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Mercury in rice (*Oryza sativa L.*) and rice-paddy soils under long-term fertilizer and organic amendment

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ABSTRACT

High levels of mercury (Hg), especially methylmercury (MeHg), in rice is of concern due to its potential of entering food chain and the high toxicity to human. The level and form of Hg in rice could be influenced by fertilizers and other soil amendments. Studies were conducted to evaluate the effect of 24 years application of chemical fertilizers and organic amendments on total Hg (THg) and MeHg and their translocation in soil, plants, and rice grain. All treatments led to significantly higher concentrations of MeHg in grain than those from the untreated control. Of nine treatments tested, chemical fertilizers combining with returning rice straw (NPK1 + S) led to highest MeHg concentration in grain and soil; while the nitrogen and potassium (NK) treatment led to significantly higher THg in grain. Concentrations of soil MeHg were significantly correlated with THg in soil ($r = 0.59^{***}$) and MeHg in grain ($r = 0.48^{***}$). Calcium superphosphate negatively affected plant bioavailability of soil Hg. MeHg concentration in rice was heavily influenced by soil Hg levels. Phosphorus fertilizer was a main source contributing to soil THg, while returning rice straw to the field contributed significantly to MeHg in soil and rice grain. As a result, caution should be exercised in soil treatment or when utilizing Hg-contaminated soils to produce rice for human consumption. Strategic management of rice straw and phosphorus fertilizer could be effective strategies of lowering soil Hg, which would ultimately lower MeHg in rice and the risk of Hg entering food chain.

1. Introduction

Mercury (Hg) has emerged to be a highly pervasive global pollutant, occurring widely in the environment in various forms. The toxicity of Hg is strongly dependent on its chemical forms (Ullrich et al., 2001; Wyn et al., 2009). Of all Hg forms, methylmercury (MeHg) receives the greatest attention because it is the most toxic to human and may be biomagnified along the food chain (Stein et al., 1996; Watras et al., 1998). The main MeHg exposure pathways to humans were often considered to be related to fish, shellfish , and sea mammals (Stein et al., 1996). Recent studies demonstrated that rice (*Oryza sativa L.*) also accumulate MeHg, which could be by average 30.9% of THg (Meng et al., 2010, 2011). Moreover, approximately 80% of MeHg in rice was located in edible white rice (Meng et al., 2014).

As an important food crop in the world, about 160 million ha of rice was planted in 2014, taking about 10.7% of cultivated field of the year (Faostat, 2014). Of those rice paddy fields, approximately 89% were flooded (Sohrabi et al., 2012). Similar to other wetlands, flooding paddy field was an important Hg methylation sites (Rothenberg and Feng, 2012). Methylation of Hg was mainly carried out by anaerobic microbes that may contain *hgc*A and *hgc*B genes (Liu et al., 2014; Parks et al., 2013). In addition, the Hg methylation in soil is also determined by a range of factors, such as pH, dissolved organic carbon, sulfur, iron, and dissolved Hg concentration (Ullrich et al., 2001). Flooding not only promotes growth of anaerobic microbes, but also enhance sulfide and polysulfide formation, which could lead to increased solubility of HgS (s) (Barnes et al., 1967; Bell et al., 2007). As a result, the bioavailability and subsequent methylation of Hg (Marvin-DiPasquale et al., 2003) were enhanced. Correlations between MeHg concentration in soil and rice grain suggested that MeHg in grain was from soil (Meng et al., 2010). Those leaded to the concentration of MeHg in rice grain being hundreds times of those in corn or wheat (Qiu et al., 2008, 2006). It has been reported that MeHg intake by human from rice consumption in Hg mine area was as high as 1.8 μg kg^{-1} of body weight per day (Feng

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et al., 2008; Qiu et al., 2008), which far exceeded the reference dose of 0.1 μg Hg kg^{-1} bw d^{-1} recommended by the Environmental Protection Agency in the United States (US-EPA) (Hassett et al., 1997).

The potential impact of MeHg in rice to human health is well recognized. Efforts have been undertaken to reduce MeHg in rice. Most attempts focused on screening and selecting rice varieties with low uptake of MeHg (Rothenberg et al., 2012). Some efforts were devoted to altering soil redox potential through water management to reduce production and uptake of MeHg (Peng et al., 2012; Wang et al., 2014a). Limited attention has been given to elucidate the effect of fertilization and organic amendments on Hg-methylation in soil and its subsequent translocation to rice plants and grain. It has been established that fertilizers and commonly used soil amendments contain considerable amount of Hg (Mortvedt, 1995; Zhao and Wang, 2010). The concentration of Hg ranged from 410 to 880 μg kg^{-1} in calcium phosphate and 40–190 μg kg^{-1} in livestock manure (Luo et al., 2009; Wang et al., 2013; Zhao and Wang, 2010). Moreover, long-term application of chemical fertilizer and organic amendments could alter soil pH and other soil properties that affect bioavailability and methylation potential of Hg (Barkay et al., 1997; Miskimmin et al., 1992). Furthermore, low-molecular-weight thiol-containing organic compounds could form complexes with Hg that influence Hg bioavailability (Barkay et al., 1997). Studies showed that Hg-methylation was greatly enhanced by low concentrations of cysteine through forming Hg-cysteine complex (Schaefer and Morel, 2009). Application of potassium sulfate has been shown to stimulate growth of sulfur-reducing bacteria, which could result in enhanced Hg-methylation (King et al., 1999). A more recent study suggested that returning rice straw to the field led to increase of MeHg concentration in the soil (Liu et al., 2016). Unfortunately, most of these studies were short-term greenhouse evaluations. Therefore, a long-term experiment was conducted in a rice paddy field to elucidate the effects of chemical fertilizers and organic amendments on accumulation and translocation of various forms of Hg in soil and rice, with the ultimately goal of lower the risk of Hg entering the food chain.

2. Materials and methods

2.1. Site description and experimental design

The study site was located in Chongqing, China (30°26′N , 106°26′E) in a region under humid subtropical climate with average annual rainfalls in the range of 900–1300 mm and an annual mean temperature of 18.3 °C. The experiment is a part of a Long-Term National Soil Fertilizer Monitoring Network that was constructed in 1990 in nine major grain production areas in China.

In 1991, wheat-rice rotation were initiated in nine treatments and an untreated control. The nine fertilizer treatment and organic amendments included *(1)* swine manure (M), *(2)* calcium superphosphate and potassium sulfate (PK), *(3)* urea and potassium sulfate (NK), *(4)* urea and calcium superphosphate (NP), *(5)* urea, calcium superphosphate and potassium sulfate (NPK1), *(6)* urea, calcium superphosphate, potassium sulfate and swine manure (NPK1 + M), *(7)* ammonium chloride, calcium superphosphate, potassium chloride and swine manure (NPK2 + M), *(8)* urea, calcium superphosphate, potassium sulfate and rice stalk (NPK2+S), and *(9)* 1.5 times than NPK1 and swine manure (NPK1 + M).

Chemical fertilizers were applied twice a year before planting wheat and rice seasons, respectively. Swine manure and rice straw were applied once a year before planting wheat. During 1991–1996, application of chemical fertilizer were N 150 kg ha^{-1}, P 32.8 kg ha^{-1}, K 62.2 kg ha^{-1}; Since fall of 1996, application of chemical fertilizer were changed to N 135 kg ha^{-1}, P 26.2 kg ha^{-1}, K 49.8 kg ha^{-1}. Organic amendments were remain unchanged with swine manure 22.5 × 10^3 kg ha^{-1} y^{-1} and rice straw (dry weight) 7.5 × 10^3 kg ha^{-1} y^{-1}. The concentration of THg in experimental fertilizers and organic amendment were shown in Supplementary information (SI), Table S1.

Measurements included THg, MeHg in soil and rice plant. Several soil properties that have been shown to impact Hg and Hg uptake by plants were also evaluated, including pH, total N (TN), total P (TP), total K (TK), organic carbon (OC), cation exchange capability (CEC), Sodium hydroxide hydrolyzable N (HN), sodium bicarbonate extractable P (EP) and K (EK), and diethylene-triaminepentaacetic acid (DTPA) extractable iron (EFe) and manganese (EMn).

2.2. Sampling

Samples were collected following maturity of rice in 2015, 24 years after initiation of the experiment. Each plot was 120 m^2 and divided into three subplots for sampling, serving as replications. The entire rice plants including the grain were collected from each subplot. Following washing off soil particles attached to rice plant, the collected materials were transported to the laboratory, and then rinsed with deionized water three times before dividing into roots, stalks, leaves, and seeds. The seeds were further separated into husk and brown rice using a huller. These samples were powered with agate mortar after freeze-dried and stored at 4 °C prior to analysis.

A five-core composite soil sample was collected from each subplot at 0–5 cm, 5–10 cm, 10–20 cm, and 20–30 cm using a five-diameter sampler probe. Each soil sample was divided into two parts. One part was freeze-dried, passed 100-mesh sieve, and stored in sealed polypropylene bags at 4 °C for subsequent mercury analyses. The other part was air-dried and passed 60-mesh sieve for the analyses of CEC and HN, EP, EK, EFe and EMn. A small portion of air-dried < 60-mesh samples was further ground and passed 100-mesh sieve for the analyses of TN, TP, and OC. All air-dried samples were stored at room temperature (15° to 25 °C) in sealed polypropylene bags.

2.3. Determination of Hg in plant and soil

For THg analysis, soil was determined with Direct Mercury Analyzer 80 (Milestone, Italy). Rice plant samples were determined using cold vapor atomic fluorescence spectrometry (CVAFS, Brooks Rand model III, USA) after digestion with in a freshly prepared mixture of HNO_3/H_2SO_4 (4:1, v/v) at 95 °C, oxidation by BrCl, reduction by $SnCl_2$, and finally thermo-reduction to Hg^0 (USEPA, 2002). For MeHg analysis, rice plant and soil samples were firstly digested using KOH-methanol/solvent extraction and the $CuSO_4$-methanol/solvent extraction, respectively (Liang et al., 1996). Methyl Hg in samples was then determined using aqueous ethylation, purge, trap, and gas chromatography-cold vapor atomic fluorescence spectrometry detection (CVAFS, Brooks Rand model III, USA) (USEPA, 2002).

2.4. Quality control

Quantification for THg and MeHg in rice tissues sample was conducted using daily calibration curves with the coefficient of variation (r^2) ≥ 0.99. The method detection limits were 0.01 μg kg^{-1} for THg and 0.002 μg kg^{-1} for MeHg in rice tissues, and 0.005 μg kg^{-1} for THg and 0.002 for MeHg in soil samples. The precision and bias for triplicate samples were less than 5% for THg and 8% for MeHg analysis. The recoveries for matrix spikes ranged from 83% to 114% for THg analysis, and from 83% to 119% for MeHg. The following certified reference materials were employed: citrus leaf (GBW10020, National Research Centre for Certified Reference Materials), soil (GBW07428, National Research Centre for Certified Reference Materials), and lobster (TORT-2, National Research Council of Canada). The results of the certified reference material analysis are shown in SI, Table S2.

2.5. Determination of selected soil properties and extractable nutrient content

Soil pH was measured using a pH electrode (soil: water = 1:2.5).