as the quantity of NH_3-N produced by 1.0 g of air-dried soil at 37°C/h.

In order to distinguish the effects of the two pesticides on soil urease distinctly, an extended range of concentrations of furadan (0.01–100 mg/kg) and chlorimuron-ethyl (0.01–20 mg/kg) were selected for this experiment. The concentration of Hg for the experiments ranged from 0.92 mg/kg to 29.74 mg/kg. Six levels for each pollutant were set in this work.

To examine the joint effects of furadan-Hg, and chlorimuron-ethyl-Hg, six levels for each combined-pollution experiments were designed. Tested concentrations of the pollutants are shown in Table 2.

1.4 Statistical analysis

Unless otherwise specified, all reported results are an average of three replications. A multiple comparison was performed to compare the discrepancy of urease activity responding to each treatment of the pollutants, using the LSD (least significant difference) test at P=0.05. Regression analysis was used to examine the relationship between the urease activity and Hg concentration added to the tested soils. For all statistical analyses, the SPSS 11.0 software package was used.

2 Results and discussion

2.1 Single-factor effects of pesticides

The soils under the stress of individual pestcides displayed the variability in urease activity (Table 3), which ranged from a minimum value of 4.35 μg/(g·h) in Soil 2 to a maximum value of 17.49 μg/(g·h) in Soil 3.

Activation effects on urease activity were observed after chlorimuron-ethyl was added to the soils (Table 4). It was found through analysis of variance that the effects were significant (P<0.05) in all of the tested soils, compared with the controls. The maximum increment in urease activity by chlorimuron-ethyl in burozem and phaeozem was up to 17% (at 20 mg/kg in Soil 1), 14% (at 20 mg/kg

Table 1 Basic properties of the tested soils

Soil number	Total carbon (mg/g)	Total nitrogen (mg/kg)	Organic matter (mg/kg)	pH	CEC (cmol/kg)	Sand (%)	Silt (%)	Clay (%)
1	13.49	2.14	26.4	5.94	13.30	54.0	30.0	16.0
2	11.00	2.10	20.7	6.19	15.35	62.0	20.0	18.0
3	19.87	2.62	31.6	6.26	28.05	29.8	38.2	32.0
4	19.15	2.57	29.4	6.71	23.00	22.0	42.0	36.0

Table 2 Concentrations of the pollutants used in the experiment (mg/kg)

Level	Chlorimuron-ethyl	Furadan	Hg	Chlorimuron-ethyl+Hg	Furadan+Hg
1	0.01	0.01	0.92	0.01+0.92	0.01+0.92
2	0.10	0.10	1.85	0.10+1.85	0.10+1.85
3	1.00	1.00	3.69	1.00+3.69	1.00+3.69
4	5.00	10.0	7.39	5.00+7.39	10.0+7.39
5	10.0	50.0	14.77	10.0+14.77	50.0+14.77
6	20.0	100.0	29.54	20.0+29.54	100.0+29.54

Table 3 Baseline urease activity of soils

Soil number	Soil type	Urease activity (μg/(g·h))*
1	Burozem	16.65 ± 1.22
2	Burozem	4.35 ± 0.27
3	Phaeozem	17.49 ± 0.43
4	Phaeozem	9.61 ± 0.89

*Value was mean of three replicates±standard error of the mean (SEM).

in Soil 2), 18% (at 0.01 mg/kg in Soil 3) and 15% (at 10 mg/kg in Soil 4), respectively. It is found that the increase in soil urease activity by chlorimuron-ethyl in Soils 2 and 4 was smaller than that in Soils 1 and 3.

When the concentration of chlorimuron-ethyl was increased, there was a discrepancy in urease activity between different soils. The urease activity generally increased in Soils 1 and 2 compared with in the control, however, for Soils 3 and 4 urease activity undulated with the increase in chlorimuron-ethyl concentration. The maximum increase was at 20, 20, 0.01 and 10 mg/kg for the Soils 1, 2, 3 and 4, respectively.

Activation effects were also observed after furadan was added to the soils (Table 5). According to the ANOVA results, it was indicated that the effect on urease activity differed significantly ($P<0.05$) in Soils 1, 2 and 4, though to a different degree because of the different concentrations of furadan used. When the concentration of furadan was increased, urease activity generally increased in Soil 1. However, for Soils 2 and 4, urease activity increased at the low concentration, then decreased with an increase in the concentration of furadan in soil. The maximum increasing extent was at 100, 10 and 0.1 mg/kg for Soils 1, 2 and 4, respectively. However, there was no significant ($P>0.05$) differed effect in Soil 3. This seems to be largely responsible for the almost constant effect at all concentrations for this soil.

The effect of furadan on urease activity was dependent on soil properties, even in the same type of soil. The maximum increase of urease activity was up to 17% (at 100 mg/kg in Soil 1), 21% (at 10 mg/kg in Soil 2) and 13% (at 0.1 mg/kg in Soil 4) compared with the control, respectively. It appears that the increase in urease activity induced by furadan in burozem was greater than that in phaeozem, and the increase was greater in soils with low organic matter than in soils with high organic matter.

The soil urease activity measured in the absence of the pesticides had a close correlation with organic matter in the same type of soils (Table 3). This is mainly contributed to association of soil urease with inorganic and organic soil colloids (Burns *et al*., 1972; Gianfreda *et al*., 1992, 1995), and the soil types in this work had the similar content of inorganic colloids (Table 1). Thus, soils with higher organic matter had higher urease activity. This viewpoint was also reported in some literature (Gianfreda and Bollag, 1996). However, according to He and Zhu (1997a, b), enzymatic activity in different soils sometimes do not vary according with the content of organic mater, because it is also affected by other environmental factors that influence soil formation, for example, temperature, moisture, and mineral composition.

Pesticides have a complex effect on soil urease activity (Sannino and Gianfreda, 2001). The activation effect was explained by three possible reasons, namely: (1) the result of direct activation of pesticides on enzymatic molecules; (2) the significant increase in cell wall permeability or cellular lysis, resulting in consequential increase in the accessibility of the substrate molecules to the intracellular enzymes (Gianfreda *et al*., 1994); or (3) a possible adsorption of the pesticides on inorganic and organic supports, and a competition between immobilized enzymes and pesticides, and subsequent release of free enzymatic molecules from matrices (Gianfreda *et al*., 1995). Soil is a very complicated system, and all situations mentioned above could occur simultaneously, so there is need for further study on the effect of pesticides on soil urease activity.

The maximum activation of soil urease activity by chlorimuron-ethyl occurred in Soils 1 and 3, which were burozem and phaeozem with higher organic matter, respectively. This phenomenon could be attributed to a possible competitive adsorption of pesticides on organic matter

Table 4 Effects of chlorimuron-ethyl on the relative activity of soil urease*

Soil number	Chlorimuron-ethyl (mg/kg)						
	0	0.01	0.1	1	5	10	20
1	1.00(0.06)^{a}	1.00 (0.00)^{a}	1.01 (0.01)^{a}	1.07 (0.02)^{a}	1.15 (0.02)^{b}	1.15 (0.04)^{b}	1.17 (0.01)^{b}
2	1.00(0.04)^{a}	1.09 (0.00)^{bc}	1.06 (0.00)^{ab}	1.03 (0.02)^{ab}	1.07 (0.01)^{b}	1.09 (0.01)^{bc}	1.14 (0.02)^{c}
3	1.00(0.03)^{a}	1.18 (0.01)^{b}	1.13 (0.02)^{c}	1.14 (0.02)^{c}	1.13 (0.01)^{c}	1.13 (0.00)^{c}	1.05 (0.02)^{a}
4	1.00(0.05)^{a}	1.06 (0.02)^{ab}	1.09 (0.01)^{bc}	1.02 (0.00)^{a}	1.10 (0.00)^{c}	1.15 (0.00)^{d}	1.11 (0.01)^{c}

*The activity is relative to the activity of urease measured in the absence of chlorimuron-ethyl; values in parentheses indicate standard error of the mean (SEM); the same below; values within each rank not marked with the same letter differ significantly ($P<0.05$), the same below.

Table 5 Effects of furadan on the relative activity of soil urease*

Soil number	Furadan (mg/kg)						
	0	0.01	0.1	1	10	50	100
1	1.00(0.06)^{ab}	0.98 (0.02)^{a}	1.03 (0.01)^{b}	1.09 (0.02)^{c}	1.06 (0.00)^{bc}	1.07 (0.01)^{c}	1.17 (0.02)^{d}
2	1.00(0.04)^{a}	0.99 (0.00)^{a}	1.14 (0.00)^{b}	1.17 (0.00)^{bc}	1.21 (0.00)^{c}	1.20 (0.00)^{c}	1.14 (0.00)^{b}
3	1.00(0.03)^{a}	1.01 (0.00)^{a}	1.01 (0.00)^{a}	0.97 (0.00)^{a}	0.99 (0.05)^{a}	1.00 (0.00)^{a}	1.02 (0.00)^{a}
4	1.00(0.05)^{a}	1.03 (0.01)^{ab}	1.13 (0.01)^{d}	1.09 (0.05)^{cd}	1.10 (0.02)^{cd}	1.07 (0.00)^{bc}	0.97 (0.03)^{a}

*The activity is relative to the activity of urease measured in the absence of furadan.

surfaces and consequent release of active urease molecules in solution. While the soil contains more organic matter, more urease molecules would be released when the other properties of soils are similar.

In contrast to chlorimuron-ethyl, urease in Soils 2 and 4 was more sensitive to furadan. This could possibly be ascribed to the adsorption of furadan by organic matter, and furadan and urease molecules adsorbing to different binding sites associated with organic matter. Thus the adsorption to furadan would not bring about the release of molecular urease, and the same type of soils with higher content of organic matter would exhibit a greater buffering of furadan on urease activity.

In spite of the observed activation of chlorimuron-ethyl and furadan on soil urease activity, the effects varied with increasing concentrations of the two pesticides. As reported previously, the activity of a soil enzyme is composed of several active components, which coincide qualitatively as well as quantitatively to the overall enzymatic activity (Lethbridge and Burns, 1976). The response of each component to the presence of a pollutant will probably differ and the observed effect will be the combination of different individual changes. To explore the mechanisms involved in the pesticide-enzyme interactions in soil, further studies are needed.

2.2 Single-factor effects of mercury

There was a significantly (P<0.01) inhibitive effect of Hg on urease activity, and the effect was promoted with increasing Hg concentration. The inhibitive effect also occurred in the same soil type at the lowest Hg concentration (0.92 mg/kg), where urease activity decreased by 13% and 12% in Soils 1 and 2, and 17% in Soils 3 and 4, respectively, compared with the control. When soil Hg was 1.85 mg/kg, a slight increase in urease activity was observed in Soils 1, 3 and 4, in particular, there was a 5% increment in Soil 3, compared with the control. The decreasing mode of urease activity varied in different soils when soil Hg concentration increased. When the concentration of Hg was 29.54 mg/kg, the decrease in urease activity was 39% and 98% in Soils 1 and 2, and 60% and 61% in Soils 3 and 4, respectively, compared with the control.

To describe the relationship between the relative urease activity and Hg concentrations, logarithmic equations were used. The relationship was significant in Soil 3, and highly significant in other soils. The regression equations were expressed as follows:

$$U_1 = -0.099\ln X + 0.913 \quad (r = 0.936, P < 0.01) \qquad (1)$$

$$U_2 = -0.243\ln X + 0.839 \quad (r = 0.991, P < 0.01) \qquad (2)$$

$$U_3 = -0.155\ln X + 0.947 \quad (r = 0.885, P < 0.05) \qquad (3)$$

$$U_4 = -0.144\ln X + 0.870 \quad (r = 0.973, P < 0.01) \qquad (4)$$

Where X is the concentration (mg/kg) of $HgCl_2$, and U_1 to U_4 is the relative urease activity in the four soils. The fit of the experimental data to these equations is shown in Fig.1. According to the 4 equations, the semi-effect dose (ED_{50}, the concentration of a toxicant which inhibits a microbe-mediated ecological process by 50%) (Babich *et al.*, 1983) based on the inhibition of urease activity by Hg was 88, 5.5, 24 and 20 mg/kg for Soils 1, 2, 3 and 4, respectively. According to the values of ED_{50} and the slopes in the Equations (1)–(4), it was found that the inhibitory effect of Hg on urease activity was the greatest in Soil 2 and the smallest in Soil 1, and there was only a little greater inhibitory effect in Soil 4 than that in Soil 3.

The inhibitory effect was related to organic matter in soil. When organic matter in soil increased, the inhibition

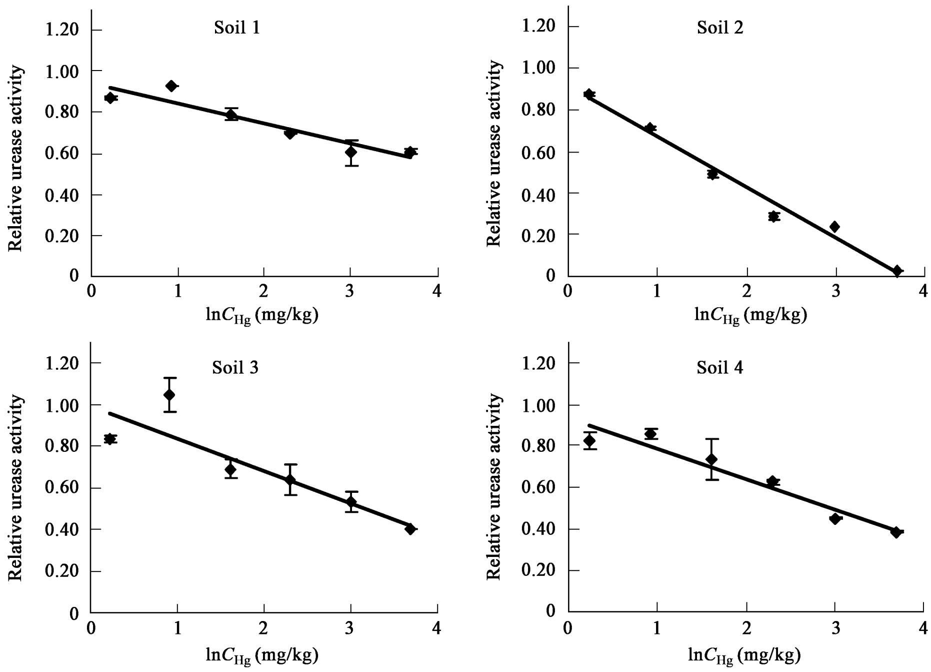


Fig. 1 Inhibitive effects of mercury on the relative activity of soil urease in Soils 1, 2, 3, and 4.

of urease activity in soils decreased. This phenomenon indicated that organic matter can protect soil urease from the toxicity of Hg in soils, which is consistent with the previous study by He *et al*. (2001).

According to Zhou *et al*. (1985) and He *et al*. (2001), Hg behaves as a typical uncompetitive inhibitor (V_{max} decreased and K_m constant with increasing Hg concentration) to soil urease, and the inhibition can be attributed to the combination of Hg and the active site of urease, and stable chemical bond formation. The recovery of urease activity in some soils is not easily explainable at present. Moreover, no general conclusion can be drawn because of the limited number of soils used in this experiment. To better understand this phenomenon, more soil types and further experiments would be needed.

2.3 Joint effects of pesticides and mercury

The joint effect of Hg and chlorimuron-ethyl could significantly reduce the activity of soil urease, and the inhibition effect was enhanced with increasing concentrations, as shown in Table 6. Under the joint stress of 29.54 mg/kg Hg and 20 mg/kg chlorimuron-ethyl, urease activity was reduced by 33%, 89%, 67% and 69% in soils 1, 2, 3 and 4, respectively. Thus, the joint effect of Hg and chlorimuron-ethyl was obvious. Comparing the data in Table 6 with that for Hg alone, it is observed that the joint effect was the similar with the effect of Hg alone, indicating that Hg played a dominant role in inhibition of urease activity.

In order to understand the interactive effect of Hg and chlorimuron-ethyl on the activity of soil urease, following equation was used to calculate the net change (ΔU) in urease activity (Zhou, 1995):

$$\Delta U = (U_{\text{Hg+Chlorimuron-ethyl}} - 1) - (U_{\text{Hg}} - 1) - (U_{\text{Chlorimuron-ethyl}} - 1) \quad (5)$$

If ΔU=0, or $|\Delta U|$<total SEM (standard error of the mean), there is no interactive effect; if ΔU>0, and ΔU>total SEM, it means an antagonistic effect; if ΔU<0, and ΔU>|total SEM|, a synergic effect takes place. The total SEM is the summation of the corresponding SEM in the three experiments of chlorimuron-ethyl, Hg and Hg+chlorimuron-ethyl. The results of these calculations are listed in Table 7.

Generally speaking, there were obviously interactive effects between Hg and chlorimuron-ethyl. According to Table 7, it was showed that there was a consistent synergic effect in Soil 3, either antagonistic or synergic effects in other 3 Soils. When the concentrations of chlorimuron-ethyl and Hg were lower than 0.10 and 1.85 mg/kg, respectively, there was a weak antagonism in Soils 1, 2 and 4. With an increase in the treatment levels, the effect was becoming mainly synergic, in which the combined effect was stronger than the additive effect of Hg and chlorimuron-ethyl individually.

The joint effect of Hg and furadan could significantly (P<0.01) inhibit urease activity in the four soils. With an increase in the concentrations of the two pollutants, the inhibitive effect increased (Table 8). The results also showed that the joint effect of Hg and furadan was also close to the treatment of Hg only. Therefore, Hg might play

Table 6 Joint effect of Hg and chlorimuron-ethyl on the relative activity of soil urease*

Soil number	Chlorimuron-ethyl+Hg (mg/kg)						
	0+0	0.01+0.92	0.10+1.85	1.00+3.69	5.0+7.39	10.0+14.77	20.0+29.54
1	1.00 (0.06)^{a}	0.91 (0.00)^{bc}	0.93 (0.00)^{b}	0.89 (0.01)^{c}	0.75 (0.00)^{d}	0.69 (0.01)^{e}	0.67 (0.00)^{e}
2	1.00 (0.02)^{a}	0.91 (0.02)^{ab}	0.81 (0.03)^{b}	0.52 (0.02)^{c}	0.36 (0.02)^{d}	0.21 (0.01)^{e}	0.11 (0.00)^{e}
3	1.00 (0.02)^{a}	0.92 (0.02)^{b}	0.84 (0.00)^{c}	0.70 (0.01)^{d}	0.49 (0.01)^{e}	0.40 (0.00)^{f}	0.33 (0.01)^{g}
4	1.00 (0.02)^{ab}	1.04 (0.02)^{a}	0.93 (0.03)^{b}	0.73 (0.04)^{c}	0.57 (0.04)^{d}	0.49 (0.03)^{d}	0.31 (0.01)^{e}

*The activity is relative to the activity of urease measured in the absence of chlorimuron-ethyl and Hg.

Table 7 Net changed amount of relative urease activity (ΔU) caused by Hg + Chlorimuron-ethyl treatment*

Soil number	Chlorimuron-ethyl+Hg (mg/kg)					
	0.01+0.92	0.10+1.85	1.00+3.69	5.0+7.39	10.0+14.77	20.0+29.54
1	0.04 (0.01)	–0.01 (0.02)	0.03 (0.05)	–0.10 (0.02)	–0.06 (0.09)	–0.10 (0.02)
2	–0.07 (0.01)	0.05 (0.03)	0.00 (0.04)	0.01 (0.03)	–0.11 (0.06)	–0.05 (0.02)
3	–0.05 (0.02)	–0.30 (0.07)	–0.09 (0.04)	–0.24 (0.05)	–0.22 (0.05)	–0.09 (0.01)
4	0.16 (0.04)	–0.02 (0.02)	0.04 (0.05)	–0.12 (0.02)	–0.11 (0.01)	–0.19 (0.02)

*The activity is relative to the activity of urease measured in the absence chlorimuron-ethyl and Hg.

Table 8 Joint effects of Hg and furadan on the relative activity of soil urease*

Soil number	Furadan+Hg (mg/kg)						
	0+0	0.01+0.92	0.10+1.85	1.00+3.69	10.0+7.39	50.0+14.77	100.0+29.54
1	1.00 (0.06)^{a}	0.93 (0.04)^{bc}	0.96 (0.03)^{ab}	0.87 (0.05)^{cd}	0.80 (0.02)^{d}	0.69 (0.01)^{e}	0.67 (0.02)^{e}
2	1.00 (0.04)^{a}	0.78 (0.01)^{b}	0.78 (0.00)^{b}	0.70 (0.09)^{bc}	0.56 (0.06)^{c}	0.30 (0.00)^{d}	0.10 (0.05)^{d}
3	1.00 (0.00)^{a}	0.97 (0.02)^{a}	0.97 (0.01)^{a}	0.77 (0.01)^{b}	0.58 (0.03)^{c}	0.42 (0.00)^{d}	0.35 (0.02)^{e}
4	1.00 (0.01)^{a}	0.95 (0.05)^{a}	0.93 (0.04)^{a}	0.74 (0.01)^{b}	0.56 (0.05)^{c}	0.35 (0.03)^{d}	0.37 (0.02)^{d}

*The activity is relative to the activity of urease measured in the absence furadan and Hg.

Table 9 The net changed amount of relative urease activity (ΔU) caused by simultaneous addition of Hg and furadan*

Soil number	Furadan+Hg (mg/kg)					
	0.01+0.92	0.10+1.85	1.00+3.69	10.0+7.39	50.0+14.77	100+29.54
1	0.08 (0.02)	–0.01 (0.01)	–0.01 (0.05)	0.04 (0.00)	0.02 (0.06)	–0.10 (0.03)
2	–0.09 (0.01)	–0.08 (0.01)	0.04 (0.02)	0.07 (0.02)	–0.14 (0.05)	–0.07 (0.00)
3	0.13 (0.02)	–0.09 (0.05)	0.11 (0.04)	–0.05 (0.10)	–0.10 (0.05)	–0.07 (0.00)
4	0.10 (0.05)	–0.06 (0.03)	–0.03 (0.10)	–0.13 (0.02)	–0.17 (0.01)	0.01 (0.04)

*The activity is relative to the activity of urease measured in the absence furadan and Hg.

the dominant role in multiple-contaminant systems.

In order to understand the interactive effect of Hg and furadan on the activity of soil urease, the corresponding equation was used to calculate the net change (ΔU) in urease activity:

$$\Delta U = (U_{\mathrm{Hg+furadan}} - 1) - (U_{\mathrm{Hg}} - 1) - (U_{\mathrm{furadan}} - 1) \quad (6)$$

According to Table 9, there was either synergism or antagonism when the exposure levels of furadan and Hg was less than 10.0 and 7.39 mg/kg, respectively. However, with an increase in the concentrations of furadan+Hg, the effect was mainly becoming synergistic in all 4 soils.

3 Conclusions

Urease activity in the tested 4 soils was obviously different and dependent on basic properties of soils. Generally speaking, urease activity was high in the soils with high organic matter. Chlorimuron-ethyl had an activating effect on urease activity. The more organic matter content in soil was, the more activating effect was. The maximum observed increment of urease activity in the 4 soils was up to 17%, 14%, 18% and 15%, respectively. Furadan could also activate soil urease. Phaeozem soils with high organic matter had smaller activating effects and stronger a buffering capacity than meadow burozem. The maximum increase in urease activity in meadow burozem was up to 17% and 21%, respectively, while the increase in urease activity in Soil 4 (phaeozem) was only 10% and there was no obvious change in urease activity in Soil 3 (phaeozem). On the contrary, Hg had a strong inhibitory effect on urease activity and the relation between Hg and urease activity can be well fitted by a logarithmic equation, in which the relative efficiency reached significant or highly significant levels. Therefore urease activity can possibly indicate the degree of Hg pollution. In according with the logarithmic equations, the ED_{50} for the Soils 1, 2, 3 and 4 was 80, 5.5, 24 and 20 mg/kg, respectively.

The combined effect of Hg and chlorimuron-ethyl in Soil 3 with the highest organic matter showed obvious synergism for all treatments. In the other 3 soils, the synergism occurred at the high concentration treatments only. The interactive effects between Hg and furadan could be either synergisic or antagonisic if the concentration of Hg + furadan was less than 10 mg/kg + 7.39 mg/kg, however, at the high concentrations of Hg and furadan the interactive effect was mainly synergistic.

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