##### Supplemental Material

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

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# Experimental

## Reagents and standards

All solutions were prepared using ultrapure water (18 MΩ cm, Millipore). Optima acids (HNO3 and HCl) from Fisher Scientific (Illkirch, France) were used for the preparation of all the samples, standards and blanks used in stable isotopic analyses. Mercury (Standard reference materials NIST-3133) and thallium standard solutions (Isotopic Standard for Thallium NIST-997) were purchased from NIST (Gaithersburg, MD, USA). Tin chloride dihydrate (SnCl2·2H2O) was purchased from Sigma-Aldrich (Seelze, Germany).

## Hg stable isotopic analysis

Hg isotopic composition was determined by cold-vapor MC-ICP-MS as detailed elsewhere ([Perrot, et al. 2010](#_ENREF_9)) (Nu Plasma, Nu Instruments), following a sample/standard (NIST-SRM-3133 reference material (National Institute of Standards and Technology)) bracketing sequence. Thallium solution (NIST-SRM-997) was introduced for instrumental mass bias correction using the exponential fractionation law. Several reference materials: sediments (IAEA 405), fish muscle (BCR-464), and UM Almadén ([Blum and Bergquist 2007](#_ENREF_1)) were used as secondary standards, values were presented in Table S3. The secondary standards solution is matrix matching the analyzed samples.

MDF is reported by using delta notation (δ202Hg) in per mil (‰) referenced to Hg standard NIST 3133, according to the following equation (Bergquist, et al, 2007):

*δ*xxxHg = [(xxx/198Hgsample/xxx/198HgNIST 3133) - 1)] x 1000 (1)

MIF signatures of 199Hg and 201Hg are calculated as follow:

Δ199Hg = *δ*199Hg - *δ*202Hg x0.252 (2)

Δ201Hg = *δ*201Hg - *δ*202Hg x0.752 (3)

# Results and discussion

## Screening of Hg containing biomolecules in rice seeds water extracts

A screening of Hg (and other elements) binding biomolecules was carried out by HPLC-ICP-MS **(Figure S1, S2)**. Two rice seeds samples of three different sites (DSX3-RS, DSX5-RS; MZX2-RS, MZX3-RS; GX2-RS, GX3-RS) were selected according to their iHg and MeHg distribution. The rice water soluble fraction was extracted by 30 s sonication at 20% max. power (100 W) followed by ultra centrifugation at 100 000 g during 20 min at 4 °C ([Pedrero et al., 2011](#_ENREF_31)). Aliquots of 100 µL of the obtained soluble fraction were directly injected in the size exclusion chromatographic column (Superdex 200) coupled to the ICP-MS (Perkin Elmer 300X).

Noticeable differences are observed between the Hg chromatographic patterns of the analysed samples. In general a wide distribution of Hg containing biomolecules is revealed. The molecular weight varies from approximately 650 to less than 10 kDa. Principally five main fractions are found in several rice seeds samples, eluting at 12, 21, 24, 27 and 29 minutes. However the proportion between the mentioned fractions is variable depending on the sample.

Regarding the identification of Hg-protein/biomolecules in plants, the literature is reduced to the recent studies ([Krupp et al., 2008](#_ENREF_15); [Krupp et al., 2009](#_ENREF_14)). 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.

It should be noticed that the Hg-containing fraction corresponding to the lowest molecular weight, co-elutes with the main peak of Cu and Zn (**Figure S2**). Contrary to Hg, the identification and characterization of such essential elements binding biomolecules is much more advanced. It is known that such (essential) metals are bound to low-molecular weight ligands, involved in mobilization from soil and translocation within the plant ([Haydon and Cobbett, 2007](#_ENREF_10)). Mugineic acid family phytosiderophores (MAs), a low molecular weight metabolite exclusively present in graminaceous plants, is associated to Fe uptake from soil ([Haydon and Cobbett, 2007](#_ENREF_10); [Masuda et al., 2008](#_ENREF_23)). However, it complexes other elements such as Cu, Zn and Ni ([Haydon and Cobbett, 2007](#_ENREF_10); [Masuda et al., 2008](#_ENREF_23)). Organic acids (citrate and malate) have been also associated to the uptake, translocation and compartmentalization of such elements in plants ([Haydon and Cobbett, 2007](#_ENREF_10)).

The co-elution of both, the fraction containing Hg and the principal fraction of essential Cu and Zn, cannot be considered as an evidence of Hg binding MAs or organic acids, further studies are required for its unambiguous identification. For the moment, such co-elution raises a question about the potential transport of Hg species through the metabolic pathways responsible of essential elements translocation within the plant. It should be noticed that the Hg bioaccumulation in shoots of aquatic plant have been linked to Cu transporters ([Regier et al., 2013](#_ENREF_38)).