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Research Article

Interactive Effects of Selenium and Mercury on Their Uptake by Rice Sedlings

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Abstract: In this study, a new modeling method based on metasynthesis is proposed from the macro and micro levels. And the system analysis and design agent-oriented based on POMDP are provided in the same time. Finally, the two case studies are given and the experimental results have shown efficiency and rationality of this modeling method. Under greenhouse conditions, rice was taken as test object and the interactive effects of selenium and mercury in rice seedlings grown in solution culture were researched by the use of quadratic-orthogonal-rotation-combination design. The results showed that low mercury concentration (<30 μg/L) promoted selenium absorption in rice roots and shoots, which may be associated with selenium's favorable effect on plant. Higher mercury concentration (>30 μg/ L) reduced selenium accumulation in rice roots and stems, as well as the transfer of selenium to the rice shoot. When the mercury concentration was fixed at 100 μg/L, Se in the growth medium significantly reduced Hg accumulation in the roots and the effect of Se on Hg accumulation in shoots displayed a similar pattern. However, with the increase of selenium concentration of the solution, the Hg transfer coefficient from the root to the shoot remained unchanged.

Keywords: Interaction, Mercury, Rice, Selenium

INTRODUCTION

In China, mercury (Hg) pollution comes mainly from unreasonable sewage irrigation, long-term use of chemical fertilizers, pesticides, veterinary drugs, feed and atmospheric deposition. The concentrations of Hg found in a survey of Chinese soils ranged from 0.001 to 45.9 mg/kg, with an average of 0.038 mg/kg (CNEMS, 1990), was higher than the average content of mercury in the world natural soil (Hong *et al*., 2007; Nakagawa and Yumita, 1998). Mercury concentrations of irrigated soil in Eastern outskirts of Beijing reached as high as 2.91 mg/kg, far more than the first standard of National Soil Environmental Quality (0.15 mg/kg). In mercury-contaminated areas, the total mercury content of brown rice was up to 1451/mg kg, organic mercury content up to 117 mg/kg (Horvat *et al*., 2003). Rice is one of China's major grain crops, 60% of the population take rice as the stable food. Paddy fields of sewage irrigation suffered mercury pollution covered 15 provinces and cities, the rice mercury content exceeded the national food hygiene standards (0.02 mg/kg) (Liu *et al*., 2000; Yathavakilla and Caruso, 2007). Compared with other dryland crops, rice was more vulnerable to mercury contamination, because flooding conditions of soil can increase the toxicity and bioavailability of mercury, with the same soil mercury concentration, mercury accumulation of rice was the strongest (Liu *et al*., 2000). Therefore, how to reduce the rice absorption of mercury has been concerned as an important issue.

Selenium (Se) is an essential nutrient to human body and it plays an important role in maintaining human immunity system and reducing the risk of cancer (Wallace *et al*., 2009). Selenium has been shown to provide protection against the toxic effects of Hg, As and possibly Pb (Mukherjee and Sharma, 1988; Jonnalagadda and Rao, 1993). This antagonism of Se and Hg was first reported by Pelletier (1985). Investigations of Se-Hg interactions in terrestrial plants have appeared recently (Shanker *et al*., 1996a). Some studies suggested that under the conditions of soil culture experiment, selenium can significantly reduce the absorption and transport of mercury of radish and tomato root, so as to reduce the mercury poison (Shanker *et al*., 1996b), this phenomenon may be due to insoluble HgSe complex formed by selenium and mercury in the soil (Shanker *et al*., 1996a, b).

At the same time, the use of size exclusion chromatography suggested that selenium hindered soybean absorbtion of Hg, mainly due to the formation of macromolecular compounds of Hg and Se in soybean root. This entity was also extracted in protein specific isolate, but it resisted enzymatic breakdown. This compound was difficult to move in plants, which was

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also discovered in Se hyperaccumulation mustard (Thangavel *et al*., 1999). However, until now the study of interaction of selenium and mercury in rice plants have not yet been reported. In this study, solution culture method was used to study the interaction between rice selenium and mercury, which was of practical significance.

MATERIALS AND METHODS

Experimental design: The test selected two factors of selenium and mercury, five levels of each factor and two sets of nested hydroponic experiments were taken to study the interaction of rice selenium and mercury. The treatment level of hydroponic experiments was determined by quadratic orthogonal rotation combination design for the five levels of two factors. The upper level of selenium treatment was 100 g/L, mercury 200 g/L, the lower levels of both mercury and selenium were 0 g/L. Then, zero (X_0) and change interval (Δ_j) of Se, Hg were calculated according to their respectively upper and lower levels and the formula was as follows:

$$X_0=(X_{up} + X_{down})/2$$

$$\Delta_j = (X - X_0)/r$$

In the formula:

- X_0 = Zero level of selenium and mercury treatment
- X_{up} = Higher level of selenium and mercury treatment
- X_{down} = Lower level of selenium and mercury treatment
- Δ_j = Change interval of selenium and mercury treatment (selenium change interval was 35.4, mercury change interval was 70.0)
- X = The actual concentration of selenium and mercury treatment, r represented code values (Table 1, codes column)

Level codes of each factor were listed in Table 1.

Experiment 1 was a group of single factor test, used to study the simple reaction between selenium and mercury in rice plants. The test process was as follows: when the impact of selenium on mercury was studied, the mercury concentration was set to zero level as 100 g/L and the selenium concentration was increased from 0 to 100 g/L; Similarly, when the impact of mercury on selenium was studied, the selenium concentration was set to zero level as 50 g/L and the mercury concentration was increased from 0 to 200 g/L. During the experiment, each treatment was repeated three times.

Table 1: The simple effects of Se and Hg on rice

Treatment level	Codes	Se (μg /L)	Hg (μg /L)
1	-1.414	0	0
2	-1	14.6	30
3	0	50	100
4	1	85.4	170
5	1.414	100	200

Table 2: Se and Hg treatment levels of interaction of Se and Hg in rice (including treatments in experiment 1)

	Codes		Treatment (μg /L)	
Serial number	Se	Hg	Se	Hg
1	1	1	85.3	170
2	1	-1	85.3	30
3	-1	1	14.6	170
4	-1	-1	14.6	30
5	-1.414	0	0	100
6	1.414	0	100	100
7	0	-1.414	50	0
8	0	1.414	50	200
9	0	0	50	100
10	0	0	50	100
11	0	0	50	100
12	0	0	50	100
13	0	0	50	100
14	0	0	50	100
15	0	0	50	100
16	0	0	50	100
17	0	-1	50	30
18	0	1	50	170
19	-1	0	14.6	100
20	1	0	85.3	100

Table 3: Biomass (g dry matter per pot) of rice seedlings exposed to various concentrations of Hg (μg/L) with or without Se in nutrient solution (mean ± SE, n = 3)

Hg concentration	Shoot biomass		Root biomass	
(μg /L)	With Se	No Se	With Se	No Se
0	0.723	0.783	0.215	0.213
30	0.695	0.534	0.195	0.191
100	0.675	0.675	0.175	0.183
170	0.576	0.595	0.093	0.0831
200	0.421	0.388	0.082	0.068
Analysis of variance				
Hg	$p<0.001$		$p<0.001$	
Se	NS		NS	
Hg×Se	NS		NS	

On the basis of experiment 1, a two-factor (selenium and mercury) experiment (experiment 2) was designed, each factor was set five levels. Quadratic orthogonal rotation combination design was used to study the interaction between selenium and mercury in the rice plants (Table 2). Experiment 2 was quadratic orthogonal rotation combination design for the five levels of the two factors, mainly studied the interaction between selenium and mercury in the rice plants. This experiment had a total of 16 treatments, 9-16 treatments were eight repeats of 9, the other experimental treatments were repeated three times (Table 3), 17-20 treatments were nested combination to experiment 1. The two experiments were carried out at the same time.

Rice seeds (Q You-1) were surface-sterilized by 10% H_2O_2 for 15 minutes, washed by clean deionized water, germinated on moist quartz sand with temperature of 25~28 ºC. During the breeding process, the pot was supplemented with water and nutrients momentarily. 7 d later, 12 healthy and growing consensus seedlings were transplanted to a plastic bucket containing 1/2 L Kimura B nutrient solutions. Stamped 2.5 L kegs were used in the experiment, each cover was equably drilled four 2 cm diameter holes, rice seedlings were fixed into the holes with sponge, 3 seedlings each hole, a total of 44 pots. Kimura B nutrient solution.

Sample determination: Concentrations of rice samples were determined using the Standard Method GB/T 5009. 93-2010 developed by the Ministry of Health of China (Zhang *et al*., 2006).

Determination of rice mercury was according the method (Rasmussen *et al*., 1991).

Data processing: Data obtained from experiment 1 was compiled and single-factor variance analyzed by Excel 2003 and DPS7.5 software, different concentrations were analyzed by Tukey method to test their significance (p<0.05). Data obtained from experiment 2 were fitted by quadratic polynomial equation (Eq.1) (Tu and Ma, 2003):

$$Y=b_0+\sum_{j=1}^{m} b_j x_j + \sum_{i\neq j}^{m} b_{ij} x_i x_j + \sum_{j=1}^{m} b_{jj} x_j^2 \quad (1)$$

RESULTS

Plant biomass: When the Se concentration was fixed at central value as 50 µg L-1, with the increase of Hg concentration, both root and shoot biomass decreased significantly with increasing Hg concentration in the growth solution (Table 3). For plant exposed to Se, a 37% reduction in root biomass occurred at Hg^{2+} concentrations between 100 and 200 µg/L; and for shoot growth and 24% reduction in shoot biomass occurred at Hg^{2+} concentrations between 100 and 200 µg/L; At the concentration of 50 µg/L Se, there was no significant effect of selenite on plant biomass, suggesting that no selenium toxicity or salt injury occurred in the rice plants. The interaction of Hg and Se had no notable impact on the biomass of rice plant stem and root.

Function model building: Based on test results, regression model equation between mercury and selenium uptake of rice root and stem and various factors was established by the application of quadratic regression general rotary combination design statistical analysis, seeing Table 4.

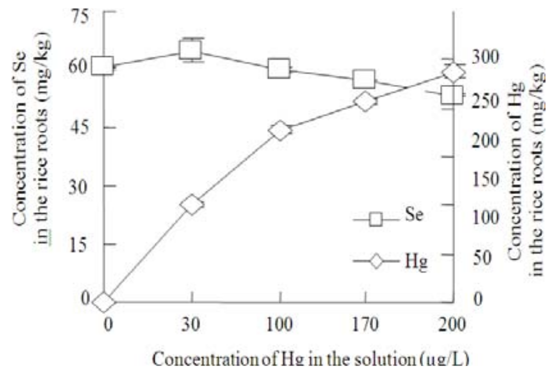
Table 4: Best-fit equations and statistical tests of the effects of independent variables Se and Hg and the uptake of Se and Hg by rice

Equations of actual variables
Hg uptake (fronds)
y_1 = 0.272-0.055 [Se]+0.1245 [Hg]-0.3594 [Se][Hg]-0.011[Se][Se]-0.045 [Hg][Hg], F = 943.821 p< 0.0001
Hg uptake (roots)
y_2 = 181.942-40.359[Se]+79.381[Hg]-20.053[Se][Hg] 13.393[Se][Se]-33.329[Hg][Hg] p<0.0001
Se uptake (fronds)
y_3 = 16.789+9.407 [Se]-2.458 [Hg]-1.278 [Se][Hg]- 1.809 [Se][Se]-0.777 [Hg][Hg], F= 569.281 p< 0.0001
Se uptake (roots)
y_4 = 58.313+29.718 [Se]-4.740[Hg]-4.832 [Se][Hg]-8.196 [Se][Se]-2.488 [Hg][Hg], F= 61.228 p< 0.0001

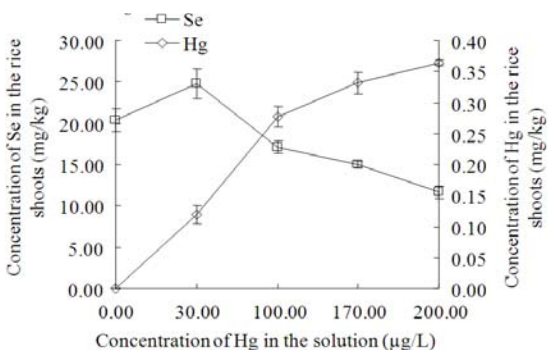
Each regression model was tested lose effectiveness of fit by Analysis of Variance, test result of each modelhad no significant difference in p = 0.05 level, F test of each regression equation was of significant level, suggesting that the model fitted well with the practical experiments, which can reflect the theory predicts of interactions of selenium and mercury in rice plants. The results of regression coefficient t showed that one degree term coefficients of Hg and Se in the equation varied significantly on 0.05 level, indicating mercury and selenium had significant influence on rice absorption of mercury and selenium. Quadratic degree term coefficients varied significantly on 0.05 level, indicating that the interaction of mercury and selenium had significant influence on rice absorption of mercury and selenium.

Simple effect of selenium and mercury in rice plants: When the Se concentration was fixed at central value as 50 µg/L, with the increase of Hg concentration, the Hg concentrations of rice root and shoot increased significantly (root p<0.001; shoot p<0.001) (Fig. 1a and b). When mercury treatment concentration increased from 0 to 100 µg/L, mercury content of rice root and shoot increased almost linearly; When mercury treatment concentration increased from 100 to 200 µg/L, with the increase of mercury concentrations, mercury content of rice root and shoot was in slowly increasing trend (Fig. 1a and b). When mercury concentration increased from 0 to 200 µg/L, with the increase of mercury concentrations, Se content of rice root and shoot increased first and then decreased, which suggested low concentration of mercury (<30 µg/L) can promote the Se content of rice root and shoot, whereas high concentration of Se (>30 µg/L) inhibited the Se content of rice root and shoot.

When the Hg concentration was fixed at central value as 100 µg/L, with the increase of Se concentration, the Se content of rice root and shoot increased significantly. It was worth noting that, with the increase



(a)



(b)

Fig. 1: (a) Impact of Hg treatment on the Se and Hg content in the rice root, (b) Impact of Hg treatment on the Se and Hg content in the rice shoot

of selenium concentrations in the solution, the Hg content of rice root and shoot was in a significant downward trend. With the selenium concentrations increased as 0, 14.6, 50, 85.3, 100, mercury content of root decreased by 10.3, 17.6, 32.6 and 53.0%, respectively mercury content of shoot increased by 12.2, 19.1, 29.2 and 48.8%, respectively.

From the respect of transfer coefficient, mercury transfer coefficient (the ratio of mercury content of shoot and root) showed different mutation characteristics with the increasing of selenium. When the Hg concentration was fixed at central value as 100 μg/L, with the increase of Se concentration, the Hg transfer coefficient had no significant change, maintaining around 0.0015, indicating that with the increase of Se concentration, the Hg content of root and shoot decreased significantly, but the transfer of Hg from root to shoot did not decrease. The level of accumulation of mercury in different plant tissues indicates that most of the mercury (89~96%) is retained in the root and only a small amount of mercury is translocated to aerial part (2~7%) (Shanker *et al.*, 1996a). When the Se concentration was fixed at 50 μg/L, with the increase of Hg concentration, the transfer coefficient of mercury increased from 0 to 0.0015, which increased first and then became stable. With the increase of Hg concentration, the transfer coefficient of Se was in fluctuate downward trend, indicating that with the increase of Hg concentration in solution reduced the transfer of rice Se from root to shoot.

Impact of interaction of Se and Hg on the absorption of Se and Hg: Figure 2a and b showed Se uptake of rice root on by the influence of selenium and mercury. When the solution was in lower mercury concentration (0 μg/L) and higher concentration of selenium (100 μg/L), rice root accumulated plenty of Se, the Se accumulation increased quickly with the increase of Se concentration and reached the maximum at higher Se concentration (100 μg/L)

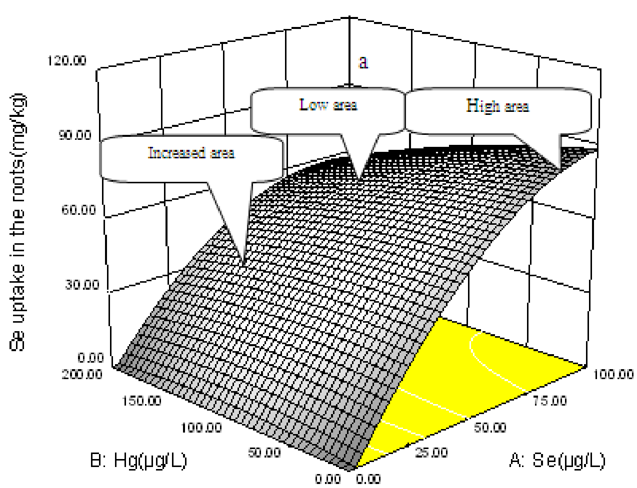


Fig. 2a: The interactive effect of Se and Hg on Se uptake in the roots

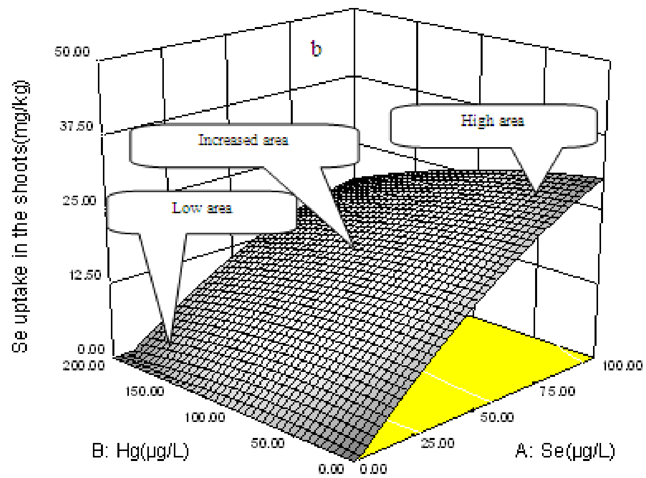


Fig. 2b: The interactive effect of Se and Hg on Se uptake in the shoots

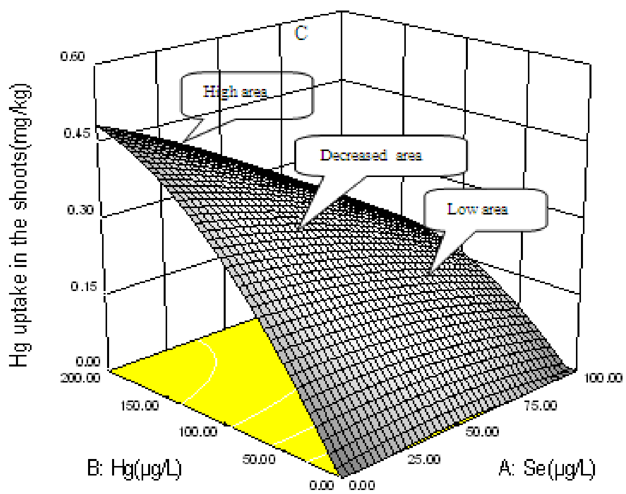


Fig. 2c: The interactive effect of Se and Hg on Hg uptake in the shoots

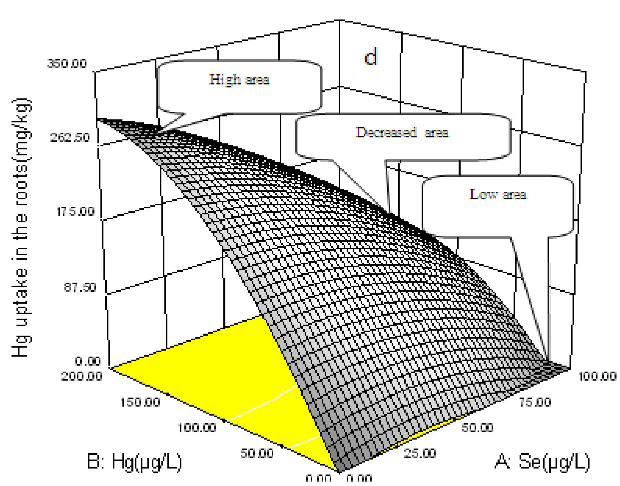


Fig. 2d: The interactive effect of Se and Hg on Hg uptake in the roots

But when selenium concentrations was higher, with the increase of the mercury concentration, the root Se accumulation decreased significantly (Fig. 2a Low area) and when mercury concentration was higher, the Se accumulation of root increased with the increasing of the concentration of selenium (Fig. 2a Increased area). Se content of rice shoot was similar to the root, but the increase rate was lower than that of root, suggesting that Se accumulation was mainly in the root.

Figure 2c showed that when the solution was in lower Se concentration (0 μg/L) and higher concentration of Hg (200 μg/L), rice root accumulated plenty of Hg, the Hg accumulation increased quickly with the increase of Hg concentration (Fig. 2c High area); when Hg concentrations was higher, with the increase of the Se concentration, the root Hg accumulation decreased significantly (Fig. 2c Low area), The higher the selenium concentration was, the more significant the Hg concentration decreased. Hg content of rice shoot was similar to the root, suggesting that Se can reduce the accumulation of Hg of rice root and shoot (Fig. 2d).

DISCUSSION

Impact of Se on rice uptake of Hg: Statistically significant reductions in mercury uptake with increasing concentrations of selenium were observed. The observed reduction in plant uptake of mercury is explained by the formation of an HgSe insoluble complex in the root circumference environment. A possible explanation, in general, for the higher reduction in Hg uptake observed in plants grown in the solution culture on the basis of the ease of formation of the Hg-Se complex. Rice roots may release more root exudates in rice root surrounding environment (organic acids) (Hale *et al*., 1978). The release of organic acids as root exudates was likely to provide the low pH (Hale and Griffin, 1974) required for the reduction of selenite. It might provide a favorable reducing environment for the formation of Se^0 or Selenide Se^{2-}. These forms of selenium can combine with Hg^{2+} to form insoluble HgSe complex in rice root surrounding environment or root surface (Hogland, 1994; Shanker *et al*., 1996b), which was stable and insoluble compound (HgSe, log Ksp = -64.5) and appeared to be unavailable to the plant, resulting in the decrease of Hg content in rice root.

Impact of Hg on rice uptake of Se: Our experiments also testify that low Hg concentration (<30 μg/L) can stimulate the Se uptake of rice root and shoot. The effect of Hg^{2+} on root uptake of SeO_3^{2-} has rarely been reported. One possible explanation for the phenomena observed in this study could be that the adsorption of Hg^{2+} increased the negative charge on the root surface, thereby enhancing SeO_3^{2-} adsorption. More SeO_3^{2-} adsorbed on root surface might stimulate its uptake by root cells subsequently.

Absorption competition existed between selenate and sulfate, showing that the two ions were absorbed through the sulfur transporter. $ZnCl_2$ inhibited the activity of cysteine synthase, to a certain extent suppressed the absorption of selenium acid, suggesting cysteine synthase played an important role in the selenium acid uptake of rice root (Zhang *et al*., 2006). While higher levels of mercury (>30 μg/L) decreased the Se uptake of rice root and stem, it could be because high levels of Hg^{2+} caused cells stress, such as cells enzyme inactivation and cell membrane permeability decrease.

High concentration of Hg may lead to protein precipitation (Patra and Sharma, 2000), thus reducing the functions of some enzymes including Se transporter. With intact rice plants, our data show that Hg^{2+} substantially reduced Se accumulation in root. However, the effect of SeO_3^{2-} on Hg^{2+} translocation from roots to shoots was no straightforward effect.

CONCLUSION

In general, this test confirmed that Hg was very toxic to rice, with the increase of mercury concentrations in the solution, the Hg content of rice root and shoot increased significantly. Lower mercury concentrations (<30 μg/L) can promote Se uptake of rice root and shoot and higher Hg concentrations (>30 μg/L) decreased Se accumulation of rice root and stem. Se in the growth medium significantly reduced Hg accumulation in the roots and the effect of Se on Hg accumulation in shoots displayed a similar pattern. But when the mercury concentrations was fixed at 100 μg/L, with the increase of selenium concentrations, selenium transfer coefficient increased and the mercury transfer coefficient was basically kept a constant.

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