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ORIGINAL PAPER

Predictive modeling of methylmercury in rice (*Oryza sativa* L.) and species-sensitivity-distribution-based derivation of the threshold of soil mercury in karst mountain areas

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Abstract The bioavailable mercury (Hg) in the soil is highly active and can affect the formulation of methyl-Hg (MeHg) in soil and its accumulation in rice. Herein, we predicted the concentration of MeHg in rice using bioavailable Hg extracted from soils; additionally, we determined the threshold value of soil Hg in karst mountain areas based on species sensitivity distribution. The bioavailable Hg was extracted using calcium chloride, hydrochloric acid (HCl), diethylenetriaminepentaacetic acid mixture, ammonium acetate, and thioglycolic acid. Results showed that HCl is the best extractant, and the prediction model demonstrated good predictability of the MeHg concentration in rice based on the HCl-extractable Hg, pH, and soil organic matter (SOM) data. Compared with the actual MeHg concentration in rice, approximately 99% of the predicted values (n = 103) were within the 95% prediction range, indicating the good performance of the rice MeHg prediction model based on soil pH, SOM, and bioavailable Hg in karst mountain areas. Based on this MeHg prediction model, the safety threshold of soil Hg was calculated to be 0.0936 mg/kg, which is much lower than the soil pollution risk screening value of agricultural land (0.5 mg/kg), suggesting that a stricter standard should be applied regarding soil Hg in karst mountain areas. This study presents the threshold of soil Hg pollution for rice safety in karst mountain areas, and future studies should target this threshold range.

Keywords Multiple linear regression analysis · Chemical extraction · Bioavailable Hg · Soil Hg thresholds · High geological background area

Introduction

Mercury (Hg) pollution is becoming a global concern due to its harmful effects on human beings and the

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environment. Hg exists in both inorganic and organic forms. Under specific conditions, inorganic Hg is converted into a more toxic form, namely, methylmercury (MeHg). Exposure to MeHg may cause nervous system disorders, cardiovascular diseases, kidney and liver damage, and visual impairment in humans (Beckers & Rinklebe, 2017), and may even reduce the intelligence quota of children (Tang et al., 2015). Presently, rice is considered a MeHg-accumulating plant, and its consumption can be a major source of MeHg intake in addition to that of fish (Qiu et al., 2008). Because rice is a staple food worldwide, ensuring the safety of its production is essential for human health (Xu et al., 2020a).

Rice MeHg primarily originates from soils; MeHg in paddy soils is absorbed by roots and translocated to the aboveground parts of rice plants, where it eventually accumulates in grains during the ripening period (Meng et al., 2011). Generally, Hg accumulation in paddy soils is subject to soil properties (Li et al., 2022), particularly soil pH and soil organic matter (SOM) (Ding et al., 2014). Under low pH conditions, Hg-methylating microorganisms exhibit high microbial activity, which is conducive to promoting Hg methylation in the soil (Tang et al., 2018). Furthermore, a high SOM content provides adequate carbon sources, increasing the activity of Hg-methylating microorganisms and promoting MeHg production in paddy soils (Hang et al., 2016; Yin et al., 2018).

Although the soil pH and the SOM can affect each other and MeHg accumulation in soil, the effects of MeHg accumulation in rice are not clearly understood. Rice Hg concentrations are generally related to the soil Hg content; rice plants mainly absorb bioavailable Hg from the soil (Zhang et al., 2018), which is defined as the fraction of contaminants in the soil that are directly or indirectly available for plant uptake and for assimilation by soil microorganisms (Scheckel et al., 2009). Bioavailable Hg also affects soil physicochemical properties; therefore, it is important to explore the interactive effect of bioavailable Hg on accumulation in rice–soil systems.

Chemical extraction methods are widely employed to assess Hg speciation in soils. Sequential extraction procedures (SEPs), including the Tessier and modified BCR methods, have played an important role in the fraction analysis of Hg in soils (Liang et al., 2019; Xu et al., 2019). The SEP-extractable soluble Hg fraction and a considerable fraction of Hg held in the solid phase of soil are considered bioavailable Hg (Huang et al., 2020). Usually, all single extraction methods can be mainly categorized into the following three groups: (1) weak or dilute solutions of strong acids (such as hydrochloric acid [HCl], acetic acid, and nitric acid [HNO_3]) (Monteiro et al., 2016); (2) neutral salts (such as calcium chloride [$CaCl_2$], sodium nitrate, and ammonium acetate [CH_3COONH_4]) (Feng et al., 2005; Shetaya et al., 2017); and (3) chelating agents (such as ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid [DTPA]) (Huang et al., 2020; Wang et al., 2017). Compared with those of SEPs, single extraction methods extract a similar amount of bioavailable Hg and are more time-free and cost-effective (Dong et al., 2019; Jing et al., 2008; Tessier et al., 1979).

Many studies suggest that the bioconcentration factor (BCF) is an effective indicator of heavy metal pollution levels in crops (Zhang et al., 2010). A higher BCF indicates a greater ability of rice plants to accumulate Hg from soils. However, to date, only a few studies have involved with quantitative models based on soil properties related to the ability of rice to accumulate MeHg. Recently, individual prediction models of rice MeHg were constructed based on soil pH and SOM to predict rice total Hg (THg) concentrations; these models measure MeHg concentrations in rice within twofold prediction intervals (Du et al., 2023). However, geochemical factors cannot control MeHg phytoavailability in different soils. A study on the extraction of accumulated MeHg from rice grains with different extractants showed a good correlation between the extracted MeHg and rice grain MeHg, establishing an extraction method for soil bioavailable Hg; ammonium thiosulfate was proven useful in screening Hg-contaminated soils in Hg mining areas (Zhu et al., 2015). Soil pH and SOM play important roles in rice MeHg accumulation. It is important to construct a suitable model based on the interactions among soil pH, SOM, and bioavailable Hg for rice MeHg accumulation in karst mountain areas. The development of good predictive models for assessing Hg transfer from soil into the edible parts of food crops is crucial for preventing rice MeHg pollution (Rodrigues et al., 2012).

Usually, an empirical model is used to determine the safety threshold of Hg in agricultural soils. The species sensitivity distribution (SSD) method is commonly used in ecological risk assessments (Yu et al., 2023). The SSD method collects the Hg enrichment factors of the soil–crop system and uses a distribution model to fit the crop enrichment factors and accumulation probability; subsequently, a target to protect 95% of the biological species in the farmland was established, and the hazardous Hg concentrations for 5% of the species (HC5 values) were considered the Hg concentration values of the contaminants (Ding et al., 2018). The SSD method is also preferred as a quality method for soil quality standards in China (Gao et al., 2021); therefore, SSD was employed to perform a risk assessment of Hg at the whole-ecosystem level and set soil thresholds.

China is enriched with karstic paddy soils. The unique hydraulic and hydrogeological characteristics of karst mountain areas render them highly vulnerable to pollution from activities, making these karstic arable soils considerably problematic (Shen et al., 2017; Zeng et al., 2011). Although the background concentration of heavy metals is high in karst areas, the bioavailable Hg content is lower than that of zinc and cadmium (Xu et al., 2016). To manage the threshold of Hg pollution in arable soils, China has set standards for agricultural land pollution; however, these standard values may not be suitable for karst areas. The safety threshold of Hg in agricultural soils is set mainly referring to the maximum safe dose or concentration of Hg in agricultural soils without harmful effects on exposed organisms in their ecosystem (Gao et al., 2021). However, owing to differences in soil and regional characteristics, only a few studies have focused on the MeHg content in rice, and a feasible standard for predicting Hg in karst mountain soils is unavailable.

The objectives of this study were: (1) to develop a prediction model for rice Hg content based on the soil pH, SOM, and bioavailable Hg, and verify the accuracy of the model to provide a new method and evidence for predicting rice MeHg accumulation; and (2) to derive the soil thresholds of Hg in paddy soils by using the SSD curve and calculate the HC5 value. The present study provides an effective model for predicting MeHg transfer from soil to rice and a new safety threshold for soil Hg in karst mountain areas.

Materials and methods

Sample collection

Guizhou province, the most important Hg production base in China, possesses an estimated Hg reserve of 888,000 tons, accounting for approximately 78% of China's total reserves (Qiu et al., 2009). Hg contents in the surface (0–25 cm depth) and deeper soils in Guizhou are 3–5 times greater than those in the other regions of China, largely due to the high geochemical background Hg levels (Xia et al., 2022). Longli is rich in sulfur and iron (S and Fe) ore mining, Dushan is characterized by antimony (Sb) ore, Kaiyang is known for rich phosphate ore (P), and Duyun and Shuicheng are characterized by lead and zinc (Pb and Zn) mining (Kong et al., 2018; Li et al., 2023). Large amounts of Hg were released during these industrial processes. In total, 103 mature-stage rice grains and corresponding rhizosphere soil samples (0–20 cm) were collected from the above five cities in 2020. Each soil sample was preserved in a polyethylene zip-locked bag, and the soil samples were completely air-dried and sieved through a 200-mesh sieve in the laboratory. Approximately 30 g of rice was weighed and rinsed with ultrapure water three times, followed by freeze-drying using a freeze drier (FDU2110, EYELA, Japan). The rice sample was powdered using a grinder (IKA-A11 basic, IKA, Germany). The powder was passed through an 80-mesh sieve (size: 177 μm) and then stored in zip-locked plastic bags until further analysis.

Sample chemical analysis

Soil pH and organic matter

The soil pH was determined at a 1:2.5 (weight/volume) soil/water ratio using a pH meter (LeiciZD-2; Shanghai, China). The soil organic matter (SOM) content was analyzed through potassium dichromate oxidation via the oil bath heating method (He et al., 2019).

THg and MeHg in rice and soil

To determine the rice THg concentration, approximately 0.5 g of rice sample was weighed in a borosilicate glass tube and digested using a mixture of HNO_3

and sulfuric acid (4:1, volume/volume) at 95 °C for 3 h in a water bath. The digested sample was subsequently diluted to 25 mL using ultrapure water, and THg was determined via cold-vapor atomic-fluorescence spectroscopy (CVAFS, Model III, Brooks Rand, USA), according to the United States Environmental Protection Agency (USEPA) method 1631E (USEPA, 2002). To determine rice MeHg, approximately 0.5 g of sample was weighed in a centrifuge tube and digested using 25% potassium hydroxide in methanol for 3 h in a water bath at 75–80 °C. Following this, the digested solutions were acidified using HCl, and MeHg in the sample was extracted using dichloromethane (CH_2Cl_2) and back-extracted into the water phase for gas chromatography–CVAFS (GC–CVAFS), according to the USEPA method 1630 (USEPA, 1998), for determining rice MeHg.

To determine soil THg, approximately 0.1–0.3 g of soil sample was heated at 95 °C for 30 min in a mixture of HCl and HNO_3 (3:1, volume/volume) using a water bath. Following this, bromium chloride was added to the sample, and the mixture was heated at 95 °C for another 30 min. Finally, an appropriate amount of digested solution was subjected to cold-vapor atomic absorption spectrometry (Brooks Rand, USA) for soil THg determination (Feng et al., 2007). To determine soil MeHg, approximately 0.2–0.5 g of soil sample was subjected to MeHg extraction using CH_2Cl_2 after leaching with a saturated solution of copper sulfate and HNO_3 (Liang et al., 1996). Following this, an appropriate amount of digested solution was subjected to GC–CVAFS, according to the USEPA method 1630 (USEPA, 1998), for determining soil MeHg. All the acids used in this study were of ultrapure grade and other reagents were of analytical grade (Sinopharm Chemical Reagent Co., Ltd, China). The CH_2Cl_2 reagent was of chromatographic grade (Tedia Company, Inc., USA). The vials were rinsed with double-distilled water and preheated in a muffle oven (500 °C, 45 min) to ensure low Hg blanks.

Bioavailable Hg extraction

The bioavailable Hg in the soil was extracted using the following five extractants: 0.1 M $CaCl_2$, 0.1 M HCl, a DTPA mixture ($DTPA_{mix}$; 0.0005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M triethylamine), 1 M CH_3COONH_4, and 0.03% thioglycolic acid (TGA) (Huang et al., 2020; Reis et al., 2015; Różański et al., 2016). The soil samples (2 g) were added to different extractants in a 10:1 ratio (liquid/solid) and shaken for 1 h at room temperature, followed by centrifugation at 3500 $r\cdot min^{-1}$ for 30 min. Finally, the supernatants were passed through a 0.45-μm filtration membrane, and the extract was stored in the refrigerator until it was used for Hg content analyses. The modified BCR method includes four steps (Table 1) (Pueyo et al., 2001).

QA/QC

The detection limits for THg and MeHg were 0.0120 μg/kg (3σ) and 0.00600 μg/kg (3σ), respectively. Both THg and MeHg analyses were validated using duplicates, method blanks, matrix spikes, and certified reference materials. The following certified reference materials were used in this study: THg estimation, citrus leaf (GSB-11) for rice and soil composition standard material (GSS-5) for soil; and MeHg estimation, lobster hepatopancreas reference material for trace metals (Tort-2) for rice and estuarine sediment Hg and MeHg (CC-580) for soil. One standard reference material and three repeats for a random sample were set for every 20 samples. For THg, the measured value of GSB-11 was 150 ± 20 μg/kg, and that of GSS-5 was 290 ± 30 μg/kg. For MeHg, the measured value of Tort-2 was 270 ± 60 μg/kg, and that of CC-580 was 150 ± 20 μg/kg. The recovery rates were 92–110%, 90–105%, 95–103%, and 90–108% for soil THg, rice THg, rice MeHg, and soil MeHg, respectively. For Hg speciation in rhizosphere soils, the recovery rate was 93.6–102.2% for soil THg speciation (F1 + F2 + F3 + F4).

Calculating the soil Hg thresholds

To determine the soil MeHg thresholds, the SSD resulting from the accumulation of Hg in rice was investigated. The prediction model for MeHg transfer from soil to rice was subsequently constructed by including the soil pH and SOM information. The prediction model was developed and verified against actual measurements of rice MeHg concentrations. Finally, SSD curves were constructed, and HC5 values were calculated as follows (Gao et al., 2021):

Table 1 The procedures of the modified BCR

Fraction	Fraction definition	Extraction reagent	Chemical extraction process
F1	Acid solube fraction	0.11 M CH_3COOH	(1) 1 g of soil was mixed with 40 ml 0.11 M CH_3COOH in a 50 ml centrifuge tube and was shaken for 24 h in an end-over-end shaker at 120 rpm at room temperature. The separation of extract from solid residue was conducted by centrifugation at 3500 rpm for 20 min. The supernatant was decanted gently into a glass test tube and stored at 4 °C. The residue was washed with 20 ml deionized water twice, and the supernatant was decanted and discarded
F2	Reducible fraction	0.1 M $NH_2OH·HCl$	(2) The residue from step 1 was extracted with 40 ml 0.1 M $NH_2OH·HCl$ (pH = 1.5 with HNO_3), shaken for 16 h, and then centrifuged at 3500 rpm for 20 min. The supernatant and residue in this step were obtained as previously described
F3	Oxidizable fraction	30% H_2O_2 + 0.5 M CH_3COONH_4	(3) The residue from step 2 was treated with 5 ml 30% H_2O_2. The digestion was proceeding at room temperature for 1 h with manual shaking at 10 min interval, and then digested at 85 °C for 1 h in a water bath until the mixture was reduced to 2–3 ml. After cooling, an additional 5 ml of 30% H_2O_2 was added and repeated the above procedure. Finally, 50 ml of 0.5 M CH_3COONH_4 (pH = 2 with HNO_3) was added to the mixture and shaken for 16 h at room temperature, then centrifuged at 3500 rpm for 20 min. The supernatant and residue in this step were obtained as previously described
F4	Residual fraction	HCl + HNO_3 + HF	(4) Soild residue from step 3 was digested in a Teflon crucible with an acid mixture of concentrated HCl (4.5 ml), HNO_3 (1.5 ml), and HF (2 ml) and heated at on a hot plate up to dryness. After cooling, the sample was dissolved in a 2 ml of high pure HCl, and diluted with deionized water to a volume of 25 ml

$$\mathrm{BCF} = \frac{C_{plant}}{C_{soil}} \tag{1}$$

$$y = \frac{a}{1 + \left(\frac{x}{x0}\right)^{b}} \tag{2}$$

$$C_{soil} = \frac{1}{BCF} \times C_{rice} \tag{3}$$

In formula (1), C_{plant} and C_{soil} are the MeHg concentrations (μg/kg) in rice and soil, respectively. In this study, we used a flexible function to fit the cumulative probability distribution of BCF data for rice to obtain the SSD curve (Du et al., 2021). In formula (2), x is the 1/BCF, y is the cumulative probability of x, and a and x_0 are the fitting parameters. The 1/BCF values were the abscissa, and the cumulative probability was the ordinate. The HC5 value in rice field was calculated based on the cumulative probability distribution curve, and formula (3) was used to calculate the MeHg concentration threshold in soil, where C_{rice} (10.2 μg/kg) was considered the limit of MeHg content in rice (Xu et al., 2020b). The thresholds of Hg in soils were calculated by the soil MeHg to soil THg ratio.

Data analyses

All the data analyses were performed using the Statistical Package for Social Sciences 25.0 (New York, USA) software package, and the Origin 2023 software was used for drawing the figures.

Results and discussion

Rice THg and MeHg concentrations

The THg content in rice ranged from 1.48 to 61.4 μg/kg (mean: 9.34 ± 10.2 μg/kg) (Fig. 1). The THg results showed that 8.74% (9/103) of the samples exceeded the Chinese National Standard Limit for rice THg (20 μg/kg), with a maximum rice THg concentration of approximately 307% of the national standard rice THg limit. Among the different cities, Dushan had the highest mean THg concentration (mean: 15.0 ± 14.6 μg/kg, range: 2.46–61.4 μg/kg),

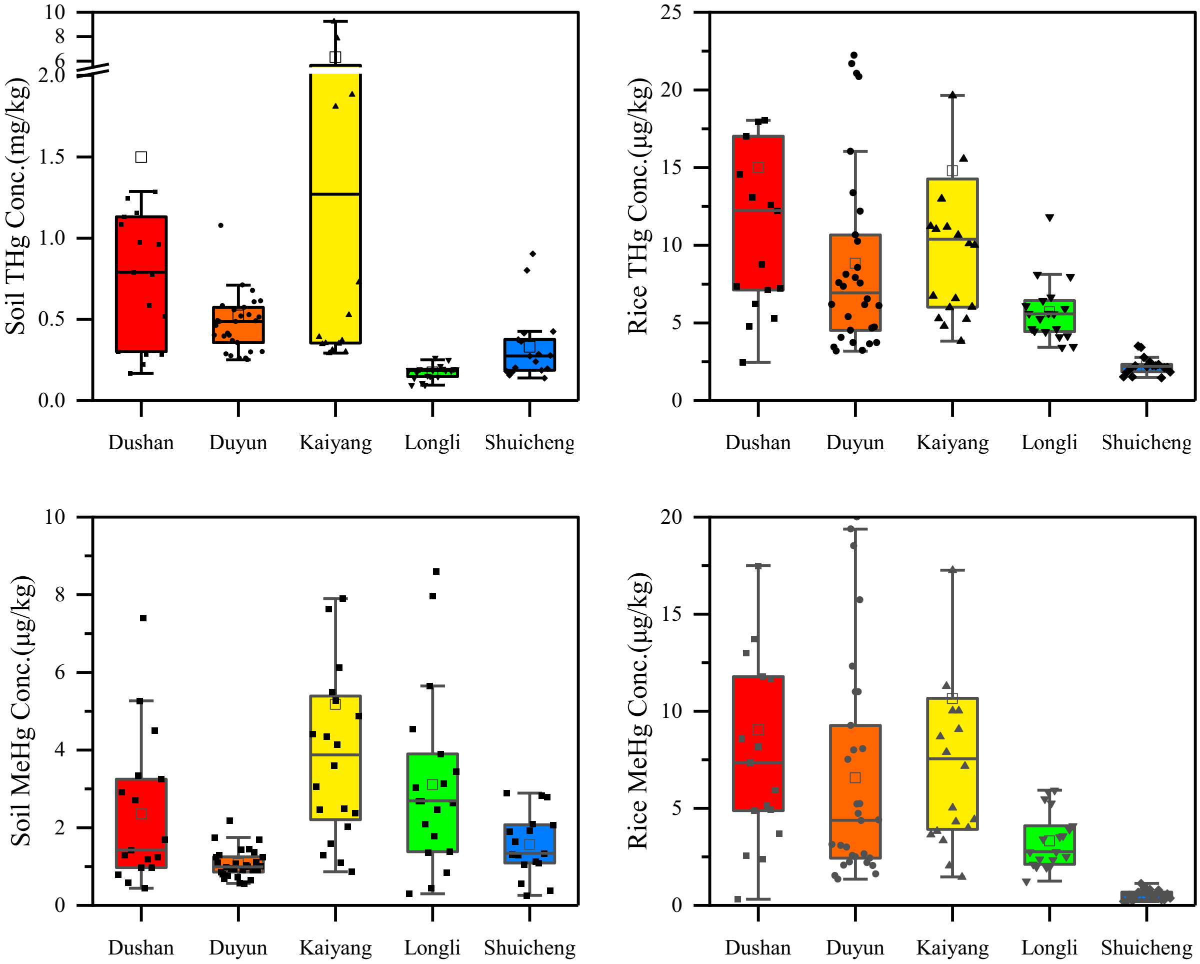

Fig. 1 THg and MeHg concentrations of soil and rice

followed by Kaiyang, Duyun, Longli, and Shuicheng. The rice THg concentrations in this study were lower than those in the Hg mining area in Wanshan, where the THg concentration of approximately 60% of the rice samples (range: 0.500–188 μg/kg) exceeded the Chinese National Standard Limit for rice THg (20 μg/kg) (Yin et al., 2018). However, some samples in this study had higher rice THg levels than those in Heilongjiang and Guangdong (Zhao et al., 2020). This could be because the study areas were located in surrounding farmlands without Hg mining; therefore, the concentrations were lower than those in Wanshan; however, the study areas experienced mining-induced pollution; therefore, the concentrations were higher than those in Heilongjiang and Guangdong.

The MeHg content in rice ranged from 0.202 to 38.3 μg/kg (mean: 6.18 ± 7.15 μg/kg). The results showed that 17.5% (18/103) of the samples exceeded the limit for rice MeHg (10.2 μg/kg), as recommended by a previous study (Xu et al., 2020a, 2020b, 2020c). The maximum rice MeHg concentration was approximately 375% of the rice MeHg limit reported in a previous study (Wang et al., 2020). The highest mean rice MeHg content was found in Kaiyang (mean: 10.7 ± 10.5 μg/kg, range: 1.47–38.3 μg/kg), followed by Dushan, Duyun, Longli, and Shuicheng. The concentrations of rice MeHg accounted for approximately 58% of the rice THg, which is similar to the proportion of MeHg in Wanshan rice (45.7%). The rice THg concentrations were lower than those of rice from mining areas in Wanshan, and the MeHg concentration was also lower than that in a previous study (38.1 μg/kg) (Li et al., 2017). The concentration of rice MeHg was positively correlated with the concentration of soil THg, suggesting that Hg accumulation in rice is associated with soil Hg pollution (Fig. 2).

Soil THg, MeHg, and bioavailable Hg concentrations

The pH of the soil ranged from 4.53 to 7.80 (mean: 6.82) (Table 2), indicating the acidic to slightly acidic nature of the soils; soils with a pH between 5.00 and 7.50 strongly affect the Hg concentrations in soil and rice (Ding et al., 2018). Regarding the distribution rate, 27 of the samples had a soil pH ≤ 6.50, accounting for 26.3% of the total; 63 had a pH between 6.50 and 7.50, accounting for 61.2% of the total; and only 12 samples had a pH > 7.50. The SOM content ranged from 2.54 to 31.0 mg/kg (mean: 14.2 mg/kg).

Table 2 The soil pH value and soil organic matter in karst mountain areas

Soil physical properties	pH	SOM
Minimum value	4.53	2.55
Median	7.02	13.9
Maximum value	7.80	31.0
Mean value	6.82	14.2
Standard deviation	0.710	6.32

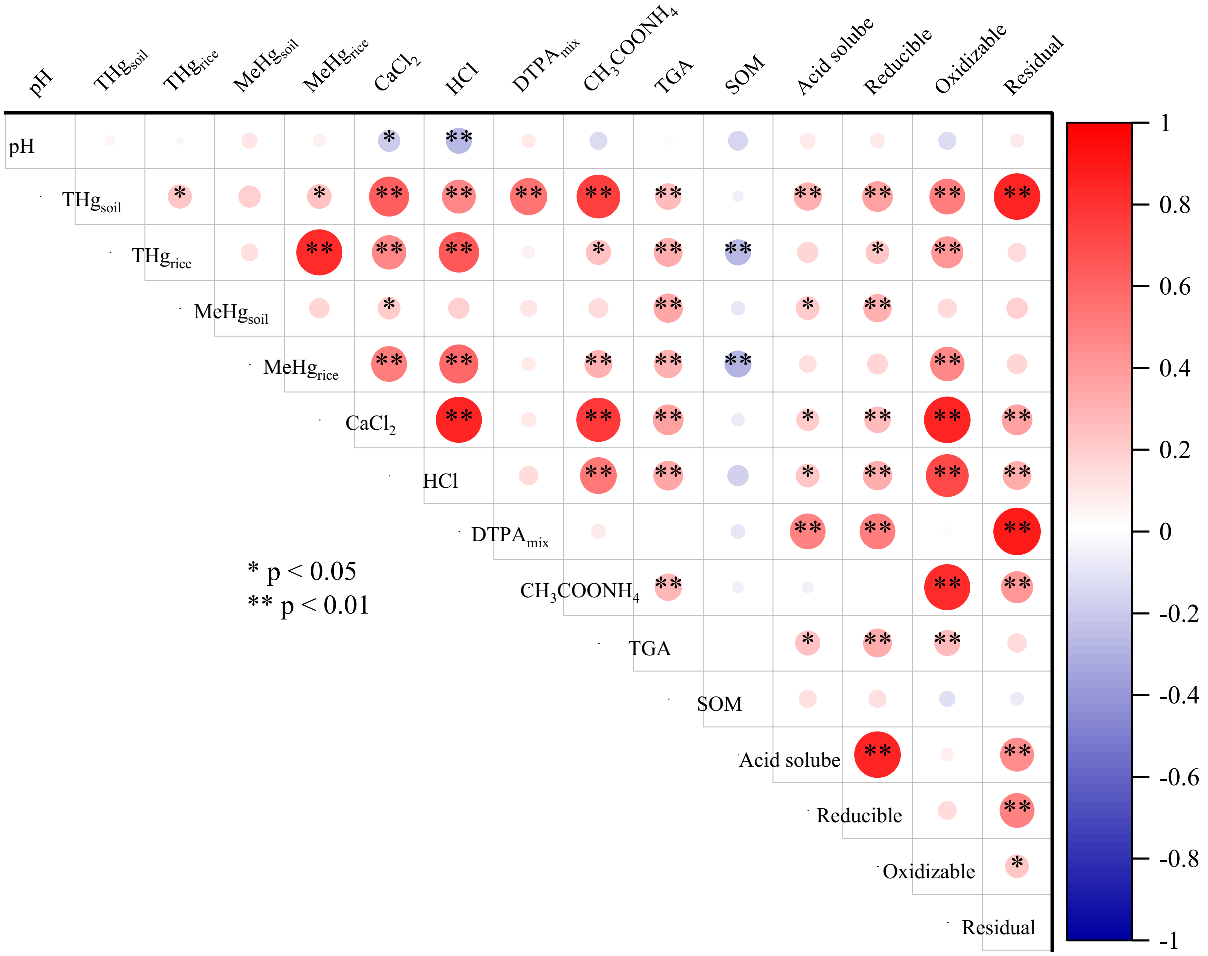

Fig. 2 The correlation between soil pH, SOM, the concentrations of bioavailable Hg extracted by different extractants, and the concentrations of different forms Hg extracted by the modified BCR

The THg and MeHg concentrations in the soil are shown in Fig. 1. The soil THg concentration varied greatly, ranging from 0.0953 to 48.0 mg/kg (mean: 1.71 mg/kg). The THg concentrations of 78.6% (81/103) soil samples exceeded the background value in Guizhou, and 29.1% (30/103) of the soil samples exceeded the soil risk screening values for agricultural land in China (Pan et al., 2021), indicating the need for preventive measures to control Hg pollution. Among the different cities, the highest mean THg value was found in Kaiyang, followed by Dushan, Duyun, Shuicheng, and Longli. Furthermore, some soil THg concentrations were higher than those in the Hg-mining areas in Wanshan (with a mean value of 2.70 ± 8.70 mg/kg), indicating severe soil THg pollution in the study area (Jin et al., 2023).

The soil MeHg concentration ranged from 0.270 to 32.3 μg/kg (mean: 2.54 μg/kg). The highest mean level of soil MeHg was observed in Kaiyang (mean: 5.17 ± 6.71 μg/kg, range: 0.870–32.2 μg/kg), followed by Longli, Dushan, Shuicheng, and Duyun. Compared with the soil MeHg concentrations in Wanshan, the soil MeHg concentrations in this study were lower (Xu et al., 2020c). Soil MeHg accounted for 0.67% of the THg in the soil, with the maximum proportion of MeHg being 4.50% and the minimum proportion being 0.950%, which is greater than that in Wanshan. This was possibly because of the favorable conditions of pH and SOM for Hg methylation (Galloway & Branfireun, 2004). The increased MeHg concentrations and their high percentages could pose potential health risks.

Herein, five commonly used methods were used to extract bioavailable Hg from the soil. The decreases in the concentrations of bioavailable Hg in soil after exposure to chemical extractants were as follows: 0.03% TGA > 0.1 M HCl > DTPA_{mix} > 0.1 M $CaCl_2$ > 1 M CH_3COONH_4, with mean bioavailable Hg values from these five extractants being 55.9 μg/kg, 2.01 μg/kg, 1.55 μg/kg, 0.889 μg/kg, and 0.378 μg/kg, respectively. The bioavailable Hg extracted by TGA, HCl, DTPA_{mix}, $CaCl_2$, and CH_3COONH_4 accounted for 11.84%, 0.4%, 0.18%, 0.12%, and 0.04% of the soil THg, respectively. Different extractants respond differently to different Hg forms (Kaninga et al., 2019). Reportedly, HCl is more effective in neutral to low-pH soil than in calcareous soil (Groenenberg et al., 2017). The soil samples in this study were acidic to slightly acidic, suggesting that the extraction efficiency of HCl was greater for bioavailable Hg. The higher the bioavailable Hg content was, the greater the uptake of Hg by the rice plants.

Relation between Hg concentration in rice and bioavailable Hg in soil

Correlations between rice MeHg concentrations and soil bioavailable Hg concentrations are shown in Fig. 2. The concentrations of TGA-, CH_3COONH_4-, $CaCl_2$-, HCl-, and DTPA_{mix}-extractable Hg exhibited highly significant correlations with soil THg (coefficient of association [r] = 0.28, $p < 0.01$; r = 0.75, $p < 0.01$; r = 0.63, $p < 0.01$; r = 0.46, p < 0.01; and r = 0.55, $p < 0.01$, respectively). A low pH in soil leads to an increase in dissolved Hg and a corresponding increase in bioavailable Hg in soil. However, the Hg concentrations extracted using different extractants exhibited different correlations with the rice MeHg concentration. Among them, the concentrations of bioavailable Hg ($CaCl_2$-, HCl-, CH_3COONH_4-, and TGA-extractable Hg) in soils were significantly positively correlated with rice MeHg ($p < 0.01$), with r = 0.30 and $p < 0.01$ for CH_3COONH_4-extractable Hg and r = 0.31 and $p < 0.01$ for TGA-extractable Hg.

Our results also showed that the different Hg forms exert different effects on soil and rice MeHg concentrations, and the pH and SOM can affect the Hg and MeHg concentrations in rice. Soil pH plays a crucial role in the soil Hg cycle, affecting the solid–liquid distribution of Hg (Ding et al., 2019; Liu et al., 2020; Mu et al., 2019).

Prediction model for rice MeHg concentration

Correlation analysis revealed that soil pH and SOM were two dominant soil properties that affected Hg uptake from paddy soil. Multiple linear regression analysis was performed to develop a predictive model for rice MeHg concentrations. The variables strongly correlated with rice MeHg concentration were selected as predictive factors for the effectiveness of the rice-use formula. The corresponding formulas are presented in Table 3, and they were used to calculate the Hg content in rice based on the soil Hg content extracted using $CaCl_2$, HCl, DTPA_{mix}, CH_3COONH_4, and TGA. Their r values were in the following order: HCl (0.655) > $CaCl_2$

Table 3 The prediction formula of rice MeHg concentrations extracted by five extractants

Extractant	Prediction formula	Coefficient of association
$CaCl_2$	MeHg-rice = − 1.292 + 1.378 × pH + 1.550 × THg ($CaCl_2$)–0.255 × SOM	R = 0.574
HCl	MeHg-rice = − 10.710 + 2.196 × pH + 2.043 × THg (HCl)–0.156 × SOM	R = 0.655
$DTPA_{mix}$	MeHg-rice = 8.878 + 0.245 × pH + 0.009 × THg ($DTPA_{mix}$)–0.312 × SOM	R = 0.291
CH_3COONH_4	MeHg-rice = 4.845 + 0.716 × pH + 1.072 × THg (CH_3COONH_4)–0.292 × SOM	R = 0.410
TGA	MeHg-rice = 6.874 + 0.165 × pH + 0.048 × THg (TGA)–0.319 × SOM	R = 0.424
THg-soil	MeHg-rice = 8.735 + 0.191 × pH + 0.000279 × THg(soil)–0.036 × SOM	R = 0.364

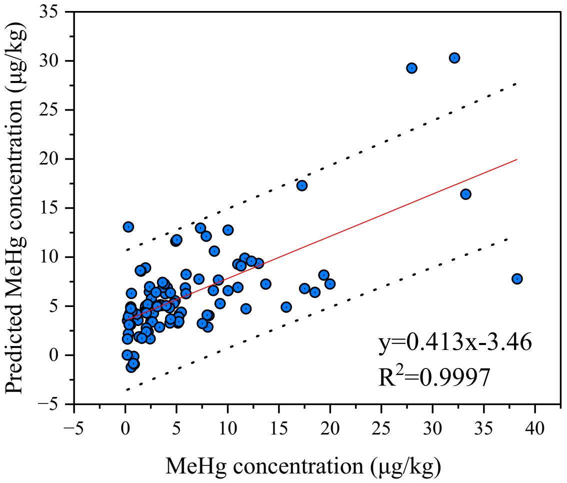

Fig. 3 Accuracy of the prediction MeHg concentration and the actual concentration

(0.574) > TGA (0.424) > CH_3COONH_4 (0.410) > $DTPA_{mix}$ (0.291). The HCl-extractable Hg exhibited the best performance, suggesting its considerable advantages in predicting the Hg concentration in rice in paddy soils. Compared with those of brown rice, the MeHg concentration prediction models consider soil pH and SOM (Du et al., 2023). The model developed in this study also considered bioavailable Hg in soil, which is a more comprehensive consideration.

The accuracy of the prediction model was determined by plotting the measured rice MeHg concentrations against the corresponding predicted Hg concentrations. Most of the predicted values were within the 95% prediction range (Fig. 3), indicating the good accuracy and applicability of the established model. The results of this study showed that the Hg concentration in rice can be predicted by multiple linear regression analysis.

Although multiple linear regression models could predict rice MeHg concentrations, they still involved some level of uncertainty. Previous studies have demonstrated that rice cultivar, water management, and soil type strongly affect the uptake of bioavailable Hg by rice. Furthermore, studies have shown that the different capacities of rice cultivars to accumulate heavy metals could result in varying Hg uptake (Wang et al., 2019; Xie et al., 2018). In the prediction model of HCl-extractable Hg, which has an r value of 0.655, the above reasons may need to be considered. Although the Hg contents in soil in this study are widely representative, considering our study areas are mainly in the karst high background area, we suggest that the established model is suitable at least in the karst high background area.

Validation of the soil Hg threshold based on rice safety

The rice uptake model indicated that BCF models can be used to extrapolate the inherent sensitivity of different rice samples. According to the rice MeHg model, the BCF data of HCl-extractable bioavailable Hg were obtained by normalizing the BCF under the combined soil pH and SOM. Similarly, the BCF was calculated by two methods: by predicting rice MeHg concentrations using the best-performing model, where bioavailable Hg was extracted with HCl, and by actually measuring rice MeHg concentrations. The BCF of the predicted MeHg concentrations varied from 0.008 to 16.6 (mean: 4.11), and the BCF of the actual rice MeHg concentrations varied from 0.007 to 24.1 (mean: 3.81).

To ensure that the rice MeHg concentration is below the maximum allowable limit, establishing an accurate soil MeHg threshold value is important. The HC5 value derived through the SSD method is an accurate approach for defining soil environmental quality benchmarks (Gao et al., 2021). Herein, the established multiple linear regression model was used to determine the soil MeHg threshold. The thresholds for soil MeHg can be back-calculated using two methods: one method is based on the predicted rice MeHg content, where the rice MeHg enrichment coefficient is calculated; second, SSD curve construction is performed using the logistics function to obtain the 1/BCF (HC5) value and, consequently, the safety threshold of soil MeHg; and, consequently, the other method is based on the determined rice MeHg content and follows the abovementioned steps to calculate the soil safety threshold of soil MeHg.

The SSD curves are shown in Fig. 4. Based on the SSD curves, the 1/BCF values were as follows:

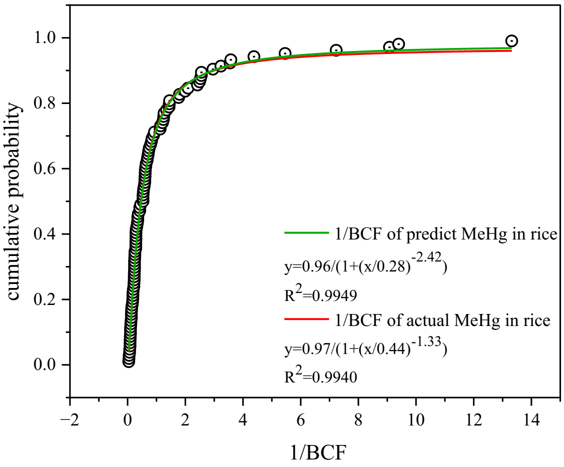

Fig. 4 The species sensitivity distribution curve conducted by the predict MeHg concentrations conducted by HCl and the rice actual MeHg concentrations

$$1/\mathrm{BCF} = 10^{\lg([0.96/y]-1)/2.42} + \lg(0.28)$$

$$1/\mathrm{BCF} = 10^{\lg([0.97/y]-1)/1.33} + \lg(0.44)$$

According to the prediction model, the soil MeHg concentration was determined to be 0.0627 mg/kg, and the soil MeHg concentration according to the actual rice MeHg concentration was found to be 0.182 mg/kg (Table 4). Accordingly, the soil THg thresholds were calculated to be 0.0936 and 0.272 mg/kg, considering that the MeHg content accounted for approximately 0.67% of the soil THg.

Compared with the Chinese national soil environment quality standard, the derived soil Hg thresholds were lower than the soil pollution risk screening value of agricultural lands (which is 0.5 mg/kg). The results indicated that a stricter soil threshold of Hg in paddy soil should be considered in typical karst areas. Additionally, rice cultivars should be considered to improve the accuracy of the threshold further.

Conclusions

Our study revealed that soil pH and SOM are important factors affecting Hg accumulation in rice. Based on the bioavailable Hg content in soils and soil properties, a multiple linear regression model was developed to quantitatively predict Hg accumulation in rice. The bioavailable Hg extracted using HCl was the most reliable factor for predicting the rice MeHg concentration (r = 0.655). The results indicate that the prediction model established in the present study has strong applicability, at least in areas with high karst backgrounds. The suggested soil Hg thresholds for paddy soils, calculated based on the MeHg concentrations from the predictive model and actual measurements (0.0936 and 0.272 mg/kg, respectively) were lower than the soil pollution risk screening value of

Table 4 The thresholds of soil Hg calculated by the predict MeHg concentration extracted by HCl and the rice MeHg actual concentration

	1/BCF	MeHg in soil (mg/kg)	Hg in soil (mg/kg)
Predict MeHg concentration conducted by HCl	6.15	0.0627	0.0936
Actual MeHg concentration	17.8	0.182	0.272

agricultural land in China, suggesting that a stricter Hg threshold value needs to be set for soils in typical karst areas.

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Author contributions All authors contributed to the study conception and design; they had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Wanbin Hao wrote the report. Xiaohang Xu, Longchao Liang, Guangle Qiu, and Zhuo Chen critically revised the report. Fang zhu, Jialiang Han, and Xian Dong performed the statistical analysis. The manuscript was written through contributions of all authors. All authors have given approval to the fnal version of the manuscript.

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Data availability Data are available upon request to the corresponding author.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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