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Ecological toxicity of reactive X-3B red dye and cadmium acting on wheat (*Triticum aestivum*)

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Abstract: Ecological toxicity of reactive X-3B red dye and cadmium in both their single form and their combined form on wheat was studied using the experimental method of seed and root exposure. The single-factor exposure indicated that the inhibitory rate of wheat root elongation was significantly increased with the increase in the concentration of the dye in the cultural solution, although seed germination of wheat was not sensitive to the dye. The toxicity of cadmium was greatly higher than that of the dye, but low concentration cadmium (< 40 mg/L) could promote the germination of wheat seed. Interactive effects of the dye and cadmium on wheat were complicated. There was no significant correlation between the inhibitory rate of seed germination and the concentrations of the dye and cadmium. Low concentration cadmium could strengthen the toxicity of the dye acting on root elongation. On the contrary, high concentration cadmium could weaken the toxicity of the dye acting on root elongation.

Keywords: ecological toxicity; reactive X-3B red dye; cadmium; combined pollution; wheat

Introduction

With the increasing varieties and quantities of dyes entering into the environment, dyes, as an important type of pollutants, have an adverse effect on water-soil-plant systems, according to some experimental results(Cheng, in press). In the eastern China, industrial wastewater from rural enterprises such as textiles, printing, dyeing, and synthetic dye factories and domestic wastewater including clothing washing water usually contain some dyes(Zhou, 1997). In the process of producing commercial organic dyes, cadmium, copper, mercury, cobalt and other transition metals are often used as catalysts(Zhou, 2001). Thus, environment polluted by wastewater from synthetic dye enterprises usually shows combined pollution of organic dyes and heavy metals(Zhou, 1995).

In recent years environmental problems from dye production are increasingly concerned and relevant studies have been focused on decolorization of wastewater containing dyes (Hao, 2000; Kang, 2000; Panswad, 2000; Willetts, 2000). As for azo dyes widely used and nearly identified as possible revulsant of cancer, investigations on mechanisms of bacterium mutation and mammal cancer-causing of dyes (Kaur, 1993; Sobti, 1992) and ecological effects of dyes on algae (Sun, 1998; Liu, 1993) in angle of medicine were made. Instead, there was a little report about environmental behavior of dyes. In particular, research on ecological effects and toxicity of dyes acting on plants including crops is scarce.

Thus, reactive X-3B red dye widely occurred in environment as an example was chosen to examine its toxicological effects on seed germination and root elongation of wheat, including single action of the dye and joint toxicity of the dye and cadmium. Perhaps, the work is useful to environmental, agricultural and food-chain safety and can provide with basic data for synthetic dye enterprises to produce dyes with low ecological risk.

1 Materials and methods

The form of the tested heavy metal, cadmium, used in this experiment was $CdCl_2 \cdot 5H_2O$(analytical grade). The tested organic dye, reactive red X-3B dye, was obtained from the Second Shenyang Factory of Synthetic Dye and its purity is 99.5%. The molecular formula of the dye is $C_{19}H_{10}C_{12}N_6Na_2O_7S_2$ and its structural formula is as follows:

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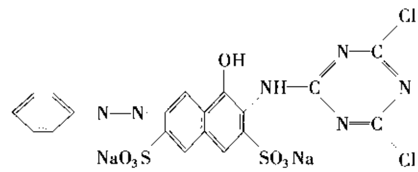

The tested crop was wheat (*Triticum aestivum*) whose variety is Liaoning Spring No. 10.

According to 10% – 50% of the inhibitory rate of wheat root elongation by the dye, the tested concentration of the dye in the formal solution culture was ascertained and was respectively equal to 0, 4500, 5000, 5500, 6000 and 6500 mg/L. According to the report by Song (Song, 2001), the tested concentration of cadmium was also determined and was respectively equal to 0, 10, 20, 40 and 60 mg/L.

Fifteen seeds of wheat were respectively exposed to reactive X-3B red dye, cadmium and the mixture of the dye and cadmium under the condition of the darkness with the temperature of 25 ± 1℃ in the culturing box (LRH-250-A, made in Guangdong). All the treatments were replicated third in order to decrease experimental errors to the maximum. When the length of the growing root cultured in the control solution without the dye and cadmium was up to 20 mm, the exposed experiment was finished, and seed germination and root elongation of all the treatments were calculated and measured. The data from the exposed experiment were statistically processed, including calculation of average values and standard deviations, correlation and regression.

2 Results and discussion

2.1 Single-factor effects of reactive X-3B red dye

The results indicated that reactive X-3B red dye had a poisonous effect on seed germination (Fig. 1a) and root elongation (Fig. 1b) of wheat under the experimental condition. In other words, it is undeniable that there is adverse ecological risk from environmental pollution of the dye once the dye is release into ecosystems. However, wheat is not sensitive to toxicological effects of reactive X-3B red dye, because the inhibition rate of seed germination was only 10% when the concentration of reactive X-3B red dye in water solution reached 4500 mg/L.

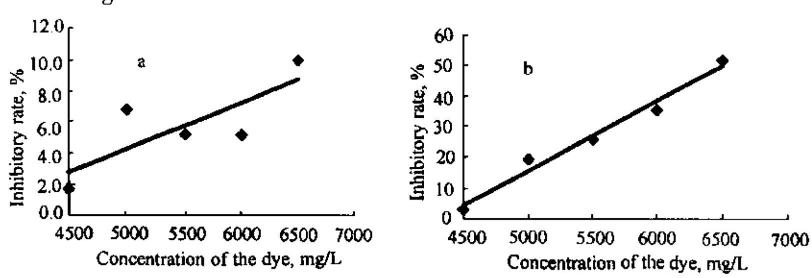

Fig.1 Toxic effect of reactive X-3B red dye on seed germination(a) and root elongation(b) of wheat

The results also pointed out that seed germination of wheat showed a descending trend and the inhibitory rate of root elongation of wheat was increased with the increasing concentration of the reactive red X-3B in the cultural solution. The corresponding relationships can be expressed using following regression equations:

$$GI_{DYE} = 0.003X - 10.72 \ (R^2 = 0.6221, \ n = 6, \ p = 0.025) \quad (1)$$

and

$$RI_{DYE} = 0.028X - 128.41 \ (R^2 = 0.9792, \ n = 6, \ p < 0.005). \quad (2)$$

Where X is the tested concentration (mg/L) of reactive X-3B red dye in water solution, GI_{DYE} is the inhibitory rate(%) of seed germination, RI_{DYE} is the inhibitory rate(%) of root elongation. According to regression equations (1) and (2) it can be calculated that half effect concentration(IC_{50}) based on the inhibition of seed germination and root elongation was equal to 20240 and 6418 mg/L, respectively.

The significance level of Equation (2) was greatly higher than that of Equation (1). Obviously, it can be deduced that there was a markedly positive relationship between root elongation of wheat and the concentration of reactive X-3B red dye in water solution. Perhaps the significant relationship could be contributed that reactive X-3B red dye penetrates the tissue of wheat seedlings and then interacts with living components of growing wheat plant. In the process, —N = N— of the dye may be deoxidized to be $—NH_2$, then changed into N. The mechanism can affect normal metabolisms of growing wheat seedlings. Nevertheless, it is difficult for reactive X-3B red dye to penetrate wheat seed with a hard capsule at the stage of seed germination, so the dye can not have evident effects on seed germination of wheat.

2.2 Single-factor effects of cadmium

The results indicated that cadmium could promote the germination of wheat seed when the tested concentration of cadmium was lower than 40 mg/L. Only after the tested concentration of cadmium exceeded 40 mg/L, cadmium could inhibit the germination of wheat seed. According to Fig. 2a, there was a curvilinear correlation between the inhibitory rate of seed germination and the tested concentration of cadmium. The corresponding regression equation is as follows:

$$GI_{Cd} = 0.0036Y^2 - 0.2757Y + 5.6474 \ (R^2 = 0.6661, n = 6, p < 0.025). \quad (3)$$

Where GI_{Cd} is the inhibitory rate(%) of the germination of wheat seed exposed to cadmium, Y is the tested concentration(mg/L) of cadmium in water solution.

Root elongation of growing wheat seedlings was increasingly inhibited with the increase in the concentration of cadmium, which obviously shows positive linear relationship (Fig. 2b). The regression equation to describe the linear relationship is as follows:

$$RI_{Cd} = 0.2219Y + 6.4481 \ (R^2 = 0.7838, n = 6, p < 0.005). \quad (4)$$

Where RI_{Cd} is the inhibitory rate(%) of root elongation of growing wheat seedlings exposed to cadmium.

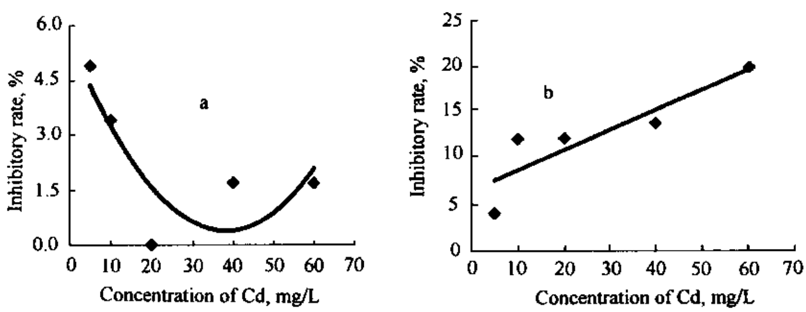

Fig.2 Toxic effects of Cd pollution on the inhibitory rate of seed germination(a) and root elongation(b) of wheat under the experimental conditions

On the one hand, wheat root may absorb the nutrient from the surroundings during the growing of wheat seedlings, and embryo provides seed with nutrient to germinate so that cadmium has more adverse influences on root elongation than on seed germination. On the other hand, it is also difficult for cadmium to penetrate wheat seed with a hard capsule at the stage of seed germination, so cadmium can not have evident effects on seed germination of wheat. According to regression equations (3) and (4) it can be calculated that IC_{50} of cadmium inhibiting seed germination and root elongation of wheat was equal to 156 and 196 mg/L, respectively. Thus, we can know that the toxicity of cadmium acting on wheat is stronger than that of reactive X-3B red dye acting on wheat, under the condition of single-factor cadmium pollution.

2.3 Joint effects of reactive red X-3B and cadmium

It can be concluded from Table 1 that there was no good correlation between the inhibitory rate of wheat seed germination and the combined concentration of reactive X-3B red dye and cadmium. However, the significant level was increased with the increase in the concentration of cadmium while wheat seeds were simultaneously exposed to the dye and cadmium. When the concentration of cadmium was up to 60 mg/L, we can believe that the inhibitory rate of wheat seed germination was significantly increased with the concentration of the dye during the combination of the dye and cadmium.

Table 1 Correlation between the inhibitory rate of seed germination and various concentration combinations of reactive X-3B red dye and cadmium

No.	Concentration, mg/L					
	Dye + Cd	Dye + Cd	Dye + Cd	Dye + Cd	Dye + Cd	R^2
1	4500 + 5	5000 + 5	5500 + 5	6000 + 5	6500 + 5	0.0990
2	4500 + 10	5000 + 10	5500 + 10	6000 + 10	6500 + 10	0.2037
3	4500 + 20	5000 + 20	5500 + 20	6000 + 20	6500 + 20	0.1376
4	4500 + 40	5000 + 40	5500 + 40	6000 + 40	6500 + 40	0.3767
5	4500 + 60	5000 + 60	5500 + 60	6000 + 60	6500 + 60	0.5828

Under the experimental conditions, combined pollution of reactive X-3B red dye and cadmium could obviously inhibit the elongation of wheat root. There was a markedly positive linear relationship between the inhibitory rate of wheat root elongation and the concentration of reactive X-3B red dye when cadmium was remained at the same concentration of 5, 10, 20, 40 and 60 mg/L, respectively. These corresponding regression equations are listed in Table 2.

According to regression equations in Table 2, IC_{50} of the dye inhibiting root elongation of wheat was calculated and listed in Table 3. It was showed that IC_{50} of the dye was changed with the concentration of cadmium in the cultural solution under the combined-pollution condition of the dye and cadmium. The toxicity of the dye was strongest when the concentration of cadmium was 5 mg/L.

Table 2 Changes of inhibitory rate (RI) of root elongation with concentration of added dye at the same concentration of added cadmium($n = 6$)

Added Cd, mg/L	Regression equation	R^2	p
5	$RI = 0.0231X - 99.67$	0.9763	< 0.005
10	$RI = 0.0254X - 95.61$	0.9581	< 0.005
20	$RI = 0.0247X - 96.26$	0.9349	< 0.005
40	$RI = 0.017X - 64.14$	0.9227	< 0.005
60	$RI = 0.0226X - 99.41$	0.9355	< 0.005

Table 3 Half effect concentration of the dye at the same concentration of added cadmium

Added Cd, mg/L	5	10	20	40	60
IC_{50}, mg/L	5732.7	5921.5	6714.1	6611.1	6479.2

It can clearly make know according to Fig. 3 that interactive effects of reactive red X-3B and cadmium on root elongation of wheat were synergic and basically squinted towards cadmium dependence, when cadmium in the cultural solution was at low concentration ($\leqslant 10$ mg/L). Probably, cadmium in low dose could enforce the toxicity of —N=N— and accelerate its deoxidization and ionization, which made the dye and cadmium in joint form more poisonous with the same concentration as in the single form to show more serious toxicity.

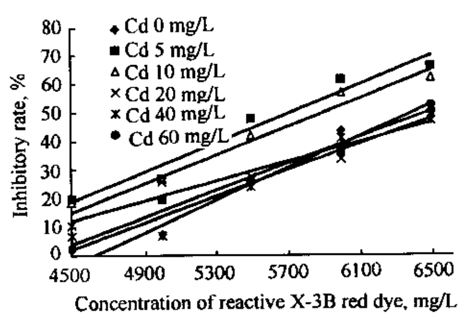

Fig.3 Jointly toxic effects of reactive X-3B red dye and cadmium on root elongation of wheat

When added cadmium in cultural solution was at the high concentration, jointly toxic effects of reactive X-3B red dye and cadmium on root elongation of wheat were not only dependent on the concentration of added cadmium, but also related to the concentration of the added dye. When cadmium was up to the same concentration of 20 mg/L and the concentration of the dye was lower than 5700 mg/L, reactive red X-3B and cadmium had a synergic effect on root elongation of wheat; when cadmium was increased to the same concentration of 40 mg/L and the concentration of the dye was lower than 5500 mg/L, reactive red X-3B and cadmium had also an synergic effect on root elongation of wheat. However, when cadmium was remained at the same concentration of 20 mg/L and the concentration of the dye was higher than 5700 mg/L, reactive red X-3B and cadmium had an antagonistic effect on root elongation of wheat; when cadmium was remained at the same concentration of 40 mg/L and the concentration of the dye was higher than 5500 mg/L, reactive red X-3B and cadmium had also an antagonistic effect on root elongation of wheat. Perhaps, cadmium in high concentration could be chelated with —OH of the dye and $—NH_2$ from the deoxidization of —N=N— in the dye, which inhibited the toxicity of cadmium, at the same time temper the action of the reactive groups of the dye so that weaken both toxicity by obstructing the combination of the groups and reactive part of enzyme taking part in metabolism and decreasing the number of N^+ .

3 Conclusions

Reactive X-3B red dye, a typical organic dye, obviously inhibited root elongation of wheat. In contrast, it had a weak effect on seed germination of wheat. IC_{50} of the dye inhibiting seed germination and root elongation was 20240 and 6417.6 mg/L, respectively. Cadmium showed more serious toxicity on wheat than reactive X-3B red dye, but the germination of wheat seed when exposed to cadmium was affected less than root elongation. IC_{50} of cadmium inhibiting seed germination and root elongation was 156 and 196 mg/L, respectively. Under the condition of the combined pollution, the germination of wheat seed was not significantly affected through changes in the concentrations of the dye and cadmium. However, the inhibition of wheat root elongation was not only dependent on the concentration of the dye, but also related to the concentration of cadmium. Combinations of low dose cadmium and the dye could behave stronger toxicity to root elongation than in their single occurrence. Combinations of high dose cadmium and the dye could display weaker toxicity to root elongation than in their single occurrence. The conclusion completely conforms to the generalized theory of joint effects put forward by Zhou (Zhou, 1995). Thus, the important significance of the new theory is very profound. It will greatly impel the development of ecotoxicology and other disciplines. The cognition on interactions among pollutants will enter into a new century.

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