#### RESEARCH ARTICLE

# Toxic effects of wastewater from various phases of monosodium glutamate production on seed germination and root elongation of crops

LIU Rui<sup>1,3</sup>, ZHOU Qixing (
)<sup>2,4</sup>, ZHANG Lanying<sup>1</sup>, GUO Hao<sup>3</sup>

College of Environment and Resources, Jilin University, Changchun 130026, China
 Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
 Liaoning Province Academy of Analytic Sciences, Shenyang 110015, China
 College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

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Abstract To make a comprehensive assessment on monosodium glutamate (MSG) wastewater pollution, a pollution exposure experiment was carried out on the seed germination and root elongation of wheat. Chinese cabbage and tomato by using the wastewater discharged from different processing phases of MSG production. The results showed that there were significantly positive linear relationships between the inhibitory rates of wheat seed germination and root elongation and the COD<sub>cr</sub> of the mother liquor scraps. The toxicity of MSG wastewater to the test crops was in the order of tomato > Chinese cabbage > wheat, indicating that tomato was the most sensitive to the wastewater, and could be considered as an ideal toxic bioindicator. The half-effect concentrations (IC<sub>50</sub>) based on the seed germination and root elongation of the test crops exposed to the wastewater discharged from various processing phases of MSG production was 22.0–32 432 and 17.3–3320 mg/L, respectively.

**Keywords** monosodium glutamate (MSG) wastewater, pollution exposure, ecotoxicology, inhibitory rate

# 1 Introduction

Monosodium glutamate (MSG) wastewater is a very refractory case of industrial wastewater containing high concentrations of organic pollutants [1]. In order to insure the sustainable development of the MSG industry, more efforts have been directed toward the acquisition of feasible,

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E-mail: zhouqx@iae.ac.cn

efficient and economical methods for treating MSG wastewater [2,3]. China has become a big country of MSG production and consumption. The annual output of MSG in this country is up to 1/2 as much as that in the whole world [4]. With the increase of MSG production in the past decade, highly efficient treatment and ecological safety evaluation of MSG wastewater had been considered as an emergency problem to be solved [5-7]. Although MSG wastewater is seen as a toxic substance, toxic effects of the wastewater on crops and their toxicological mechanisms are obscure [8–11]. In particular, the quantitative evaluation about ecological risk of MSG wastewater is scarce. Even though many researches have been conducted on MSG wastewater treatment, little information is available on ecological behavior and ecotoxicological effects of the wastewater discharged into the environment [12–16]. In order to understand the whole pollution process of MSG wastewater discharged into the environment, ecotoxicological effects of MSG wastewater was thus examined in this paper. Undoubtedly, this work would be very significant to clean production of the MSG Industry [6], to efficient treatment, safe discharge, and ecological risk assessment of MSG wastewater [7,11,17].

# 2 Materials and methods

# 2.1 Materials

MSG wastewater used in the study was collected from four different discharge phases in MSG production of a big MSG factory in the suburbs of Shenyang, the most important old industrial base in China. The four different discharge phases included mother liquor scraps (Discharge 1 in Fig. 1,  $COD_{cr} = 18\,555$  mg/L), pretreatment liquor (Discharge 2,

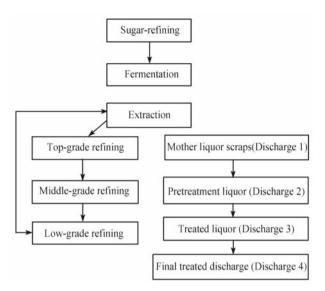


Fig. 1 Whole process of monosodium glutamate production, wastewater discharge, and sampling sites

 ${\rm COD_{cr}}=10~603~{\rm mg/L})$ , treated liquor discharge (Discharge 3,  ${\rm COD_{cr}}=30~{\rm mg/L})$ , and final treated discharge (Discharge 4,  ${\rm COD_{cr}}=720~{\rm mg/L})$ . Seeds of the tested crops including wheat (*Triticum aestivum*), Chinese cabbage (*Brassica chinensis*) and tomato (*Lycopersicon esculentum*) were bought from the Liaoning Academy of Agricultural Sciences.

# 2.2 Toxicity experiments

In view of the fact that the rate of seed germination treated by the mother liquor scraps (Discharge 1) and the pretreatment liquor (Discharge 2) was 0.0 due to their extremely high COD<sub>cr</sub> concentration and high toxicity, MSG wastewater was diluted 2, 5, 10, 25, and 50 times with distilled water, respectively. Correspondingly, the tested concentration of COD<sub>cr</sub> in the formal cultural solution from the mother liquor scraps (Discharge 1) was 9 278, 3 711, 1 856, 742, and 371 mg/L, and that from the pretreatment liquor (Discharge 2) was 5 302, 2 121, 1 060, 424, and 212 mg/L, respectively. The seed germination and root elongation of crops were carried out by using the diluted solutions, the treated liquor discharge (Discharge 3), and the final treated discharge (Discharge 4).

Under darkened conditions at  $(25\pm1)^{\circ}$ C in the culturing box (LRH-250-A, made in Guangdong), 15 sterilized seeds of wheat, 25 sterilized seeds of Chinese cabbage, and 25 sterilized seeds of tomato were exposed to MSG wastewater, respectively. In order to decrease experimental errors to the minimum, all the treatments were replicated for three times. When the length of the growing roots cultured in the control solution without the addition of MSG wastewater was up to 20 mm, the exposed experiment was finished, and then seed germination and root elongation under all the treatments were calculated and measured [7].

# 2.3 Data processing and statistical analysis

The data from the experiment were calculated, processed, and regressed using Microsoft Office Excel 2003 and Origin 7.0 in a computer.

#### 3 Results and discussion

#### 3.1 Toxic effects of MSG wastewater on seed germination

The results showed that there were visible inhibitory effects of MSG wastewater on seed germination of wheat, Chinese cabbage, and tomato under the experimental conditions. It is shown in Fig. 2 that the inhibitory rate of Chinese cabbage had an increasing trend with increasing COD<sub>cr</sub> in MSG wastewater. When the COD<sub>cr</sub> concentration in the mother liquor scraps was higher than 400 mg/L, the inhibitory rate of wheat and tomato seed germination increased with increasing COD<sub>cr</sub> concentration. However, the inhibitory rate of wheat seed germination decreased with increasing COD<sub>cr</sub> concentration when the COD<sub>cr</sub> concentration in the mother liquor scraps was 0-400 mg/L. It suggested that MSG wastewater had no poisonous effects on seed germination of wheat and tomato when the COD<sub>cr</sub> concentration in the mother liquor scraps was low. In particular, seed germination could be promoted by the low concentration of NH<sub>4</sub>+-N in MSG wastewater.

The interrelationships between the inhibitory rate of seed germination of wheat, Chinese cabbage, and tomato and the COD<sub>cr</sub> concentration in MSG wastewater can be expressed using the following regression equations (Fig. 2)

$$Y(I_1) = 0.012X-7.20$$
  $(R^2 = 0.987, n = 5, P < 0.005)$  (1)

$$Y(II_1) = 0.011X + 6.34$$
  $(R^2 = 0.889, n = 5, P < 0.01)$  (2)

$$Y(III_1) = 0.012X + 11.46 \quad (R^2 = 0.664, n = 5, P > 0.05)$$
 (3)

where X was the COD<sub>cr</sub> concentration (mg/L) in MSG wastewater;  $Y(I_1)$  was the inhibitory rate (%) of wheat seed germination;  $Y(II_1)$  was the inhibitory rate (%) of Chinese cabbage seed germination;  $Y(III_1)$  is the inhibitory rate (%) of tomato

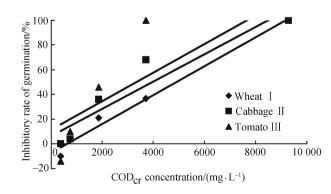


Fig. 2 Effects of MSG wastewater on seed germination of wheat, Chinese cabbage, and tomato

seed germination. The results showed that there was a markedly positive linear relationship between the inhibitory rate of wheat seed germination and the COD<sub>cr</sub> concentration of MSG wastewater. The linear relationship between the inhibitory rate of Chinese cabbage seed germination and the COD<sub>cr</sub> concentration was weak, but it was still positive. The positive linear correlation for tomato seed germination was not found. The different linear relationships among three crops might be responsible for different tissues and structure of crops, because they affected toxic effects of MSG wastewater on crop seed germination.

As shown in Fig. 3, the inhibitory rate of wheat, Chinese cabbage and tomato increased with increasing COD<sub>cr</sub> concentration in the pretreatment liquor. However, there were no good correlations between them, according to the following regression equations

$$Y'(I_1) = 0.0114X' + 50.51$$
 ( $R^2 = 0.563$ ,  $n = 5$ ,  $P > 0.05$ ) (4)  
 $Y'(II_1) = 0.0165X' + 30.00$  ( $R^2 = 0.572$ ,  $n = 5$ ,  $P > 0.05$ ) (5)  
 $Y'(III_1) = 0.0082X' + 66.27$  ( $R^2 = 0.429$ ,  $n = 5$ ,  $P > 0.05$ )(6)

where X' was the  $COD_{cr}$  concentration (mg/L) in the diluted solution from the pretreatment liquor;  $Y'(I_1)$ ,  $Y'(II_1)$ , and  $Y'(III_1)$  were the inhibitory rate (%) of seed germination of wheat, Chinese cabbage, and tomato, respectively. To a certain extent, the noncorrelation between them might be attributed to the action mechanism that the toxicity of polyacrylate sodium added to the pretreatment liquor had some influences on the linear relationship between the seed germination rate of the three tested crops and the  $COD_{cr}$  concentration of MSG wastewater. Therefore, the inhibitory rate of

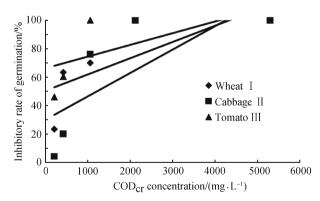


Fig. 3 Effects of MSG wastewater on seed germination of wheat, Chinese cabbage, and tomato

seed germination did not increase markedly with increasing COD<sub>cr</sub> concentration in the pretreatment liquor.

However, the inhibitory rate of seed germination by the pretreatment liquor (Discharge 2) was higher than that by the mother liquor scraps (Discharge 1), when the mother liquor scraps and the pretreatment liquor were diluted at the same time. As shown in Figs. 2 and 3, the inhibitory rate of wheat seed germination by Discharge 2 was up to 70.0% (COD<sub>cr</sub> = 1.060 mg/L), and that by Discharge 1 was only 21.0% (COD<sub>cr</sub> = 1.855 mg/L). It could be thus concluded that the toxicity of flocculants added in the process of wastewater treatment could not be neglected for the toxicity of MSG wastewater.

It is shown in Fig. 4 that the inhibitory rate of seed germination of the three crops by the treated liquor was higher than that by the final treated discharge. The increased toxicity of the treated liquor might be responsible for multiple flocculants added to the treated liquor. Moreover, the final treated discharge was the mixture of wastewater including washing water, cooling water, and wastewater after treating mother liquor scrap. There were no other chemical substances added in this process. Therefore, the inhibitory rate of seed germination by Discharge 4 was low.

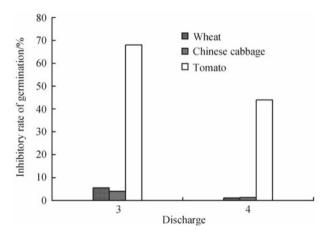


Fig. 4 Effects of MSG wastewater on seed germination of wheat, Chinese cabbage, and tomato

According to the linear regression equations in Figs. 2, 3, and 4, half-effect concentrations ( $IC_{50}$ ) of MSG wastewater were calculated and listed in Table 1. The results show that there was a big difference in  $IC_{50}$  of seed germination of the 3 crops by various discharging phases of wastewater.  $IC_{50}$ 

Table 1 Half-effect concentrations (IC<sub>50</sub>) based on seed germination by various discharging phases of MSG wastewater

Crop		$IC_{50}/(m_{\tilde{s}})$	$g \cdot L^{-1}$			
	Mother liquor scraps (Discharge 1)	Pretreatment liquor (Discharge 2)	Treated liquor (Discharge 3)	Final treated discharge (Discharge 4)		
Wheat	3659	< 10 603	270	32 432		
Chinese cabbage	3969	1212	375	27 068		
Tomato	3323	< 10 603	22	818		

of tomato seed germination was at the lowest level, only 22.0 mg/L by Discharge 3. Correspondingly, the toxicity of MSG wastewater in this phase was the strongest. On the other hand, tomato seeds were considered as the most sensitive to MSG wastewater among the three tested crop seeds.

#### 3.2 Toxic effects of MSG wastewater on root elongation

As shown in Table 2, the inhibitory rate of root elongation of wheat, Chinese cabbage, and tomato seedlings by the mother liquor scraps and the pretreatment liquor decreased with a

decrease of the COD<sub>cr</sub> concentration in MSG wastewater. However, the decreasing extent of the inhibitory rate by the pretreatment liquor was weak compared with that by the mother liquor scraps.

It is shown in Table 3 that there is a significant positive relationship between the inhibitory rate of wheat and Chinese cabbage root elongation and the COD<sub>cr</sub> concentration in the mother liquor scraps, but a good correlation between them was not found in the pretreatment liquor. In other words, the inhibitory rate of root elongation of the 3 tested crops was not only dependent on the COD<sub>cr</sub> concentration in MSG

 Table 2
 Effects of MSG wastewater on root elongation of crops

Crop			Mother liquor sci	raps/(mg·L <sup>-1</sup> ) (Disch	narge 1)		
	18 555	9278	3711	1856	742	371	
Average of wheat contrasted/cm				$3.07 \pm 0.18$			
Average of wheat tested/cm	0	0	$1.26 \pm 0.05$	$1.57 \pm 0.06$	$2.40 \pm 0.12$	$2.61 \pm 0.10$	
Wheat inhibitory rate/%	100	100	59.07	48.70	21.76	15.03	
Average of Chinese cabbage contrasted/cm				$3.88 \pm 0.19$			
Average of Chinese cabbage tested/cm	0	0	$0.71 \pm 0.02$	$1.31 \pm 0.06$	$1.87 \pm 0.07$	$1.90 \pm 0.11$	
Chinese cabbage inhibitory rate/%	100	100	81.68	66.15	51.86	50.93	
Average of tomato contrasted/cm				$1.27 \pm 0.05$			
Average of tomato tested/cm	0	0	0	$0.38 \pm 0.01$	$0.56 \pm 0.02$	$0.88 \pm 0.04$	
Tomato inhibitory rate/%	100	100	100	70.25	55.83	30.98	
Crop			Pretreatment liqu	or/(mg·L <sup>-1</sup> ) (Discha	arge 2)		
	10 603	5302	2121	1060	424	212	
Average of wheat contrasted/cm			$3.07 \pm 0.18$				
Average of wheat tested/cm	0	0	0	$1.11 \pm 0.03$	$1.59 \pm 0.03$	$1.62 \pm 0.06$	
Wheat inhibitory rate/%	100	100	100	63.73	51.81	47.15	
Average of Chinese cabbage contrasted/cm			$3.88 \pm 0.19$				
Average of Chinese cabbage tested/cm	0	0	0	$1.18 \pm 0.05$	$1.20 \pm 0.05$	$2.33 \pm 0.14$	
Chinese cabbage inhibitory rate/%	100	100	100	69.57	69.10	40.06	
Average of tomato contrasted/cm			$1.27 \pm 0.05$				
Average of tomato tested/cm	0	0	0	0	$0.28 \pm 0.01$	$0.48 \pm 0.02$	
Tomato inhibitory rate/%	100	100	100	100	77.61	62.27	
Crop	Treated	l liquor/(mg·L	·1) (Discharge 3)	Final treated	d discharge/(mg·L <sup>-1</sup>	) (Discharge 4)	
		30			720		
Average of wheat contrasted/cm		$3.07 \pm 0.$	18	$3.07 \pm 0.18$			
Average of wheat tested/cm		$1.37 \pm 0.07$		$1.83 \pm 0.08$			
Wheat inhibitory rate/%	55.37		40.28				
Average of Chinese cabbage contrasted/cm	$3.88 \pm 0.19$		$3.88 \pm 0.19$				
Average of Chinese cabbage tested/cm	$0.74 \pm 0.02$		$1.38 \pm 0.06$				
Chinese cabbage inhibitory rate/%		80.80		64.43			
Average of tomato contrasted/cm		$1.27 \pm 0.$	05	$1.27 \pm 0.05$			
Average of tomato tested/cm		$0.17 \pm 0.01$			$0.47 \pm 0.03$		
Tomato inhibitory rate/%		86.61			62.99		

Table 3 Relationships between the inhibitory rate of crop root elongation and COD<sub>Cr</sub> in MSG wastewater

Crop	Mother liquor scraps				Pretreatment liquor			
	Regression equation	$R^2$	n	P	Regression equation	$R^2$	n	P
Wheat	$Y(I_2) = 0.009X + 20.1$	0.9388	5	< 0.005	$Y(I_2) = 0.0104X + 53.6$	0.7056	5	>0.05
Chinese cabbage	$Y(II_2) = 0.0055X + 52.6$	0.9217	5	< 0.005	$Y(II_2) = 0.0094X + 58.6$	0.6063	5	> 0.05
Tomato	$Y(III_2) = 0.0065X + 50.8$	0.6297	5	> 0.05	$Y(III_2) = 0.0053X + 78.3$	0.4078	5	> 0.05

Table 4	Half-effect concentrations	(IC50) based on roo	t elongation by various	s discharging phases of MSG was	stewater

Crop	$IC50/(mg \cdot L^{-1})$					
	Mother liquor scraps (Discharge 1)	Pretreatment liquor (Discharge 2)	Treated liquor (Discharge 3)	Final treated discharge (Discharge 4)		
Wheat	3320	<212	27.1	894		
Chinese cabbage	< 371	<212	18.6	559		
Tomato	< 371	<212	17.3	572		

wastewater, but also related to the flocculants added to the pretreatment liquor. In particular, polyacrylate sodium could increase the toxicity of MSG wastewater. Thus, it was an important influencing factor on root elongation of crops.

It can also be seen in Table 2 that the inhibitory rate of root elongation of the 3 tested crops by the treated liquor from Discharge 3 are all higher than that by the final treated discharge (Discharge 4). The inhibitory rate of tomato root elongation by the treated liquor was the highest among the 3 tested crops, up to 86.61%. Posteriorly, the inhibitory rate of Chinese cabbage root elongation by the treated liquor was 80.80%. Meanwhile, the inhibitory rate of root elongation of Chinese cabbage and tomato by the final treated discharge was almost equal, and wheat inhibitory rate was the lowest (40.28%). Moreover, the inhibitory rate of crop root elongation by Discharge 3 (COD<sub>cr</sub> = 30 mg/L) and Discharge 4  $(COD_{cr} = 720 \text{ mg/L})$  did not increase with an increase in the COD<sub>cr</sub> concentration, because the multiple flocculants added to treat MSG wastewater changed the toxicity of MSG wastewater.

The half-effect concentrations (IC $_{50}$ ) based on root elongation by MSG wastewater from various discharging phases were calculated according to the linear regression equations between the inhibitory rate of root elongation of the 3 tested crops and the COD $_{cr}$  concentration in MSG wastewater and listed in Table 4. It indicated that there was a big difference in the IC $_{50}$  of root elongation of the tested crops by the 4 discharging phases of MSG wastewater. The IC $_{50}$  of wheat root elongation by the mother liquor scraps was the highest, up to 3 320 mg/L. In other words, wheat seedlings were the most tolerable to the toxicity of MSG wastewater among the 3 tested crop seedlings. This experiment can provide a scientific basis for reusing MSG wastewater resources.

It is also shown in Table 4 that the  $IC_{50}$  of Chinese cabbage and tomato root elongation was both lower than that of wheat root elongation by the treated liquor and the final treated discharge. In other words, Chinese cabbage, and tomato seedlings were more sensitive to the toxicity of MSG wastewater, and could be considered as its toxic bioindicators. According to the comprehensive influences of MSG wastewater on seed germination and root elongation of crops, tomato was the most sensitive to MSG wastewater. Therefore, tomato plants could be considered as an ideal toxic bioindicator of MSG wastewater.

# 4 Conclusions

The linear relationships between the inhibitory rate of seed germination and root elongation and the COD<sub>cr</sub> concentration in the mother liquor scraps were in the order of wheat>Chinese cabbage>tomato; in particular, there was a markedly positive correlation between the inhibitory rate of wheat seed germination and root elongation and COD<sub>cr</sub> in MSG wastewater.

There was a big difference in the IC<sub>50</sub> of seed germination and root elongation of the 3 tested crops by the 4 discharging phases of MSG wastewater, and the changing range was 17.3–32 432 mg/L. According to the comprehensive influences of MSG wastewater on seed germination and root elongation of the tested crops, the toxicity of MSG wastewater was in the order of tomato > Chinese cabbage > wheat, indicating that tomato was the most sensitive crop responding to the toxicity of MSG wastewater and could be considered as an ideal toxic bioindicator.

The toxicity of MSG wastewater on seed germination and root elongation of the tested crops was not only dependent on the COD<sub>cr</sub> concentration in MSG wastewater, but also related to the flocculants added in the process of MSG wastewater treatment.

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