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Environmental Contamination and Toxicology

Binary-Joint Effects of Acetochlor, Methamidophos, and Copper on Soil Microbial Population

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Eco-toxicological effects and ecological risk assessment of single pollutant and single target organism were well documented. Many studies have paid more and more attention to combined pollution of more than one pollutant in ecosystems. Joint toxicity of different metals, organic chemicals and agrochemicals to plants, algae, microbial respiration rate, and aquatic organisms was examined (Zhou 1999; Wang et al. 2001; Cheng and Zhou 2002). Many models for theoretically predicting expected joint toxicity have been used to evaluate interactive effects between different chemicals in actual ecosystems (Zhou et al., in press). Compared with individual pollutant, many experimental results about combination forms of organic-organic, organic-inorganic and inorganic-inorganic contaminants are very confused and usually bring about more serious pollution problems. This should be more significant for revealing interactive mechanism of their joint action than that of individual pollutants (Kungolos et al. 1999). Study on combined pollution rather than single one is more important for further understanding of ecological behavior of pollutants, and for assessing potential ecological risk in real systems (Prakash et al. 1996; Hadjispyrou et al 2001; Zhou and Chen 2001).

In recent years, various agrochemicals have been increasingly on the market, and their consumption has been greatly expanded to ensure the high yield of agricultural production in China. Usually, agrochemicals were applied in combinations. In other words, it is very prevalent that herbicide, pesticide, bactericide, fungicide are simultaneously applied on the same site in one growing season. Combined pollution of agrochemicals is thus becoming one of widespread environmental pollution phenomena. According to Hu (1998) and Wang (1999), the consumption of acetochlor and methamidophos is more than 10,000 tons per year in China, and in particular, they are widely used in the same growing season for the high-yield production of crops such as maize, soybean and wheat in northeastern China. At least once a year, acetochlor is applied to agricultural soils to selectively control most annual grasses, certain wild broadleaf weeds, and yellow nutaedge. Methamidophos is always applied more than once a year to selectively control pests during growth season. As a result, acetochlor and

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methomidophose are simultaneously applied to agricultural soils at the same growth season, resulted in a combined pollution of the two agrochemicals in water environment and agricultural soil systems. Copper, as an essential trace metal for living crops and as a bactericide for protecting crops against pathogen bacteria, was widely used in agricultural production. In addition, copper, as a growth promoter for pigs and other breeding animals, was finally discharged to agricultural soils through application of organic manure and surface runoff. However, copper at high concentration showed potential toxicity for all organisms including soils microorganisms (Zhuang et al. 2000; Zheng et al. 2001). Therefore, the ecological risk from interaction between agrochemicals and at low concentration copper is an important subject to be examined.

As the most popular agrochemicals, their behavior and fate after application, toxicity to plants and animals, and ecological risk to soil organisms have been studied in great details when individually applied. However, few papers published deal with their joint effects with other pollutants (Zhuang et al., 2000; Zheng et al., 2001). The toxicity of acetochlor, methamidophos, and copper, and the joint toxicity of their binary combinations including acetochlor-methamidophos, methamidophos-Cu and acetochlor-Cu on the growth of soil indigenous microorganisms was investigated. Interactive actions of their binary combinations were evaluated using the model of P(E) (Hadjispyrou et al. 2001).

MATERIALS AND METHODS

The test microbial populations were enriched from the soil samples from a field of the Hailun Agroecological Experimental Station, Hailun County, Heilongjiang Province, China. Five replicate soil samples were collected from different parts of the sampling field, thoroughly mixed, homogenized, and stored at 4℃ prior to being treated for enrichment.

The sampling plot located at 47°27'N, 126°55'E was prevented from applying any agrochemicals, and rotated in the mode of corn-wheat-soybean. Some physical and chemical characteristics of the soil tested are listed in Table1. The background concentrations of acetochlor, methamidophos and copper in the soil were 15.0 $\mu g \cdot kg^{-1}$, 7.8 $\mu g \cdot kg^{-1}$ and 24.8 $mg \cdot kg^{-1}$, respectively.

The enrichment medium for microbial population was the minimal salt medium (MSM) consisted of 0.6 $g \cdot l^{-1}$ Na_2HPO_4, 0.4 $g \cdot l^{-1}$ KH_2PO_4, 0.2 $g \cdot l^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.1 $g \cdot l^{-1}$ NaCl, 0.01 $g \cdot l^{-1}$ $CaCl_2$, 1.0 $g \cdot l^{-1}$ glucose, 0.005 $g \cdot l^{-1}$ $FeSO_4$, and 1.0 $g \cdot l^{-1}$ NH_4NO_3, supplemented with 250 $g \cdot l^{-1}$ water extractants of test soil samples. pH was adjusted to 7.0 by using a phosphate buffer (10mM KH_2PO_4, 10mM Na_2HPO_4). 45 ml of MSM in a flask was inoculated with 5.0 g fresh soil sample (10 %), and incubated aerobically by shaking at 28℃ with 180 r/min for 48 hr. Aliquots of the

Table 1. Some physical and chemical characteristics of test soil

Sampling depth (cm)	pH	OM ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	Total K ($g \cdot kg^{-1}$)	Particle size (%)		
						Sand	Silt	Clay
0-20	6.58	37.83	2.56	0.61	26.00	33.8	39.6	26.6

first enrichments mentioned above were sub-cultured (5 ml into 45 ml fresh medium) every 48hr for 3 times in succession, and those of the final subcultures were used to determine the IR (inhibitory rate to the growth of indigenous microbial population) and IC_{50} of acetochlor, methamidophos, copper and their binary combinations by the MIC method.

The test agrochemicals, acetochlor and methamidophos, were purchased from the Institute of Chemical Industry, Shenyang, China, which were standard chemicals for qualitative and quantitative analyses of acetochlor (herbicide) and methamidophos (pesticide) residues. Their purity was 99.00% and 99.50%, respectively. The $CuSO_4$ solution was prepared using the analytically pure chemical of $CuSO_4$ and distilled water.

A series of MSM (each contains 2 ml) was prepared in tubes. Each of them was added different concentrations of acetochlor, methamidophos and $CuSO_4$ or their binary mixtures, inoculated with 0.2 ml final fresh enrichment subcultures of indigenous microorganism population in soils (IMPS), incubated at 28℃ for 48hr. The toxicity of different agrochemicals to the growth of IMPS was determined by the OD_{600} changes of the liquid cultures in different treatments. There were 3 single-factor (acetochlor, methamidophos and copper) and 3 binary combination (acetochlor-Cu, methamidophos-Cu and acetochlor-methamidophos) treatments, respectively. Each treatment group was three replicated. The control treatments included No Growth Control (NGC), namely, the OD_{600} of the treatment by adding 0.2 ml sterilized water to liquid MSM instead of 0.2 ml suspension of soil indigenous microbe enrichment, and General Growth Control (GGC), namely, the OD_{600} of treatment by adding 0.2 ml suspension of soil indigenous microbial enrichment in liquid MSM and equal volume sterilized water instead of the agrochemicals tested.

The joint effects of each binary combination between acetochlor, methamidophos and copper were determined using the inhibitory rate of the growth of IMPS in liquid SMS. The study of joint effects of each binary combination was based on such assumption that chemicals act simultaneously, but not successively on soil microorganisms. The theoretically expected additive IR P(T), which has been described in details (Kungolos et al., 1997, 1999; Hadjispyrou 2001), was given by

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

Where P_1 - the inhibition caused by chemical A_1, P_2- the inhibition of chemical A_2 and P_n- the inhibition of chemical A_n.

RESULTS AND DISCUSSION

Acetochlor had a strong inhibitory effect on the growth of IMPS at the range of 41.1-125.0 $mg \cdot l^{-1}$. The inhibitory rate of IMPS was sharply increased from 0 to 78.7 $\pm$3.3%, and then, kept a moderate increase with the increasing concentration at the range of 125.0-500.0 $mg \cdot l^{-1}$. According to Fig.1, it could be deduced that IC_{50} of acetochlor was 58.3 $mg \cdot l^{-1}$. However, when the concentration of acetochlor was lower than 41.1 $mg \cdot l^{-1}$, its application might be beneficial to the growth of IMPS. In other words, 41.1 $mg \cdot l^{-1}$ was the critical concentration whether acetochlor could promote or inhibit the growth of IMPS. When the concentration of acetochlor was 31.3 $mg \cdot l^{-1}$, its promotion to IMPS reached the highest point (IR = –22.73$\pm$ 6.43%).The action of acetochlor on the growth of IMPS was thus a dual effect being dependent on its concentration. Some of microorganisms which can tolerate or utilize low concentratin of acetochlor mighe be responsible for the promotion on growth of IMPS.

Methamidophos showed a relatively mild toxic effect on the growth of IMPS at the range of 26.6-500.0 $mg \cdot l^{-1}$, and the inhibitory rate was slowly increased from 0 to 97.5$\pm$0.40%. In particular, when the concentration of methamidophos was 31.3-125.0 $mg \cdot l^{-1}$, the inhibitory rate was kept relatively stable. It was deduced from Fig.1 that IC_{50} of methamidophos was equal to 256.5 $mg \cdot l^{-1}$. However, when the concentration of methamidophos was lower than 24.8 $mg \cdot l^{-1}$, the inhibitory rate of IMPS became a negative value, namely, methamidophos could promote the growth of IMPS. Thus, the critical concentration of methamidophos to stimulate or inhibit IMPS was 26.6 $mg \cdot l^{-1}$. When its concentration was 7.8 $mg \cdot l^{-1}$, there was the highest growth promotion (IR = –36.4$\pm$7.91%) of IMPS. The action of methamidophos on the growth o IMPS was also a dual effect being dependent on its concentration. Some of microorganisms that can tolerate or utilize low concentration of methamidophos might be responsible for the promotion on growth of IMPS.

The action of single copper on the growth of IMPS was also complicated (Fig.2). Copper had an acute toxic effect on the growth of IMPS at the concentration of higher than 1.40 $mg \cdot l^{-1}$, and it could be deduced that IC_{50} of copper was equal to 1.76 $mg \cdot l^{-1}$. However, when the concentration of copper was lower than 1.40 $mg \cdot l^{-1}$, the inhibitory rate values became negative, and copper had a stimulating or promoting effect on the growth of IMPS. Therefore, 1.40 $mg \cdot l^{-1}$ could be the critical value whether copper showed toxic or beneficial effects on the growth of IMPS.

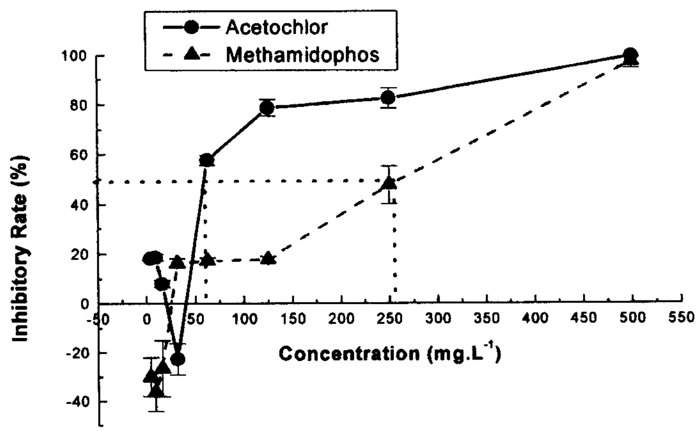


Figure 1. Dose-response curves of acetochlor and methamidophos to the growth of IMPS.

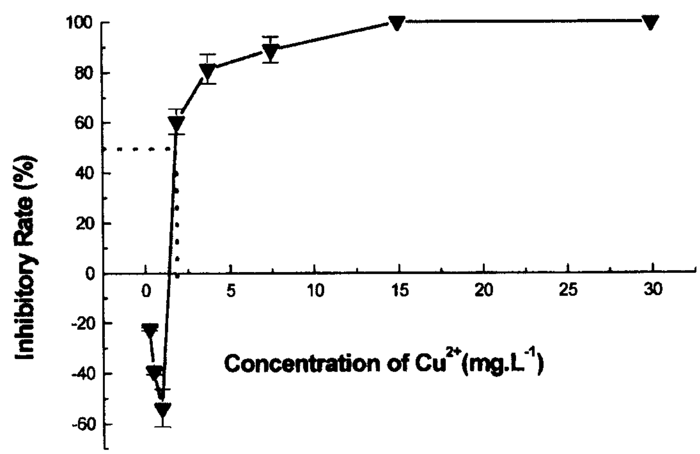
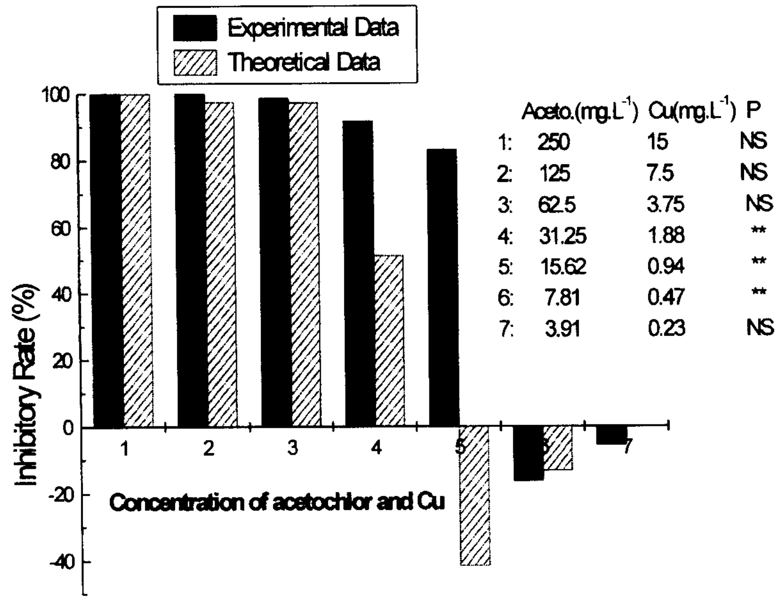


Figure 2. Dose-response curve of copper to the growth of IMPS

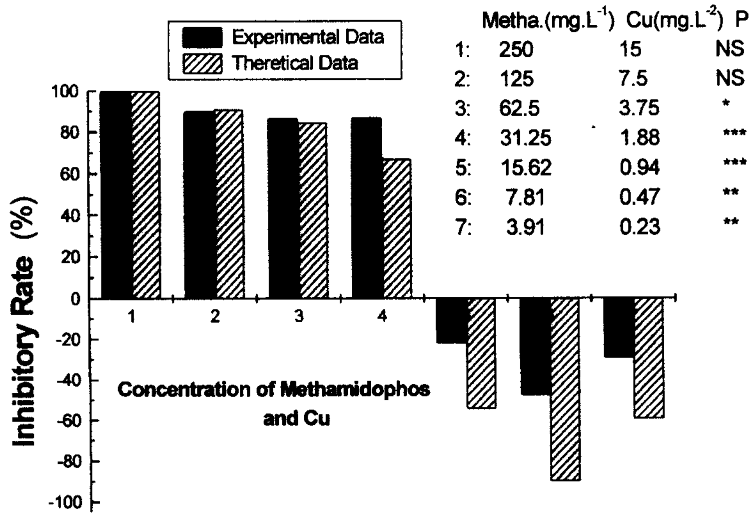
When copper concentration was 0.94 mg·l^{-1}, there was the maximum promotion on the growth of IMPS. In other words, as an important essential trace element for living organisms, copper could not only stimulate the growth of IMPS at its low concentration, but also had a toxic effect when its concentration exceeded the critical value above-mentioned.



NS: not significant; ** $p<0.01$.

Figure 3. Experimental and theoretical IR under the condition of acetochlor-Cu combined pollution

Under the condition of acetochlor-copper combined pollution, the action of these two chemicals on the growth of IMPS was still dual. Low concentration combinations of acetochlor and copper could promote the growth of IMPS. When the concentration of acetochlor and copper was respectively 7.81 $mg \cdot l^{-1}$ and 0.47 $mg \cdot l^{-1}$, the growth of IMPS reached their peak (IR = -16.44%), While their concentrations exceeded 8.36 $mg \cdot l^{-1}$ and 0.58 $mg \cdot l^{-1}$ respectively, there were inhibitory effects of the two binary-combined chemicals on the growth of IMPS. The IC_{50} of acetochlor and copper jointly acting on IMPS calculated on the basis of concentration-effect relationships was 13.26 $mg \cdot l^{-1}$ and 0.81 $mg \cdot l^{-1}$, respectively. It can be known according to the IC_{50} that the toxicity of these two chemicals under the condition of combined pollution was higher than that of single-factor pollution. In other words, the toxicity of these two chemicals was strengthened due to their interaction. The theoretical IR [$IR_{(t)}$] which was calculated using the model suggested by Kungolos et al. (1999) and Hadjispyrou (2001) was compared with the experimental IR [$IR_{(e)}$] of the binary combination of acetochlor and copper with various concentrations (Figure 3). $IR_{(e)}$ and $IR_{(t)}$ of most concentration concentration combinations (acetochlor-Cu = 250-15, 125-7.5, 62.5-3.75, and 3.91-0.23 $mg \cdot l^{-1}$, respectively) were close each other. However, both of a few concentration



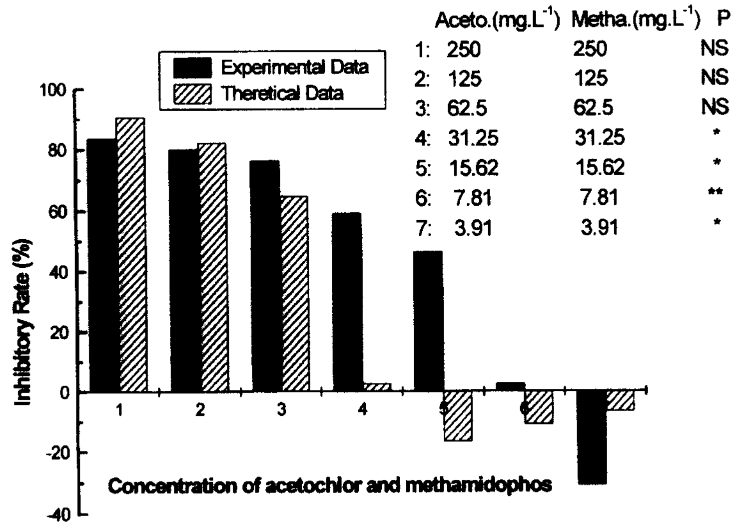
NS: not significant; * $p<0.05$; **: $p<0.01$; ***; $p<0.001$.

Figure 4. Experimental and theoretical IR under the condition of methamidophos-Cu combined pollution

combinations (acetochlor-Cu = 31.25-1.88, 15.62-0.94 and 7.81-0.47,mg·l^{-1}, respectively) were different.

The binary-joint effects of methamidophos and copper on the growth of IMPS were also investigated. When the concentration of methamidophos and copper was respectively lower than 18.38 and 1.11 mg·l^{-1}, the growth of IMPS was promoted due to their interaction. The concentration of these two chemicals with the maximum growth (IR = - 47.29 %) of IMPS was 7.31 and 0.47 mg·l^{-1}, respectively. The toxicity of methamidophos and copper at their high concentrations were strongly increased due to the interaction of their binary combination, and dependent on their different concentration combinations. Except combinations 1 and 2, other combinations of these two chemicals resulted in synergic effects on the growth of IMPS (Figure 4). It was calculated that IC_{50} of methamidophos and copper jointly acting on IMPS was 26.18 and 1.54 mg·l^{-1}, respectively. $IR_{(e)}$ and $IR_{(t)}$ of the binary-joint of methamidophos and copper were compared. The difference in combinations 1 and 2 (methamidophos-Cu = 250-15 and 125-7.5 mg·l^{-1}) was not significant. As for other combinations, $R_{(e)} > IR_{(t)}$ (Fig. 4).

Acetochlor and methamidophos are the two agrochemicals with mild toxicity. Binary-joint toxicity on the growth of IMPS were also strengthened due to their interaction when their concentrations exceeded the critical concentrations of 7.17



NS: not significant; * : p<0.05; ** ; p<0.01.

Figure 5. Comparison between experimental and theoretical IRs under the condition of acetochlor-methamidophos combined pollution.

$mg \cdot l^{-1}$.It was calculated that IC_{50} of acetochlor and methamidophos at their equal proportion of concentration combinations was equal to19.48 $mg \cdot l^{-1}$. However, low dose mixture of these two chemicals could promote the growth of IMPS. When the concentration of the chemicals mixed was respectively 3.91 $mg \cdot l^{-1}$, the growth of IMPS reached its growth peak. The differences between $IR_{(e)}$ and $IR_{(t)}$ of mixed methamidophos and acetochlor was changing along with different concentration combinations. They were not significant between $IR_{(e)}$ and $IR_{(t)}$ for combinations 1, 2 and 3, $IR_{(e)} > IR_{(t)}$ for combinations 4, 5 and 6, and $IR_{(e)} < IR_{(t)}$ for combination 7.

IC_{50} under the condition of each binary combination between acetochlor, methamidophos and copper was calculated and compared with that when they occurred individually. Obviously, IC_{50} of the binary-joint chemicals was much lower than their corresponding IC_{50} values of the individual agrochemicals. In other words, the toxicity of all these three agrochemicals under the condition of combined pollution was greatly promoted when their concentrations were higher than their critical values, even though the individuals were not toxic at the same concentrations.

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