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Single and Joint Toxicity of Sulfamonomethoxine and Cadmium on Three Agricultural Crops

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*In order to evaluate ecological risk of agrochemicals in an agricultural environment, single and joint toxic effects of sulfamonomethoxine (SMM) and cadmium (Cd) on seed germination of wheat (*Triticum aestivum L.*), Chinese cabbage (*Solanum lycopersicum*), and radish (*Raphanus sativus L.*) were investigated. The results showed that the root and shoot elongation were significantly affected under the contamination condition (*$P < 0.05$*). In the single-factor experiments, the toxic effect of SMM on crops was much stronger than that of Cd. In the joint effects of SMM and Cd, the interactive effects of the two pollutants on root/shoot elongation of the three crops were complicated, while SMM and Cd had significantly (*$P < 0.05$*) synergic effects on root/shoot elongation of the three crops. At the low concentration of SMM, SMM and Cd had significantly (*$P < 0.05$*) synergic effects on root/shoot elongation of the three crops, but antagonistic effects were found when the concentration of SMM was high.*

Keywords Sulfamonomethoxine, cadmium, joint toxicity, inhibitory rate, crops

Introduction

Veterinary antibiotics are physiologically highly active substances and are used on a large scale as therapeutic drugs and feed additives in modern agricultural practice for the prevention and treatment of bacteria-borne diseases and improved growth rates (Boxall *et al.*, 2002; Jørgensen and Halling-Sørensen, 2000; Klaus, 2009a; 2009b). But it has been reported that only a fraction of the drugs are absorbed by animals after oral administration; a large percentage are excreted and released into the environment; for example, via manure, sludge, and waste water used as fertilizer or irrigation water in agricultural lands (Jørgensen and Halling-Sørensen, 2000; Kong and Zhu, 2007; Nicole, 2008; Wei *et al.*, 2011). The residual concentration of veterinary drugs in soil has ranged from μg/kg to g/kg (Kong and Zhu, 2007); this poses an important potential threat to the ecosystem and to human health. Therefore, the effect of residual veterinary drugs on the ecosystem is a growing environmental concern (Boxall *et al.*, 2002; Thiele-Bruhn, 2003).

Sulfonamides, as one of the selective veterinary antibiotics for human and animal

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treatment, are widely used for the control of most gram-positive and gram-negative microorganisms by inhibiting the multiplication of bacteria as competitive inhibitors of p-aminobenzoic acid in the folic acid metabolism cycle (Accinelli *et al*., 2007; Baran *et al*., 2006). In the 1990s, a survey in several countries revealed that sulfonamides account for 11–23% of the applied veterinary antibiotics in the European Union (Thiele-Bruhn, 2003). According to Thiele-Bruhn *et al*. (2004), as much as 90% of sulfonamides are excreted after consumption. Some research has indicated that sulfonamides would not be easily degradable in a soil environment (Ingerslev and Halling-Srensen, 2000; Holger *et al.*, 2008). In the past several years, sulfonamides have been widely detected in manure, soils, and surface waters (Boxall *et al*., 2003; Christian *et al*., 2003; Nicole, 2008). Other investigations have also demonstrated the potential for sulfonamides to contaminate subsoil (Boxall *et al*., 2002). Like other veterinary antibiotics, sulfamonomethoxine (SMM), a very common sulfonamide drug, entered agricultural soils mainly by fertilizing, grazing (Dagnac *et al*., 2002; Haller *et al*., 2002; Jørgensen and Halling-Sørensen, 2000), transportation through soil (Christian *et al*., 2003; Hamscher *et al*., 2005; Wojciech *et al*., 2006), surface runoff (Davis *et al*., 2006), and uptake by plants (Dolliver *et al*., 2007).

On the other hand, cadmium (Cd) is widely used in modern industry. A total of 2.2×10^4 tons of Cd has been discharged into the environment during the past half-century (Singh *et al*., 2003). According to Zhou *et al*. (2004a), the concentration of Cd in some severely contaminated soils (a result of mining, smelting, and wastewater irrigation) was higher than 100 $mg{\cdot}kg^{-1}$. Since Cd is not degradable, the accumulation of Cd in the soil may have durable effects on the plants growing on the soil. As a result, the pollution of water-soil-plant systems is becoming the focus of ecological studies (Zhou, 2003; Wang and Zhou, 2006). It is not difficult to understand that there is more frequent coexistence of SMM and Cd in agricultural lands. Increasing attention has been paid to the pollution problems associated with sulfonamides and Cd, respectively; however, little information is available about the joint toxic effects of SMM and Cd.

Seed germination and root elongation tests have been used as acute phytotoxicity tests to provide valuable information about the toxicity of contamination in the environment and other parameters (Liu *et al.*, 2009). This test has several advantages (sensitivity, simplicity, low cost, and suitability for unstable chemicals or samples) over other toxicity tests and is suitable as a standby test method as well as a rapid tool to evaluate the ecological risk of hazardous chemicals (Wang *et al*., 2002; Lin and Xing, 2007). Thus, in this study, wheat, radish, and Chinese cabbage, the main crops in north China, were chosen as target testing plants to examine the single and joint effects of SMM and Cd on seed germination and shoot and root elongation.

Materials and Methods

Materials

All reagents used in this study were of analytic grade. The tested form of cadmium was $CdCl_2{\cdot}2.5H_2O$. Sulfamonomethoxine (SMM) was obtained from Boyahua Science and Technology Company in Beijing, China, and its type is 92.9% of dispersible granule. The molecular formula of SMM is $C_{11}H_{12}N_4O_3S$ and its structure is shown in Figure 1.

The seeds of wheat (*Triticum aestivum L.*), Chinese cabbage (*Solanum lycopersicum*), and radish (*Raphanus sativus L.*) were purchased from Seed Corporation of Xinxiang City. The crop seeds were immersed in 10% sodium hypochlorite solution for

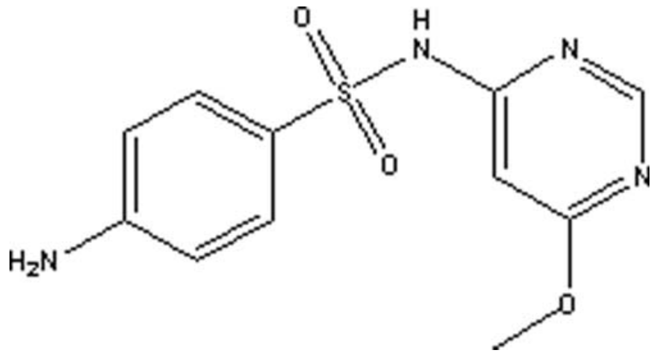

Figure 1. Molecular structure of SMM.

10 min to ensure sterility, and then they were washed several times with deionized water. The surface soil samples (0–20 cm) were collected from an experimental plot at the College of Life Sciences, Henan Normal University (35°31′N and 113°85′E), Xinxiang, Henan province, China, which had not been fertilized for almost 30 years. The fresh soil samples were air-dried and ground to pass a sieve of 1.0 mm before use. The physicochemical prosperities of the soil were: pH, 7.83; OM, 1.65%; CEC, 12.26 cmol/kg; total N, 0.09 mg·kg^{-1}; total P, 0.04 mg·kg^{-1}; total K, 0.18 mg·kg^{-1}; total Cd, 0.066 mg·kg^{-1}.

Toxicity Experiments

A 50 g sample amended with different concentrations of tested pollutions (SMM and Cd) was put into a culture dish. The soil moisture was adjusted with deionized water until it reached 60% of maximal holding capacity, and then mixed well. Fifteen sterilized seeds of three crops were scattered with a tweezer in soil in a culture dish and covered, then the dishes were put into the culturing box (HG-303-3, made in Nanjing, China) under dark conditions with temperature being controlled at 25 $\pm$ 1°C. All treatments were replicated three times to minimize experimental errors. When the length of the growing root cultured in the control soil without SMM and Cd reached 20 mm (December 13, 2012), the exposed experiment was finished, and the seed germination and the shoot and root elongation of all of the treatments were measured and calculated.

Single-Factor Experiments

According to 10–50% of the root elongation inhibitory rate of three crops by Cd and SMM, the tested concentrations of Cd were 0, 100, 200, 300, 400, 500, 600 mg·kg^{-1}, and the tested concentrations of SMM were equal to 0, 2, 5, 10, 20, 40, and 60 mg·kg^{-1} for wheat; 0, 0.5, 1, 2, 4, 6, and 8 mg·kg^{-1} for Chinese cabbage; and 0, 0.5, 1, 2, 4, 8 and 16 mg·kg^{-1} for radish.

Combined-Pollution Experiments

According to the results from the single-factor experiments, the tested concentration of SMM was 0, 2, 5, 10, 20, 40, and 60 mg·kg^{-1} for wheat; 0, 0.5, 1, 2, 4, 6, and 8 mg·kg^{-1} for Chinese cabbage; and 0, 0.5, 1, 2, 4, 8, and 16 mg·kg^{-1} for radish. The tested concentration of Cd was set to be 0, 200, and 500 mg·kg^{-1} in the joint effect experiment of SMM and Cd.

Data Reliability

The inhibition rate (IR,%) was calculated by Equation (1) as follows:

$$\mathrm{IR} = \frac{A - B}{A} \times 100\% \tag{1}$$

where A is the root or shoot length of contrast (mm) and B is root or shoot length of different concentration treatments (mm). Statistical analysis, including calculation of average values, standard deviation (S.D.), and regression, was performed on the data obtained in the tests with SPSS12.0. The multiple comparison procedure (LSR test) was used to compare shoot and root elongation under the combined pollution of SMM and Cd and statistical significance was set at $P < 0.05$.

Results

Toxic Effects of SMM on Three Crops

The statistical analysis showed that there were no significant differences ($P > 0.05$) in germination rate of the three crops (wheat, Chinese cabbage, and radish) exposed to the seven experimental concentrations of SMM, respectively. By contrast, SMM had a significantly ($P < 0.05$) toxic effect on root and shoot elongation of these crops under the experimental conditions (Figure 2). The inhibitory rate of root and shoot elongation of the three crops increased with the increasing concentration of SMM in soil. As shown in Figure 2, for each one of the three crops, there was a significant linear correlation between the inhibitory rate of root and shoot elongation and the tested concentration of SMM. The corresponding relationship can be expressed using the regression equations in Table 1. According to the regression equations based on the inhibition of root elongation and shoot elongation, IC_{50} (the concentration of SMM) corresponds to the 50% inhibitory rate of RI (the inhibitory rate (%) of root elongation) or SI (the inhibitory rate (%) of shoot elongation), mg·kg^{-1}) of SMM was calculated. The calculation showed that IC_{50} of SMM based on the inhibitory rates of root elongation of wheat, Chinese cabbage, and radish was 33.7, 4.41, and 8.82 mg·kg^{-1}, respectively, which meant that the toxic effect of SMM on the three crops was in the following sequence: Chinese cabbage>radish>wheat. For IC_{50} of SMM based on the inhibitory rates of shoot elongation of the three crops, the order was the same. Thus, Chinese cabbage was the most sensitive to the toxicity of SMM.

As shown in Figure 2, under the same concentration of SMM, the inhibitory rate of root elongation of wheat was higher than that of shoot elongation; for example, when the concentration of SMM was 2 mg·kg^{-1}, the inhibitory rates of root elongation and shoot elongation were 9.6% and 3.4%, respectively; when the concentration of SMM was 20 mg·kg^{-1}, the inhibitory rates of root elongation and shoot elongation were 49.3% and 28.5%, respectively. The same results were found for Chinese cabbage and radish. The inhibitory effect on shoot elongation of SMM was weaker than that on root elongation, which meant that root was more sensitive than shoot under the pollution of SMM, while the statistical analysis showed that differences between root elongation and shoot

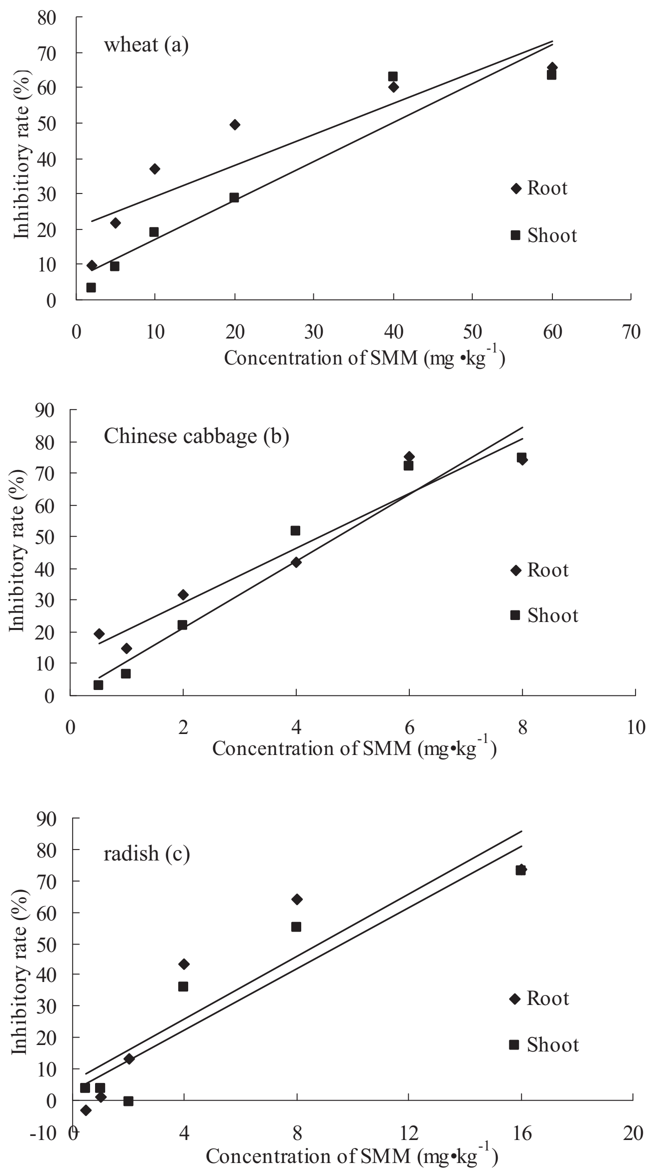

Figure 2. Toxic effects of SMM on the root and shoot elongation of the three crops (wheat, Chinese cabbage, and radish).

elongation were not significant ($P > 0.05$) at the high concentration of SMM. When the concentration of SMM in soil reached the highest for each crop in this experiment, the inhibitory rates of root elongation and shoot elongation of wheat, Chinese cabbage, and radish were 65.6% and 63.3%, 74.1% and 74.6%, 73.8%, and 73.1%, respectively.

Toxic Effects of Cd on Three Crops

Similar to the effects of SMM, root elongation and shoot elongation were correlated with the concentration of Cd ($P < 0.05$), and seed germination was not sensitive to Cd at the

Table 1
Relationships between inhibitory rate of root elongation (RI) and shoot elongation (SI) of the three crops and the concentration of SMM in soil

Crop Species	Regression Equations[a]	R^2	*P*	IC_{50}[b]
Wheat	$RI = 0.8709C_{SMM}+20.657$	0.8188	<0.05	33.69
	$SI = 1.1012C_{SMM}+5.9302$	0.9211	<0.01	40.02
Chinese cabbage	$RI = 8.5443C_{SMM}+12.331$	0.9263	<0.01	4.41
	$SI = 10.491C_{SMM}+0.6209$	0.9461	<0.01	4.71
Radish	$RI = 5.0153C_{SMM}+5.7707$	0.8122	<0.05	8.82
	$SI = 4.8835C_{SMM}+2.9261$	0.8732	<0.01	9.64

[a]C_{SMM} was the tested concentration of SMM, mg/kg.
[b]IC_{50} was the concentration of SMM correspond to 50% inhibitory rate of RI or SI, mg/kg.

tested concentration ($P > 0.05$). As shown in Figure 3, there were positive linear relationships between the inhibitory rate of root elongation, shoot elongation, and the concentration of Cd. The inhibitory rates of root and shoot elongation increased with increasing concentration of Cd in the tested soil. The corresponding regression equations are listed in Table 2. According to Table 2, Cd had obviously toxic effects on root and shoot elongation. Toxic effects on growth of the three crops were the strongest when the concentration of Cd was 600 $mg \cdot kg^{-1}$; the inhibitory rates of root elongation of wheat, Chinese cabbage, and radish were 65.9%, 58.5% and 42.1%, respectively. According to Figure 3 and Table 2, it can be concluded that IC_{50} of Cd based on the inhibition of root elongation of wheat, Chinese cabbage, and radish was equal to 507, 550, and 781 $mg \cdot kg^{-1}$, respectively, which meant that the tolerance of radish was stronger than the other crops to the toxicity of Cd, and wheat was the most sensitive among the three crops.

As shown in Figure 3, when the concentration of Cd was 100 $mg \cdot kg^{-1}$, the inhibitory rates of root elongation of wheat and radish were −4.2% and −6.1%, respectively. That means that Cd had a promoted effect on the growth of wheat and radish when the concentration of Cd was low in soil.

Toxic Effects of SMM and Cd on Three Crops

It was shown by the variance analysis that there were markedly positive correlations between the inhibitory rate of root and shoot elongation and the concentration of SMM when the concentration of Cd remained at 0, 200, and 500 $mg \cdot kg^{-1}$, respectively (Figures 4 and 5). As shown in Table 3, SMM and Cd had synergic effects on the inhibition of root and shoot elongation of wheat. The root elongation of wheat was significantly reduced with increasing concentration of Cd in the tested soil at low concentrations of SMM ($P < 0.05$), which meant that the toxicity was mainly caused by Cd when the concentration of SMM was low. However, the synergic effects were not significant at high concentrations of SMM ($P > 0.05$). When SMM concentration was 40 $mg \cdot kg^{-1}$ in soil and Cd was 0, 200, and 500 $mg \cdot kg^{-1}$, respectively, the inhibitory rate of root elongation of wheat was 59.9%, 66.7%, and 69.2%, respectively.

The root and shoot elongation of Chinese cabbage and radish were also severely inhibited under the combined pollution of SMM and Cd (Tables 4 and 5). Joint effects of SMM and Cd on seeding growth of Chinese cabbage and radish showed a similar

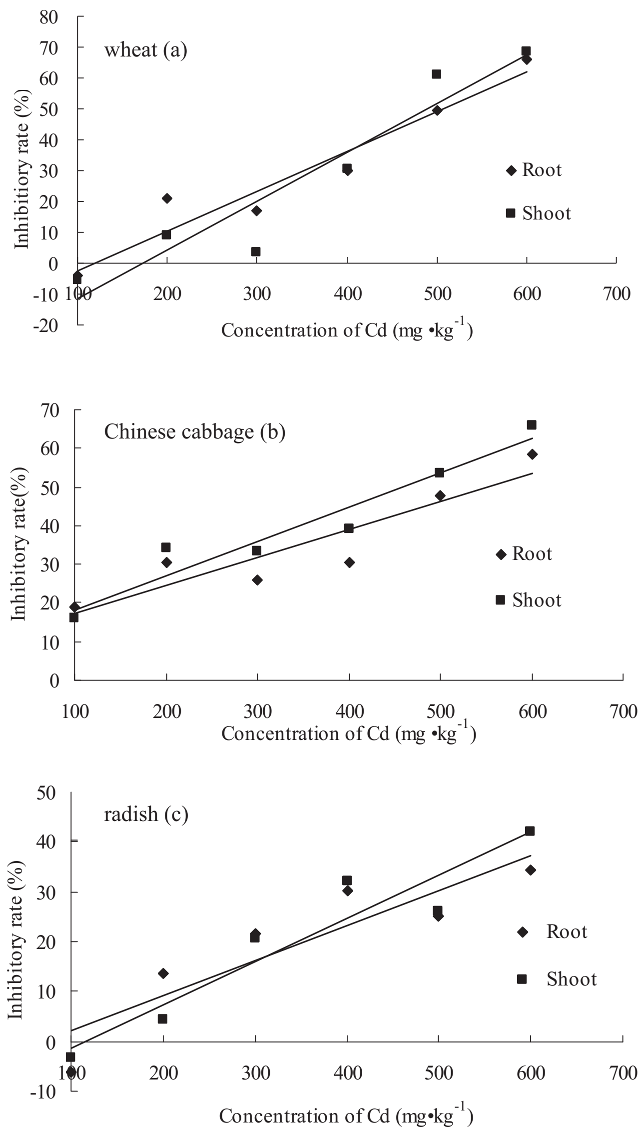

Figure 3. Toxic effects of Cd on the root and shoot elongation of the three crops (wheat, Chinese cabbage, and radish).

tendency. As can be seen from Figures 4 and 5, about a 60% inhibitory rate was a dividing point. The synergic effect played a major role when the inhibitory rate was lower than 60%, but an antagonistic effect was observed when the inhibitory rate was higher than 60%. For example, when SMM concentration was 8 mg·kg^{-1} in soil and Cd was 0, 200, and 500 mg·kg^{-1}, respectively, the inhibitory rate of root elongation of Chinese cabbage was 74.1%, 67.1%, and 66.1%, respectively. The inhibitory rate was gradually decreased with increasing concentration of Cd in the tested soil at the same concentration of SMM.

The corresponding regression equations can be expressed as those listed in Tables 6 and 7. Calculated from the regression equations, IC_{50} of SMM inhibiting root and shoot elongation of these crops was listed. It is revealed that the value of IC_{50} decreased

Table 2

Relationships between inhibitory rate of root elongation (RI) and shoot elongation (SI) of the three crops and the concentration of Cd in soil

Crop Species	Regression Equations[a]	R^2	*P*	IC_{50}[b]
Wheat	$RI = 0.1284C_{Cd} - 15.134$	0.9319	<0.01	507.27
	$SI = 0.1580C_{Cd} - 27.442$	0.9045	<0.01	490.14
Chinese cabbage	$RI = 0.073C_{Cd} + 9.8104$	0.8438	<0.01	550.54
	$SI = 0.0894C_{Cd} + 9.1129$	0.9271	<0.01	457.35
Radish	$RI = 0.0701C_{Cd} - 4.7733$	0.8122	<0.05	781.36
	$SI = 0.0869C_{Cd} - 10.096$	0.9025	<0.01	691.55

[a]C_{Cd} was the tested concentration of SMM, mg/kg.
[b]IC_{50} was the concentration of Cd correspond to 50% inhibitory rate of RI or SI, mg/kg.

obviously when the concentration of Cd remained at 0, 200, and 500 $mg{\cdot}kg^{-1}$, as shown in Tables 6 and 7.

Discussion

In the single-factor experiment of SMM or Cd, seed germination of the three crops was not sensitive to the toxicity of the two pollutants. That may be explained by the protection of the seed coat. The seed coat plays a very important role in protecting the embryo from harmful external factors. Seed coats can have selective permeability (Malgorzata and Jolanta, 1998). Pollutants, though having an obviously inhibitory effect on root growth, may not affect germination if they cannot pass through seed coats. These results are consistent with those of Song *et al*. (2002) and Cheng and Zhou (2002). They identified, in their studies on the toxicity of heavy metals (Cd, Cu, Pb and Zn) and a chemical (X-3B red dye) reactive to wheat, that the inhibitory rate of root elongation was higher than that of germination rate at the same concentration of pollutants.

Cd inhibition on root elongation has been observed for some plants (Cheng and Zhou, 2002; Zhou, 2003). Cd could reduce ATPase activity of oat root, which hampered the absorption of potassium (K) by plant roots (He *et al.*, 1998). The reason for this inhibition of root elongation may lie in the encumbrance of K absorption. However, many studies have confirmed that Cd could promote the growth of plants when the concentration of Cd is low (Lin *et al.*, 2007; Aina *et al.*, 2007; Robert and Joanna, 2003). In this study, Cd had a promoted effect on the root and shoot elongation of wheat and radish when Cd concentration was 100 $mg{\cdot}kg^{-1}$ in soil. However, the mechanisms of stimulatory effects are not well understood (Lin *et al.*, 2007). This phenomenon is normally related to a so-called "hormetic effect" that probably represents an "overcompensation" response to a disruption in the homeostasis of the organism (Aina *et al.*, 2007). According to Robert and Joanna (2003), a low dose of Cd could stimulate cell proliferation, thus the growth of plants will be stimulated.

IC_{50} was often used to evaluate the ecotoxic effects of the pollutants (Zhou *et al.*, 2004a). Tables 1 and 2 illustrate that the effect of IC_{50} of SMM on root elongation of wheat, Chinese cabbage, and radish was 33.7, 4.41, and 8.82 $mg{\cdot}kg^{-1}$, respectively, whereas IC_{50} of Cd was 507, 550, and 781 $mg{\cdot}kg^{-1}$, respectively, which meant that the toxic effects of SMM on crops were much stronger than that of Cd. According to IC_{50}, different plants have

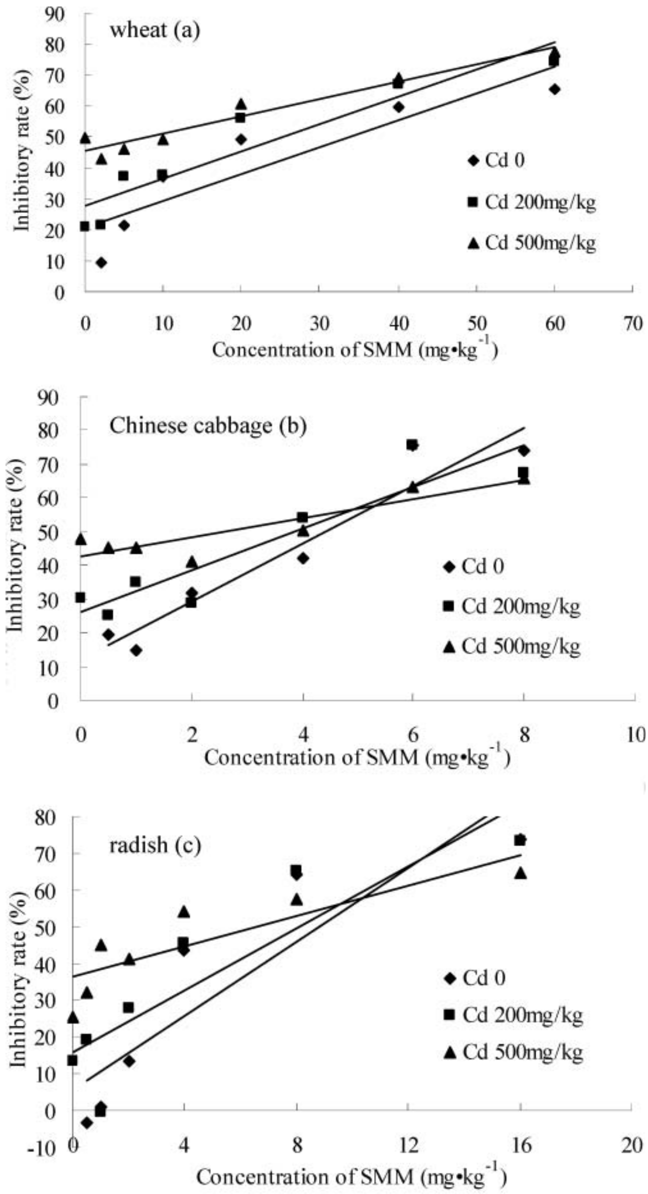

Figure 4. Joint toxic effects of SMM and Cd on root elongation of wheat, Chinese cabbage, and radish.

different sensitivities under the various pollutants. In this study, Chinese cabbage was the most sensitive to SMM and wheat was the most sensitive to the toxicity of Cd, which could be explained by the fact that different plants have their resistant mechanism to each kind of pollutant and the target molecule affected by each pollutant was different (Zhou *et al.*, 2004b). The mechanism of SMM on plants remains unknown; however, it would be closely related to the chemical composition and the nature of the molecules of SMM. Further research should focus on the specific effect mechanism.

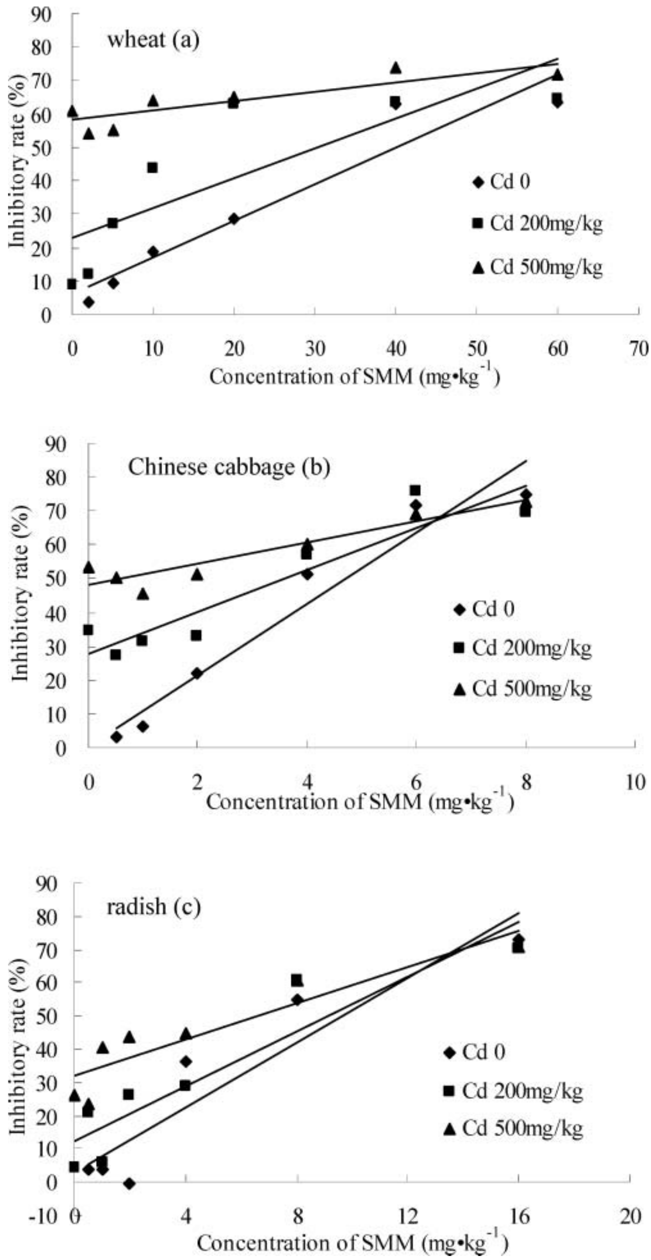

Figure 5. Joint toxic effects of SMM and Cd on shoot elongation of wheat, Chinese cabbage, and radish.

The interactive effects of the SMM and Cd on root and shoot elongation of the three crops were very complicated. SMM and Cd had significantly ($P < 0.05$) synergic effects on root and shoot elongation of the three crops at the low concentration of SMM. However, the synergic effects were not significant ($P > 0.05$), and even antagonistic effects were found when the concentration of SMM was high, which meant the toxicity of SMM

Table 3

Difference in average root/shoot elongation (mean, mm) of wheat under joint pollution of sulfamonomethoxine and cadmium

	Cd (mg/kg)	Sulfamonomethoxine(mg/kg) (Mean ± SD)						
		0	2	5	10	20	40	60
root	0	26.32 ± 2.13	23.79 ± 3.16	20.64 ± 0.68	16.53 ± 2.05	13.34 ± 1.49	10.55 ± 0.92	9.05 ± 0.86
	200	20.83 ± 1.32	20.65 ± 2.81	16.47 ± 2.50	16.34 ± 1.76	11.53 ± 0.90	8.76 ± 0.63	6.73 ± 0.72
	500	13.24 ± 1.53	15.01 ± 1.42	14.23 ± 1.45	13.36 ± 1.83	10.34 ± 0.86	8.10 ± 0.74	5.92 ± 0.65
shoot	0	8.13 ± 1.40	7.85 ± 1.24	7.36 ± 0.93	6.59 ± 0.89	5.81 ± 0.71	3.03 ± 0.63	2.99 ± 0.14
	200	7.40 ± 0.57	7.18 ± 1.45	5.92 ± 0.56	4.60 ± 0.84	3.02 ± 0.82	2.98 ± 0.58	2.87 ± 0.70
	500	3.17 ± 0.38	3.73 ± 0.96	3.65 ± 0.84	2.91 ± 0.79	2.84 ± 0.69	2.11 ± 0.42	2.31 ± 0.53

Table 4

Difference in average Chinese cabbage root/shoot elongation (mean, mm) under joint pollution of sulfamonomethoxine and cadmium

	Cd (mg/kg)	Sulfamonomethoxine(mg/kg) (Mean ± SD)						
		0	0.5	1	2	4	6	8
root	0	31.33 ± 2.91	25.18 ± 0.76	26.72 ± 0.98	21.34 ± 1.15	18.18 ± 1.29	7.70 ± 1.52	8.13 ± 0.96
	200	21.77 ± 1.82	23.44 ± 1.45	20.33 ± 2.51	22.23 ± 1.26	14.47 ± 1.90	7.57 ± 1.93	10.30 ± 1.12
	500	16.34 ± 3.20	17.17 ± 1.52	17.11 ± 1.75	18.39 ± 2.13	15.54 ± 1.53	11.50 ± 1.34	10.63 ± 1.41
shoot	0	17.38 ± 2.70	16.85 ± 1.68	16.24 ± 1.45	13.59 ± 2.51	8.42 ± 1.24	4.89 ± 0.56	4.42 ± 1.06
	200	11.41 ± 1.62	12.61 ± 0.96	11.92 ± 0.78	11.66 ± 2.31	7.47 ± 1.08	4.21 ± 0.39	5.28 ± 1.35
	500	8.07 ± 3.01	8.61 ± 1.34	9.44 ± 1.18	8.47 ± 1.95	6.89 ± 0.89	5.38 ± 1.15	4.73 ± 0.72

Table 5

Difference in average root/shoot elongation (mean, mm) of radish under joint pollution of sulfamonomethoxine and cadmium

	Cd (mg/kg)	Sulfamonomethoxine(mg/kg) (Mean ± SD)						
		0	0.5	1	2	4	8	16
root	0	40.57 ± 4.13	41.90 ± 2.37	40.10 ± 3.59	35.12 ± 3.12	22.92 ± 2.91	14.59 ± 1.56	10.63 ± 0.98
	200	33.20 ± 3.26	32.69 ± 1.98	40.76 ± 4.01	29.35 ± 2.17	22.15 ± 1.46	14.10 ± 1.24	10.81 ± 1.01
	500	20.57 ± 1.75	27.53 ± 2.04	22.31 ± 2.56	23.91 ± 1.27	18.65 ± 2.42	17.23 ± 1.95	14.39 ± 1.25
shoot	0	21.51 ± 2.31	20.71 ± 1.56	20.69 ± 0.91	21.59 ± 1.78	13.71 ± 1.24	9.70 ± 1.19	5.79 ± 0.99
	200	20.57 ± 2.42	17.00 ± 2.01	20.19 ± 1.21	15.91 ± 1.42	15.29 ± 1.51	8.45 ± 0.91	6.34 ± 1.95
	500	15.89 ± 1.95	16.43 ± 1.78	12.78 ± 1.56	12.12 ± 2.19	11.88 ± 0.95	8.48 ± 1.34	6.22 ± 1.05

Table 6
Relationships between inhibitory rate of root elongation (RI) and the concentration of SMM at the same concentration of added Cd

Crop Species	Cd[a]	Regression Equations[b]	R^2	*P*	IC_{50}[c]
Wheat	0	$RI = 0.8709C_{SMM}+20.657$	0.8188	<0.05	33.69
	200	$RI = 0.8861C_{SMM}+27.663$	0.8854	<0.01	25.21
	500	$RI = 0.5597C_{SMM}+45.511$	0.9404	<0.01	8.02
Chinese cabbage	0	$RI = 8.5443C_{SMM}+12.331$	0.9263	<0.01	4.41
	200	$RI = 6.1668C_{SMM}+26.301$	0.8439	<0.01	3.84
	500	$RI = 2.8312C_{SMM}+42.666$	0.8049	<0.01	2.59
Radish	0	$RI = 5.0153C_{SMM}+5.7707$	0.8122	<0.05	8.82
	200	$RI = 4.2274C_{SMM}+15.847$	0.7903	<0.01	8.08
	500	$RI = 2.0763C_{SMM}+36.297$	0.7229	<0.05	6.60

[a]The concentration of added Cd, mg/kg.
[b]C_{SMM} was the tested concentration of SMM, mg/kg.
[c]IC_{50} was the concentration of SMM corresponding to 50% inhibitory rate of RI, mg/kg.

and Cd to plants was less than each of them. This fact demonstrated that, in soil conditions, as an organic pollutant, SMM could change the surface characteristics of the soil by adsorption to the constituent soil, such as organic matter and clay, which made the soil adsorb more Cd and then reduce its bioavailability (Liu *et al.*, 2009). In addition, this may be related to the interactive effects of the two pollutants. SMM and Cd would form precipitation of Cd when the concentration of Cd was high in the solution, which would reduce the bioavailability of Cd and SMM. Nevertheless, the joint effects of chemicals and heavy metals could be determined by the interactive mode and the ratio of the two substances (Sun *et al.*, 2009).

Table 7
Relationships between inhibitory rate of shoot elongation (SI) and the concentration of SMM at the same concentration of added Cd

Crop Species	Cd[a]	Regression Equations[b]	R^2	*P*	IC_{50}[c]
Wheat	0	$SI = 1.1012C_{SMM}+5.9302$	0.9211	< 0.01	40.02
Chinese cabbage	200	$SI = 0.8998C_{SMM}+22.708$	0.6836	< 0.05	30.33
	500	$SI = 0.2855C_{SMM}+58.022$	0.7235	< 0.05	—[a]
Radish	0	$SI = 10.491C_{SMM}+0.6209$	0.9461	< 0.01	4.71
	200	$SI = 6.1868C_{SMM}+27.936$	0.8717	< 0.01	3.57
	500	$SI = 3.136C_{SMM}+47.963$	0.8769	< 0.01	0.65
	0	$SI = 4.8835C_{SMM}+2.9261$	0.8732	< 0.01	9.64
	200	$SI = 4.1065C_{SMM}+12.598$	0.8616	< 0.01	9.11
	500	$SI = 2.7276C_{SMM}+32.066$	0.8452	< 0.01	6.58

[a] The concentration of added Cd, mg/kg.
[b] C_{SMM} was the tested concentration of SMM, mg/kg.
[c] IC_{50} was the concentration of SMM corresponding to 50% inhibitory rate of RI, mg/kg.

These results will help to further understand the phytotoxicity of an important antibiotic (SMM) and a common heavy metal (Cd), and their joint toxic effects. Future studies should be directed to phytotoxicity mechanisms; for example, possible uptake and translocation of antibiotics by plants, and the physical and chemical properties in the rhizosphere and on the root surface.

Conclusion

There were significant ($P < 0.05$) positive relationships between root and shoot elongation of the three crops (wheat, Chinese cabbage, and radish) and concentrations of the two pollutants (SMM and Cd), but seed germination of the three crops is not sensitive to the toxicity of the two pollutants. As far as root elongation was concerned, Chinese cabbage was the most sensitive to the toxicity of SMM and wheat was the most sensitive to the toxicological effects of Cd. Phytotoxicity of SMM on the three crops was much stronger than that of Cd. In the joint effects of SMM and Cd, the interactive effects of the two pollutants on root and shoot elongation of the three crops were very complicated. At the low concentration of SMM, SMM and Cd had significantly ($P < 0.05$) synergic effects on root and shoot elongation of the three crops. However, the synergic effects were not significant ($P > 0.05$), and antagonistic effects were found when the concentration of SMM was high.

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