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Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (*Triticum aestivum*)

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Abstract

Single and joint toxic effects of polycyclic musks including 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronapthalene (AHTN) and cadmium (Cd) on seed germination and seedling growth of wheat (*Triticum aestivum*) were investigated. The results showed that the toxicity sequence of HHCB toxic to wheat seed germination and seedling growth was similar to that of AHTN, that is, germination rate > shoot elongation > root elongation, while the toxicity of Cd was in the sequence of root elongation > shoot elongation > germination rate, according to the LC_{50} and EC_{50} values. It is suggested that polycyclic musks and Cd had different toxicological mechanisms. Root and shoot elongation of wheat might be good bioindicators for the contamination of polycyclic musks and Cd in soil. The mixture of polycyclic musks and Cd had synergistic effects on *T. aestivum* according to the equi-toxic mixture approach when root elongation was selected as the toxicological endpoint. Thus, the joint toxicity of HHCB and Cd was significantly higher than the single toxicity of HHCB or Cd, which was also confirmed by the $EC_{50\,mix}$ value of the mixture ($EC_{50\,mix}$ = 0.530 TU_{mix}). The $EC_{50\,mix}$ value of the mixture of AHTN and Cd was 0.614 TU_{mix}, which indicated that the mixture toxicity was strengthened when AHTN coexisted with Cd.

Key words: ecotoxicology; polycyclic musk; emerging pollutant; cadmium; wheat (*Triticum aestivum*); joint toxic effect
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Introduction

Polycyclic musks have been widely used as fragrances in the cosmetics and detergents since the 1950s because of their pleasant odor, inexpensive synthesis and high affinity to bind to fabrics in comparison with other synthetic musk compounds. The two main polycyclic musks used in consumer products are 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronapthalene (AHTN). In 1996, the worldwide production of polycyclic musks, 90%–95% of which were HHCB and AHTN, was up to 5600 tons (Rimkus, 1999).

The toxicity of HHCB and AHTN to aquatic biota has been well documented. Inhibition of larval development in the crustacean *Acartia tonsa* was reported with 5-day-EC_{50}-values of 0.059 mg/L HHCB, 0.026 mg/L AHTN and 0.160 mg/L ADBI (Wollenberger et al., 2003). Using sensitive *in vitro* reporter gene assays, Schreurs et al. (2002) found that HHCB and AHTN act as selective estrogen receptor modulators (SERMs), inducing both estrogenic and antiestrogenic activity being dependent on the cell line and the ER subtype targeted. Inhibiting the activity of multidrug efflux transporters responsible for multixenobiotic resistance (MXR) in gills of the marine mussel *Mytilus californianus* by HHCB and AHTN was also reported (Luckenbach and Epel, 2005). Noticeably, the inhibitory effects of a brief 2-hr exposure to musks were only partially reversed after 24- to 48-hr recovery period in clean seawater, which are relevant to human health, because they raise the possibility that exposure to common xenobiotics and pharmaceuticals could cause similar long-term inhibition of these transporters and lead to increased exposure to normally excluded toxicants (Luckenbach and Epel, 2005). The results of these studies indicated that polycyclic musks posed potential risks to aquatic organisms and human beings. However, there is still a lack of ecotoxicological information available for assessing the risks of compounds to terrestrial ecosystems and agricultural crops.

Sewage irrigation and land application of biosolids may be major routes, through which the residual synthetic musks enter terrestrial ecosystems and agricultural soils, because synthetic musks are discharged into sewage and enter municipal wastewater treatment plants (WWTPs) through down-the-drain disposal of consumer products. It is reported that the dominant removal mechanism of synthetic musks with WWTPs was not biological

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transformation but the adsorption of synthetic musks to activated sludge (Koplin et al., 2002; Gatermann et al., 1998; Simonich et al., 2000, 2002). The concentrations of HHCB and AHTN in the biosolids from a municipal wastewater treatment plant were up to 177 and 477 mg/kg, respectively (Kinney et al., 2008). Therefore, their concentrations in soil may have built up over repeated sewage irrigation and sludge applications.

The combined pollution of polycyclic musks and Cd can often occur in soils, because more Cd is being found in soil due to the application of sludge and sewage irrigation, chemical fertilizers and atmospheric precipitation (McLaughlin et al., 1999; Adams et al., 2004; Ranieri et al., 2005). Although there is a growing concern because Cd in soils can be transferred to plants, resulting in phytotoxicity and threats to animal and human health through the food chain (Wang and Zhou, 2005), little is known about joint effects of polycyclic musks and Cd on plants when they occurred in soils simultaneously.

The present study selects wheat (*Triticum aestivum*) for testing as it is the main staple cereal in northern China, Canada, USA, Russian and other countries in the world. Moreover, wheat has been widely used in ecotoxicological tests to investigate the toxic effects of pollutants such as personal care products and heavy metals (Wang and Zhou, 2005; An et al., 2009). Phytotoxicity of a chemical can be assayed using seed germination and plant growth test, which has been widely used to examine the toxic effects of chlorimuronethyl and heavy metals (Wang and Zhou, 2005). Therefore, root and shoot elongation and seed germination are selected to examine the ecotoxicity of polycyclic musks and Cd on wheat plants in this study.

The objective of this study was to assess the single and joint toxic effects of polycyclic musks and Cd on plants, and to determine the direction and extent of their interaction in soils. Results from this study will be helpful to understand the phytotoxicity and joint toxicity mechanism of polycyclic musks and Cd on plants in soils.

1 Materials and methods

1.1 Tested soil and crop species

The soil samples were collected from the surface layer (0–20 cm) of an uncultivated and unpolluted field in Tianjin, samples were air dried and sieved (< 2 mm). All physicochemical properties of soil were: pH, 6.23; organic matter, 3.86%; CEC, 19.88 cmol/kg; soil texture, 48.90% clay, 36.20% silt, and 14.90% sand.

Seeds of *T. aestivum* (winter wheat 3214) were obtained from the Tianjin Academy of Agricultural Sciences, China. Seed germination tests using control soils without adding pollutants indicated that the germination rate of the tested seeds was greater than 90%.

1.2 Chemicals

All reagents used in the study were of analytical grade. The tested form of Cd was $Cd(CH_3COO)_2 \cdot 2H_2O$ which was obtained from Tianjin Tianke Chemical Co. Ltd., China. HHCB (77.4% purity) and AHTN (99.0% purity) were obtained from Tianjin Flavors and Fragrance Corporation and Shanghai Achiever Biochemical Co. Ltd., China, respectively. Stock solutions of polycyclic musks were prepared and diluted with acetone to obtain suitable concentrations of the tested chemicals, while that of Cd with high purity water.

1.3 Single toxicity experiment

In the single toxicity experiment, the tested concentrations of pollutants were determined according to 10%–60% of the inhibitory rates of wheat root and shoot elongation. The nominal concentration of Cd^{2+} was set as 0 (control), 1200, 1440, 1728, 2074, and 2488 mg/kg, when the nominal concentrations of HHCB and AHTN were 0 (control), 39, 77, 194, 387, 775, 1936, 3873 mg/kg and 0 (control), 10, 50, 100, 500, 1000, 2500, 5000 mg/kg, respectively, which were added into the tested soil as pure HHCB and AHTN. Three replicates were used for each concentration to minimize the experimental errors.

1.4 Joint toxicity experiment

In the ecotoxicological studies, joint effects of multiple chemicals are generally analyzed using the toxic unit (TU) approach. According to the single toxicity data and the equi-toxic mixture approach, the mixed toxicant experiment was prepared as Eqs. (1) and (2) and the equi-toxic mixture was set as 0, 0.04, 0.1, 0.2, 0.4, 0.8, and 1.2 TU. The equi-toxic mixture approach is more reasonable than the equi-concentration one because that it is supposed that $0.5n \times EC_{50}$ Cd and $0.5n \times EC_{50}$ HHCB had same toxicity and their mutual toxicity should be $1n \times TU_{mix}$ (Cao et al., 2007).

$$TU = \frac{Con}{EC_{50}} \text{ (for single toxicant)} \quad (1)$$

$$TU_{mix} = \sum TU = TU_{Cd} + TU_{HHCB} \quad (2)$$

TU is a non-dimensional value calculated by the ratio of the concentration (Con) of a pollutant and its EC_{50}. In the TU model, the concentrations of the individual pollutants in the mixtures are converted to TUs. According to the resulting dose-response relationships, the sum of the toxic unit at 50% inhibition for the mixture ($EC_{50\,mix}$) was calculated by regression equations. The combined effect was defined as being concentration additive ($EC_{50\,mix}$ = 1 TU), or as being greater or less than additive ($EC_{50\,mix}$ > 1 TU or <1 TU) (An et al., 2004; Charles et al., 2006).

1.5 Plant culture

After having spiked with different concentrations of polycyclic musks dissolved in the same volume of acetone, 50 g soil samples for each treatment were transferred to 90 mm Petri dishes. Then different concentrations of Cd in high purity water were added to the soil. The moisture of the tested soil was maintained at 24% (*V/m*). Ten wheat seeds were sowed into surface soil. After the Petri dishes were covered and sealed to avoid the volatilization of polycyclic musks, they were incubated in a biochemical culturing box (HPS-250, Harbin Donglian Electronic & Technology Development Co., Ltd., China) and set with

a temperature of (25 ± 2)°C. When at least 65% of the seeds in the control had germinated, and developed roots grew above 20 mm long, the test period was finished. In the meantime, the seed germination and the shoot and root elongation of wheat in all the treatments were measured and calculated. The standard of seed germination was considered that shoot length attained 3 mm.

1.6 Statistical analysis

Statistical analysis including calculation of average values, standard deviation (SD) and regression was performed using Microsoft Excel and SPSS 13.0. The value of EC_{50} was obtained from the Probit regression, and the confidence interval of regression results was set at 95%. The variance analysis was performed by the one-way ANOVA.

2 Results and discussion

2.1 Toxic effects of HHCB on wheat

HHCB had an important influence on the germination of wheat seeds. Figure 1a describes changes in the germination of wheat seeds under different HHCB treatments. The percentage germination based on the results of the bivariate analysis had significantly ($p < 0.05$) negative correlations with the concentration of HHCB. The results of one-way ANOVA analysis showed that the percentage germination of wheat seeds was not affected significantly compared to the control when the concentration of HHCB was less than 194 mg/kg. However, when the concentration of HHCB was greater than 194 mg/kg, it decreased significantly compared to the control with the increasing concentration of HHCB ($p < 0.01$). Generally speaking, the median lethal concentration (LC_{50}) is considered as an important parameter to evaluate the toxicity of a pollutant, which is obtained from the regression analysis of experimental data. The LC_{50} value of HHCB toxic to seed germination was 846.6 mg/kg according to the regression results in Table 1.

There was an adverse effect of HHCB on the root and shoot elongation of wheat seedlings. The inhibition rates of wheat root and shoot elongation were significantly ($p < 0.05$) correlated with the concentration of HHCB in soil. Moreover, the inhibition degree of HHCB on the elongation of wheat shoots was greater than that on the elongation of wheat roots. According to Table 1, the median effective concentration (EC_{50}) of HHCB toxic to the root and shoot elongation was 2123.0 and 928.5 mg/kg, respectively. In other words, HHCB at that concentration could result in 50% inhibition rate of root and shoot elongation. These results are different from our previous study (An et al., 2009), in which the EC_{50} value of HHCB toxic to root and shoot elongation of wheat in a hydroponic experiment was 422.3 and 143.4 mg/L, respectively. This might be related to the exposure of wheat seeds to different media spiked with HHCB. Moreover, HHCB can display strong adsorption onto the soil particles because of the high normalized sorption constants (Litz et al., 2007). Thus, HHCB bioaccessible to living organisms in soil might be less than that in solution.

According to LC_{50} and EC_{50} values (Table 1), the toxicity of HHCB to wheat was in the following sequence: germination rate > shoot elongation > root elongation. The conclusion means that wheat shoot elongation was more sensitive to HHCB than wheat root elongation, which was also elicited in our previous study by An et al. (2009). Many studies pointed out that the toxicities of organic pollutants and heavy metals to wheat root elongation were higher than that to wheat shoot elongation (Wang and Zhou, 2005; An et al., 2009). Different types of pollutants have different ecotoxicological effects on a crop, which are related to the physicochemical characteristics of a chemical. HHCB is a kind of highly lipophilic synthetic musk, which has similar characteristics with the hormone (An et al., 2009). It is well known that hormones have a biphasic effect on the plant growth which is characterized by low-dose stimulation, or a beneficial effect, and a high dose inhibitory or toxic effect (Li, 2006). In addition to concentration effects, the spectrum of biological activities

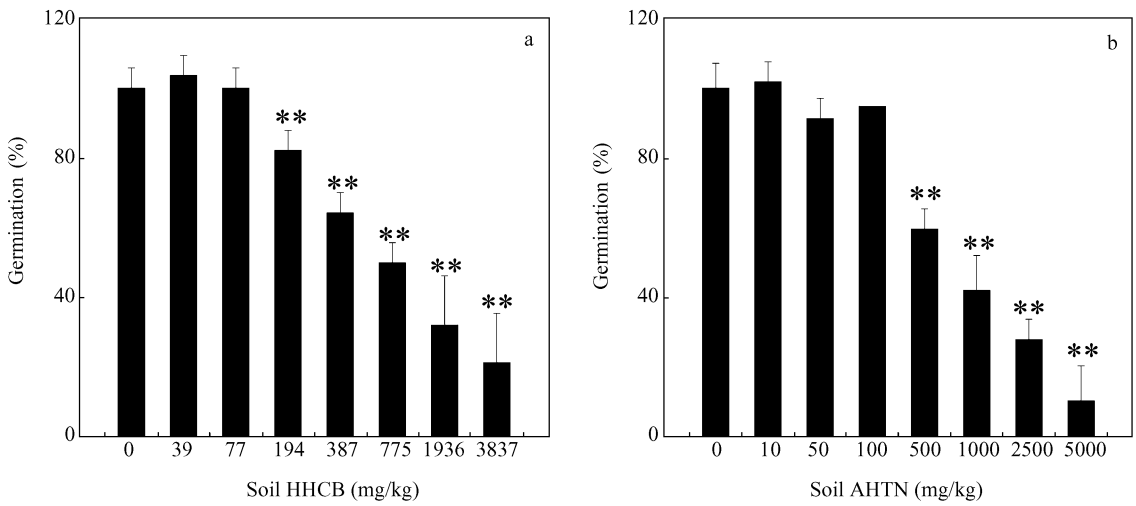

Fig. 1 Toxic effects of HHCB (a) and AHTN (b) on seed germination of wheat. Seed germination as a percentage of the mean of the control (no pollutants added) treatments. Bars represent one standard deviation of the mean of three replicates. Significant differences from controls ($p < 0.01$) are marked with two asterisk.

Table 1 Regression analysis between pollutants concentration and the inhibition rate of root and shoot elongation

Pollutant	Seed germination[a]		Root elongation[a]		Shoot elongation[a]	
	LC_{10} (mg/kg)	LC_{50} (mg/kg)	EC_{10} (mg/kg)	EC_{50} (mg/kg)	EC_{10} (mg/kg)	EC_{50} (mg/kg)
HHCB	84.1	846.6	55.7	2123.0	25.0	928.5
AHTN	90.6	762.9	33.9	2256.5	9.9	944.9
Cd	–[b]	–[b]	1260.1	1840.5	1419.9	2153.6

[a] Confidence interval of regression results is 95%.
[b] LC_{10} and LC_{50} values of Cd toxic to seed germination cannot be calculated in this study.

of hormones depends on tissue sensitivity, which is determined by the type of tissue, physiological stage and plant species, and is probably mediated by differentially elicited signal transduction pathways (Grossmann, 2009). The growth inhibition of cleavers (*Gallium aparine* L.) by herbicides has been reported, which certified that the growth inhibition of shoot was more severe than root (Grossmann, 2009).

2.2 Toxic effects of AHTN on wheat

The toxic effects of AHTN on the germination of wheat seeds and the root and shoot elongation of wheat seedlings were basically similar to those of HHCB. The changes in the germination of wheat seeds under different AHTN treatments are depicted in Fig. 1b. According to the results of the bivariate analysis, the percentage of germination had significantly ($p < 0.05$) negative correlations with the concentration of AHTN added to soils. The results of the one-way ANOVA analysis showed that the percentage germination of wheat seeds was significantly ($p < 0.01$) lower than that in control when the concentration of AHTN was ⩾ 500 mg/kg. The elongation of wheat roots and shoots was also inhibited by AHTN. The inhibition rate of wheat root and shoot elongation was elevated with the increase in AHTN concentration (Fig. 2b). According to the regression results in Table 1, AHTN could result in 50% inhibition rates of wheat root and shoot elongation at the concentrations of 2256.5 and 944.9 mg/kg.

According to the LC_{50} and EC_{50} values (Table 1), the toxicity of AHTN to wheat was in the following sequence: germination rate > shoot elongation > root elongation. These results indicated that the toxic effect of AHTN on wheat was similar to that of HHCB, because they have similar physicochemical properties. The HHCB or AHTN toxicity at median effective concentration to wheat elongation was nearly twice as much as that to wheat elongation, which indicated that wheat shoot elongation was more susceptible to polycyclic musks than wheat root elongation.

The concentration of HHCB inducing 50% inhibition of root growth was lower than that of AHTN. It indicated that HHCB was more toxic than AHTN to wheat root elongation, which might be related to their different physicochemical properties. The solubility of HHCB and AHTN is 1.75 and 1.25 mg/L, respectively (Rimkus, 1999). It has been found that the lower solubility was correlated with the higher partition coefficient (Dai, 1997). This is confirmed by partition coefficients as determined by Wrinkle et al. (1998), which are two fold higher for AHTN than for HHCB (K_p of AHTN = 9550 L/kg, K_p of HHCB = 4490 L/kg). Moreover, Litz et al. (2007) found that AHTN generally exhibited stronger adsorption on soil materials than HHCB. The normalized sorption constants of AHTN and HHCB were 4839–13,550 L/kg and 4229–7875 L/kg in different soils. Therefore, AHTN is easier to be adsorbed on soils than HHCB, which might lead to the lower bioavailability of AHTN than HHCB in the same concentration. Furthermore, the transfer factor (the ratio of the concentration in a plant to the concentration in soil) of HHCB (0.095) was higher than that of AHTN (0.082),

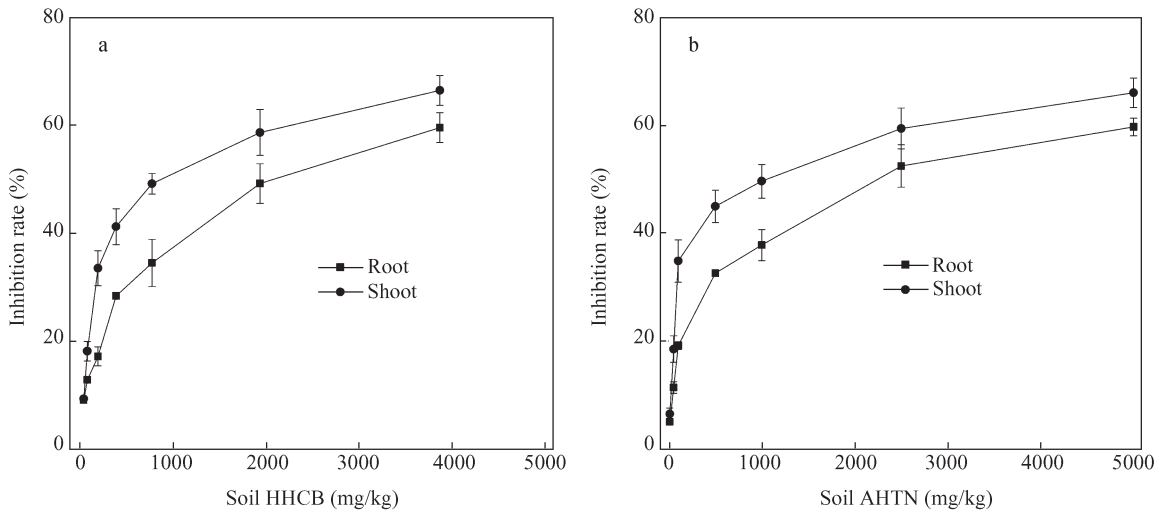

Fig. 2 Toxic effects of HHCB (a) and AHTN (b) on the elongation of wheat root and shoot. Bars represent one standard deviation of the mean of three replicates.

therefore, HHCB is easier to be transferred into various plants than AHTN. All of these results demonstrated that HHCB had a higher toxicity than AHTN in this study.

According to the EC_{50} values of HHCB and AHTN in Table 1, it could be seen that the acute toxicity of HHCB or AHTN was not high. The EC_{10} values of HHCB and AHTN were 9.9–55.7 mg/kg, nearly two orders of magnitude lower than the EC_{50} values, which were generally considered as the toxicity threshold. Thus, more attention should be paid to soil contamination with low concentrations of polycyclic musks and long-time exposure. In addition, these contaminants can be easily adsorbed by plants and may pose risks to all the components of a terrestrial ecosystem and human health through the food chain.

2.3 Toxic effects of Cd on wheat

Changes in the germination of wheat seeds under different Cd treatments are depicted in Fig. 3a. It showed that most of Cd treatments could not affect the wheat seed germination significantly, except that 2488 mg/kg Cd treatment induced a significant decrease in the percentage of wheat seed germination based on the results of the one-way ANOVA analysis ($p < 0.01$). Thus, seed germination was not a suitable indicator to assess the toxicity of Cd in soil. This trend was consistent with the result by An et al. (2004).

Cd had significantly adverse effects on wheat root and shoot elongation. As shown in Fig. 3b, the inhibition rates of wheat root and shoot elongation had significantly ($p < 0.05$) positive correlations with the concentration of Cd. The EC_{50} values of Cd toxic to root and shoot elongation were 1840.5 and 2153.6 mg/kg (Table 1), respectively. It indicated that wheat roots were more susceptible to Cd than wheat shoots. The EC_{50} value for Cd toxicity to root elongation in this study was higher than that in other studies (An, 2004; Cao et al., 2007). The reason for this phenomenon might be that different soil physicochemical properties including pH, CEC, organic matter and soil texture might lead to different bioavailabilities and phytotoxicities of Cd in soil. Besides, the anions also could affect the phytotoxicity of Cd.

According to the LC_{50} and EC_{50} values (Table 1), the toxicity of Cd to seed germination and root and shoot elongation was in the following sequence: root elongation > shoot elongation > germination rate. Therefore, in this study wheat root elongation could be considered as the most sensitive bioindicator for Cd pollution in soil. These results have been certified by other researchers (An, 2004; Cheng and Zhou, 2002; Song et al., 2002; Liu et al., 2008). The available evidence indicated that Cd can readily penetrate into the root cortex (Yang et al., 1998), consequently wheat roots are likely to undergo Cd damage first (di Toppi and Gabbrielli, 1999). However, the toxicity sequence of polycyclic musks was opposite to Cd, which suggested the toxicity mechanisms of the two different groups of chemicals were different because of their different physicochemical properties.

In the single toxicity experiments, wheat seed germination was sensitive to HHCB and AHTN, but not to Cd. It is known that seed germination relies almost exclusively on seed reserves for the supply of metabolites for respiration as well as other anabolic reactions. Starch is quantitatively the most abundant storage material in seeds and available evidence indicated that in germinating seeds starch is degraded predominantly via the amylolytic pathway (Juliano and Varner, 1969; Waghorn et al., 2003). Since synthetic musks are highly lipophilic chemicals with high octanol/water partition coefficients ($\log K_{ow}$) (5.9 for HHCB, 5.7 for AHTN), they might penetrate into the wheat seed coat more easily to inhibit the amylase activities at the stage of seed germination. Therefore, HHCB and AHTN have more evident effects on seed germination.

2.4 Joint toxic effects of HHCB and Cd on wheat

An equi-toxic combined experiment for soil spiked with HHCB and Cd was conducted based on the single toxicant exposure data. According to the sensitivity of seed

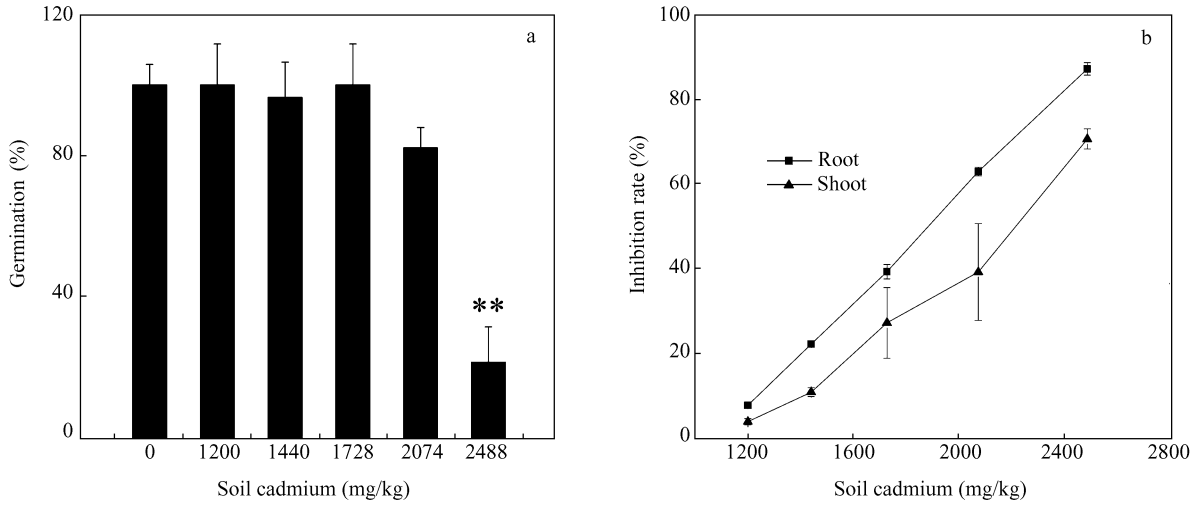

Fig. 3 Toxic effects of Cd on seed germination and seedling of wheat. Seed germination as a percentage of the mean of the control (no pollutants added) treatments. Bars represent one standard deviation of the mean of three replicates. Significant differences from controls ($p < 0.01$) are marked with two asterisk.

Table 2 Composition of combined test of polycyclic musk and Cd

Treatment (TU_{mix})	HHCB conc. (TU_{HHCB}) + Cd conc. (TU_{Cd}) (mg/kg)	AHTN conc. (TU_{AHTN}) + Cd conc. (TU_{Cd}) (mg/kg)
1 (0)	0 (0) + 0 (0)	0 (0) + 0 (0)
2 (0.04)	42.4 (0.02) + 36.8 (0.02)	45.1 (0.02) + 36.8 (0.02)
3 (0.1)	106.1 (0.05) + 92.0 (0.05)	112.8 (0.05) + 92.0 (0.05)
4 (0.2)	212.3 (0.1) + 184.0 (0.1)	225.6 (0.1) + 184.0 (0.1)
5 (0.4)	424.6 (0.2) + 368.0 (0.2)	451.3 (0.2) + 368.0 (0.2)
6 (0.8)	847.8 (0.4) + 736.0 (0.4)	902.5 (0.4) + 736.0 (0.4)
7 (1.2)	1273.7 (0.6) + 1104.0 (0.6)	1353.8 (0.6) + 1104.0 (0.6)

germination, root and shoot elongation to single HHCB and Cd, the root elongation was selected as the endpoint of combined toxicity. The combined toxicity test was performed according to Table 2.

The combined toxicity effects of HHCB and Cd on root elongation are depicted in Fig. 4. The regression results of the combined toxicity of HHCB and Cd to root elongation indicated that the EC_{50} was 0.530 TU_{mix}, which means that a mixture contamination of 562.6 mg/kg HHCB (0.265 TU_{HHCB}) and 487.7 mg/kg Cd (0.265 TU_{Cd}) would inhibit root elongation by 50% when compared with that in control. The EC_{50} value under the mixture contamination was less than 1 TU. Therefore, HHCB and Cd had a more than additive toxicity to wheat root in the soil, that is to say, HHCB could interact with Cd and could strengthen their toxicity.

2.5 Joint toxic effects of AHTN and Cd on wheat

The study on joint toxic effects of AHTN and Cd on wheat was performed based on Table 2 and the root elongation was the endpoint of toxicity. Their EC_{50} value was 0.614 TU_{mix} (Fig. 4), which was significantly lower than 1 TU_{mix}. That means a mixture contamination of 692.8 mg/kg AHTN (0.307 TU_{mix}) and 565.0 mg/kg Cd^{2+} (0.307 TU_{mix}) reduced root growth by 50%, therefore they also had a more than additive toxicity to wheat root elongation. The mode of interaction for AHTN and Cd might be similar to that for HHCB and Cd, because HHCB and AHTN have the similar physicochemical properties and toxic effect to wheat seed germination and root and shoot elongation. The EC_{50} value of the joint contamination with AHTN and Cd was lower than that of HHCB and Cd, which might be related to the lower toxic effect of AHTN on wheat root elongation when compared with that of HHCB.

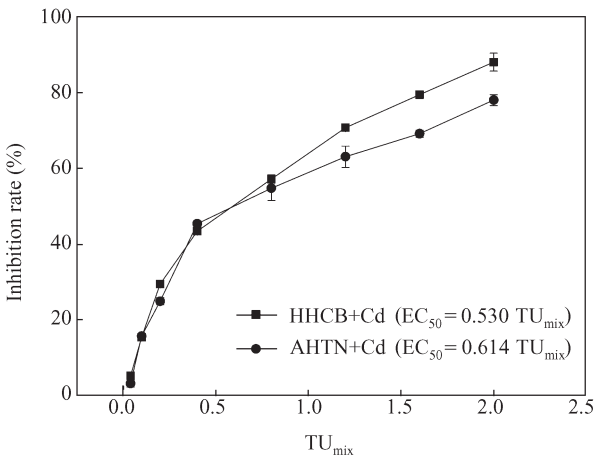

Fig. 4 Joint effects of polycyclic musks and Cd on root elongation of wheat.

The $EC_{50\,mix}$ value for the mixtures containing polycyclic musks and Cd was nearly half of the predicted value, which indicated that polycyclic musks and Cd interacted with each other and then strengthen their toxicity. There might be three reasons for this result. One reason was that polycyclic musks and Cd could affect the multixenbiotic system. It has been reported that a vast group of environmental contaminants including the artificial musks, pesticides and heavy metals could affect organisms through interference with multidrug/multixenobiotic resistance (MDR/MXR) efflux transporters (Luckenbach and Epel, 2005; Eufemia and Epel, 2000; Achard et al., 2004). The activity of these transporters provides a first line of defense to prevent the accumulation of xenobiotics in cells. Inhibition of this cellular defense mechanism increases the sensitivity of cells to xenobiotics by permitting normally excluded toxicants to enter the cell (Epel, 1998; Kurelec, 1992). A characteristic feature of these efflux transporters is affinity for a diverse array of substrates. For instance, P-glycoprotein acts on a large number of chemically unrelated substrates whose common properties are small size, moderate hydrophobicity, and positively charged domains (Bain and LeBlanc, 1998). It is reported that polycyclic musks and Cd could inhibit MXR effectively (Luckenbach and Epel, 2005; Achard et al., 2004). However, because of the higher affinity of polycyclic musks to the transporters, firstly polycyclic musks might inhibit the activity of transporters responsible for multixenobiotic resistance in wheat *T. aestivum*, so that Cd can enter the plants more easily (Luckenbach and Epel, 2005). The second reason was that polycyclic musks might compete the sorption site with Cd when polycyclic musks coexist with Cd in soil, then the weaker competing pollutant would been replaced from soil particles into soil solution, in which the pollutant is easy to be absorbed by a plant. Therefore, the bioavailability of polycyclic musks and Cd might be changed (Shen et al., 2005). In addition, the surface of biomembranes always have negative charges, thus positively charged Cd^{2+} can be easily adsorbed by biomembranes. The complexation of positively charged Cd with anionic groups such as phosphate, amino acid and acetate in biomembranes might increase the hydrophobicity of biomembranes, therefore, making it easier for highly hydrophobic polycyclic musks to reach the root surfaces and increasing the chance of being taken up by plant roots (Cao et al., 2007). This may

also be a reason why polycyclic musks and Cd had a more additive toxicity.

3 Conclusions

In this study, the single and joint toxic effects of polycyclic musks and Cd on seed germination, and root and shoot elongation of wheat were investigated. The results showed that polycyclic musks and Cd had different toxicity mechanisms and polycyclic musks and Cd had synergic effects on wheat according to the equi-toxic mixture approach. Further studies should be conducted to clearly understand the mechanisms of interaction between HHCB or AHTN and Cd in the soil environment. In order to assess the environmental risk of pollutants, more attention should be paid to low concentrations and long-time exposure of polycyclic musks and Cd to crops.

Acknowledgments

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