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Retrospective study of methylmercury and other metal(loid)s in Madagascar unpolished rice (*Oryza sativa* L.)

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A B S T R A C T

The rice ingestion rate in Madagascar is among the highest globally; however studies concerning metal(loid) concentrations in Madagascar rice are lacking. For Madagascar unpolished rice ($n = 51$ landraces), levels of toxic elements (e.g., total mercury, methylmercury, arsenic and cadmium) as well as essential micronutrients (e.g., zinc and selenium) were uniformly low, indicating potentially both positive and negative health effects. Aside from manganese (Wilcoxon rank sum, $p < 0.01$), no significant differences in concentrations for all trace elements were observed between rice with red bran ($n = 20$) and brown bran ($n = 31$) (Wilcoxon rank sum, $p = 0.06–0.91$). Compared to all elements in rice, rubidium (i.e., tracer for phloem transport) was most positively correlated with methylmercury (Pearson's $r = 0.33$, $p < 0.05$) and total mercury ($r = 0.44$, $p < 0.05$), while strontium (i.e., tracer for xylem transport) was least correlated with total mercury and methylmercury ($r < 0.01$ for both), suggesting inorganic mercury and methylmercury were possibly more mobile in phloem compared to xylem.
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1. Introduction

Asian rice (*Oryza sativa* L.) is a staple food in Madagascar, comprising more than 50% of the daily caloric intake (FAO, 2014). Globally, the average rice consumption rate ranks in the 95th percentile (289 g/day/capita), which is exceeded by 9 countries, including Bangladesh, Lao, Cambodia, Vietnam, Myanmar, Thailand, Indonesia, the Philippines, and Guinea (range: 290–475 g/day/capita, from FAO, 2014). In rural areas of Madagascar, average rice ingestion may be higher, reaching 380 g/day/capita (Alain Ramanantsoanirina, FOFIFA, personal communication). Despite one of the highest rice ingestion rates, studies reporting metal(loid) concentrations in rice grain are lacking, which are necessary to better understand potential deficiencies in the diet and exposure to toxic elements. Additionally, Madagascar is a biodiversity hotspot for rice (Mather et al., 2010; Radanielina et al., 2013); therefore a survey of metal(loid)s among multiple rice varieties is informative to rice breeders seeking sources of genetic variability for hybrid rice cultivars.

The primary focus of this study is to survey total mercury (THg) and methylmercury (MeHg) concentrations in rice cultivated in Madagascar. Fish consumption is considered the primary human exposure pathway for MeHg, a potent neurotoxin (Clarkson and Magos, 2006); however, MeHg exposure also occurs through rice ingestion (e.g., Feng et al., 2008; Rothenberg et al., 2011a, 2011b, 2013, 2014; Windham-Myers et al., 2014a, 2014b, 2014c). Worldwide approximately 90% of rice is cultivated under standing water (Kirk, 2004), creating anoxic conditions that favor microbial conversion of less toxic inorganic Hg(II) to more toxic MeHg (Rothenberg and Feng, 2012; Windham-Myers et al., 2014b), which is subsequently bioaccumulated in rice grain (Rothenberg et al., 2011a, 2014; Zhang et al., 2010). Despite the importance of rice as a staple food for half the global population (Khush, 2005), there are few studies addressing MeHg exposure through rice ingestion outside Asia (Rothenberg et al., 2014).

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Madagascar is a signatory to the Minamata Convention on Mercury (UNEP, 2014), which requires development of a baseline Hg inventory by the Madagascar National Focal Point of Mercury Programme. To meet this obligation, Hg releases to the air, water, and land in Madagascar were estimated using the United Nations Environment Programme Hg Toolkit (Randrianomenjanahary, undated; UNEP, 2011). Madagascar imports many Hg-containing products, including dental amalgam (2368 tons/year), soaps with Hg (8288 tons/year), paint (4291 tons/year), and Hg-containing batteries (22.3 tons/year), and highest environmental Hg releases are related to their disposal or incineration (Randrianomenjanahary, undated; UNEP, 2011). Unlike neighboring South Africa, which depends on coal-fired power plants for energy (e.g., Dabrowski et al., 2008), 95% of Madagascar households rely on wood, charcoal or coal for domestic energy due to the lack of electricity or high costs associated with electricity (UNEP, 2011). Biomass burning competes with coal-fired power plants as one of the most important sources of Hg atmospheric emissions (Streets et al., 2009); biomass burning is also more difficult to regulate than industrial power plants. Like other sub-Saharan African nations (e.g., van Straaten, 2000), artisanal and small-scale gold mining is practiced in Madagascar (UNEP, 2011); however, Hg is banned from this process (Randrianomenjanahary, undated). Additionally, mining of sapphire and rubies replaced gold mining as a more lucrative source of income (Duffy, 2007). Other potential sources of Hg pollution include production of cement (8000–40,000 tons/year) and lime (2200 tons/year) (Randrianomenjanahary, undated). Calculations from the Hg Toolkit predict a maximum of 98.5 tons Hg are released annually to the environment in Madagascar (UNEP, 2011). When estimated Hg emissions are normalized by population, results suggest a moderate-to-high per capita Hg footprint in Madagascar compared to other nations, where Hg environmental emissions are known (see Table 1). However this table does not weight the magnitude of health impairments associated with each Hg pollution source.

In addition to quantifying THg and MeHg, concentrations of arsenic (As), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd), rubidium (Rb) and strontium (Sr) were also analyzed as part of the rice ionome, or collection of mineral nutrients (Salt et al., 2008). Metal(loid)s are often studied in isolation; however, chemical similarities, competition for ligands, and plant–soil interactions may alter rice grain content (e.g., Impa and Johnson-Beebout, 2012; Li et al., 2012; Williams et al., 2009a; Zhang et al., 2012). In addition, some trace elements are nutritious (e.g., Zn and Se) while others are toxic (e.g., As and Cd), making it difficult to assess the nutritional quality of rice grain without considering both.

Table 1
Mercury (Hg) releases to air, water and land for Madagascar and other nations.

	Hg releases (tons/year)[a]	Population[b]	Hg emissions (g/capita)
Yemen	0.80	26,052,966	0.000031
Pakistan	10.8	196,174,380	0.000055
Panama	0.40	3,608,431	0.00011
Dominican Republic	2.1	10,349,741	0.00020
New Zealand	1.4	4,401,916	0.00032
Australia	24.6	22,507,617	0.0011
Ecuador	76.3	15,654,411	0.0049
Burkina Faso	2.60	17,812,961	0.145
Cambodia	14.9	15,205,539	0.980
Madagascar	98.5	22,599,098	4.36
Mexico	1560	116,220,947	13.4
Philippines	1670	105,720,644	15.6

[a] Maximum annual amount of Hg released to air, water, and land from UNEP (2011).
[b] Population statistics from CIA (2014).

Madagascar rice samples ($n = 51$) were obtained from the International Rice Genebank (located at the International Rice Research Institute (IRRI) in Los Baños, Philippines), which included rice samples with both red bran and brown bran. This is a retrospective study of archived rice germplasm, and other matrices (e.g., paddy soil or irrigation water) were not available. Other researchers reported a strong positive correlation between rice THg and MeHg concentrations for rice cultivated in China, California, USA and Cambodia (Rothenberg et al., 2014), while an inverse correlation was observed between rice Hg species and Se (Zhang et al., 2012). We hypothesize interactions will be similar among these Madagascar rice varieties. Differences in bran color may signify a source of genetic variability, contributing to the accumulation and assimilation of metal(loid)s (Norton et al., 2009). We hypothesize concentrations of some elements will differ in red rice compared to brown rice. This is the first study to the best of our knowledge to document concentrations of metal(loid)s in rice from this African island nation.

2. Materials and methods

2.1. International Rice Genebank

IRRI houses the International Rice Genebank, which holds in trust the world's most comprehensive collection of rice genetic resources, including more than 117,000 germplasm samples (i.e., accessions) from 124 countries collected since 1962 (IRRI, 2014). The Base Collection is comprised of rice germplasm cultivated in the country of origin, which is stored frozen (−18 °C–−20 °C), and not typically available for researchers due to limited supplies. An exception was made for these Madagascar rice samples because there was sufficient quantity in the Base Collection.

2.2. Fumigation test

The Seed Health Unit at IRRI uses the fumigant, Phostoxin™, i.e., aluminum phosphide, for all samples shipped to the U.S. The effect of fumigation on concentrations of all trace elements was tested using two Philippine rice varieties cultivated in five paddies at IRRI. Philippine rice varieties were used because there were insufficient quantities of rice samples from Madagascar; however, the same fumigation methods were employed and any residue remaining was the same, regardless of the rice variety. Half of each sample was fumigated with Phostoxin™, then all samples were dehulled under pressure between two rollers (Huller, JLGJ4.5, China), and unpolished rice samples were ground to a powder using a stainless steel Wiley mill, which was cleaned with ethanol between samples to prevent carryover. MeHg concentrations were measured on-site at IRRI, and other trace elements were quantified at the University of South Carolina (see methods below). Fumigation residues did not affect trace element concentrations ($n = 10$, including 5 fumigated and 5 non-fumigated; two-sided paired t-test, THg $p = 0.88$; MeHg $p = 0.22$; As $p = 0.64$; Cd $p = 0.29$; Cu $p = 0.20$; Mn $p = 0.23$; Rb $p = 0.41$; Se $p = 0.35$; Sr $p = 0.81$, and Zn $p = 0.34$). This comparison provided more confidence in our analyses of IRRI rice samples from Madagascar, which were fumigated with Phostoxin™ prior to export to the U.S.

2.3. Madagascar rice samples

Data for Madagascar rice samples discussed below were made available by IRRI (G.L. Capilit, IRRI, personal communication) and FOFIFA (A. Ramanantsoanirina, FOFIFA, personal communication).

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