

# Prediction of Methyl Mercury Uptake by Rice Plants (*Oryza sativa* L.) Using the Diffusive Gradient in Thin Films Technique

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## S Supporting Information

**ABSTRACT:** Rice consumption is the primary pathway for methyl mercury (MeHg) exposure at inland mercury (Hg) mining areas of SW China. Mechanistic information on MeHg accumulation in rice is, however, limited. The process of MeHg exchange between paddy soil and rice plants predominantly occurs in pore water. The detection of bioavailable MeHg in pore water is therefore important to predict MeHg uptake by rice plants (*Oryza sativa* L.). This study investigated MeHg dynamics and spatial MeHg trends in pore water during the rice growing season using the diffusive gradient in thin films (DGT) technique and tested the ability of DGT to predict MeHg uptake by rice. The MeHg uptake flux from soil to rice plants via roots was significantly correlated with the DGT-measured MeHg flux ( $R = 0.853$ ,  $p < 0.01$ ). Our study implies that DGT can predict the bioavailability of MeHg in rice paddy soil and that the DGT method can provide quantitative description of the rate of uptake of this bioavailable MeHg. The DGT technique is demonstrated as a useful indicator of the likely ecotoxicological risk that might be apparent where paddy rice is grown in MeHg contaminated soil.



## 1. INTRODUCTION

The mercury compound methyl mercury (MeHg) is highly toxic to mammals and causes a number of adverse health effects. In aquatic systems, MeHg is bioavailable and can biomagnify in the food chain.<sup>1</sup> Diet is the main pathway of MeHg exposure to humans. When consumed, close to 100% of ingested MeHg is absorbed. Due to its high lipid solubility, the compound can rapidly cross the blood-brain and placental barriers to reach its principal targets, the brain and the fetus. Therefore, exposure of women to MeHg-contaminated food during pregnancy is of high concern.<sup>2,3</sup> Based on the findings of studies conducted in North America and Europe, the most common MeHg exposure route to humans is believed to be the consumption of fish, shellfish, and sea mammals.<sup>2,4</sup> However, a series of studies in Guizhou province, Southwestern China, have indicated that rice is a bioaccumulator of MeHg,<sup>5–7</sup> suggesting that at inland Hg mining areas, away from significant water bodies, rice consumption may be the main MeHg exposure pathway.<sup>8,9</sup> The physiochemical conditions of paddy soils promote the formation of MeHg,<sup>10</sup> which can then be taken up by rice plants and translocated to the above-ground parts (leaf and stalk) and eventually to grain.<sup>11,12</sup> Therefore, in the context of risk assessment, it is important to accurately assess the potential bioavailability of MeHg in paddy soils.

Generally, bioavailable metal is considered to be that fraction of total soil metal that is in soil solution or is weakly adsorbed to soil surfaces (the exchangeable phase). This fraction of soil

metal is defined as labile.<sup>13–15</sup> A widely published technique, the diffusive gradients in thin films technique (DGT), has been considered by some researchers to be an accurate measure of the concentration of labile metal species in soil solution.<sup>16–18</sup> A DGT probe is composed of a diffusive layer (a filter membrane and a diffusive gel) and a binding layer. The labile metals pass through the diffusive layer and bind with the binding layer, inducing a concentration gradient that is established in the diffusive gel during the deployment time. Once the DGT probe is retrieved, the mass of metal in the binding layer can be determined analytically, and the labile metal concentration in the soil quantified. When deployed into a soil, the DGT device can mimic the physical process of plant metal uptake by locally lowering the soil solution concentration. This will subsequently induce metal resupply from labile metal complexes in solution and from the labile metal pool on the soil solid phase.<sup>19,20</sup>

The DGT technique has been successfully used to predict the metal concentration in plants grown under pot<sup>13,17,21</sup> and field conditions.<sup>22</sup> For example, in the study of Tian et al.,<sup>22</sup> the DGT technique was used to measure the metal concentration in the rhizosphere soil of rice plants at the time of crop harvest. The DGT technique was shown to be a

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superior indicator of metal bioavailability in soil relative to soil–solution measurements or chemical extractions. Each indicator was assessed through correlation of measured bioavailable metal concentration with the metal concentration in plant roots and unpolished grain. The superior predictive ability of DGT was attributed to the ability of this measurement to quantitatively account for the main factors affecting bioavailability. However, the study only focused on soil sampled at crop maturation and therefore did not consider metal dynamics during the rice growing season.

The physiochemical conditions of a paddy soil and pore water, and the MeHg concentration in these environments, may fluctuate during the rice growing season. These fluctuations may affect the rate and magnitude of MeHg uptake by rice plants. Uptake of MeHg from soil is a complex process that involves not only a dynamic interaction between the solid phase, soil solution and root but also the methylation processes (abiotic and biotic) that account for changing mercury speciation. Therefore, to quantify the MeHg dynamics in pore water and to better predict the potential for this mercury to accumulate in rice plants, the flux of MeHg uptake throughout the rice growing season, as well as the spatial profile of MeHg in soil, must be investigated.

In the current study, the depth dependent MeHg concentration (spatial profile) in contaminated rice-paddy soil was field measured using DGT probes throughout a rice growing season. The relationship between the DGT-measured bioavailable flux of MeHg in soil throughout the growing season and the flux of MeHg uptake by rice plants was thereby examined. The potential usefulness of DGT to predict MeHg uptake into rice grain was also examined in order to assess if this technique could describe the potential risk for MeHg contamination of food.

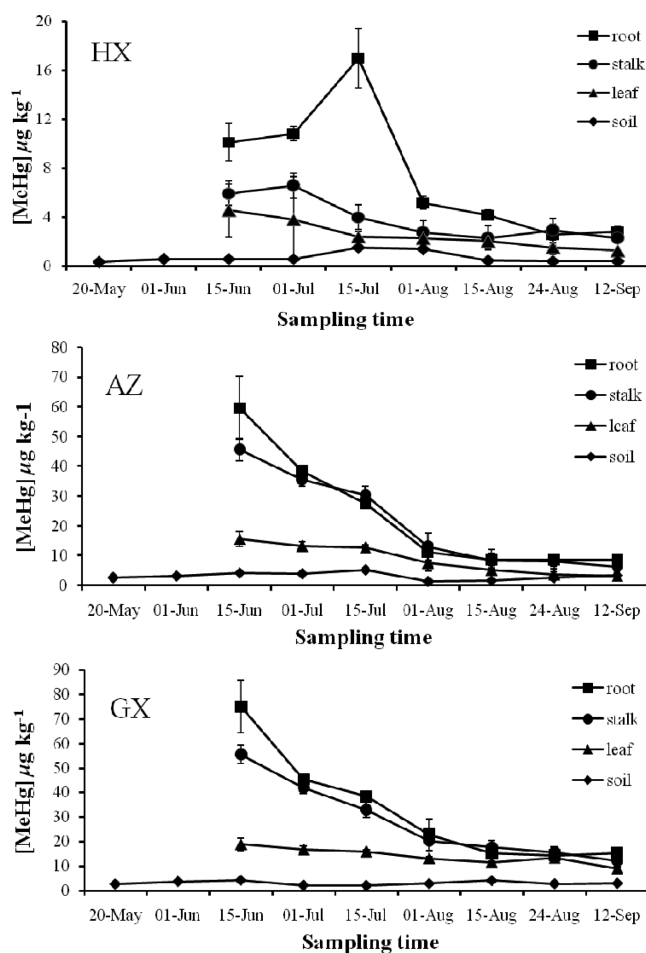
## 2. MATERIALS AND METHODS

**2.1. Study Area.** Guizhou province, in southwestern China, is one of the most historically important Hg-producing areas in China. Mercury mining has occurred in Guizhou for more than 3000 years. At the peak level of activity, Hg emissions from mining and refining activities into the atmosphere reached 11 t/year.<sup>5,23</sup> Wanshan is not only the most extensive Hg mining area in Guizhou province but also the third largest in the world.<sup>24</sup> Although large-scale mining activities were officially closed in 2004, small-scale artisanal mining activities continue to take place at locations throughout the province. Mercury discharge from historic large-scale Hg retorting combined with that from current artisanal mining activities has resulted in serious Hg contamination of air, water, and soil throughout the Wanshan mining area.<sup>5,25,26</sup> Total Hg and MeHg concentrations in soil at Wanshan have been reported to range from 0.33 to 790 mg kg<sup>-1</sup> and 0.19 to 20 μg kg<sup>-1</sup>, respectively.<sup>27,28</sup> The most common current-day agricultural land use throughout the Wanshan area is rice growing in paddy-fields. There is currently great concern over the potential for mercury contamination of rice grown at Wanshan.<sup>11</sup>

For the current research, total and MeHg concentrations in rice paddy soil were investigated at three field locations. Two of these were at Wanshan; an artisanal Hg mining site (Gouxi, GX), and a now-closed commercial Hg mining site (Jinjia Chang in Aozai, AZ). The third site was a control site (Huaxi, HX) located southwest of Guiyang city (about 367 km away from Wanshan), which has no direct point sources of Hg contamination.<sup>11</sup> Paddy rice farming is the primary land use at

each of the three locations, and investigations for the current study were performed through sampling a field plot at each location with dimensions of 10 m × 13 m.

**2.2. Sample Collection and Preparation.** Soil and plant sampling was conducted on seven consecutive occasions during the rice growing season. Sampling was initiated one month after rice plants were transplanted into paddies on June 15th, 2010, and thereafter, sampling was carried out once every 15 days until the rice harvest on September 12th, 2010 (with the single exception of the sixth sampling which was conducted 7 days after the fifth) (Figure 1). For each study area, six rice plants, as



**Figure 1.** Temporal variation of methyl mercury (MeHg) concentration in paddy soil and rice plants during the rice growing season. Plant sampling commenced on June 15th, one-month after rice was transplanted at each location. The final sampling on September 12th coincided with rice harvest.

well as the corresponding soil from the root zone (10–20 cm depth), were collected at each sampling. Soil samples were collected by hand using disposable polyethylene gloves, preserved in the field using a chiller, transported to the laboratory, and freeze-dried. Each rice plant was separated from soil using a small shovel, washed, and divided into three fractions: root, stalk, and leaf. At the final sampling time, rice grains were also separated using a scalpel. All vegetative samples were rinsed with ultrapure water (Milli-Q, 18.2 M Ω) in an ultrasonic bath and then freeze-dried.

Samples of soil pore water were collected using an in situ DGT sampler on the same sampling dates, as described for soil

and rice plants. The temperature of soil and time of sampling were recorded. The DGT probe consisted of a 3-mercaptopropyl functionalized silica gel binding layer (0.4 mm thick), a polyacrylamide diffusive gel (0.8 mm thick), and a cellulose nitrate membrane filter (Whatman, 0.22  $\mu\text{m}$  pore size, 100  $\mu\text{m}$  thick). The binding layer, diffusive gel, and the filter were assembled in a sediment DGT probe molding (1.8 cm  $\times$  15 cm window, DGT Research Ltd., Lancaster, U.K.). The details of binding layer and diffusive gel preparation have been described previously.<sup>29,30</sup> Before deployment, the DGT probes were prepared and deoxygenated for 24 h with argon gas in a container filled with precleaned (using 3-mercaptopropyl-functionalized silica) NaCl (0.01%). The container was then sealed and transported to the field.<sup>18</sup> At the time of first sampling (June 15th), one DGT probe was inserted into the root zone of each of three replicate plants. The probes were left in place until the next sampling interval when they were retrieved and replaced by fresh probes. This procedure was repeated on each successive sampling, until the final sampling on August 24th. Following this design, DGT monitoring was made of soil solution throughout the rice growing season. At each sampling, the DGT probes were retrieved, rinsed in the field using Milli-Q water (18.2 M  $\Omega$ ) and preserved at 4  $^{\circ}\text{C}$  in a sealed polythene bag. In the laboratory, the DGT probes were opened, and the resin gels were sliced into sections of 1 cm interval before elution. The sections were individually soaked in 2 mL of a 1.31 mM thiourea and 0.1 M HCl solution for at least 24 h, before the eluents were diluted and analyzed for MeHg. The concentration of labile MeHg in pore water measured by DGT ( $[\text{MeHg}]_{\text{DGT}}$ ) was calculated according to eq 1.<sup>30,31</sup>

$$[\text{MeHg}]_{\text{DGT}} = \frac{1}{t} \int_0^t [\text{MeHg}](t) dt = (M\Delta g)/(ADt) \quad (1)$$

where  $t$  is the deployment time (s),  $M$  is the mass of MeHg accumulated in the resin gel (ng),  $A$  is the surface area of the DGT probe ( $\text{cm}^2$ ),  $\Delta g$  is the thickness of the diffusive gel layer (cm), and  $D$  is the diffusion coefficient of MeHg ion in the diffusive gel ( $\text{cm}^2 \text{s}^{-1}$ ). The constant parameter  $D$  is dependent on the temperature of the soil at the time of sampling. The  $D$  value of MeHg is  $5 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$  at 20  $^{\circ}\text{C}$ ,<sup>29</sup> and the diffusion coefficient ( $D_T$ ,  $\text{cm}^2 \text{s}^{-1}$ ) at any temperature ( $T$ ,  $^{\circ}\text{C}$ ) can be calculated by the Stoke-Einstein eq 2:<sup>32</sup>

$$\log D_T = [1.37023(T - 25) + 8.36 \times 10^{-4}(T - 25)^2] / (109 + T) + \log[D_{25}(273 + T)/298] \quad (2)$$

Biofilm development can be a problem for long duration deployments of DGT. In the current study, there was biofilm development on the membrane filters above the interface between soil and overlaying water for some probes. The affected section of such probes was discarded when the gel was sliced in preparation for depth-dependent MeHg analysis.

**2.3. Analytical Methods.** For MeHg analysis, rice plant samples were digested using the KOH-methanol/solvent extraction technique,<sup>33,34</sup> whereas soil samples were prepared using the  $\text{CuSO}_4$ -methanol/solvent extraction technique.<sup>34</sup> MeHg in all samples, including the DGT eluates, was extracted with methylene chloride, then back-extracted with water and ethylated into methylethyl mercury ( $\text{CH}_3\text{HgCH}_2\text{CH}_3$ ). This phase of MeHg was purged onto a Tenax trap in a stream of  $\text{N}_2$ . This effectively preconcentrated MeHg for analysis using

cold-vapor atomic fluorescence spectrometry (CVAFS), following U.S. EPA method 1630.<sup>35</sup>

The root surface area of the sampled rice plants was determined based on the adsorption of the cationic dye Methylene Blue ( $\text{C}_{16}\text{H}_{18}\text{N}_3\text{SCl} \cdot 3\text{H}_2\text{O}$ ),<sup>36,37</sup> where root surface area is calculated based on the residual concentration of dye in solution after a period of exposure and the assumption that 1 mg of Methylene Blue can form a monomolecular adsorption layer with area of 1.1  $\text{m}^2$  on the surface of roots. The concentration of Methylene Blue was measured by visible spectrophotometer (722, China) at a wavelength of 660 nm.

**2.4. Quality Control.** Quality control (QC) for THg and MeHg determination was conducted using duplicate analysis, method blanks, matrix spikes, and certified reference materials. Details of the QC procedures adopted are described in the Supporting Information.

**2.5. Data Analysis.** Statistical analysis was made using SPSS for Windows version 16.0. The flux of MeHg uptake by rice plants ( $F_{\text{uptake}}$ , expressed as  $\text{ng MeHg cm}^{-2} \text{root h}^{-1}$ ) was calculated following the work of Degryse et al.<sup>38</sup> and Oporto et al.,<sup>39</sup> as shown in eq 3:

$$F_{\text{uptake}} = M_{\text{pl}} \times \text{RGR}/(\text{RWR} \times \text{SRA}) \quad (3)$$

where  $M_{\text{pl}}$  represents the MeHg concentration in the whole rice plant ( $\text{ng g}^{-1}$ ), RWR is the ratio of root to whole rice plant (g root per g plant), RGR is the relative growth rate ( $\text{d}^{-1}$ ) calculated from the mass difference of shoots in a defined growth period ( $\text{RGR} = (\Delta \ln W)/\Delta t$ ), and SRA is the specific root area ( $\text{cm}^2 \text{g}^{-1}$ ) measured by the methylene blue absorption method.<sup>36,37</sup>

The flux of MeHg measured by the DGT technique ( $F_{\text{DGT}}$ , expressed as  $\text{ng MeHg cm}^{-2} \text{DGT h}^{-1}$ ) was calculated according to the method of Zhang et al.,<sup>40</sup> as shown in eq 4:

$$F_{\text{DGT}} = M/(At) \quad (4)$$

where  $M$  is the mass of MeHg accumulated by DGT (ng),  $A$  is the surface area of the DGT unit ( $\text{cm}^2$ ), and  $t$  is the deployment time (h).

### 3. RESULTS AND DISCUSSION

**3.1. THg and MeHg in Soil.** The total mercury concentration (THg) in soil was elevated at the two contaminated sites relative to the control site (Figure S1, Supporting Information). The THg concentration in soil at all sites was consistent throughout the rice growing season, showing no significant variation between sampling times. The average MeHg concentration at sites AZ and GX was  $3.33 \pm 1.42$  and  $3.10 \pm 0.89 \mu\text{g kg}^{-1}$ , respectively, values that were significantly higher than the concentration for the control site ( $0.77 \pm 0.48 \mu\text{g kg}^{-1}$ ) (Figure 1).

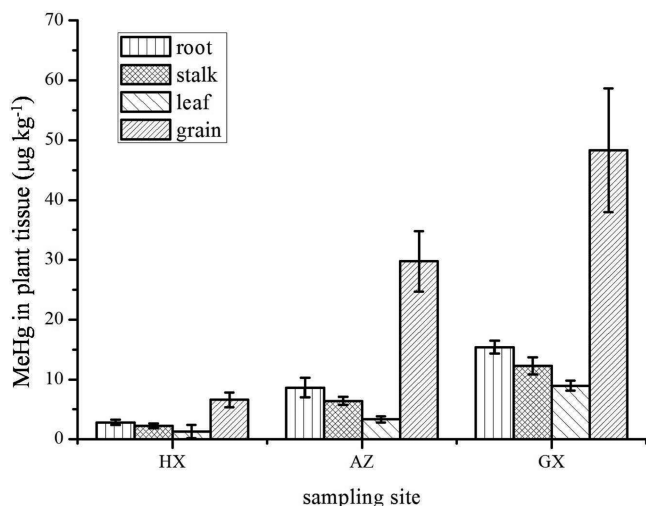
In contrast to THg, the soil MeHg concentration was not consistent throughout the rice growing season. This was more apparent for sites AZ and GX, with a higher level of soil Hg. This variation may be due to a number of factors. Increasing temperature and changing redox parameters over the rice season may have affected the process of Hg methylation. Specifically, the flooding state of each paddy may have been an important factor influencing MeHg production. Flooded conditions enhance anaerobic microbial activities and increase MeHg yields.<sup>41</sup> During a rice growing season, drying the paddy field is an important treatment for controlling the process of rice plant tillering and for increasing yield. In the current study,



drying occurred between July 15th and August 1st at AZ, and this likely contributed to a decreasing trend in soil MeHg concentration between these dates. Subsequent flooding after August 1st may have led to an increase in methylation and an increase in the soil MeHg concentration. At site GX, paddy field drying occurred between June 15th and July 15th, with subsequent flooding between July 15th and August 15th and drying again after 15th August.

### 3.2. Accumulation of MeHg in Rice Plant Tissues.

MeHg was accumulated by the roots, stalk, and leaf of rice plants at each sampling time and at all three locations (Figure 1). The MeHg concentration in the harvested plant tissues decreased in the order grain > root > stalk > leaf (Figure 2).



**Figure 2.** Distribution of MeHg in rice plant tissues for samples collected at rice harvest.

The apparent distribution of MeHg through rice plants was in agreement with the findings of Meng et al.<sup>11</sup> The MeHg concentrations in rice grain were 17, 8.3, and 16 times higher than those in the corresponding soil at locations HX, AZ, and GX, respectively, showing that rice grain has a strong ability to accumulate MeHg from the soil.<sup>7</sup> Significant and positive correlations between the MeHg concentration in the soil and that in root, stalk and leaf tissues were observed for samples collected throughout the rice growing season (Figure S2, Supporting Information), suggesting that the MeHg concentration in soil may play an important role in limiting MeHg accumulation in rice grain.<sup>12</sup>

### 3.3. Profiles of MeHg in Pore Water during the Rice Growing Season.

The MeHg concentration in pore water was monitored using DGT sediment probes over five successive time intervals from the day of planting (Figure 3). The mean DGT-measured MeHg concentration in each profile across the three sampling locations is correlated with the MeHg concentration in the soil ( $p < 0.001$ ), and that in plant root ( $p_1 < 0.01$ ), stalk ( $p_2 < 0.01$ ), and leaf ( $p_3 < 0.01$ ) (Figure S3, Supporting Information). The MeHg concentration in pore water at locations AZ and GX was much higher than at HX, which is consistent with the total MeHg concentration in the soils. Figure 3 shows variation in the pore water MeHg concentration with depth for each sampling location at each sampling time. In every vertical profile, the maximum MeHg concentration was at a depth of approximately 2 cm below the surface (defined for a rice paddy soil as the exposed water

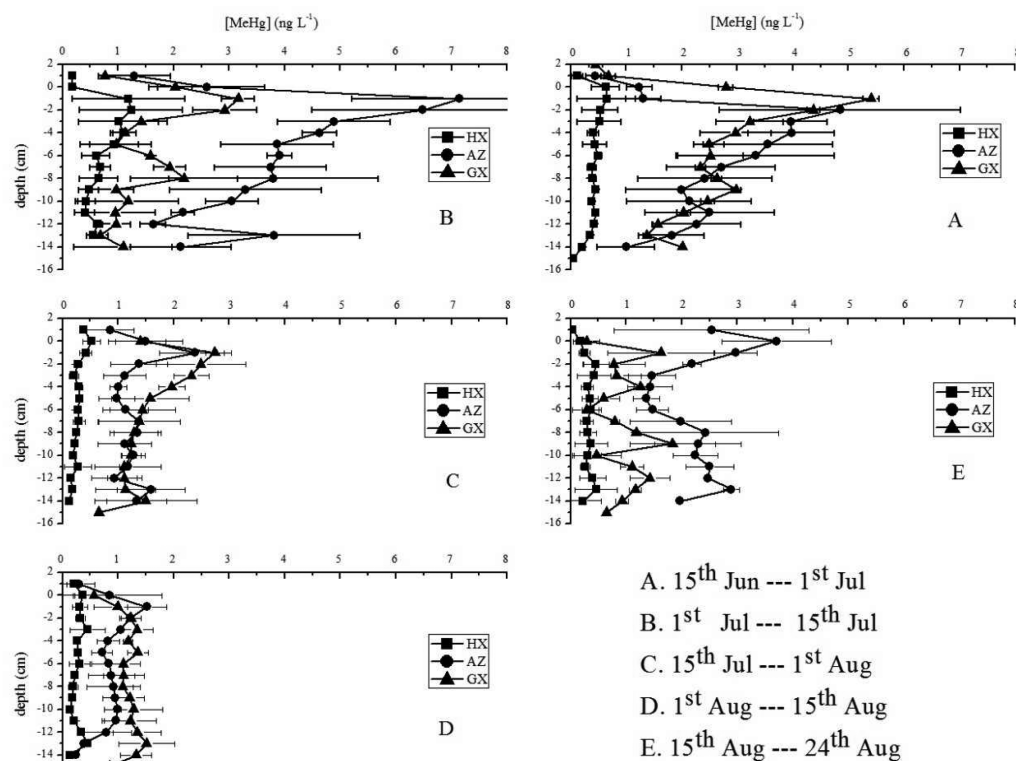
surface under flooded conditions). The 2 cm depth corresponds to the water–soil interface and is a zone of organic matter enrichment<sup>42</sup> that would represent an energy source for methylating bacteria and could stimulate microbial activity and thus the methylation of mercury.<sup>43,44</sup> Throughout the rice growing season, the pattern of temporal and spatial variation of MeHg concentration in pore water was similar at all three sites. The vertical heterogeneity in DGT-measured MeHg concentration indicates that uptake of potentially labile MeHg may vary with depth.

### 3.4. Relationship between DGT-Measured MeHg Flux and the Uptake Flux of MeHg by Rice Roots.

To better understand the correlation between the DGT-measured MeHg concentration in soil and the MeHg concentration in rice, the average concentration of MeHg measured by DGT for each profile at each sampling time was calculated. A significant and strong positive correlation exists between the DGT-measured MeHg concentration in the pore water and the MeHg concentration in rice grain ( $R = 0.768$ ,  $p < 0.05$ ) (Figure 4a). This implies that DGT-measured MeHg is bioavailable to rice plants. However, to investigate the potential of the DGT technique to provide a mechanistic information on the process of MeHg accumulation by plants, flux parameters were derived to model MeHg bioavailability in the soil ( $F_{DGT}$ ) and MeHg uptake by the rice plants ( $F_{uptake}$ ). These two flux parameters have units of  $\text{ng cm}^{-2} \text{h}^{-1}$ . A significant and positive correlation was observed between DGT-measured MeHg flux in soil and that derived for rice roots ( $R = 0.853$ ,  $p < 0.01$ , Figure 4b). The correlation between these two flux parameters implies that DGT can predict the bioavailability of MeHg in rice paddy soil, and that the DGT method can provide a quantitative description of the rate of uptake of this bioavailable MeHg.

In this study, DGT was deployed in the rice paddy soil for about two weeks at each sampling time. When used over this length of time, there can be considerable depletion of the pore water concentration near the DGT device.<sup>31</sup> Exchangeable metal will subsequently desorb from the solid phase to resupply the soil solution.<sup>45</sup> In this scenario, the DGT-measured MeHg flux will estimate not only free ion activity alone, but will provide quantitative data on the extent to which MeHg is complexed with ligands in soil solution. Many studies have demonstrated that metal complexes may contribute to metal uptake by plants.<sup>15,38,46,47</sup> Methyl mercury that crosses the root membrane has been shown to be readily transported to the above-ground parts of rice plants.<sup>12</sup> Therefore, as a result of uptake, the MeHg concentration near root tissues will decrease, and depletion of the free ion concentration near the root surface may induce the subsequent dissociation of MeHg complexes that may exist in soil solution.<sup>48</sup> The DGT technique can therefore be considered to mimic the physical process of rice plant uptake of MeHg from soil.

However, the linear regression in Figure 4b shows that the MeHg root uptake flux from soil was much lower than that measured by DGT. On average, the DGT-measured MeHg uptake flux was 6875 times higher than the corresponding uptake flux by the plant. This indicates that the root surface of rice plants functions differently than DGT with respect to accumulation of MeHg. Several reasons can be proposed to account for this differential function. (1) The DGT technique used in this study is based on Fick's law and is limited by the diffusion of MeHg in soil solution. When a DGT unit is deployed in the paddy environment, labile species of MeHg in soil can freely diffuse through the diffusive layer of the probe to



**Figure 3.** Spatial profiles of labile MeHg measured by DGT in pore water from three sites (HX, AZ, GX) at different sampling dates during the rice growing season.

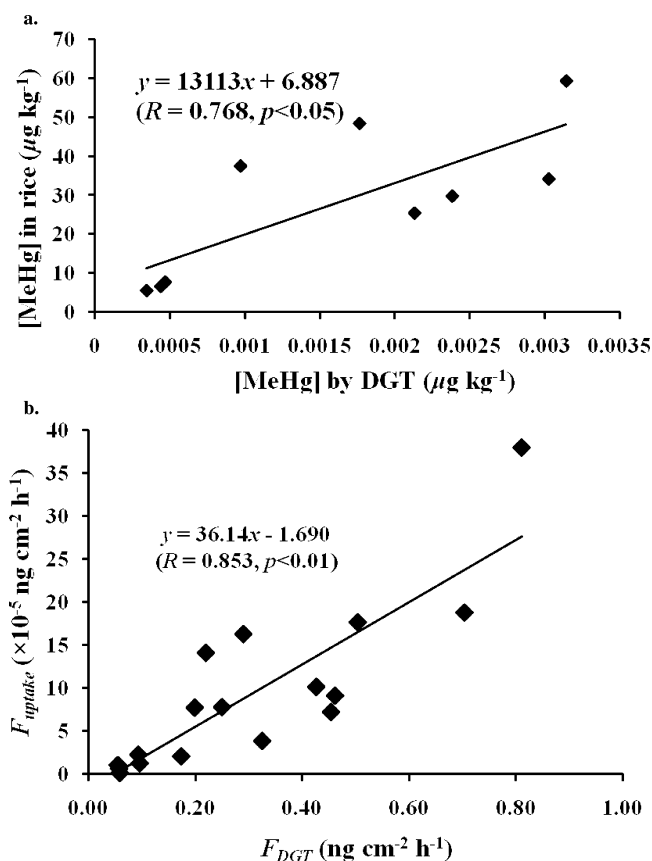
become strongly bound with the component resin gel (3-mercaptophyll-functionized silica resin), which has a strong affinity for MeHg.<sup>29</sup> However, the first step of the plant uptake process, which is the penetration of the root membranes, is likely a markedly different process that might be regulated by biota factors,<sup>49</sup> resulting in much slower uptake. (2) The DGT unit was a simple device that has uniform pore size in the diffusive layer. However, the root system is complex, and its volume and surface area decreases with soil depth.<sup>50</sup> Furthermore, the root system can secrete compounds such as organic acids, enzymes, and alkaloids,<sup>51,52</sup> which may affect rhizosphere soil microorganism communities and subsequently MeHg concentrations near the root system. (3) Plant root systems may produce phytochelatins (PCs) upon metal exposure to detoxify physiologically harmful agents. In the rice plant root system, some MeHg ions may form MeHg-PC complexes, which may not be transported to shoots and instead be sequestered upon the root surface.<sup>53</sup> As a consequence of this sequestration, the diffusion gradient between the soil and the root will be weakened and diffusion will be relatively slowed. (4) Only free ion and small MeHg complexes can be transported from roots to shoots. However, the DGT-flux is also affected by the presence of labile complexes and the extent of solid-phase buffering, as 3-mercaptophyll-functionized silica resin acts as a zero sink even at elevated MeHg concentrations unless the resin approaches saturation.

Although the root surfaces of rice plants function differently than DGT with respect to accumulation of MeHg, the correlation coefficient between DGT-measured flux and the root uptake flux is above 0.8. In theory, if uptake by roots is rapid enough to locally deplete the MeHg concentration in pore water with limited resupply via mass flow and/or diffusion, then plant uptake is limited by diffusion. Plant uptake in this

scenario will correlate well with DGT-measured flux, as DGT effectively measures a diffusion-limited flux.<sup>45</sup> The strong correlation may suggest that diffusive transport of MeHg to the rice plant roots is the rate-determining process for MeHg uptake, a hypothesis that is consistent with literature.<sup>45</sup> However, the MeHg flux for roots was much lower than the DGT-measured flux, which may indicate that MeHg is not diffusion limited. It is therefore difficult to conclusively describe the kinetics of MeHg uptake by rice plants using the data from the current study. A future modeling study, which incorporates desorption of MeHg, MeHg transport in soil, and MeHg flux to rice plants, is needed to assess the real rate-limiting step of MeHg uptake.

Using the data from our study, we can propose reasonable explanations for the variation between the magnitude of the bioavailable and uptake MeHg fluxes. However, biotic factors affecting MeHg uptake by plants are not explained by DGT. The DGT technique should be considered useful in the prediction of the bioavailability of MeHg in soil, which is related to abiotic factors only.

**3.5. Application of DGT-measured MeHg Flux to Ecotoxicological Risk and Food Safety.** From the perspective of food safety, the translocation of MeHg from leaf and stalk tissues to developing rice grain is a physiological mechanism of concern. There are no guidelines for MeHg in rice, as the presence of any MeHg in a food crop is an undesirable situation. However, neither is there a described relationship between the MeHg concentration in soil and that in rice. Therefore, risk assessment is a difficult task. The DGT-measured MeHg flux is shown in this work to describe uptake flux. Could the technique provide a similar description of the risk of MeHg contamination in rice grain? We propose that assuming the root uptake flux is constant over time, the



**Figure 4.** (a) Correlation between the MeHg concentration in soil measured by DGT and the MeHg concentration in rice. (b) Correlation between the measured uptake flux of MeHg for rice plants and that estimated by the DGT-measured MeHg concentration in soil solution. Correlations are made using combined data points for both polluted sites and the control site.

concentration of MeHg in rice can be related to root uptake flux ( $F_{\text{uptake}}$ ), according to eq 5:

$$[\text{MeHg}]_{\text{rice}} = (F_{\text{uptake}} \times \text{RWR} \times \text{SRA} \times t)k \quad (5)$$

where  $[\text{MeHg}]_{\text{rice}}$  represents the total MeHg concentration in the rice of a single plant ( $\text{ng g}^{-1}$ ), RWR is the ratio of root to whole plant biomass ( $\text{g root/g plant}$ ), SRA is the specific root area ( $\text{cm}^2 \text{g}^{-1}$ ),  $t$  is a set growing period (h), and  $k$  is the % of MeHg in the plant that is stored in rice. The parameter  $k$  has been calculated previously,<sup>12</sup> to be 81.56%.

Based on the correlation between DGT flux and root uptake flux (Figure 3b), the parameter  $F_{\text{uptake}}$  can be expressed as a function of  $F_{\text{DGT}}$ , generating eq 6:

$$[\text{MeHg}]_{\text{rice}} = [(36.14F_{\text{DGT}} - 1.690) \times 10^{-5}] \times \text{RWR} \times \text{SRA} \times t \times k \quad (6)$$

Equation 6, therefore, quantifies the MeHg content of the grain of a rice plant as a function of the DGT-measured flux of MeHg in soil. This relationship may be useful for the prediction of ecotoxicological risk that might be apparent from MeHg contaminated soil where paddy rice is the dominant land use.

Further research is needed to experimentally test this relationship and to establish critical levels of MeHg in soil based on the DGT-measured bioavailable concentration. However, the reported use of DGT-measured mercury flux to predict uptake flux throughout a rice growing season could see

the field deployment of DGT probes prior to rice seed formation. Analysis of these probes could allow for assessment of the risk for food contamination that might become apparent if the crop is allowed to reach maturity. This would be a relatively inexpensive monitoring tool that could result in the action of crop removal and destruction before the contaminated food crop became ready for harvest. This would effectively mitigate the risk of MeHg-contaminated rice entering the food chain in areas where rice is known to be the main pathway for human MeHg exposure.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Description of the quality control; three additional figures; and one table. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### 📝 Notes

The authors declare no competing financial interest.

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