Interactive effects of mercury and arsenic on their uptake, speciation and toxicity in rice seedling

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HIGHLIGHTS

- Hg inhibited As uptake, translocation and transformation in rice roots.
- As inhibited Hg uptake at low level, but enhanced Hg uptake at high level.
- As(III) and inorganic Hg were the predominant species in rice roots.
- As and Hg caused root cell damage, lipid peroxidation and iron plaque formation.

GRAPHICAL ABSTRACT

As and Hg uptake, speciation and toxicity in rice seedling.

ABSTRACT

Rice can take up and translocate more As and Hg than other cereal crops. A hydroponic experiment was conducted to investigate their interactive effects on their uptake and toxicity in rice seedling after exposing to As(III) (0.1, 0.5 or 2.5 mg L⁻¹) and Hg (0.05, 0.25 or 1.25 mg L⁻¹) for 14 d. Rice was much more effective in taking up Hg than As and sequestered both in the roots. As and Hg reached 339 and 433 mg kg⁻¹ in the roots, and 48.5 and 16.1 mg kg⁻¹ in the shoots at As₂.₅ + Hg₁.₂₅. Though Hg inhibited As uptake and translocation, it enhanced As(III) toxicity to rice seedling. However, As inhibited Hg uptake at Hg₀.₀₅, but the opposite was observed at Hg₀.₂₅ and Hg₁.₂₅. Arsenite (54–100%) and inorganic Hg (100%) were the predominant form in the plant based on speciation analysis via HPLC–ICP–MS. Malondialdehyde in the roots and shoots increased with increasing As and Hg concentrations, with the highest being 54 μmol g⁻¹ at As₀.₅ + Hg₁.₂₅ in the roots. Root cell structural damage and organelles number reduction with increasing As and Hg concentration were observed based on TEM. As and Hg transformation and toxicity can help to understand the metabolic mechanisms of As and Hg in rice plant when co-present.

1. Introduction

As is a toxic metalloid and its contamination in the environment is of great concern (Zhao et al., 2010). Arsenic contamination in
groundwater has been reported worldwide, and is of increasing public health concern in Bangladesh, India and Thailand. Groundwater in many regions of Bangladesh contains up to 9.0 mg L\(^{-1}\) As (Ahsan and Del Valls, 2011). For example, arsenic contamination affected 17 villages in Nadia, India, with As concentration in irrigation water being 0.11–0.76 mg L\(^{-1}\) (Arunakumara et al., 2013). It exceeds the World Health Organization limit of 10 mg L\(^{-1}\) As in drinking water (Ahsan and Del Valls, 2011) and the permissible limit for irrigation water set by Food and Agriculture Organization is 100 μg L\(^{-1}\) (Arunakumara et al., 2013). As-contaminated groundwater has been used to irrigate crops continuously, especially rice, which may elevate As concentrations in the plants.

Similar to As, Hg is one of the most toxic pollutants in the environment. As and Hg were used extensively in US agriculture for more than a century from the 1860s to the 1970s (Cooper and Gillespie, 2001). The co-presence of As and Hg has been reported in soils in China and other countries, so their interaction may affect their uptake and accumulation in plants (Heeraman et al., 2001; Fu et al., 2010; Rodrigues et al., 2010). For instance, addition of 20 mg L\(^{-1}\) Hg in solution decreased As uptake in rice roots by 75% during 20 min exposure to 7.5 mg L\(^{-1}\) As(III) or As(V) (Meharg and Jardine, 2003). However, addition of As increased the Hg concentration in the roots by ~20% when exposed to 0.5 mg L\(^{-1}\) As and 2.5 mg L\(^{-1}\) Hg for 20 d (Du et al., 2005). But little information is available on the interactive effect of Hg and As uptake by rice.

Rice is a major food crops for 3 billion people, especially in Asian countries. It is more efficient in As uptake and translocation than wheat and barley (Williams et al., 2007; Su et al., 2009). So rice consumption has been implicated as a major route for As exposure. Paddy soil is often flooded during growing season and arsenite (As(III)) is the dominant and most mobile As species in soil pore water (Abedin and Meharg, 2002). So in paddy soil, As(III) is more dominant than arsenate (As(V)), which exists mainly in aerobic soils. As(III) mainly exists as H\(_3\)AsO\(_3\)\(^{-}\) at pH < 9.2, which is taken up by aquaporin channels in rice roots (Meharg and Jardine, 2003) and transported into xylem through Si transporter (Ma et al., 2008). Hg inhibits aquaporin activity and thus affects As(III) uptake by rice under As and Hg co-exposure. Once taken up, As(V) is reduced to As(III) in plant cells by arsenate reductase (Xu et al., 2007). In addition, Hg reduces plant uptake of K, Mg and Mn, which are essential for enzyme synthesis including As(V) reductase and As(III) oxidase (Cargnelutti et al., 2006).

As a waterlogged plant, rice growing in anaerobic environment releases oxygen and oxidizes into rhizosphere through developed aerenchyma (Winkel et al., 2013). The oxygen oxidizes Fe\(^{2+}\) to form Fe plaques coating on the root surfaces (Zhao et al., 2010). Fe plaque may be responsible for As(III) oxidation to As(V) and act as a filter for toxic metals, preventing their uptake and reducing As toxicity (Zhao et al., 2009). Radical oxygen loss from plant roots is considered the most important biotic factor controlling Fe plaque formation (Wu et al., 2012). Under As and Hg stress, rice plant produces more ROS than under normal physiological conditions, which may outflow from cells to the rhizosphere and oxidize Fe\(^{2+}\) to form Fe plaque (Lee et al., 2012). In addition, excessive ROS disrupt cell structure and cause lipid peroxidation. So the interactive effect of As and Hg on rice metabolism needs further investigation (Ozturk et al., 2010; Chen et al., 2012).

The overall goal of this study was to investigate the interactive effects of Hg and As on their toxicity, uptake, translocation and speciation in rice seedling. Our specific objectives were to: (1) investigate Hg and As uptake, distribution and speciation in rice seedling; (2) evaluate the toxicity of Hg and As in rice seedling in response to single and co-contamination of Hg and As; and (3) examine the relationship between Fe plaque formation and As and Hg exposure in rice seedling.

2. Materials and methods

2.1. Plant culture

Rice seeds (Oryza sativa L., Fengyou 22) were surface sterilized in 30% H\(_2\)O\(_2\) solution for 15 min followed by washing with Milli-Q water. Seeds were then soaked in Milli-Q water for 48 h, and germinated on moistened filter papers, which were placed in petri-dishes. After germination, they were transferred to a 96-orifice plate floating on 0.5 mM Ca(NO\(_3\))\(_2\) in a plastic container. At one-leaf stage, they were grown in 0.25-strength nutrition solution recommended by International Rice Research Institute (Wu et al., 2011). At three-leaf stage, they were grown in 0.5-strength nutrition solution. After one week, full strength nutrient solution was used. The nutrient solution pH was adjusted to 5.5–5.8 with KOH. The solution was changed twice a week. The seedlings were allowed to grow for 3-wk before treatment. Plants were grown in a plant growth chamber with 16 h light period (180–240 μmol m\(^{-2}\) s\(^{-1}\)) and 8 h dark period, 28/20 °C day/night temperature, and 60–70% relative humidity.

2.2. As(III) and Hg uptake by rice plants

After 3-wk cultivation, uniform seedlings were transferred in pots containing 550 mL nutrient solution. After 1-wk acclimation, rice seedlings were exposed to nutrient solution containing NaAsO\(_2\) (0, 0.1, 0.5 or 2.5 mg L\(^{-1}\) As(III)) and HgCl\(_2\) (0, 0.05, 0.25 or 1.25 mg L\(^{-1}\) Hg) for 14 d before harvesting. Thereafter, they were referred to As\(_0\), As\(_{0.1}\), As\(_{0.5}\) and As\(_{2.5}\) and Hg\(_0\), Hg\(_{0.05}\), Hg\(_{0.25}\) and Hg\(_{1.25}\).

The treatment solution was replenished with Milli-Q water every 2 d and renewed every 3 d. Aliquots of 10 mL of the growth media were taken daily during the exposure. Total As and As speciation in the growth media were determined. After harvest, all roots were rinsed with an ice-cold phosphate buffer solution including 1 mM K\(_2\)HPO\(_4\), 5 mM MES and 0.5 mM Ca(NO\(_3\))\(_2\) for 20 min to remove apoplastic As, and then washed thoroughly with Milli-Q water. Rice was flash-frozen in liquid N\(_2\) and stored at −80 °C for further analysis.

2.3. DCB extraction of Fe plaque

To analyze Fe concentrations in the Fe plaque, fresh root surfaces were extracted using dithionite–citrate–bicarbonate (DCB) (Liu et al., 2004). After extraction, the roots were rinsed three times with Milli-Q water, which was added to the DCB extract. The resultant solution was made up to 50 mL with Milli-Q water. The Fe concentration was measured by an atomic absorption spectrophotometer (PerkinElmer 900T, USA). After DCB extraction, some roots and shoots were oven-dried at 70 °C for 3 d for total elemental analysis, and some fresh samples were stored at −80 °C for analysis of metal speciation.

2.4. Total As and Hg in plants

Oven-dried and frozen fresh plant samples were grounded to powder in an acid-cleaned agate mortar and passed through 1-mm sieve for total As and Hg concentration analysis. For As measurement, ~0.2 g of ground plant samples were weighed into digestion vials, mixed with 10 mL of 1:1 concentrated HNO\(_3\)/H\(_2\)O and left overnight. All samples were digested according to EPA Method 3050B using the Hot Block Digestion System (Environmental Express, USA; Srivastava et al., 2010). They were incubated at 105 °C for 2 h, removed from the block and then cooled for 3 min. The samples were mixed with 1 mL of 30% H\(_2\)O\(_2\) slowly,
and then heated another 15 min. The samples were cooled completely, and then diluted up to 50 mL with Milli-Q water. For Hg measurement, ~0.5 g of ground fresh plant samples were weighed into digestion vials, mixed with 4 mL of HNO₃ and 2 mL of H₂O₂, heated for 2 h at 85 °C and then diluted up to 50 mL.

Blank and certified reference material for rice samples (GBS-21, Chinese geological reference materials) were used for quality control. The Hg and As mean ± standard error were 4.3 ± 0.5 μg kg⁻¹ and 0.114 ± 0.013 mg kg⁻¹, which was comparable to the certified values (4.8 ± 0.8 μg kg⁻¹ and 0.114 ± 0.018 mg kg⁻¹). Arsenic concentrations in the plants were measured by an inductively coupled plasma mass spectrometry (ICP–MS; PerkinElmer NexION 300X, USA). The internal standards were carried to ensure accuracy and precision. Standard solution at 1 μL⁻¹ As and Hg were measured every 20 samples to monitor the stability of the ICP–MS.

2.5. As and Hg speciation in plants

The frozen fresh samples were crushed in a mortar under liquid nitrogen for As and Hg speciation. ~0.5 g of sample was weighed into a 50 mL polypropylene centrifuge tube and 10 mL of 1:1 methanol/water mixture was added to extract As. All samples were extracted ultrasonically for 2 h and repeated three times at 25 °C (Srivastava et al., 2010). The three extracts were mixed, and then diluted to 50 mL with Milli-Q water. After filtering, extracts were stored at −80 °C and analyzed within 24 h. For Hg speciation in fresh samples, ~0.2 g of sample was weighed into a 15 mL polypropylene centrifuge tube, mixed with 10 mL solution containing 0.1% v/v HCl, 0.05% m/v L-cysteine and 0.1% v/v 2-mercaptoethanol. The mixture was shaken at 150 rpm and 30 °C for 12 h and then sonicated for 15 min (Wang et al., 2007; Batista et al., 2011). Extracts were centrifuged and then collected in a 50 mL centrifuge tube.

As and Hg speciation was measured by high performance liquid chromatography (HPLC; Waters 2695, USA) coupled with ICP–MS. A guard column (Hamilton, UK) connected to a PRP-X100 10 μm anion-exchange column (Hamilton, UK) was used to separate As species using 7.5 mM (NH₄)₂HPO₄ and NH₄NO₃ mobile phase at pH 6.2. A C₁₈ column (Waters, USA) was used to separate Hg species. The mobile phase contained 5% v/v methanol, 0.1% v/v L-cysteine and 0.06 mM ammonium acetate, adjusted to pH 6.5 using ammonia. Sample injection volume was 50 μL at a flow rate of 1 mL min⁻¹. A 10 μg L⁻¹ standard mix of As(III), As(V), dimethylarsinic acid (DMA), monomethylarsenic acid (MMA), mercury and methylmercury was run to obtain retention time. All working standards were measured at 532 nm using spectrophotometer (UV Probe, Shimadzu, Japan). Concentrations of TBARS were calculated using a molar extinction coefficient of 1.56 × 10⁶ M⁻¹ cm⁻¹ and expressed as μmol TBARS g⁻¹ FW.

2.7. Data analysis

All results were presented as mean ± standard deviation (SD) of three replicates. Statistical analysis was performed using PASW statistics 18.0, including one-way ANOVA and the least significant difference. Difference was considered significant at p < 0.05 and highly significant as p < 0.01.

3. Results and discussion

During 14 d exposure, As and Hg concentration and speciation in the nutrient solution were determined (Table 1). As concentration in the nutrient solution decreased by 20–33% (25.2–741 μg L⁻¹) and almost all As(III) was oxidized to As(V) after 3 d (94–100%) (Table 1). Since some of the As(III) was oxidized to As(V), it was probably adsorbed on the iron plaque of the root surface (~100 mg kg⁻¹ in the roots), increasing the negative charge on the root surface, thereby enhancing Hg adsorption and uptake by the roots (Du et al., 2005). Compared to As, rice seedling took up more Hg, with Hg concentration in the growth media being decreased by 95–99% (48.9–1228 μg L⁻¹, Table 1), but no speciation change was observed in the nutrient solution after 3 d (data not shown). These results emphasized the need to monitor As speciation in the media even in short-term experiments, as As(III) oxidation occurred rapidly.

3.1. Effect of As and Hg on rice seedling biomass

After 14 d exposure to As and/or Hg, rice seedlings were still alive in all treatments. Both root and shoot biomass increased initially and then decreased with increasing As and Hg concentrations (Fig. 1). Low concentrations of As (<0.5 mg L⁻¹) and Hg (<0.05 mg L⁻¹) stimulated plant growth, resulting in 8–11% and 13–15% increase in the root and shoot biomass compared with As₀ and Hg₀. However, high level of As (As₂.₅) and Hg (Hg₁.₂₅) reduced plant biomass by 40–46% and 35–54% in the roots and shoots compared with As₀ and Hg₀. There was a synergic effect on plant growth under co-contamination of low As and Hg (Fig. 1). At low As exposure, the root biomass increased from 0.85 at As₀₁ to 1.13 g plant⁻¹ at As₀₁ + Hg₀₁ (Fig. 1A), and similar trend was found in the shoots. However, at high As exposure, there was an inhibitive effect. For example, the root biomass was reduced from 1.07 at As₀₅ to 0.66 g plant⁻¹ at As₀₅ + Hg₁.₂₅ (Fig. 1A).

In this study, As and Hg stimulated the growth of rice plant at low concentration (<0.5 mg L⁻¹ As or <0.05 mg L⁻¹ Hg), which was probably due to hormesis effect and was common in plants (Ozturk et al., 2010). At higher concentrations of As and/or Hg, cell membrane and functionality of transporters in rice seedling were impacted, leading to less transport of important nutrients. Several studies have shown that Hg decreases the levels of photosynthetic electron transport chain where photosystem II is the most sensitive target and inhibits the chlorophyll biosynthesis, subsequently inhibiting plant growth (Cargnelutti et al., 2006).

3.2. Hg inhibited As uptake and translocation by rice seedling

Arsenic concentrations in rice plants increased with increasing As concentrations (Fig. 2). For example, As concentration was 35 times higher at As₂.₅ than As₀₁ in the roots (312 vs. 9.20 mg kg⁻¹; Fig. 2A). Arsenic was mainly retained in the roots, with small amount being translocated to the shoots. Take As₂.₅ for example,
As concentrations in the roots and shoots were 312 and 49.1 mg kg\(^{-1}\) (Fig. 2). It was consistent with Su et al. (2009) and it was probably due to complexation of As(III) by thiols, which is sequestered in the root vacuoles (Zhao et al., 2009).

Hg addition decreased As uptake and translocation by the rice roots at As\(^{0.1}\) and As\(^{0.5}\). For example, As concentrations in the roots and shoots at As\(^{0.1}\) (9.20 and 0.87 mg kg\(^{-1}\)) were 2.4 times higher than that at As\(^{0.1} + \text{Hg}_{1.25}\) (6.07 and 0.43 mg kg\(^{-1}\)). Although As concentrations in the roots increased from 312 at As\(^{2.5}\) to 339 mg kg\(^{-1}\) at As\(^{2.5} + \text{Hg}_{1.25}\) (Fig. 2A), total As content decreased from 18.0 to 13.1 mg plant\(^{-1}\) due to reduction in root biomass (Fig. 1A). It was documented that As influx was decreased by 75% in excised rice roots when 20 mg L\(^{-1}\) Hg was added to 7.5 mg L\(^{-1}\) As(III) (Meharg and Jardine, 2003). The high Hg concentration probably affected enzyme activities, causing protein precipitation.

**Table 1**

<table>
<thead>
<tr>
<th>As + Hg</th>
<th>As(III)</th>
<th>As(V)</th>
<th>As(III)</th>
<th>As(V)</th>
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<th>As(V)</th>
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<td>Average</td>
<td>After 1 d</td>
<td>Average</td>
<td>After 1 d</td>
<td>Average</td>
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<tr>
<td>0.1 + 0</td>
<td>28.0 ± 5.12</td>
<td>ND</td>
<td>43.0 ± 6.53</td>
<td>43.5 ± 5.85</td>
<td>74.8 ± 12.3</td>
<td>59.2 ± 9.08</td>
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<td>–</td>
</tr>
<tr>
<td>0.1 + 0.05</td>
<td>42.0 ± 7.65</td>
<td>ND</td>
<td>47.8 ± 5.78</td>
<td>22.8 ± 4.32</td>
<td>67.0 ± 13.9</td>
<td>44.9 ± 9.11</td>
<td>1.66 ± 0.225</td>
<td>0.800 ± 0.143</td>
</tr>
<tr>
<td>0.1 + 0.25</td>
<td>28.0 ± 4.24</td>
<td>ND</td>
<td>43.3 ± 4.35</td>
<td>33.4 ± 65.2</td>
<td>257 ± 47.2</td>
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<td>–</td>
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<tr>
<td>0.1 + 0.05</td>
<td>199 ± 30.1</td>
<td>ND</td>
<td>235 ± 3.5</td>
<td>332 ± 26.9</td>
<td>235 ± 24.8</td>
<td>0.980 ± 0.172</td>
<td>0.800 ± 0.143</td>
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<tr>
<td>0.1 + 1.25</td>
<td>231 ± 29.5</td>
<td>17.3 ± 3.29</td>
<td>249 ± 35.6</td>
<td>249 ± 39.5</td>
<td>232 ± 42.4</td>
<td>1.66 ± 0.256</td>
<td>0.700 ± 0.132</td>
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<tr>
<td>0.5 + 0.05</td>
<td>307 ± 54.2</td>
<td>ND</td>
<td>270 ± 40.1</td>
<td>397 ± 49.2</td>
<td>252 ± 37.2</td>
<td>10.0 ± 1.89</td>
<td>497 ± 73.2</td>
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<tr>
<td>0.5 + 0.25</td>
<td>28.0 ± 4.24</td>
<td>ND</td>
<td>43.3 ± 4.35</td>
<td>332 ± 26.9</td>
<td>235 ± 24.8</td>
<td>0.980 ± 0.172</td>
<td>0.800 ± 0.143</td>
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</tr>
<tr>
<td>0.5 + 1.25</td>
<td>370 ± 81.5</td>
<td>ND</td>
<td>1043 ± 160</td>
<td>1013 ± 149</td>
<td>1769 ± 168</td>
<td>1769 ± 149</td>
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<tr>
<td>0 + 0.05</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>1.66 ± 0.256</td>
<td>0.700 ± 0.132</td>
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<tr>
<td>0 + 0.25</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>13.5 ± 2.49</td>
<td>6.70 ± 1.39</td>
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<tr>
<td>0 + 1.25</td>
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<td>–</td>
<td>65.6 ± 15.9</td>
<td>39.0 ± 6.79</td>
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ND – not detected.

Fig. 1. The effect of interactive As and Hg exposure on root (A) and shoot (B) biomass of rice seedlings after exposing to 0.1, 0.5 or 2.5 mg L\(^{-1}\) As and 0.05, 0.25 or 1.25 mg L\(^{-1}\) Hg for 14 d under hydroponics. Significant differences for a given Hg treatment are indicated as *(p < 0.05) and **(p < 0.01) according to one-way ANOVA.

Fig. 2. As concentrations in the roots (A) and shoots (B) of rice seedling after exposing to 0.1, 0.5 or 2.5 mg L\(^{-1}\) As and 0.05, 0.25 or 1.25 mg L\(^{-1}\) Hg for 14 d under hydroponics. Significant differences for a given Hg treatment are indicated as *(p < 0.05) and **(p < 0.01) according to one-way ANOVA.
and inhibiting As uptake by rice (Du et al., 2005). Aquaporin transporter (Lsi2) for silicic acid in the xylem transports As(III) from the roots to shoots in rice (Ma et al., 2008). Hg addition can inhibit Lsi2 activity, thereby reducing As translocation in rice.

The Fe plaque formation on root surface increased with increasing As and Hg concentrations (Fig. 3). Lee et al. (2012) also reported that As exposure to rice seedling enhanced Fe plaque formation on the roots. Addition of Hg to rice seedling probably increased ROS production in the roots. These ROS were released outward from the roots, causing oxidation of Fe$^{2+}$ to Fe$^{3+}$ and subsequent formation of Fe plaque (Lee et al., 2012). Due to its strong affinity for As, the presence of Fe plaque may decrease As uptake by rice seedling (Fig. 3). For example, extractable Fe concentration was 1.8-fold higher at As$_{0.1}$ + Hg$_{1.25}$ than As$_{0.1}$ (1285 vs. 696 mg kg$^{-1}$), but As concentration in the roots was 1.5-fold lower (6.07 vs. 9.20 mg kg$^{-1}$). We concluded that Fe plaque may reduce As uptake by rice seedling except under As and Hg co-exposure.

### 3.3. As inhibited Hg uptake at Hg$_{0.05}$ but enhanced Hg uptake at Hg$_{0.25}$ and Hg$_{1.25}$

Hg was readily taken up by rice roots, which was mainly retained in the roots with limited amount being translocated to the shoots. For example, Hg concentrations were 382 mg kg$^{-1}$ in the roots and 10.9 mg kg$^{-1}$ in the shoots at Hg$_{1.25}$ (Fig. 4), similar to Meng et al. (2010). Phytochelatins, small peptides that detoxify heavy metals in rice plants, can sequester Hg in the roots (Krupp et al., 2009). Compared to As, rice seedling was much more effective in taking up Hg than As, but only 1.2–3.8% of the Hg was translocated to the shoots (Fig. 4B). This was consistent with solution As and Hg concentrations, with Hg concentration decreasing by 95–99% and As decreasing by 20–33% after 3 d of growth (Table 1).

With increasing As concentration, Hg uptake in rice roots decreased at Hg$_{0.05}$, but increased at Hg$_{0.25}$ and Hg$_{1.25}$ (Fig. 4A). For example, Hg concentration decreased from 14.5 mg kg$^{-1}$ at Hg$_{0.05}$ to 12.0 mg kg$^{-1}$ at Hg$_{0.05}$ + As$_{0.5}$. Hg uptake into root cells was possibly through the same channels as essential micronutrients, such as Fe, Cu or Zn (Chen and Yang, 2012). These transport systems depend on metabolic energy and root transporter in rice plant (Esteban et al., 2008; Chen and Yang, 2012). As(III) can bind with –SH groups of proteins and then alter protein structure and interfere with the catalytic sites of enzymes (Zhao et al., 2009). So As(III) probably inhibited the activity of Hg transporters in the roots.

As(V) can replace phosphate in energy transfer phosphorylation reactions, forming ADP-arsenate instead of ATP, which probably slowed down Hg uptake process. So addition of As inhibited Hg influx into cells. However, with Hg concentration increasing, addition of As enhanced Hg uptake by the roots. For example, Hg concentration increased from 62.6 mg kg$^{-1}$ at Hg$_{0.25}$ to 89.3 mg kg$^{-1}$ at Hg$_{0.25}$ + As$_{2.5}$ (Fig. 4A). Similar results were obtained by Du et al. (2005). This was because cell membranes were fluidized and destroyed at high As and Hg concentration, enhancing their permeability and Hg transport across the membrane (Tuan et al., 2008). This was supported by MDA content (Fig. 5) and TEM micrographs of the roots (Fig. 6A).

### 3.4. As–Hg co-exposure caused lipid peroxidation and root damage

To better understand As and Hg toxicity to rice, we measured TBARs in rice roots. At As$_{0.1}$ and As$_{0.5}$, As had little effect on plasma membrane of the roots, as indicated by MDA content (Fig. 5). The results were consistent with TEM micrographs at As$_{0.5}$. The cell structure and cell membrane were intact and continuous, and the endoplasmic reticulum and mitochondria were abundant in the cytoplasm (Fig. 6A). Ozturk et al. (2010) found that at 75 mg L$^{-1}$ As(III), the MDA content was similar to the control for watercress. The antioxidant defense system in plant removes, neutralizes and scavenges the ROS, thus protecting the plasma membrane. The MDA level in the rice seedling depended on the As and Hg concentration. At As$_{0.5}$, MDA content in the roots and shoots (34.5 and
Increased As and Hg uptake by rice seedlings led to higher MDA content in roots compared to the control (8.67 and 5.31 µmol g⁻¹) (Fig. 5A). In addition, the MDA content was higher in the roots than shoots since more As and Hg were accumulated in the roots (Figs. 2 and 4).

Exposure to high concentrations of As and Hg in rice seedling induced more ROS including H₂O₂, which caused serious lipid peroxidation and degradation of organelles (Henzler and Steudle, 2000). At As₂转变 and Hg₁转变, the number of endoplasmic reticulum decreased and the cell structure was seriously degraded, including mitochondrion. The mitochondrion is another site of ROS production besides chloroplast (Mittler, 2002). Mitochondrion is a key regulator of programmed cell death in plants so enhanced ROS levels in the mitochondria may trigger programmed cell death (Lam et al., 2001).

Though Hg decreased As uptake by rice, it increased the cell structure damage. This was consistent with the fact that Hg increased MDA content at As₀转变 and As₀转变 in the roots and shoots (Fig. 5). Hg probably disrupted the normal metabolic balance, enhanced ROS load and increased the rate of MDA formation. The highest MDA was 53.8 and 18.6 µmol g⁻¹ at As₀转变 + Hg₁转变 in the roots and shoots, increasing by 421% and 158% compared to As₀转变. Wang et al. (2009) also indicated that H₂O₂ and MDA level are increased 4 times in rice leaves after exposing to 40 mg L⁻¹ Hg for 48 h. In cucumber seedlings, MDA levels are enhanced 2.5 times after 15 d exposure to 100 mg L⁻¹ Hg (Cargnelutti et al., 2006). However, the MDA content in rice seedling decreased at As₂转变 + Hg₁转变 compared to As₂转变 or Hg₁转变. Similar results were obtained in other studies. Ozturk et al. (2010) found the MDA content decreased at 3.75 mg L⁻¹ As(III) compared to 0.75 mg L⁻¹ As(III). Arsenic may modify the activities of the enzyme lipoxigenase, which catalyzes polyunsaturated fatty acid oxidation and produces peroxide (Chakrabarty et al., 2009). Another reason for MDA content decrease may relate to the subcellular damage of membrane structure in cells exposed to high As and Hg concentration. The TEM micrographs showed that mitochondria and...
endoplasmic reticulum appeared to reunite in the roots, which was important organelle to synthesize and transport protein and lipid molecules (Fig. 6D). So it was possible that biosynthesis of polyunsaturated fatty acid was inhibited because of serious cell structure and function damage.

3.5. Hg inhibited As transformation, but As had no effect on Hg speciation in rice plants

After Hg exposure to rice seedling, only inorganic Hg was found in plant, with no methylmercury being detected. This is consistent with the observation that rice cannot methylate Hg (Qiu et al., 2008; Zhang et al., 2010).

After 14 d exposure to As(III), more As(III) than As(V) were accumulated in the roots and shoots. However, little MMA and DMA were detected in rice seedling (Table 1). After 3 d of growth, most As(III) in the growth media was oxidized to As(V), with <6% As(III) concentration in the growth media increased, more As(III) was accumulated in the roots. As(III) accounted for 55–92% and 60–100% of As in the roots and shoots, with less As(III) in the roots than shoots (Fig. 7). The results suggested that rice roots and shoots exhibited strong As(V) reduction activities, with As(V) reduction being more important in the roots than shoots. Xu et al. (2007) observed that rice roots contained 92% As(III) and 8% As(V) after exposure to 1.54 mg kg⁻¹ at As_{0.1} to 0.87 mg kg⁻¹ at As_{0.5} and 0.25 mg kg⁻¹ at As_{2.5} or from 72% to 55% in the roots (Fig. 7A). As(V) in rice roots can be reduced to As(III) by As reductase, which plays an important role in rice roots (Xu et al., 2007). Addition of Hg inhibited As(V) reduction, possibly due to that fact that Hg can induce protein precipitation and deactivate As reductase, which have been identified in rice (Cargnelutti et al., 2006; Bhaduri and Fulekar, 2011). Therefore, Hg decreased As(V) reduction and as a result, As(III) concentration decreased in rice plant.

4. Conclusion

In conclusion, Hg inhibited As uptake, translocation and transformation in rice. Arsenic inhibited Hg uptake at low Hg concentration, and then enhanced Hg uptake and translocation at high concentration, but did not affect Hg transformation in rice. Both MDA content in the rice and Fe plaque formation in the root surface increased with increasing As and Hg concentration, which was consistent with the results of transmission electron microscope. As and Hg co-exposure aggravated cell toxicity compared with single treatment.

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