JOURNAL OF ENVIRONMENTAL SCIENCES 119 (2022) 119–129

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Assessing the factors impacting the bioaccessibility of mercury (Hg) in rice consumption by an *in-vitro* method

Ze Wu[1,2], *Ping Li*[3], *Xinbin Feng*[3,*]

[1] *College of Eco-Environmental Engineering, Guizhou Minzu University, Guiyang 550025, China*
[2] *Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang 550025, China*
[3] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China*

ARTICLE INFO

Article history:
Received 30 November 2021
Revised 11 April 2022
Accepted 21 April 2022
Available online 2 May 2022

Keywords:
Rice
Bioaccessibility
Mercury
Impact factors

ABSTRACT

Mercury (Hg) in rice is drawing mounting concern since methylmercury (MeHg) was found capable of accumulating in rice. *In-vitro* bioaccessibility is a feasible and reliable method to assess the health effects of Hg in rice and has been utilized in a number of studies. This study was done to investigate the impact of cultivar, planting location, and cooking on the total mercury (THg) and MeHg bioaccessibility of rice, for which multiple statistical analysis methods were used to analyze the significance of their effects. The THg concentrations of rice samples taken from non-Hg contaminated areas of China were all below 15 ng/g and their MeHg concentrations were below 2 ng/g. Cooking could significantly reduce the MeHg bioaccessibility of rice because the MeHg was mainly combined with protein and the protein will be denatured during the cooking process, and then the denatured MeHg is difficult to be dissolved into the liquid phase. Indica- and japonica-type rice cultivars did not show significant differentiation in either the concentration of Hg or its bioaccessibility. However, the glutinous rice type differed significantly from the above rice types, and it showed greater bioaccessibility of THg and MeHg due to its distinct protein contents and starch properties. Planting location can affect the Hg concentration in rice and THg bioaccessibility but has a limited impact on MeHg bioaccessibility. Based on these results, two macro factors (rice cultivar, planting location) are presumed to impact Hg bioaccessibility by how they affect micro factors (i.e., Hg forms).

Introduction

Mercury (Hg) is a heavy metal toxic to organisms and a threat to environmental health. It can cause negative impacts on humans through various exposure routes, namely inhalation, skin contact, and ingestion of Hg-contaminated food (UNEP, 2013). Fish is an important dietary source of protein, however, significant concentrations of Hg have been found in fish tissues and so fish was initially considered the chief pathway by which Hg enters the human body (Afanso et al., 2015a; Siedlikowski et al., 2016). To protect human health, safe limits for Hg ingestion were formulated, for example, the World Health Organization (WHO) suggested a provisional tolerable weekly intake (PTWI) of 4 μg/kg bw for inorganic

---

[*] Corresponding author.
E-mail: fengxinbin@vip.skleg.cn (X. Feng).

https://doi.org/10.1016/j.jes.2022.04.028

mercury (IHg) and that of 1.6 μg/kg bw for methylmercury (MeHg) (JECFA, 2010). Similarly, the European Food Safety Authority (EFSA) also suggested a MeHg-tolerable weekly intake (TWI) of 1.3 μg/kg bw (EFSA, 2012). To accurately calculate the ingestion amount of Hg from consumed fish, *in-vitro* methods were applied to obtain the bioaccessibility data. Siedlikowski et al. (2016) investigated the bioaccessibility of MeHg in eight seafood species, finding that it ranged from 50.1 ± 19.2% up to 100%. The total mercury (THg) bioaccessibility of chinook salmon, sockeye salmon, and butter clams respectively were 49%, 46%, and 50% (Laird and Chan, 2013). In another work, Wang et al. (2013) investigated 10 species of freshwater fish and marine fish sold in a Hong Kong market, their THg and MeHg bioaccessibility values ranged from 21.4% to 51.7% and 19.5% to 59.2%, respectively. These studies collectively indicate that the Hg in fish tissue is not entirely bioaccessible, accordingly, using Hg concentrations to calculate the ingestion amount may overestimate potential health risks.

Rice, however, has also been found to contain high levels of THg and MeHg. Feng et al. (2008) reported mean THg and MeHg concentrations of 36.2 and 8.5 μg/kg in rice samples they collected from the Wanshan Hg mining area, in Guizhou Province, China. Similar findings were also reported by Li et al. (2008) and Qiu et al. (2008). Based on those reports, combined with the large consuming amounts, rice was deemed the dominant pathway of MeHg ingestion for inhabitants of Guizhou (Feng and Qiu, 2008). Zhang et al. (2010a) also suggested that for residents of inland China, eating rice is the major pathway for MeHg exposure. Since then, increasing attention has been paid to Hg in rice, with some studies investigating the quantity of Hg in rice (Meng et al., 2010; Zhang et al., 2010b) and others evaluating the associated potential health risk (Qian et al., 2010; Fang et al., 2013; Wang et al., 2017).

Recently, several studies that addressed Hg bioaccessibility in rice reported that THg bioaccessibility in rice is $<$ 50%, which implies a limited health impact caused by rice consumption (Liao et al., 2019; Gong et al., 2018; Lin et al., 2019). Other researchers have focused on the factors influencing the Hg bioaccessibility in fish; e.g., the cooking mode used can decrease the Hg bioaccessibility, because the heat applied denatures the protein structures (Torres-Escribano et al., 2011; Ouedrago and Amyot, 2011; Maulvault et al., 2011; Matos et al., 2015; Afanso et al., 2015a, 2015b). Yet, concerning rice, the factors impacting THg and MeHg bioaccessibility are seldom reported. To fill this knowledge gap, in this study we collected 34 rice samples to investigate their THg and MeHg bioaccessibility, and different sample groups were set to explore the affection of Hg bioaccessibility. Three factors of interest were tested: rice cultivar, planting location, and cooking, with appropriate statistical analyses implemented to investigate their effects.

1. Material and method

1.1. *Samples*

Table 1 – Sampling sites and rice cultivar.

Sample number	Sampling sites	Rice cultivar
ZJ-1~ZJ-8	Zhejiang province	Indica rice
DB-1~DB-8	Heilongjiang province	
ZJ-9~ZJ-15	Zhejiang province	Japonica rice
DB-9~DB-15	Heilongjiang province	
NM-1	Zhejiang province	Glutinous rice
NM-2	Jiangsu province	
NM-3	Guangdong province	
NM-4	Fujian province	

In total 34 samples were analyzed in this study which included three rice cultivars (14 of japonica rice, 16 of indica rice, and 4 of glutinous rice). Of the samples, 16 were collected from Zhejiang province, 15 were from Heilongjiang province and the remaining 3 samples were respectively taken from 3 other provinces in China. All details of these samples were listed in Table 1. Each rice sample was obtained directly from a rice paddy and consisted of an entire rice plant removed, but only the rice grains were analyzed in this study. Rice grains of each sample were first separated into brown rice and husk parts, after which the brown rice portion was polished into white rice. These white rice samples were then milled into to pass through 100-mesh filters and stored in polyethylene bags.

To simulate its food conditions, cooked rice samples were prepared in this way: milled rice samples were weighed (1 g) and put into 50 mL centrifuge tubes, to which 5 mL of deionized water was added. The centrifuge tubes were capped and slightly shaken to homogenize the rice powder and water, then they were placed in a 100 °C water bath for 30 min. Besides that, milled rice samples were directly used in experiments as raw samples without any treatment.

1.2. In-vitro *experiment*

In our previous study (Wu et al., 2018), we showed that a physiologically based extraction test (PBET), first introduced by Ruby et al. (1996), is more accurate for quantifying the Hg bioaccessibility of rice. Consequently, the PBET *in-vitro* method was applied in this study. The parameters for the simulated gastric and intestinal juice used in the PBET method followed those of Ng et al. (2013) and showed in Table 2. Simulated gastric juice (50 mL) was added to the cooked rice samples and shaken at 120 r/min at 37 °C. Then, the mixtures were centrifuged at 3000 r/min for 20 min, and the supernatants were collected and moved into another set of centrifuge tubes. Simulated intestinal juice was added into the residue to 50 mL

Table 2 – Ingredients and parameters of PBET *in vitro* methods.

Digestion stage	Ingredients (per L)	pH	Extraction time (hr)
Gastric	1.25 g pepsin, 0.5 g sodium malate, 0.5 g sodium citrate, 420 μL lactic acid, 500 μL acetic acid	1.5	1
Intestinal	1.75 g bile, 0.5 g pancreatin	7.0	4

and shaken for 4 hr (at 120 r/min, 37 °C), then centrifuged at 3000 r/min for 20 min. From there, the collected supernatants were frozen at −18 °C until analysis.

1.3. *Hg determination*

To detect and quantify Hg in samples, (1) for rice samples:1 g of each rice sample was weighed and put inside a 15 mL centrifuge tube. Then the tubes for cooked samples added deionized water to 1 mL and placed in a 100 °C water bath for 30 min. The raw rice samples were directly digested by the procedures described below; (2) for supernatants: 10 mL of each supernatant was added into a 15 mL centrifuge tube and then treated as the description below. Two different digestion procedures were applied: (1) For the THg analysis, 5 mL of HNO_3 was added to each sample and these were placed in a 95 °C water bath for 3 hr; (2) For the MeHg analysis, 5 mL of KOH-CH_3OH solution (25%, W/V) was added to each sample and put into a water bath (75 °C) for 4 hr. After either procedure, each sample was cooled to room temperature and topped to 15 mL with deionized water. To determine the MeHg and THg, the procedures of the United States Environmental Protection Agency method 1630 (USEPA, 1998) and 1631 (USEPA, 2002) were followed, respectively. Cold vapor atomic fluorescence system (CVAFS) was applied for THg determination and gas chromatography (GC)-CVAFS for MeHg (Model III detector, Brooks Rand Instruments, the USA).

1.4. *Calculation and statistical methods*

To calculate bioaccessible fraction, the following Eq. (1) was applied:

$$\text{Bioaccessibile fraction (ng)} = C_{ext} \times V_{ext} \quad (1)$$

In this equation, C_{ext} (ng/mL) is the Hg concentration of the extraction solution from the PBET experiment; V_{ext} (mL) is the volume of extraction solution;

And the bioaccessibility of Hg in rice was calculated by Eq. (2) introduced by the USEPA (2012).

$$\text{Bioaccessibility} = \frac{\text{Bioaccessible fraction}}{C_{sample} \cdot M_{sample}} \cdot 100\% \quad (2)$$

In this equation, C_{sample} (ng/g) is the Hg concentration in a given sample; and M_{sample} (g) is the mass of a given sample.

To describe the IHg concentration, the following Eq. (3) was applied:

$$\text{IHg(ng)} = \text{THg} - \text{MeHg} \quad (3)$$

And the MeHg and IHg fraction (MeHg% and IHg%) were calculated by following Eqs. (4) and (5):

$$\text{MeHg\%} = \frac{\text{MeHg}}{\text{THg}} \times 100\% \quad (4)$$

$$\text{IHg\%} = \frac{\text{IHg}}{\text{THg}} \times 100\% \quad (5)$$

In this equation, THg (ng), MeHg (ng) and IHg (ng) are concentrations in samples.

To statistically analyze the data, we used SPSS software (IBM Corporation, v26.0) to perform parametric or nonparametric test methods, based on the outcome of normality testing. Two-way analysis of variance and multivariate analysis of variance were also applied to determine the effects of different factors on the concentration and bioaccessibility of Hg

1.5. QA/QC

Here, the limits of detection (LODs) for THg and MeHg respectively were 0.03 ng/L and 0.02 ng/L, these corresponding to the procedures introduced by the USEPA method 1630 (1998) and 1631 (2002). The relative standard deviation (RSD) values ranged from 2.3% to 7.8% for duplicate samples, which were added in every 5 samples during Hg concentration determination in rice. The THg and MeHg concentrations in certified reference material (CRM) were determined with three duplicate samples. These measured values are in Table 3.

2. Results

2.1. *Hg concentrations*

As seen in Table 4, all the rice samples met the Chinese national standard (20 ng/g) for the THg concentration in foodgrain (GB 2762-2005). Most of their MeHg concentrations were lower than 2 ng/g. In contrast to reported data from Wanshan (Feng et al., 2008; Li et al., 2008; Qiu et al., 2008), both THg and MeHg concentrations in our study were much lower, likely because all of our sampling sites were in non-mercury contaminated areas. Low Hg concentrations were also reported for commercial rice sold in China (Gong et al., 2018; Xv et al., 2020), Europe (Brombach et al., 2017), and Canada (Lin et al., 2019), which together suggests a small probability of Hg pollution in rice grown in a non-mercury contaminated area. In contrast to the THg concentrations of our data, relatively low MeHg concentrations were found, which indicated the rice samples contained a high IHg fraction. The mean IHg% was also shown in Table 4.

2.2. *Hg bioaccessibility*

Two parallel tests were utilized in the Hg bioaccessibility experiment. The bioaccessible fraction in the DB and ZJ rice samples can be found in Table 5 and are depicted in Fig. 1. The data showed that the THg and MeHg in rice were both more bioaccessible in the gastric than the intestinal stage for all rice samples. From the mean bioaccessible fraction data shown in Table 5, compared with indica and japonica rice cultivars, the mean THg and MeHg bioaccessible fractions in both the gastric and intestinal digestion stage were relatively higher in the glutinous rice group.

Hg bioaccessibility values were calculated (Fig. 2). With a greater accumulating Hg bioaccessible fraction, Hg bioaccessibilities after the gastric and intestinal stages were higher than those in the gastric stage. The means of THg bioaccessibility in the gastrointestinal stage and the gastric stage

Table 3 – The reference value and measured value of THg and MeHg in CRM.

CRM	Material type	THg (ng/g)		MeHg (ng/g)	
		reference value	measured value	reference value	measured value
GBW-10020	Tangerine leaf	150±20	145±6	—	—
TORT-2	Lobster hepatopancreas	—	—	152±13	140±5

Table 4 – Hg concentrations and ratios in rice samples (ng/g).

		THg	MeHg	IHg	MeHg (%)	IHg (%)
ZJ	Mean	5.91	1.06	4.85	18.35	81.65
	STD	0.63	0.15	0.52	1.63	1.63
	Max	13.2	2.64	10.5	30.79	93.49
	Medium	5.48	0.90	4.35	17.60	82.40
	Min	2.56	0.45	1.83	6.51	69.20
DB	Mean	2.75	0.73	2.01	29.61	70.38
	STD	0.24	0.06	0.23	4.25	4.25
	Max	4.39	1.10	3.61	82.28	83.46
	Medium	2.71	0.73	1.90	26.80	73.20
	Min	1.29	0.33	0.23	16.53	17.72
NM	Mean	3.87	1.40	2.47	38.15	61.85
	STD	0.56	0.36	0.58	8.86	8.86
	Max	4.76	2.35	3.94	54.41	84.84
	Medium	-	-	-	-	-
	Min	2.32	0.70	1.06	15.16	45.60

were 48.04% ± 3.35% (23%–82%) and 30.46% ± 2.41% (12%–61%), respectively. The means of MeHg bioaccessibility were 52.89% ± 3.14% (27%–87%) and 35.09% ± 2.31% (14%–67%), respectively.

For the ZJ and DB sample groups, the mean value of THg bioaccessibility was 37% ± 15% and 59% ± 14% while that of MeHg was 52% ± 19% and 53% ± 16%, respectively. Liao et al. (2017) and Lin et al. (2019) reported less than 50% for the bioaccessibility of THg in commercial rice of China and Canada, respectively, and Gong et al. (2018) reported a mean MeHg bioaccessibility of 40.5% ± 9.4% for rice in China. Similar results were obtained in the present study for the Hg bioaccessibility of rice in ZJ and DB. However, among rice types, when compared with either indica or japonica samples, the bioaccessibility of glutinous rice samples was much higher, being 72% ± 11% and 95% ± 3% for THg and MeHg bioaccessibility, respectively (Fig. 3).

To investigate the impact of the cooking procedure on Hg bioaccessibility in rice, raw and cooked rice were also tested in this study. The resulting Hg concentrations appear in Table 6, clearly showing differential variation in the THg and MeHg concentrations in rice before versus after the cooking procedure. Fig. 4 shows the Hg bioaccessibility (after gastrointestinal digestion) of raw and cooked rice, which evidently decreased after the cooking procedure. For THg bioaccessibility, cooking reduced the mean percentage of the ZJ and DB sample group by 8% and 22%, respectively. Similar results were also revealed by the MeHg data, for which the corresponding reductions in its bioaccessibility were 7% and 5%. Similarly, Liao et al. (2019) reported that the average THg bioaccessibility fell from 69.74% to less than 47% after cooking rice using a different procedure.

3. Discussion

3.1. *The relations of Hg bioaccessible fraction in gastric and intestinal stage*

For the bioaccessible fraction data Section 2.2, both THg and MeHg have higher bioaccessible fractions in the stage of gastric digestion than those of the intestine. As shown in Table 4, the selected rice samples contained high IHg fractions, and

Table 5 – Hg bioaccessible fraction in rice sample groups (ng).

Sample	Rice cultivar	Test group	THg bioaccessible fraction		MeHg bioaccessible fraction		IHg bioaccessible fraction	
			Gastric	Intestinal	Gastric	Intestinal	Gastric	Intestinal
ZJ	Indica	1	1.08 ± 0.10	0.68 ± 0.03	0.25 ± 0.06	0.18 ± 0.04	0.83 ± 0.08	0.50 ± 0.05
		2	1.09 ± 0.09	0.63 ± 0.05	0.26 ± 0.05	0.18 ± 0.05	0.83 ± 0.08	0.45 ± 0.07
	Japonica	1	1.46 ± 0.23	0.87 ± 0.11	0.44 ± 0.13	0.28 ± 0.09	1.02 ± 0.12	0.58 ± 0.10
		2	1.40 ± 0.25	0.80 ± 0.10	0.45 ± 0.11	0.27 ± 0.09	0.95 ± 0.16	0.53 ± 0.09
DB	Indica	1	1.03 ± 0.04	0.58 ± 0.06	0.30 ± 0.03	0.12 ± 0.01	0.73 ± 0.03	0.45 ± 0.06
		2	0.96 ± 0.05	0.60 ± 0.06	0.31 ± 0.02	0.13 ± 0.01	0.64 ± 0.04	0.47 ± 0.05
	Japonica	1	0.89 ± 0.03	0.50 ± 0.07	0.22 ± 0.02	0.09 ± 0.01	0.67 ± 0.05	0.41 ± 0.08
		2	0.91 ± 0.02	0.58 ± 0.07	0.23 ± 0.02	0.08 ± 0.01	0.68 ± 0.03	0.50 ± 0.08
NM	Glutinous	1	1.51 ± 0.23	1.29 ± 0.17	0.83 ± 0.23	0.53 ± 0.13	0.68 ± 0.18	0.76 ± 0.16
		2	1.53 ± 0.23	1.13 ± 0.13	0.78 ± 0.25	0.51 ± 0.09	0.75 ± 0.19	0.61±0.10

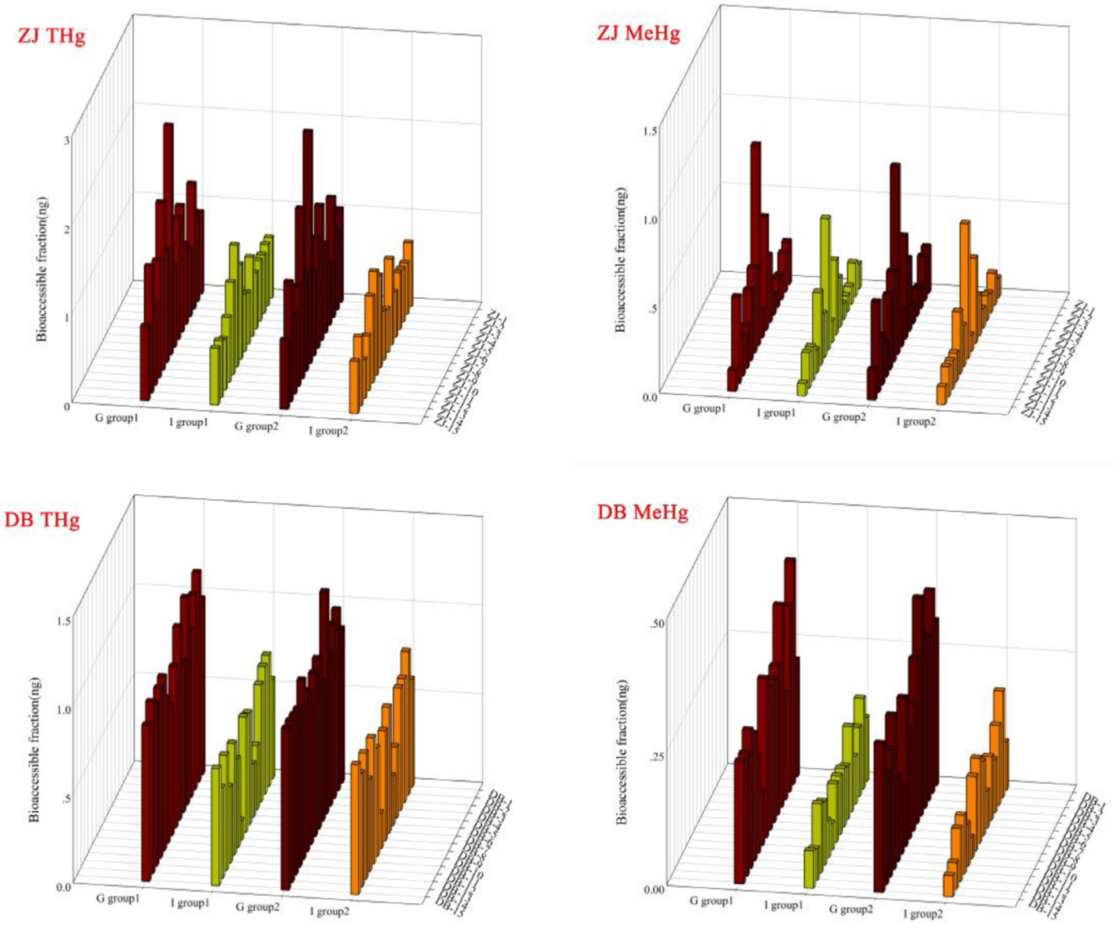

Fig. 1 – THg and MeHg bioaccessible fraction in ZJ and DB sample groups.
*** G and I means gastric and intestinal digestion stage;**
*** Group 1 and 2 are two parallel test groups.**

then the samples have higher initial IHg and MeHg concentrations in the gastric digestion stage, thus the higher IHg and MeHg concentration lead to larger dissolution into gastric juice. Also, based on the paired-sample non-parametric test (Wilcoxon test, $F = 0.000 < 0.05$ in two parallel tests) results for the MeHg and IHg bioaccessible fraction, the means of both MeHg and IHg were higher in the gastric than in the intestinal digestion stage, and the IHg bioaccessible fraction were reflected into THg bioaccessible fraction. This may be the reason why both THg and MeHg showed more bioaccessible in gastric than in intestinal digestion stage. But the mean value of the IHg bioaccessible fraction exceeded that of the MeHg bioaccessible fraction (Table 5) both in gastric and intestinal digestion stages, which is possibly caused by the different combinations. Meng et al. (2014) reported that IHg and MeHg in rice grains are mainly combined with cysteine, yet they are associated with different ingredients (IHg of phytochelatins and MeHg of protein), thereby leaving IHg largely immobile when compared with MeHg in rice grain. But in our experiment, rice grain was milled into rice powder, facilitating the digestion of both IHg and MeHg in stomach-like conditions and largely released into gastric juice and showed a higher Hg bioaccessible fraction. The phenomenon of our experiment implies that Hg bioaccessibility was influenced by the Hg concentration of different Hg forms rather than the digestion stage, e.g. both IHg and MeHg were largely released into gastric juice due to the higher initial concentration. Yet the mechanism of the differences between IHg and MeHg bioaccessibility was not well explained in this study, which deserves further investigation.

3.2. *The influence of rice cultivar on Hg concentration and bioaccessibility*

Different statistical analyses were applied to test whether the rice cultivar affected the THg and MeHg concentrations of rice. For the DB rice group, both its THg and MeHg concentrations were normally distributed ($p > 0.05$, Kolmogorov–Smirnov test), thus ANOVA was applied. The results showed the two rice cultivars did not differ significantly in their THg and MeHg concentrations ($p > 0.05$). For the ZJ rice group, the THg and MeHg concentrations were not normally distributed ($p < 0.05$, Kolmogorov–Smirnov test). According to the Wilcoxon signed-rank tests, the difference in THg concentrations was statistically significant ($p < 0.05$), with THg concentrations being higher in japonica than in indica rice type; however, their MeHg concentrations were similar ($p > 0.05$).

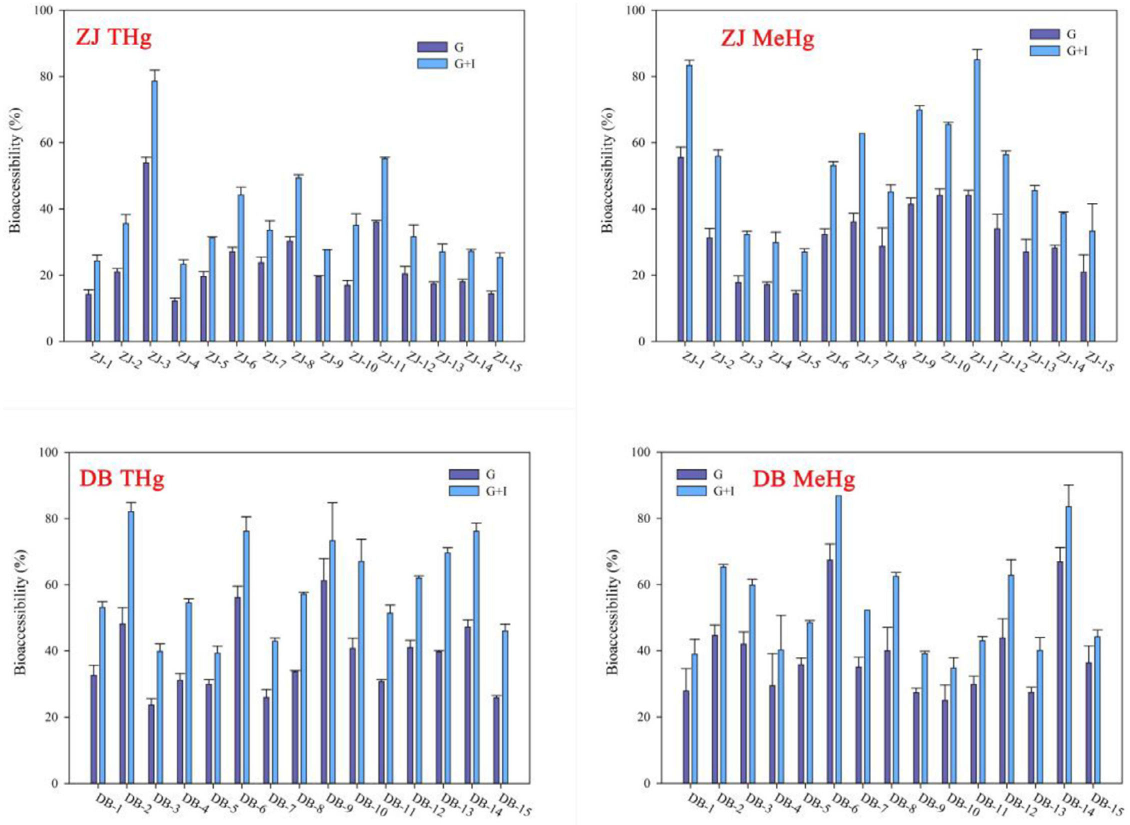

Fig. 2 – THg and MeHg bioaccessibility in ZJ and DB sample groups.
***G and G+I in this figure means bioaccessibility after gastric and gastrointestinal digestion.**

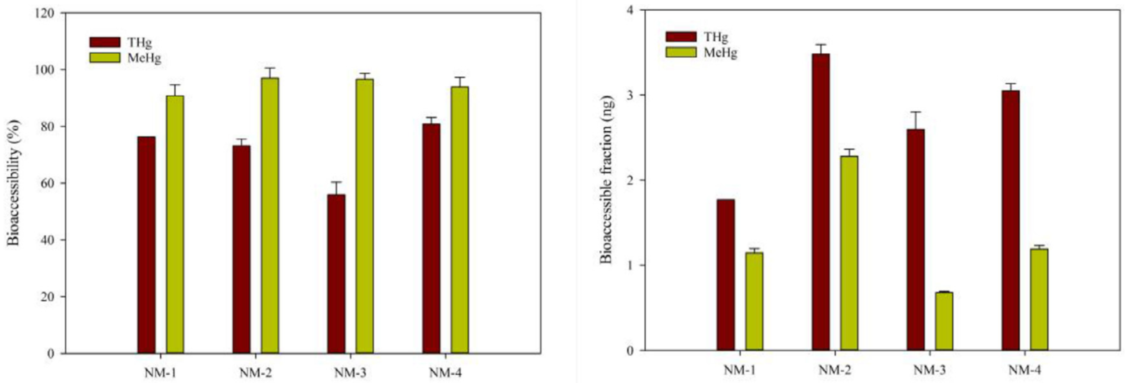

Fig. 3 – THg and MeHg bioaccessible fraction and bioaccessibility in glutinous rice samples.

Brombach et al. (2017) also reported a non-significant difference in the Hg concentration among six commercial rice types sold in European markets, with similar results reported for commercial rice sold in Canadian markets (Lin et al., 2019). Combined with the Hg concentration data here, this indicated that rice type may not be the dominant factor influencing the Hg concentration in rice grain. The Mann–Whitney test was applied to determine whether rice cultivar type affects the Hg bioaccessibility. The differentiation of japonica and indica rice type within ZJ and DB rice sample groups in terms of their THg bioaccessibility data was not statistically significant ($p > 0.05$), with similar results found for MeHg.

A multivariate nonparametric test was applied to compare the three rice cultivars, that is, now including the glutinous rice cultivar (Fig. 3). Based on the results of the Jonckheere–Terptra test, both THg and MeHg concentrations in three rice cultivars were not significantly different, nonetheless, the p-value (0.051) of MeHg concentration was very close to 0.05, which suggests the rice cultivar might not be main impact factor affecting the Hg concentration in rice. Moreover, the p-value of THg bioaccessibility was 0.498, thus rice cultivar might not strongly affect THg bioaccessibility either. But MeHg bioaccessibility showed different outcomes, given its p-value was significant at 0.032. This suggests the type of rice

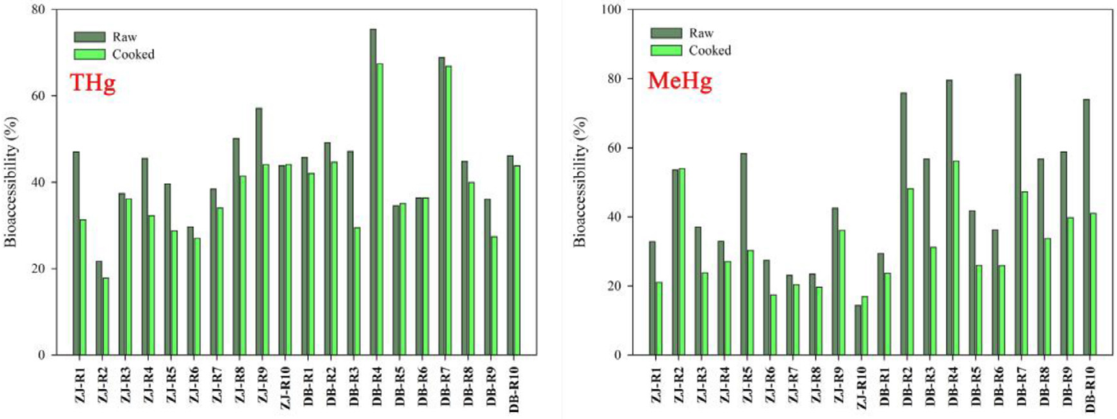

Fig. 4 – Bioaccessibility (after gastrointestinal digestion) in raw and cooked rice samples.

Table 6 – Hg concentrations in raw and cooked rice (ng/g).

	THg_{RAW}	THg_{COOKED}	$MeHg_{RAW}$	$MeHg_{COOKED}$
ZJ-R1	3.89	4.37	0.68	0.75
ZJ-R2	2.67	2.56	0.69	0.73
ZJ-R3	5.34	5.48	1.71	1.69
ZJ-R4	5.40	5.42	1.23	1.22
ZJ-R5	2.98	3.14	0.58	0.64
ZJ-R6	5.17	5.22	0.81	0.94
ZJ-R7	6.13	6.00	0.78	0.65
ZJ-R8	13.0	13.2	2.79	2.64
ZJ-R9	5.20	5.80	1.05	0.93
ZJ-R10	6.88	7.00	0.98	1.12
DB-R1	4.28	4.39	0.83	0.78
DB-R2	2.32	2.28	0.96	0.90
DB-R3	3.10	2.89	0.53	0.88
DB-R4	1.47	1.45	0.57	0.44
DB-R5	4.00	3.94	0.66	0.73
DB-R6	3.39	3.45	0.63	0.70
DB-R7	2.03	1.99	0.45	0.33
DB-R8	2.80	2.71	0.78	0.60
DB-R9	2.31	2.28	0.83	0.91
DB-R10	2.19	2.26	0.65	0.60

cultivar, especially glutinous rice, can influence the MeHg bioaccessibility. Some studies found large differences in the protein content in different rice cultivars and demonstrated that this can influence the properties of cooked rice (Martin and Fitzgerald, 2002). Du et al. (2010) showed glutinous rice differs substantially from japonica and indica rice in terms of its starch properties. These differences in the properties of glutinous rice might affect the form of MeHg, which may explain why the MeHg bioaccessibility of this rice type was statistically significant to the other two rice cultivars.

3.3. *The influence of planting location on Hg concentration and bioaccessibility*

Given normality testing results, the Mann–Whitney test was applied to investigate the effect of planting location. Due to the glutinous rice samples were not planted in the same province, thus glutinous rice group was neglected in this section to avoid misleading the outcomes of statistical analysis. The results showed the THg and MeHg concentrations were significantly affected by planting location, for which the p-values were 0.008 and 0.031, respectively. Although the planting locations selected in this study were all in non-mercury contaminated areas, the provinces of Zhejiang and Heilongjiang are located in southeast China and northeast China, respectively, implying large differences between them on a geographical scale. Thus, the soil type, planting methods, and weather between these two provinces were different and this probably influenced the Hg accumulation in their rice grains.

For Hg bioaccessibility, Mann–Whitney test results showed large differences in terms of THg and MeHg bioaccessibility values. Firstly, MeHg bioaccessibility (raw vs. cooked) was not significant (respectively, $p = 0.940$ and 0.364, > 0.05), which implied the planting location negligibly influenced MeHg bioaccessibility. However, the THg bioaccessibility of raw and cooked rice showed significant differences (respectively, $p = 0.016$ and 0.008, < 0.05) between the two planting locations. As mentioned above, the THg and MeHg concentrations were strongly affected by planting location; when considered alongside the results of Hg bioaccessibility, the possible reason for that result is that planting location may affect the form of IHg, thereby indirectly influencing THg bioaccessibility via the relatively high IHg concentration percentage. But for MeHg bioaccessibility, planting location mattered little due to the relatively low MeHg concentration percentage. The effect of planting location on the MeHg form thus deserves more in-depth study.

3.4. *The influence of cooking on Hg concentration and bioaccessibilty*

Paired-sample nonparametric tests (Wilcoxon test) were used to assess differences in the Hg concentration and bioaccessibility in raw and cooked rice. For the THg and MeHg concentrations, the results were not statistically significant (respectively, $p = 0.255$ and 0.837, > 0.05); similarly, the test for THg bioaccessibility also gave a non-significant result ($p = 0.067 > 0.05$). Therefore, cooking might not change the

Table 7 – Between-subjects effect tests of multiple factors on Hg concentration.

Source	Dependent Variable	Type III Sum of Squares	*df*	Mean Square	*F*	Sig.[¶]
Corrected Model	THg[†]	143.055[a]	7	20.436	5.870	.000
	MeHg[†]	2.386[b]	7	.341	1.426	.229
Intercept	THg	698.150	1	698.150	200.519	.000
	MeHg	32.132	1	32.132	134.408	.000
Factor 1[‡]	THg	14.992	1	14.992	4.306	.046
	MeHg	.101	1	.101	.423	.520
Factor 2[‡]	THg	90.582	1	90.582	26.017	.000
	MeHg	1.983	1	1.983	8.293	.007
Factor 3[‡]	THg	.039	1	.039	.011	.916
	MeHg	.000	1	.000	.001	.970
Factor 1*2	THg	39.022	1	39.022	11.208	.002
	MeHg	.301	1	.301	1.260	.270
Factor 1*3	THg	.001	1	.001	.000	.990
	MeHg	.016	1	.016	.068	.796
Factor 2*3	THg	.076	1	.076	.022	.884
	MeHg	.000	1	.000	.002	.965
Factor 1*2*3	THg	.000	1	.000	.000	.993
	MeHg	.002	1	.002	.008	.930
Error	THg	111.415	32	3.482		
	MeHg	7.650	32	.239		
Total	THg	980.033	40			
	MeHg	43.105	40			
Corrected Total	THg	254.470	39			
	MeHg	10.036	39			

a. *R* Squared = .562 (Adjusted *R* Squared = .466)
b. *R* Squared = .238 (Adjusted *R* Squared = .071)
¶. Sig. is a value indicates a significant influence when less than 0.05.
†. THg and MeHg in this table represents THg and MeHg concentration.
‡. Factor 1, 2 and 3 in this table represents rice cultivar, planting location and cooking respectively.

Hg concentration in rice and also has a limited influence on its THg bioaccessibility. However, the p-value of MeHg bioaccessibility was 0.000, indicating an extremely significant difference, in that the cooking procedure decreases the MeHg bioaccessibility in rice. Similar findings were also reported in research using fish, where it was inferred this phenomenon arises because protein will undergo denaturation under the high temperatures of cooking (Matos et al., 2015; Afanso et al., 2015a, 2015b). As such, the binding of Hg to amino acids and proteins will be impaired, rendering difficult their dissolution into the liquid phase. Meng et al. (2014) proved that MeHg was associated with protein, thus MeHg bioaccessibility was strongly affected by cooking. However, the rice samples in our study have large IHg concentrations relative to their MeHg concentration, thus the change of MeHg bioaccessibility was covered by IHg bioaccessibility, this may be the reason why THg bioaccessibility in rice showed unaffected by the cooking procedure.

3.5. *The combined influence of multiple factors on the concentration and bioaccessibility of Hg*

Multivariate analysis of variance was applied to investigate the combined influences of different impact factors upon Hg concentration and bioaccessibility. These results are presented in Tables 7 and 8.

As shown in Table 7, only rice cultivar and planting location jointly affected the THg concentration whereas they had no impact on MeHg concentration in rice. However, as evinced by Table 7, neither the combined influence of two factors nor three factors was statistically significant for the THg and MeHg bioaccessibility of rice. Recently, Liu et al. (2021) demonstrated that soil and atmosphere were both sources of IHg in rice, while its MeHg was mainly absorbed from the soil. Meng et al. (2010) proved that MeHg mainly bioaccumulates in the grain tissue of rice while IHg bioaccumulates in its leaf tissue. The two sampling locations in this study are geographically disparate, and their differing rice cultivars should be physiologically distinct; consequently, these two factors probably jointly influenced the accumulation of IHg in rice, leading to the statistical difference found in THg concentration. Overall, single factor influences the Hg bioaccessibility through its affection on micro factors (e.g. Hg forms), thus the differentiation can show statistically significant. But when the combined effects of multiple factors were taken into account, it seems likely to have little influence on both MeHg and THg bioaccessibility. The possible reason was implied that the affections of single factors were too microscopic and easily covered by the affection of macro factors in combined factors, thus the statistical analysis showed mostly unaffected when compared with single factors discussed above.

Table 8 – Between-subjects effect tests of multiple factors on Hg bioaccessibility.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig. [¶]
Corrected Model	MeHg[†]	2850.127[a]	7	407.161	2.067	.077
	THg[†]	6225.457[b]	7	889.351	3.612	.006
Intercept	MeHg	103828.345	1	103828.345	526.975	.000
	THg	95251.851	1	95251.851	386.841	.000
Factor 1[‡]	MeHg	122.445	1	122.445	.621	.436
	THg	224.319	1	224.319	.911	.347
Factor 2[‡]	MeHg	215.262	1	215.262	1.093	.304
	THg	4823.341	1	4823.341	19.589	.000
Factor 3[‡]	MeHg	1833.005	1	1833.005	9.303	.005
	THg	187.006	1	187.006	.759	.390
Factor 1*2	MeHg	425.031	1	425.031	2.157	.152
	THg	993.462	1	993.462	4.035	.053
Factor 1*3	MeHg	41.050	1	41.050	.208	.651
	THg	4.199	1	4.199	.017	.897
Factor 2*3	MeHg	57.914	1	57.914	.294	.591
	THg	80.933	1	80.933	.329	.570
Factor 1*2*3	MeHg	60.491	1	60.491	.307	.583
	THg	15.844	1	15.844	.064	.801
Error	MeHg	6304.871	32	197.027		
	THg	7879.352	32	246.230		
Total	MeHg	115775.687	40			
	THg	110301.530	40			
Corrected Total	MeHg	9154.997	39			
	THg	14104.809	39			

a. R Squared = .311 (Adjusted R Squared = .161)
b. R Squared = .441 (Adjusted R Squared = .319)
¶. Sig. is a value indicates a significant influence when less than 0.05.
†. THg and MeHg in this table represents THg and MeHg bioaccessibility.
‡. Factor 1, 2 and 3 in this table represents rice cultivar, planting location and cooking respectively.

4. Conclusion

Driven by mounting concerns about exposure to Hg through the food chain, Hg bioaccessibility is of interest because it is implicitly associated with potential health risks. In this study, rice cultivars, planting locations, and cooking were considered as three candidate factors impacting the Hg bioaccessibility of rice. The results showed (1) the three rice cultivars (japonica, indica, and glutinous rice) differed little in their Hg concentration and Hg bioaccessibility, and apart from glutinous rice with respect to MeHg bioaccessibility, these differences were not significant in the statistical tests. This means the method of partition of rice cultivar was too general and subdivision on rice type may be necessary to investigate the differentiation of Hg bioaccessibility. (2) Cooking can reduce MeHg bioaccessibility considerably, due to the denaturation of proteins and amino acids at high temperatures, but this had limited influence on IHg bioaccessibility. Given the relatively high IHg concentrations in the rice samples, their THg bioaccessibility was negligibly affected by cooking. (3) THg bioaccessibility differed between the two rice sample groups from different provinces; hence, planting location is presumably capable of affecting the IHg forms and this was responsible for that outcome. (4) Statistically, the three studied impact factors had a limited joint effect on THg and MeHg bioaccessibility of rice. Our results showed that Hg bioaccessibility in rice may be affected by micro factors, such as the different IHg and MeHg forms, with macro factors (rice cultivar and planting location) mainly modulating the micro ones to impact the overall Hg bioaccessibility. Thus if the impact factors are too general, their differentiation would fail to reveal statistically relevant patterns. Instead, subdividing these factors into their measurable and testable components may be needed to figure out their effects on Hg bioaccessibility in rice.

Acknowledgments

This study was supported by the research fund of Guizhou Minzu University (No. GZMUZK[2021]YB14) and the open research fund of Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education of the People's Republic of China (No. QJH-KY[2020]247).

REFERENCES

Afonso, C., Costa, S., Cardoso, C., Oliveira, R., Lourenço, H.M., Viula, A., et al., 2015a. Benefits and risks associated with consumption of raw, cooked, and canned tuna (*Thunnus* spp.) based on the bioaccessibility of selenium and methylmercury. Environ. Res. 143, 130–137.

Afanso, C., Costa, S., Cardoso, C., Bandarra, N.M., Batista, I., Coelho, I., et al., 2015b. Evaluation of the risk/benefit associated to the consumption of raw and cooked farmed meagre based on the bioaccessibility of selenium, eicosapentaenoic acid and docosahexaenoic acid, total mercury, and methylmercury determined by an in vitro digestion model. Food Chem. 170, 249–256.

Brombach, C.C., Manorut, P., Kolambage-Dona, P.P.P., Ezzeldin, M.F., Chen, B., Corns, W.T., et al., 2017. Methylmercury varies more than one order of magnitude in commercial European rice. Food Chem. 214, 360–365.

Du, S.K., Yang, H.D., Yu, X.Z., Zhang, Y.L., Li, Z.X., 2010. Quality properties of commercial rice. Food Science 36 (5), 78–81.

Fang, Y., Sun, X., Yang, W., Ma, N., Xin, Z., Fu, J., et al., 2013. Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China. Food Chem. 147, 147–151.

Feng, X., Qiu, G., 2008. Mercury pollution in Guizhou, Southwestern China - an overview. Sci. Total Environ. 400, 227–237.

Feng, X.B., Li, P., Qiu, G., Wang, S.F., Li, G.H., Shang, L.H., et al., 2008. Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou province, China. Environ. Sci. Technol. 42, 326–332.

Gong, Y., Luis, M.N., Greenfield, B.K., Zhen, Q., Yang, Q.Q., Huang, L., et al., 2018. Bioaccessibility-corrected risk assessment of urban dietary methylmercury exposure via fish and rice consumption in China. Sci. Total Environ. 630, 222–230.

Laird, B.D., Chan, H.M., 2013. Bioaccessibility of metals in fish, shellfish, wild game, and seaweed harvested in British Columbia, Canada. Food Chem. Toxicol. 58, 381–387.

Li, P., Feng, X., Qiu, G., Shang, L., Wang, S., 2008. Mercury exposure in the population from Wuchuan mercury mining area, Guizhou, China. Sci. Total Environ. 395, 72–79.

Liao, W., Wang, G., Li, K., Zhao, W., Wu, Y., 2019. Effect of cooking on speciation and in vitro bioaccessibility of hg and as from rice, using ordinary and pressure cookers. Biol. Trace Elem. Res. 187, 329–339.

Lin, H.F., Santa-Rios, A., Barst, B.D., Basu, N., Bayen, S., 2019. Occurrence and bioaccessibility of mercury in commercial rice samples in T Montreal (Canada). Food Chem. Toxicol. 126, 72–78.

Liu, J., Meng, B., Poulain, A.J., Meng, Q., Feng, X., 2021. Stable isotope tracers identify sources and transformations of mercury in rice (*Oryza sativa* L.) growing in a mercury mining area. Fundam. Res. 1 (3), 259–268.

Martin, M., Fitzgerald, M.A., 2002. Proteins in rice grains influence cooking properties. J. Cereal Sci. 36, 285–294.

Matos, J., Lourenço, H.M., Brito, P., Maulvault, A.L., Martins, L.L., Afanso, C., 2015. Influence of bioaccessibility of total mercury, methyl-mercury and selenium on the risk/benefit associated to the consumption of raw and cooked blue shark (*Prionace glauca*). Environ. Res. 143, 123–129.

Maulvault, A.L., Machado, R., Afanso, C., Lourenço, H.M., Nunes, M.L., Coelho, I., et al., 2011. Bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab. Food Chem. Toxicol. 49, 2808–2815.

Meng, B., Feng, X.B., Qiu, G.L., Cai, Y., Wang, D.Y., Li, P., et al., 2010. Distribution patterns of inorganic mercury and methylmercury in tissues of rice (*Oryza sativa* L.) plants and possible bioaccumulation pathways. J. Agric. Food Chem. 58, 4951–4958.

Meng, B., Feng, X.B., Qiu, G.L., Anderson, C.W.N., Wang, J., Zhao, L., 2014. Localization and speciation of mercury in brown rice with implications for Pan-Asian public health. Environ. Sci. Technol. 48, 7974–7981.

Ng, J.C., Juhasz, A., Smith, E., Naidu, R., 2013. Assessing the bioavailability and bioaccessibility of metals and metalloids. Environ. Sci. Pollut. Res. doi:10.1007/s11356-013-1820-9.

Ouedraogo, O., Amyot, M., 2011. Effects of various cooking methods and food components on bioaccessibility of mercury from fish. Environ. Res. 111, 1064–1069.

Qian, Y., Chen, C., Zhang, Q., Li, Y., Chen, Z., Li, M., 2010. Concentrations of cadmium, lead, mercury and arsenic in Chinese market milled rice and associated population health risk. Food Control 21, 1757–1763.

Qiu, G.L., Feng, X.B., Li, P., Wang, S.F., Li, G.H., Shang, L.H., et al., 2008. Methylmercury accumulation in rice (*Oryza sativa* L.) grown at abandoned mercury mines in Guizhou, China. J. Agric. Food Chem. 56, 2465–2468.

Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M., 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ. Sci. Technol. 30, 422–430.

Siedlikowski, M., Bradley, M., Kubow, S., Goodrich, J.M., Franzblau, A., Basu, N., 2016. Bioaccessibility and bioavailability of methylmercury from seafood commonly consumed in North America: *in vitro* and epidemiological studies. Environ. Res. 149, 266–273.

Torres-Escribano, S., Ruiz, A., Barrios, L., Vélez, D., Montoro, R., 2011. Influence ofmercury bioaccessibility on exposure assessment associated with consumption of cooked predatory fish in Spain. J. Sci. Food Agric. 91, 981–986.

EFSA, 2012. Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. URL: https://www.efsa.europa.eu/en/efsajournal/pub/2985, Last accessed date: May, 2022.

USEPA, 2012. Standard operating procedure for an *in vitro* bioaccessibility assay for lead in soil. URL: https://nepis.epa.gov/Exe/ZyPDF.cgi/P100GESL.PDF?Dockey=P100GESL.PDF, Last accessed date: May, 2022.

USEPA, 2002. Method 1631, revision e: mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. URL: https://www.epa.gov/sites/default/files/2015-08/documents/method_1631e_2002.pdf, Last accessed date: May, 2022.

UNEP, 2013. Mercury: time to act. URL: http://cwm.unitar.org/cwmplatformscms/site/assets/files/1254/mercury_timetoact.pdf, Last accessed date: May, 2022.

USEPA, 1998. Method 1630: methyl mercury in water by distillati on, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry. URL: https://www.epa.gov/sites/default/files/2015-08/documents/method_1630_1998.pdf.

Wang, H.S., Xu, W.F., Chen, Z.J., Cheng, Z., Ge, L.C., Man, B.Y., et al., 2013. *In vitro* estimation of exposure of Hong Kong residents to mercury and methylmercury via consumption of market fishes. J. Hazard. Mater. 248-249, 387–393.

Wang, G., Gong, Y., Zhu, Y.X., Miao, A.J., Yang, L.Y., Zhong, H., 2017. Assessing the risk of hg exposure associated with rice consumption in a typical city (Suzhou) in Eastern China. Int. J. Environ. Res. Public Health doi:10.3390/ijerph14050525.

Wu, Z., Feng, X.B., Li, P., Lin, C.J., Qiu, G.L., Wang, X., et al., 2018. Comparison of *in vitro* digestion methods for determining bioaccessibility of Hg in rice of China. J. Environ. Sci. 68, 185–193.

Xu, X.H., Han, J.L., Pang, J., Wang, X., Lin, Y., Wang, Y.J., et al., 2020. Methylmercury and inorganic mercury in Chinese commercial rice: implications for overestimated human exposure and health risk. Environ. Pollut. 258, 113706.

Zhang, H., Feng, X.B., Larssen, T., Qiu, G., Vogt, R.D., 2010a. In inland China, rice, rather than fish, is the major pathway for methylmercury exposure. Environ. Health Perspect. 118, 1183–1188.

Zhang, H., Feng, X.B., Larssen, T., Shang, L.H., Li, P., 2010b. Bioaccumulation of methylmercury versus inorganic mercury in rice (*Oryza sativa* L.) grain. Environ. Sci. Technol. 44, 4499–4504.