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Investigation of Hg uptake and transport between paddy soil and rice seeds combining Hg isotopic composition and speciation

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Abstract

Human consumption of rice constitutes a potential toxicological risk in mercury (Hg) polluted areas such as Hg mining regions in China. It is recognized to be an important source of Hg for the local human diet considering the efficient bioaccumulation of methylmercury (MeHg) in rice seed. To assess Hg sources and uptake pathways to the rice plants, Hg speciation and isotopic composition were investigated in rice seeds and their corresponding paddy soils from different locations within the Wanshan Hg mining area (Guizhou Province, China). A large variation of Hg speciation is observed in rice seeds and paddy soils irrespective of the sampling location. Mass dependent fractionation (MDF) of Hg in rice seeds differs by up to ~4.0 ‰ in δ^{202}Hg values, while mass independent fractionation (MIF) of Hg isotopes remains constant (Δ^{199}Hg ~ 0‰). Hg isotopic composition in rice seeds covaries with that of paddy soils but exhibits lighter isotopic signature (δ^{202}Hg). Such isotopic offset is mainly attributed to plant uptake and translocation processes. Also, seeds containing higher MeHg (MeHg/total Hg > 50%) have significantly heavier Hg isotopes suggesting that MeHg uptake and transport to the seed in such rice plants is facilitated compared to inorganic Hg.

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Introduction

Mercury (Hg) is a pollutant of major concern that can be bioaccumulated and biomagnified in the trophic web, resulting in potential negative effects on human health (Driscoll et al., 2013; Mergler et al., 2007; WHO, 1990). Rice, the dominant global crop, is recognized to be an important source of Hg in human diet considering the soil uptake and transport to the edible part (Feng and Qiu, 2008; Horvat et al., 2003). It constitutes a potential risk in Hg polluted areas, such Hg-contaminated mining regions, where the most important methylmercury (MeHg) exposure source is not fish, but rice consumption (Feng et al., 2008). This is the case of Hg mining areas in Guizhou province (China). Considering that Hg concentration in rice from this region can reach values up to 500 ng g^{-1} (Horvat et al., 2003) and the daily consumption of this staple cereal, the dietary intake has been estimated around 400 g. MeHg intake through rice ingestion has been reflected on the levels of MeHg in hair of inhabitants of such areas (Horvat et al., 2003; Li et al., 2015).

Chinese Hg geological reserve is ranked third in the world, being Guizhou province the largest Hg producer. Wanshan district, located in the mentioned province, is worldwide recognized by its important Hg mining activity. Despite the large-scale production officially finished in 2001, small-scale and artisanal production still takes place in the area (Feng et al., 2008; Zhang et al., 2010). The impact of Hg in the surrounding ecosystem, due to the historic (and illegal) Hg mining activity has been largely described (Feng et al., 2008; Horvat et al., 2003). Drinking water and food products of agricultural origin have been identified as considerable Hg dietary sources (Feng et al., 2008).

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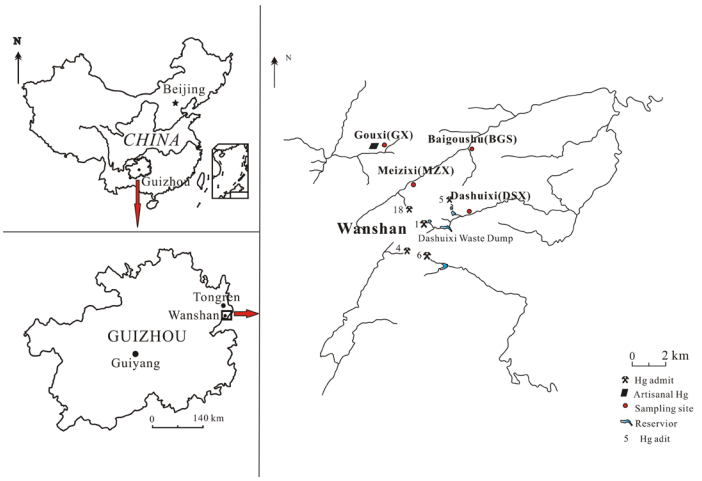


Figure 1
Study area.

High Hg concentrated rice seeds and their corresponding paddy soil samples were collected from Wanshan Hg mining areas, Guizhou, China. Sampling was specifically carried out at: Dashuixi (DSX), Meizixi (MZX), Baigoushu (BGS) and Gouxi (GX).

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Less attention has been paid to the fate and metabolism of Hg in crop plants compared to fish and other aquatic organisms. The mechanisms of uptake, distribution and transport of Hg by rice plants are not fully elucidated. Rice (*Oryza sativa* L.) has the particularity to accumulate between 10 and 100 times more MeHg than other crops such as corn, cabbage and tobacco, exposed to identical Hg exposure conditions (Qiu et al., 2008), principally in the rice seeds (Horvat et al., 2003; Li et al., 2010, 2013; Meng et al., 2011, 2014; Qiu et al., 2008; Zhang et al., 2010). It has been recently demonstrated that the addition of rice residues to the (contaminated) paddy soil significantly affect the biogeochemistry of Hg species, resulting in an increased production of MeHg (Zhu et al., 2015).

Hg isotopic signature, a powerful tool for the investigation of biogeochemical processes and the potential identification of pollution sources (Feng et al., 2010; Perrot et al., 2010; Sonke, 2011), can also be used to investigate biological processes (Feng et al., 2015; Kritee et al., 2008; Kwon et al., 2012; Perrot et al., 2015; Rodŕiguez-Gonźalez et al., 2009). Hg contains seven stable isotopes (Hg^{196}, Hg^{198}, Hg^{199}, Hg^{200}, Hg^{202}, Hg^{201} and Hg^{204}) that can undergo mass dependent fractionation (MDF) due to specific reactions like methylation/demethylation, reduction as well as transport and metabolic processes (Bergquist and Blum, 2007; Kritee et al., 2008; Yin et al., 2010, 2013b). Hg can additionally exhibit mass independent fractionation (MIF) (Bergquist and Blum, 2007). Although the mechanism is not fully elucidated, its origin is mainly associated to photochemical reactions (Bergquist and Blum, 2007). So far, this powerful tool has been applied to rice in exclusively one study providing unambiguous information about both soil and atmospheric Hg uptake by the plant (Yin et al., 2013a).

In this work, Hg isotopic composition is combined to Hg speciation in order to constrain Hg biogeochemical pathways between paddy soils and rice seeds. To achieve this goal, 14 rice seeds samples and their corresponding paddy soils were selected from different locations in Whanshan Hg-mining area (Guizhou Province, China). Hg isotopic composition is analysed and different speciation approaches are used for both seeds and soils.

Experimental

Study area

High Hg concentrated rice seeds and their corresponding paddy soil samples were selected from Wanshan Hg-mining areas. Samples come from different origins (Figure 1) and are impacted by diverse sources. In brief, the Dashuixi (DSX), Meizixi (MZX) and Baigoushu (BGS) watersheds are mainly impacted by the calcine tailing from its headwater large-scale Hg mining activities, where the sampling sites are just around 100 m nearby Hg mine adits (Zhang et al., 2010). The Gouxi (GX) watershed was impacted by the distillation practices of small scale illegal artisanal Hg retorts along the stream, representing an atmospheric Hg source (Qiu et al., 2008). More details were described elsewhere (Feng et al., 2008; Li et al., 2008a; Qiu et al., 2005, 2008; Zhang et al., 2014). The sampling period and locations are summarized in Table S1. All the rice seeds samples correspond to white rice, except samples originally from GX, where the hull was not completely removed.

Hg speciation and stable isotopic analyses

Hg species (iHg and MeHg) quantification in rice seeds and paddy soils was carried out using species specific isotope dilution by adding the adequate amount of Hg isotopically enriched species (^{199}iHg and ^{201}MeHg)

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(Monperrus et al., 2008). Isotope pattern deconvolution was used for data treatment (Clémens et al., 2011). A GC-IPC-MS (Thermo Electron GC, model Trace Ultra, coupled to a Thermo Electron ICP-MS X series XII) was employed for such speciation analyses. Validation of our results was carried out by comparison of the certified and experimentally obtained values of several certified reference materials (CRM) (Table S2). CRM digestion was carried out with TMAH (tetramethylammonium hydroxide) or HNO_3 for biota and sediments, respectively, in an analytical microwave (Discover SP-D, CEM) as detailed elsewhere (Clémens et al., 2011; Monperrus et al., 2008; Rodríguez Martin-Doimeadios et al., 2003).

Regarding Hg isotopic composition, it was determined by cold-vapor MC-ICP-MS (Nu Plasma, Nu Instruments) as detailed elsewhere (Perrot et al., 2012). Analytical uncertainty was determined as the largest 2SD corresponding to the sample or to the measurement of the UM-Almadén secondary standard during each session.

Basically, the concentrations of samples and bracketing standards (NIST 3133) were matched within maximum 10% difference, also demonstrating a quantitative Hg recovery and minimized interferences of the cold vapor generation for all samples analyzed in this work.

In Table S3, the Hg isotopic results for several reference materials and secondary standards (UM Almaden: δ^{202}Hg= -0.54±0.12, Δ^{199}Hg= 0.01±0.12, n=34; sediments (IAEA 405): δ^{202}Hg= -0.44±0.03, Δ^{199}Hg= -0.04±0.13, n=3; fish muscle (BCR-464): δ^{202}Hg= 0.69±0.16, Δ^{199}Hg= 2.35±0.15, n=6) corresponding to this work and previous publications is included. It demonstrates a good precision and accuracy of our measurements compared to other groups using different Hg extraction techniques (Sherman and Blum, 2013) and CVG-MC-ICP-MS systems (Sonke et al., 2010).

The sample treatment was optimized according to the matrix, as described below.

Rice seeds

Sample preparation was optimized for both Hg speciation and Hg isotopic composition analyses. The optimized condition for Hg species (iHg and MeHg) extraction from rice seeds consist on the digestion of 0.1g of rice sample and 5 mL TMAH. The microwave (Discover SP-D, CEM) digestion (Clémens et al., 2011; Monperrus et al., 2008) step was repeated three times after manual shaking between them due to the jelly texture of the mixture. The quantitative Hg species extraction was validated by the good agreement found between the sum of the Hg species and the total Hg (THg) concentration in the rice seeds samples (Table S4). THg content in rice seeds sample was determined both in the acid extract (HNO_3 and H_2SO_4 (4:1, v: v)) by cold vapour atomic fluorescence spectrometry (CVAFS, Tekran 2500, Tekran Instruments) and by using thermal decomposition and Zeeman Atomic Absorption Spectrometry (Lumex, Russia). Good Hg recoveries (85%–115% for CRM GBW10020-orange foliage) and comparability were obtained with these two methods.

Regarding sample preparation for Hg isotopic composition determination, the best results were obtained by heating 0.2–1 g of sample with 5mL HNO_3:H_2SO_4 (4:1) in a hot block (Environmental Express) at 90°C for 3 hours (Hg recovery >90%).

Paddy soil

THg concentration in paddy soil was determined by using an advance mercury analyzer (AMA-254 LECO). Meanwhile, 0.1g soil was digested with 5mL 6 mol L^{-1} HNO_3 in a Microwave (Discover SP-D, CEM) as detailed elsewhere (Monperrus et al., 2008; Rodríguez Martin-Doimeadios et al., 2003) for the total quantification of MeHg in soil, as well as the acid extractable fraction (AEF) of iHg in the bulk soil. This relatively mild acid extraction of iHg can also be used to check for Hg chemical availability from Hg mining contaminated areas (Rodríguez Martin-Doimeadios et al., 2000). In opposition a non-acid extractable fraction (NAEF) was defined by subtracting Hg-AEF concentration to THg content.

The Hg water soluble fraction (WSF) was extracted by following the protocol described by (Yin et al., 2013b) which was adapted with regard to the soil: water ratio and the extraction time (Reis et al., 2014). In brief, 5g soil and 15 mL pure water (Mill-Q water, 18.2 MΩ cm) were mixed in centrifuge tubes and rotated in a shaker over 24 hours. The mixture was centrifuged (3500 rpm) during 20 min and the supernatant was filtrated by using 0.45µm micro-filter (PVDF, Milli pore). An adequate amount of ultra pure HCl was added to the extracted fraction in order to reach 1% HCl (v v^{-1}).

For Hg isotopic composition determination, 0.2-1 g soil sample was digested by 4mL HNO_3: HCl (3:1) in hot block at 90°C heating for 3 hours (Hg recovery >90%). The obtained extracts were stored at -20°C before analysis.

Results and discussion

Hg concentration and species distribution in rice seeds

THg concentrations in rice seeds samples (Table S4) vary in a wide range between 87 and 579 ng Hg g^{-1}. This variation is, in general, irrespective to the sampling site, except for samples from DSX that exhibits a similar Hg level. The concentration found in the rice seed samples is in good agreement with values previously

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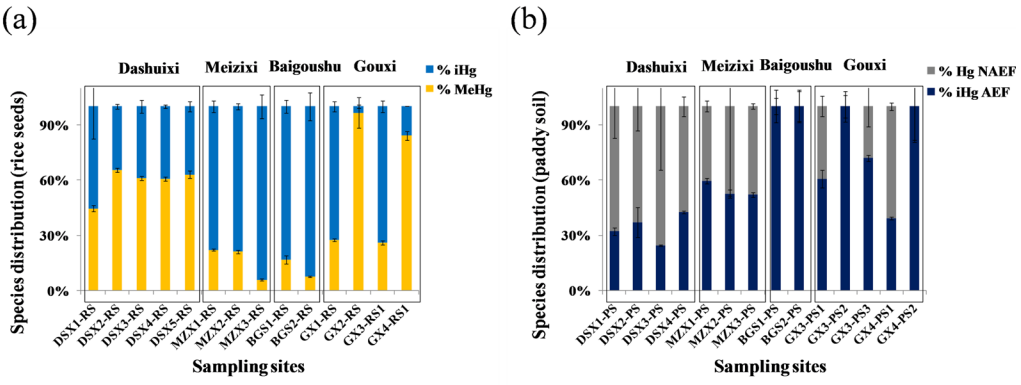


Figure 2

Hg species distribution in a) rice seeds and b) paddy soil samples.

Rice seeds (RS) and paddy soil (PS) samples were collected from different locations of Wanshan Hg mining areas: Dashuixi (DSX), Meizixi (MZX), Baigoushu (BGS) and Gouxi (GX). For paddy soil samples, Hg concentration in non-acid extractable fraction (Hg_{NAEF}) is defined as THg concentration (determined by AMA) subtracts iHg concentration of acid (6 mol L^{-1} HNO_3) extractable fraction (iHg_{AEF}).

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reported in rice seeds from Hg mining areas of China (Horvat et al., 2003; Meng et al., 2014). According to the classification of "seriously contaminated" (iHg: 7-464 ng g^{-1} and MeHg: 1-43 ng g^{-1}) and "less impacted" (iHg: 6-167 ng g^{-1} and MeHg: 1–24 ng g^{-1}) areas giving by Zhang et al., (2010), the samples of our study should be included in the first group. The obtained values exceed the Chinese National Standard Agency permissible limit (20 ng g^{-1}) (CNSA, 1994), indicating the impact of mining and smelting activity in the study area.

A large Hg species distribution (Figure 2a, Table S4) variation is also observed in rice seeds from this Chinese region, as previously reported in the literature (Horvat et al., 2003; Qiu et al., 2008; Rothenberg et al., 2014; Zhang et al., 2010). The percentage of MeHg (%MeHg) fluctuates between 6 and 97% (Figure 2a, Table S4) and it is not related to the THg concentration in rice seeds ($p>0.05$). The lack or poor correlation between THg and MeHg (Qiu et al., 2008) in rice seeds is understood as a reflex of the differences on the uptake mechanism for both MeHg and iHg, as discussed below. Significant variations on Hg levels and its species distribution have been reported among different rice cultivars (Li et al., 2013).

A screening of the biomolecules binding Hg (see Supplementary material Text S1) in the water soluble fraction of selected rice seeds samples was performed by HPLC-ICP-MS by using a size exclusion chromatographic column (Figure S1). Hg chromatographic profile varies regardless of the sampling site and the ratio between iHg and MeHg in the samples. It confirms the complexity of the mechanisms of Hg species uptake, translocation and accumulation in rice seeds, in total agreement with the isotopic and speciation data discussed below.

Regarding the identification of Hg-protein/biomolecules in plants, the literature is reduced to the recent studies (Krupp et al., 2008, 2009). In the mentioned work, novel Hg peptide complexes-phytochelatins have been identified in roots of rice plants, where their principal role is the detoxification of Hg through its in vivo sequestration. However, despite the large number of research work focused on iHg and MeHg quantification in rice seeds, the current study perform for the first time a screening of the Hg binding biomolecules in such samples.

Hg concentration and species distribution in paddy soil

THg concentration in paddy soils (THg_T) (Table S5) corresponding to the regions where rice seeds samples were collected varies between 1 and 160 μg g^{-1}. The THg_T content, in general higher than the domestic environmental quality standard for agricultural soil (1.5 μg g^{-1}) (CNEPA, 1995), diverges irrespectively to the sampling site and is in good agreement with values previously reported in this region (Horvat et al., 2003; Qiu et al., 2005; Zhang et al., 2010).

Although the sum of both species (iHg and MeHg) of the analyzed CRM matches the THg concentration value (Table S2), however, as shown in Table S5, Figure 2b, in the paddy soil from the studied area the acid extractable fraction Hg (Hg_{AEF}) provide much lower concentration than for THg_T. The difference between the sum of Hg species in the AEF and the THg content in the soil can be attributed to a partial extraction of iHg. The investigation of the chemical availability of Hg by sequential extraction in sediments from a cinnabar mining area (Almaden, Spain) reveals that Hg is principally associated to sulfides in the form of cinnabar or altered minerals (Rodríguez Martin-Doimeadios et al., 2000), limiting its leaching by a mild acid extraction procedure. Considering the large extension of cinnabar in Wanshan, the study area (Horvat et al., 2003), the non-extractable Hg fraction should also be associated to sulfides. It should be noticed that differences between THg content in (bulk) paddy soil and AEF are observed in all the analyzed samples (Figure 2b, Table S5), except in the group with Hg content lower than 2 μg g^{-1}. It could be attributed to the association of Hg to a less reactive fraction in cinnabar or calcines but further studies are required to better characterize Hg-complex in the soil samples leading to a partial extraction.

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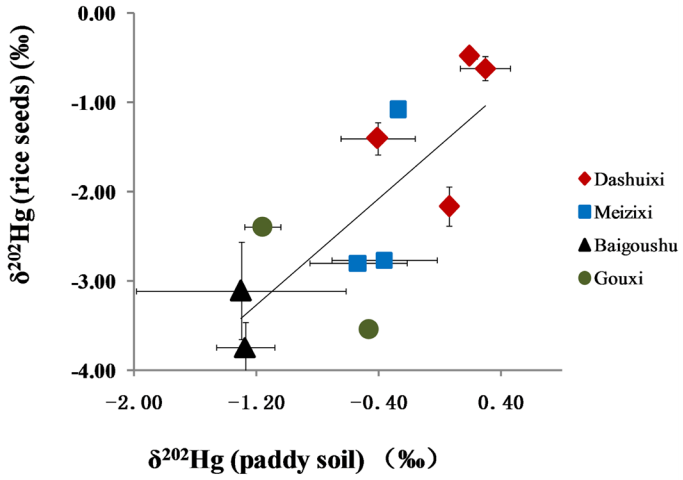


Figure 3

Comparison of Hg isotopic compositions between rice seeds and paddy soil samples.

δ^{202}Hg isotopic compositions in rice seeds and paddy soil show a covariation.

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The acid digestion of the paddy soil under the conditions described in the experimental section allows the quantitative extraction of MeHg in the soil (bulk) ($MeHg_T$) and the iHg associated to the mineral fraction. Regarding $MeHg_T$, the values are in general lower than 0.6% of the THg_T.

Several approaches have been used to determine the Hg bioavailable fraction from soil, based on water soluble fraction (WSF) (Meng et al., 2014), and the diffusive gradient in films technique (Liu et al., 2012) among others. In the current study the first one was adopted and THg content in the WSF (THg_{WSF}) is found generally lower than 0.003% respects to the soil THg_T content (Table S5). The portion of bioavailable Hg from soil previously reported in the literature varies according to the applied technique, but in general the Hg level in such fractions is lower than 0.18% of the THg_T content (Meng et al., 2014; Wang et al., 2014). No correlation is observed between THg level in the soil WSF and the soil bulk ($p > 0.05$), while previous studies found more positive relationships (Meng et al., 2014; Yin et al., 2013b).

There is also no clear correlation observed for Hg (or MeHg) concentration between rice seeds and soil for bulk or bioavailable fractions ($p > 0.05$). This is in good agreement with previous results (Horvat et al., 2003) obtained in the vicinity of a Hg mining area, although a significant positive correlation is observed in other studies (Meng et al., 2014; Zhang et al., 2010). It evidences the complexity of the Hg accumulation process in rice seeds and the influence of several environmental factors. Among the variables that affect such mechanisms there is the rice cultivar (Horvat et al., 2003; Li et al., 2013) and the contribution from atmospheric deposition (Horvat et al., 2003; Meng et al., 2010, 2012; Yin et al., 2013a). The latter one principally influenced the top soil (0–10 cm) of rice paddies (Li et al., 2008b), which is evidenced by one order of magnitude decrease of Hg concentration with in 1km distance from the artisanal Hg mining sites (Meng et al., 2011).

Hg isotopic signature in paddy soils and rice seeds

Hg MDF (δ^{202}Hg) in paddy soil varies between -1.31 and 0.30 ‰ (Table S6, Figure S3a), which partially match with the ones already reported in Wanshan Hg mining area (Yin et al., 2013a, 2013b). In the current study, the mean value (-0.59 ± 0.55 ‰, n=14) overlaps mean values from previous works (-0.34 ‰ and -0.53 ‰ in DSX and GX (Yin et al., 2013a), -0.02± 0.16 ‰ (n=8) (Yin et al., 2013b), respectively). It should be considered that in both studies the number of soil samples is limited in comparison with the vast extension of Wanshan area. Hg MDF in rice seeds (δ^{202}Hg) exhibit a negative signature that varies significantly between -3.74 and -0.48 ‰ (Figure S3a, Table S7). The previously reported δ^{202}Hg values of rice seeds (-2.24 and -2.51 ‰ in DSX and GX, respectively) (Yin et al., 2013a) are within the range determined in our study. Comparing both set of data, MDF values (δ^{202}Hg) of rice seeds and paddy soils show a covariation (Figure 3).

MIF signature in top soils (Δ^{199}Hg) averages -0.03± 0.06 ‰ (n=14), and excellently coincides with the values from previous studies in this Chinese region (Yin et al., 2013a, 2013b). Concerning Hg MIF (Δ^{199}Hg) in rice seeds, it remains constant regardless of the sampling site and the Hg species distribution, exhibiting a mean value of -0.01± 0.05 ‰, (n=14). Similar values were previously reported in rice seeds from the same mining area (Yin et al., 2013a). MIF signature deconvolution in seeds carried out by Yin et al., considering atmospheric and soil Hg sources, reveals a contribution of about 20% from atmospheric Hg (Yin et al., 2013a). In general, the isotopic signature (MDF and MIF) does not depend on the sampling sites (Figure S3) in both rice seeds and paddy soil samples.

Despite the large number of studies focused on Hg bioaccumulation in rice, most of them are based on total concentration and speciation analyses. The application of Hg isotopic signature as a complementary

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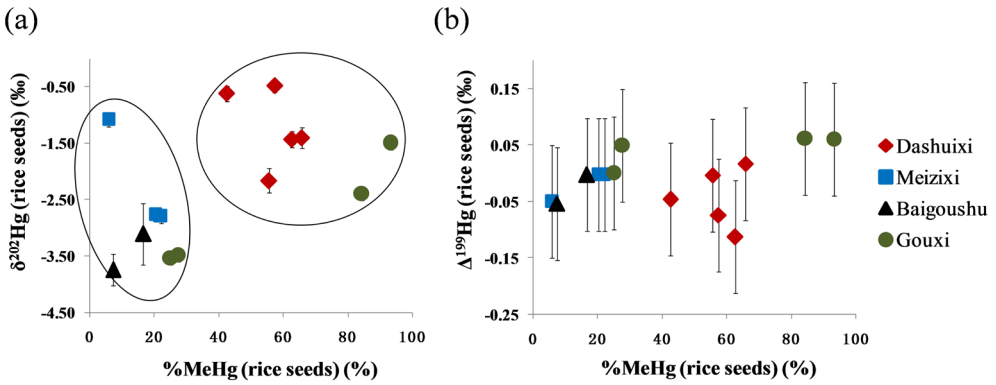


Figure 4

Hg isotopic compositions relative to %MeHg in rice seeds samples.

a) δ^{202}Hg and **b)** Δ^{199}Hg isotopic compositions relative to %MeHg in rice seeds samples. The variation of δ^{202}Hg isotopic composition as function of %MeHg in rice seeds samples shows two groups, which is significantly different ($p<0.01$ for both δ^{202}Hg and %MeHg; t-test, data with normal distribution).

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tool is relatively recent. The following MDF trend is observed in the previous study by (Yin et al., 2013a): roots>seeds>stem>leaves exhibiting a net variation of approximately 1 ‰ (δ^{202}Hg) between roots and seeds. The current study also demonstrates that rice seeds are enriched in lighter isotopes (MDF) in comparison to the paddy soil (Figure S3a). This enrichment of seeds in lighter isotopes can be associated to Hg translocation from roots to above-ground plant tissues as already observed for other elements like Zn and Cu (Jouvin et al., 2012). It should be mentioned, that in addition to such transport inducing MDF, the resulting isotopic pattern can also be influenced by other physiological processes considering the important contribution of Hg atmospheric deposition in leaves and steam (Yin et al., 2013a). The occurrence of several (microbial mediated) processes and agricultural activities leading to Hg species transformation also influences the net Hg MDF signature in soil. Therefore, all the mentioned processes and parameters can contribute to the enrichment of paddy soil in heavier isotopes (MDF) in comparison to rice seeds. Considering the limited available data, the contribution of each MDF source cannot be assessed.

Hg isotopic signature (MDF and MIF) in plant tissues reported in this and previous studies corresponds to the bulk Hg isotopic composition. However, it has been largely demonstrated that Hg metabolization is strongly dependent on its speciation resulting in Hg species specific isotopic signature.

Combination of speciation and Hg isotopic pattern for the investigation of plant uptake and transport

The variation of MDF (δ^{202}Hg) as a function of the percentage of MeHg (Figure 4a) shows two groups of rice seeds significantly different ($p<0.01$ for MDF and %MeHg), while MIF remain invariable (Figure 4b). The observed trend exhibits a specific enrichment in lighter isotopes for seeds containing lower proportion of MeHg (MeHg < 30% of THg) when compared to seeds containing higher proportion of MeHg (> 50%). This result suggests a different MDF extent for MeHg and iHg in the rice seeds probably induced by species specific plant uptake, metabolization and/or translocation.

A contrasting pattern of uptake and plant distribution of Hg species has been largely documented (Horvat et al., 2003; Meng et al., 2010, 2011, 2012; Rothenberg et al., 2011). Soil contribution appears as the main source of iHg in roots (Meng et al., 2012). Meanwhile in stalks and leafs, Hg is principally coming from atmospheric deposition (Meng et al., 2010, 2012), and its translocation through the plant seems to be quite limited.

Soil is definitively considered the main MeHg source in rice plant (Meng et al., 2011) with a negligible contribution of this species from atmospheric Hg sources (Horvat et al., 2003; Rothenberg et al., 2011). Once absorbed by roots, it is transported to leaf and stalk, where it is accumulated in the premature plant. Finally, during the ripening period, MeHg is translocated to the rice seeds (Meng et al., 2011). A similar plant mobility pattern is observed for major nutrients (Ogawa et al., 1979), which appears concentrated in the edible part at harvesting period (Meng et al., 2011).

Microbial Hg methylation is considered the main source of this organomercurial species in paddy soils. The traditional rice culture practice involves several flooding process, which lead to anaerobic conditions facilitating iHg methylation by sulphate reducing bacteria (Meng et al., 2010). An alternative practice to traditional culture alternating wetting and drying implies a reduction of freshwater supply without affecting the rice production. As a consequence of the periodic drainage, anaerobic conditions are reduced, leading to a decrease of methylating microbial activity and MeHg in rice (up to 10 folders) (Rothenberg et al., 2011). The higher MeHg proportion in rice than in other local crops evidences the influence of paddy soil in the formation of MeHg, latter uptake by the rice plant (Qiu et al., 2008).

MeHg produced by microbial activity, is expected to be preferentially found in the soil bioavailable fraction. It is supported by the Hg species distribution in the WSF of the current study, which shows a higher %MeHg in soil WSF than in the soil (bulk) (Table S5). As a product of iHg methylation reaction, the

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MeHg in soil should exhibit a lighter MDF signature in comparison to the residual iHg (Perrot et al., 2015; Rodŕiguez-Gonźalez et al., 2009). However the trend observed in rice seeds revealed that Hg is enriched in heavier isotopes for higher content of MeHg (> 50%). Such isotopic pattern (δ^{202}Hg) could be attributed to the divergent uptake and distribution between iHg and MeHg, since a clear dissimilar bioaccumulation factor is observed for these species (Rothenberg et al., 2011, 2014; Zhang et al., 2010) being 800 fold higher for MeHg than for iHg (Rothenberg et al., 2014; Zhang et al., 2010). However, further studies are required to establish an unambiguous link between Hg processes and the resulting isotopic signature. Hg species are involved in several not-well characterized processes that influence Hg MDF as plant adsorption/desorption, specied transformation before uptake and metabolic processes,

The resulting MeHg in soil bioavailable fraction is easily uptake by the rice plant, being preferentially accumulated in rice seeds in comparison to other tissues (Meng et al., 2010, 2011, 2012; Qiu et al., 2012; Rothenberg et al., 2011; Zhang et al., 2010). In contrast, iHg is principally retained in roots, complexed by phytochelatins (Krupp et al., 2008, 2009) and other potential ligands (Figure S1), which could be a rate-limiting step responsible of larger MDF and lighter Hg isotopes in the seed (δ^{202}Hg). Considering the reduced transport of iHg from soil to seeds, in samples where it is the main species (example from Meizixi and Baigoushu), the contribution of atmospheric Hg cannot be neglected. Further Hg speciation and isotopic studies are absolutely needed to better understand the mechanisms of uptake and accumulation of Hg species in rice plants.

Conclusions

This work evidenced that the combination of Hg speciation and isotopic composition, allows investigating the dissimilarity between Hg species uptake and translocation pathways from paddy soil to rice seeds. Total Hg isotopic signature and offset between soils and seeds closely link the contribution soil Hg and its uptake and transport to the seed. The specific MeHg isotopic signature in seeds, as well as its larger abundance in the potentially soil bioavailable fraction, reflects its facilitated transport from roots to the edible part after the formation of this species in the paddy soil-roots system.

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Contributions

- Contributed to conception and design: CF, ZP, DA, XF
- Contributed to acquisition of data: All
- Contributed to analysis and interpretation of data: CF, PL, MM, ET, SB, ZP, DA
- Drafted and/or revised the article: CF, ZP, DA, XF
- Approved the submitted version for publication: CF, ZP, PL, BD, XF, MM, ET, SB, DA

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Competing interests

The authors have declared that no competing interests exist.

Supplemental material

- **Text S1. Investigation of Hg uptake and transport between paddy soil and rice seeds combining Hg isotopic composition and speciation**
  doi: 10.12952/journal.elementa.000087.s001
- **Table S1. Sampling year and location**
  doi: 10.12952/journal.elementa.000087.s002
- **Table S2. Hg speciation in certified reference materials used for method validation (GC-ICP-MS)**
  doi: 10.12952/journal.elementa.000087.s003
- **Table S3. Hg isotopic composition in reference material samples**
  doi: 10.12952/journal.elementa.000087.s004
- **Table S4. Hg species distribution in rice seeds samples**
  doi: 10.12952/journal.elementa.000087.s005
- **Table S5. Total Hg concentration and speciation in paddy soil samples**
  doi: 10.12952/journal.elementa.000087.s006
- **Table S6. Hg isotopic composition in paddy soil samples**
  doi: 10.12952/journal.elementa.000087.s007
- **Table S7. Hg isotopic composition in rice seeds samples**
  doi: 10.12952/journal.elementa.000087.s008

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Investigation of Hg uptake and transport in rice plant

- **Figure S1. Screening of water soluble Hg binding biomolecules in rice seeds samples**
  Rice seeds (RS) samples were selected from different locations of Wanshan Hg mining areas: Dashuixi (DSX), Meizixi (MZX) and Gouxi (GX). Screening of biomolecules binding ^{202}Hg are performed by HPLC-ICP-MS using a size exclusion chromatographic column. The molecular weight varies from approximately 650 to less than 10 kDa. Principally five main fractions are found when samples are eluted at 12, 21, 24, 27 and 29 minutes. doi: 10.12952/journal.elementa.000087.s009
- **Figure S2. Screening of biomolecules binding different elements in rice seeds samples**
  Rice seeds (RS) samples are selected from different locations of Wanshan Hg mining areas: Dashuixi (DSX), Meizixi (MZX) and Gouxi (GX). Screening of biomolecules binding elements (^{202}Hg, ^{82}Se, ^{112}Cd, ^{65}Cu and ^{64}Zn) are performed by HPLC-ICP-MS using a size exclusion chromatographic column. The molecular weight varies from approximately 650 to less than 10 kDa.
  doi: 10.12952/journal.elementa.000087.s010
- **Figure S3. Hg isotopic compositions in rice seeds and paddy soil samples**
  a) Hg even isotopes mass dependent fractionation; **b)** Hg odd isotopes mass independent fractionation. RS: rice seeds. PS: paddy soil. (DOC)doi: 10.12952/journal.elementa.000087.s011

Data accessibility statement

All data are included within this manuscript.

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