Uptake of mercury (Hg) by seedlings of rice (*Oryza sativa* L.) grown in solution culture and interactions with arsenate uptake

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Abstract

We investigated the mercury (Hg) uptake by seedlings of rice (*Oryza sativa* L.) grown in solution and interactions between Hg and arsenate uptake. The results showed that increasing Hg^{2+} concentrations in the nutrient solution decreased both root and shoot biomass. Hg^{2+} at concentrations of 1.0 and 2.5 mg L^{-1} caused 50% reduction in root biomass. A 50% reduction in shoot biomass occurred at Hg^{2+} concentrations of around 0.5 mg L^{-1} . Nevertheless, 0.5 mg As L^{-1} has no significant effect on plant yield. Hg accumulated in rice roots, and the Hg concentration factor in roots reached nearly 1900 at 2.5 mg Hg L^{-1} . The addition of As slightly increased the Hg concentration in the roots. However, As concentrations in the roots decreased significantly with increasing Hg concentration in the growth solution to 1.0 or 2.5 mg Hg L^{-1} . Shoot As concentrations decreased with increasing Hg concentrations in the growth solution, but increased again with further increase in Hg concentration to 2.5 mg L^{-1} . Possible mechanisms of Hg uptake and interactions between Hg and As in the uptake process are also discussed.

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1. Introduction

Mercury (Hg) is a potentially toxic element that can accumulate in living organisms. There is no known beneficial biological functions of Hg. Elevated Hg in soils has been observed in gold and silver mining sites, copper and zinc mining and smelting areas, and in ar-

eas close to coal burning and other industrial activities. Sewage irrigation is another source of soil Hg contamination. It has been estimated that Hg concentrations in sewage are usually around 5–10 mg kg^{-1} and occasionally up to 100 mg kg^{-1} (Nriagu, 1979). Hg in soils can be readily transferred up the food chain to plants, herbivores and carnivores (Gnamus et al., 2000). Soil toxicity testing using invertebrates showed that the EC_{50} was around several mg kg^{-1} (Lock and Janssen, 2001), but the toxicity of soil Hg depends greatly on the organisms or enzymes used in bioassays (Sheppard et al., 1993).

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The concentrations of Hg found in a survey of Chinese soils ranged from 0.001 to 45.9 mg kg⁻¹, with an average of 0.038 mg kg⁻¹ (CNEMS, 1990). Soil contamination with Hg has occurred widely in China, mainly due to sewage irrigation (for example in the suburbs of Beijing and Tianjing Municipalities) and Hg mining activities (for example in Guizhou Province in southwest China), and soil Hg contamination has elevated Hg concentrations in rice to over 0.02 mg kg⁻¹ (China National Standard for Foodstuff, GB2762-94). It was estimated that emissions of Hg from Guizhou Province to the global atmosphere accounted for 12% of the world total anthropogenic emissions. Furthermore, concentrations of Hg in rice grains in Guizhou were found up to 569 g kg⁻¹, of which 145 g kg⁻¹ was in the form of methylmercury compounds (MeHg) (Horvat et al., 2003). On the other hand, soil contamination with arsenic (As) due to irrigation with As-contaminated groundwater and mining/smeltering activities poses long-term risks to humans and animals (Abedin et al., 2002), and has recently received increasing attention. Recent surveys from Bangladesh showed that irrigation with As-contaminated groundwater is leading to the elevation of arsenic in paddy soils (Alam and Sattar, 2000). Paddy rice (*Oryza sativa*) growing on As-contaminated soils in China had high As levels in grain (Xie and Huang, 1998). It is generally accepted that soil–plant transfer of As is one of principal pathways of human exposure to As contamination (Juhász et al., 2003). Furthermore, the co-existence of As and Hg in soils has been reported in some contaminated areas in China (Haiyan and Stuanes, 2003).

Plant uptake of Hg is affected by various factors including selenium (Se). Shanker et al. (1996) reported that Se (as selenate) in the growth solution

could effectively inhibit Hg uptake by tomato. Arsenate is chemically similar to selenate, and therefore may influence plant uptake of Hg. Hg, on the other hand may influence plant uptake of As. Meharg and Jardine (2003) recently reported that Hg treatment could substantially inhibit the uptake of both arsenite and arsenate by rice seedlings. Rice is a major staple food crop in China and other Southeast Asian countries. However, there is little information available on the mechanisms of, and factors affecting Hg uptake by rice plants and interactions with As uptake. The present study was therefore conducted to investigate Hg toxicity and accumulation in rice plants grown in solution culture, and to study possible interactions between Hg and As in the uptake process.

2. Materials and methods

2.1. Plant culture

Rice seeds were disinfected in 30% (w/w) H₂O₂ solution for 15 min followed by thorough washing with de-ionized water. The seeds were germinated in moist perlite, and at three-leaf stage, uniform seedlings were selected and transplanted in PVC pots (7.5 cm in diameter and 14 cm in height, one plant per pot) containing 500 ml nutrient solution. The composition of the nutrient solution is shown in Table 1 (modified Long Ashton formula after Zhu et al., 1999). The nutrient solution was changed twice a week, and its pH value was adjusted to 5.5 using 0.1 M KOH or HCl solutions (Trostle et al., 2001). The seedlings were allowed to grow in the nutrient solution for three weeks before treatment.

Table 1
Composition of the nutrient solutions

Macronutrients concentration in growth solution (mM)	Salt	Micronutrients concentration in growth solution (μM)	Salt
1.7	NH ₄ NO ₃	50.0	Fe(II)-EDTA
4.0	KH ₂ PO ₄	5.0	H ₃ BO ₄
1.3	CaCl ₂	0.5	ZnSO ₄ ·7H ₂ O
0.5	MgSO ₄ ·7H ₂ O	0.5	CuSO ₄ ·5H ₂ O
0.7	K ₂ SO ₄	2.5	MnSO ₄ ·H ₂ O
		0.2	Na ₂ MoO ₄ ·2H ₂ O
		0.1	CoSO ₄ ·7H ₂ O

2.2. Treatments with Hg and As

Some (Series 1) seedlings were exposed to nutrient solutions with a series of concentrations of Hg^{2+} (0, 0.1, 0.5, 1.0, 2.5, 5.0 mg Hg L^{-1} as HgCl_2), but without As in the solution; and others (Series 2) were exposed to Hg^{2+} (0.1, 0.5, 1.0, 2.5 mg Hg L^{-1} as HgCl_2) and 0.5 mg As L^{-1} as Na_3AsO_4 . The pH value was adjusted to 5.5 according to Trostle et al. (2001). Each treatment was set up in triplicate. The entire nutrient solution was changed twice a week. The Series 1 was used to examine the toxicity of Hg to rice seedlings; and the Series 2 was employed in combination with Series 1 to investigate the interactions between Hg and As in uptake process. Treatment with 0.5 mg As L^{-1} and nil Hg was omitted since As uptake by rice seedlings in solution culture has been widely studied (Meharg and Jardine, 2003; Liu et al., 2004). Plants were grown in these nutrient solutions for a further 20 days before harvesting.

2.3. Plant analysis

The plants were harvested and the roots were washed with de-ionized water and blotted dry. The plants were then separated into shoots and roots and the fresh weights of the roots and shoots were recorded. Shoots and roots were freeze-dried and dry weights were recorded. 0.05–0.1 g of homogenized (by cutting into small pieces) samples were digested with 3 mL HNO_3 in closed Teflon vessels which were heated at 100 °C for 1 h and at 160 °C for 6 h after digestion

overnight. The samples were then diluted to 50 mL with high-purity water. Hg and As concentrations were determined using an atomic fluorescence spectrometer (AFS, model AF-610A from Beijing Ruili Analytical Instrument Co., Beijing, China). A personal computer with was AFS 610 software installed was used to control the integration of the peak areas. After mixed with HNO_3 or HCl , the generated mercury or arsenic hydrides were separated in a gas–liquid separator by argon gas and carried to a quartz atomizer warmed by infrared radiation. Atomization was achieved by argon–hydrogen flame and mercury or arsenic was detected by AFS (He et al., 2002).

2.4. Data analysis

Analysis of variance (ANOVA) was performed using Genstat for Windows on a personal computer (NAG Ltd., England). Two-way analysis of variance was carried out on plant biomass and Hg uptake, one-way analysis was carried out on As uptake data.

3. Results

3.1. Plant biomass

Both root and shoot biomass decreased significantly with increasing Hg concentration in the growth solution (Table 2). For plant exposed to As, a 50% reduction in root biomass occurred at Hg^{2+} concentrations between 1.0 and 2.5 mg L^{-1} ; and for shoot growth and

Table 2

Biomass (g dry matter per pot) of rice seedlings exposed to various concentrations of Hg (mg L^{-1}) with or without 0.5 mg L^{-1} As in nutrient solution (mean $\pm$ SE, $n = 3$)

Hg concentration (mg L^{-1})	Shoot biomass		Root biomass	
	With As	No As	With As	No As
0.0	–	0.521 $\pm$ 0.03	–	0.194 $\pm$ 0.01
0.1	0.413 $\pm$ 0.01	0.386 $\pm$ 0.02	0.161 $\pm$ 0.00	0.151 $\pm$ 0.01
0.5	0.234 $\pm$ 0.02	0.277 $\pm$ 0.02	0.148 $\pm$ 0.01	0.169 $\pm$ 0.01
1.0	0.251 $\pm$ 0.01	0.219 $\pm$ 0.01	0.141 $\pm$ 0.01	0.121 $\pm$ 0.01
2.5	0.174 $\pm$ 0.01	0.173 $\pm$ 0.01	0.071 $\pm$ 0.01	0.077 $\pm$ 0.00
5.0		0.190 $\pm$ 0.02		0.068 $\pm$ 0.01
Analysis of variance				
Hg		$P < 0.001$		$P < 0.001$
As		NS		NS
Hg $\times$ As		$P = 0.079$		$P = 0.078$

Fig. 1. Hg concentration (mg kg^{-1} DW) in roots of plants grown at various Hg^{2+} concentrations (mg L^{-1}) in the growth solution with or without arsenate. Each point is the mean of three replicates. Error bars are $\pm$ S.E.

a 50% reduction in shoot biomass occurred at Hg^{2+} concentration of around 0.5 mg L^{-1} ; for those plants not exposed to As, a 36% reduction in root biomass occurred at Hg^{2+} concentrations between 1.0 and 2.5 mg L^{-1} . At the concentration of 0.5 mg arsenate L^{-1} , there was no significant effect of arsenic on plant biomass.

3.2. Mercury uptake

Hg concentrations in plant tissues increased significantly with increasing Hg concentration in the growth solution (Figs. 1 and 2, $P < 0.001$). Hg accumulated in rice roots with the root concentration at external Hg concentration of 2.5 mg Hg L^{-1} reaching

Fig. 2. Hg concentration (mg kg^{-1} DW) in shoots of plants grown at various Hg^{2+} concentration (mg L^{-1}) in growth solution (Hg^{2+} below 2.5 mg L^{-1}) with or without As. Each point is the mean of three replicates. Error bars are $\pm$ S.E.

4734 mg kg⁻¹. The concentration factor (i.e. Hg concentration in roots divided by the external Hg concentration) reached nearly 1900. Addition of As slightly increased the Hg concentration in the roots ($P < 0.001$). The relationship between root Hg concentration and external concentration followed a saturation trend with or without As addition (Fig. 1). Hg concentration in the shoots was around two magnitudes lower than in the roots (Fig. 2). At external Hg concentration between 0 and 2.5 mg L⁻¹, the relationship between shoot and external Hg concentration also followed a saturation trend. Between 2.5 and 5.0 mg Hg L⁻¹ in the growth solution there was a sharp increase in shoot Hg concentration.

3.3. Arsenate uptake

Root arsenic concentration decreased significantly with increasing external Hg concentration (Fig. 3). The As concentration in the roots exposed to 2.5 mg Hg L⁻¹ was less than one-third of that exposed to 0.1 mg Hg L⁻¹ (107 mg kg⁻¹ versus 364 mg kg⁻¹). As concentrations in the shoots displayed a different pattern of changes with increasing external Hg concentration from 0.1 to 1.0 mg L⁻¹ (Fig. 4), but further increase in Hg concentration to 2.5 mg L⁻¹ resulted in an increase in As concentration in the shoots.

Fig. 3. Arsenic (As) concentrations (mg kg⁻¹ DW) in roots of plants grown at various Hg²⁺ concentrations (mg L⁻¹) in the growth solution. Each point is the mean of three replicates. Error bars are $\pm$ S.E.

Fig. 4. As concentrations (mg kg⁻¹ DW) in shoots of plants grown at various Hg²⁺ concentrations (mg L⁻¹) in the growth solution. Each point is the mean of three replicates. Error bars are $\pm$ S.E.

4. Discussion

It is generally accepted that plant uptake of Hg²⁺ might be via the same uptake processes that move essential micronutrients (Patra and Sharma, 2000). Mercury accumulated is mainly retained in roots. The present data show that Hg accumulated substantially in the roots of rice plants with a concentration factor exceeding 1900. Since at harvest no attempt was made to desorb Hg bound to the cell walls, we would expect that the net uptake of Hg into root cells to be lower than the total Hg accumulated in roots measured in this

study. Our data also show that the Hg concentration in the root was generally 20 times that in the shoot, which was in agreement with studies on other plant species (Patra and Sharma, 2000). Although we did not attempt to grow the plants up to maturity (with grains), it is suggested that translocation of Hg to grains would be very low, since it has been indicated that Hg concentration in the grains could be three to ten times lower than that in the straw (Patra and Sharma, 2000). Thus the accumulation of Hg in rice grains may not be a significant issue if soil Hg contamination is with a few mg kg^{-1} soil. The toxicity of Hg to rice plants has seldom been investigated and data from the present study show that the presence of Hg^{2+} at only 0.1 mg L^{-1} in the growth solution inhibited the growth of rice plants. Hg is considered as one of the most readily accumulated toxic metal elements by living organisms. It is generally considered that human exposure to Hg is mainly through fish products (Parr et al., 1992), but information on Hg accumulation in food crops is rather limited in the literature. It was recently reported that in some contaminated areas terrestrial food chain transfer could be significant, such as Hg accumulation in rice grains in Guizhou, China (Horvat et al., 2003).

It is generally known that Hg (HgCl_2) can inhibit water flow, mainly through the blockage of water channels (Maurel, 1997; Zhang and Tyerman, 1999). Arsenate is taken up via the phosphate (P) transport systems (Wang et al., 2002). Using excised rice roots, Meharg and Jardine (2003) demonstrated that HgCl_2 could inhibit the uptake of both arsenite and arsenate, possibly due to general cellular stress imposed by Hg^{2+} , such as enzyme activities and photosynthesis. High concentration of Hg may lead to protein precipitation (Patra and Sharma, 2000), thus reducing the functions of some enzymes including P transporters. With intact rice plants, our data also show that Hg^{2+} substantially reduced arsenate accumulation in roots. However, the effect of Hg^{2+} on arsenate translocation to shoots was not straightforward. Between concentrations of 0.1 and 1.0 mg L^{-1} , Hg^{2+} showed a significant inhibitory effect on arsenate accumulation in the shoots. However, at $2.5 \text{ mg Hg}^{2+} \text{ L}^{-1}$ in the growth solution, arsenate accumulation in shoots was enhanced up to a similar level to that at $0.1 \text{ mg Hg}^{2+} \text{ L}^{-1}$, despite the general decrease in root As concentration. It has been reported that arsenate accumulated in the roots can be reduced to arsenite via arsenate reductase (Wang et al., 2002;

Quaghebeur and Rengel, 2004), and is transported to shoots at least partly as arsenite. High concentration of Hg might decrease the conversion of arsenate to arsenite, and likely higher proportion of As was transported via the same pathway as that of P leading to more As accumulation in shoots. The presence of arsenate in the growth solution slightly but significantly increased Hg^{2+} concentrations in roots ($P < 0.001$), but not in shoots ($P = 0.115$). The effect of arsenate on root uptake of Hg^{2+} has rarely been reported. One possible explanation for the phenomena observed in this study could be that the adsorption of arsenate increased the negative charge on the root surface, thereby enhancing Hg^{2+} adsorption. More Hg^{2+} adsorbed on root surface might stimulate its uptake by root cells subsequently.

In conclusion, the present study indicated that Hg was highly toxic to rice plants and the relationship between Hg in the growth medium and Hg accumulation in rice plants followed a saturation pattern. Hg in the growth medium significantly reduced As accumulation in the roots. However, the effect of Hg on As accumulation in shoots displayed a different pattern. Between Hg concentrations of 0 and 1.0 mg L^{-1} , As concentration decreased with increasing Hg concentrations in the growth medium, but a further increase in Hg concentration to 2.5 mg L^{-1} resulted in a sharp increase in As concentration in the shoots.

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