Heavy Metal Contamination in Agricultural Soil, Food Crops and Associated Gastric Cancers Risks for Residents in S County of Hebei Province

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Abstract. Because of the anatomic location of stomach and its acid environment which is different from the other organs, food exposure to heavy metals is a well-known risk factor for human gastric cancers. To evaluate the potential risks, we analyzed the concentrations of heavy metals (Cr, Cd, Pb, and As) in agricultural soil and grains in a high incidence area of gastric cancer, S county of Hebei province. Potential carcinogenic risk index for heavy metals in grains were evaluated. The results indicate that the concentrations of Cr, Cd, Pb and As in soils were 36.44, 0.13, 28.01 and 13.72 mg/kg, respectively; and 2.372, 0.051, 0.494 and 0.026 mg/kg in grains, respectively. The potential carcinogenic risk index of Cr, Pb and As in grain exceeded the safety limit level of $10^4$ and Cd exceeded the acceptable limit level of $10^6$. Therefore, long-term low dose exposure of heavy metals may play an important role in tumorigenesis of human gastric cancers.

1. INTRODUCTION

The presence of heavy metals in agricultural soils is of increasing concern due to their health risks to human, with the continuous development of metallurgy, mining, materials and chemical industry. Due to the obvious toxic effect, almost no degradability, bioaccumulation effect and high durability, heavy metal elements has become a dangerous pollutant in the ecosystem [1]. Each year, there were more than 1 million new gastric cancer patients and more than 780000 people died of gastric cancer in the world [2], and more than 40% of the total number of new gastric cancer patients in China [3,4]. The pathogenesis of gastric cancer is a multi-factor and multi-stage process. These factors include Helicobacter pylori, chemical carcinogens, genetic factors, body aging, lifestyle and social environment [5]. Because of the anatomic location of stomach and its acid environment, food exposures to heavy metals become a nonnegligible risk factor for human gastric cancers. S County of Hebei province has a very high incidence rate of gastric cancer (108.16/10^5), far exceeding the average incidence rate of gastric cancer of all of our country [3]. In 2018, S county's grain planting area is 14718 hectares, with a total annual grain output of 56683 tons; the total meat output of 14481 tons, and the egg output of 29620 tons [6]. Therefore, it is important to study the heavy metal pollution and its impact on the incidence rate of gastric cancer in S County. In this study, High-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) was employed to measure the contents of these four metals (Cr, Cd, Pb and As) in agricultural soil and grains from S county, Hebei province. And the human health risks posed by heavy metals (Cr, Cd, Pb and As) were assessed.
This study will contribute to the evaluation of the instructive effects of food security and food safety on the agriculture and human health.

2. MATERIALS AND METHODS

2.1 Study area
S county is located between 113°26'–114°00' N and 36°17'–36°55' E, covering an area of 1509 square kilometers, with grain planting area of 14718 hectares and a total population of 430261 in 2018. The landform of the whole area is divided into four types: the middle mountain area in the north and west, the low mountain area in the southeast, the Zhanghe River Valley and the Loess basin in the middle. S county has a warm temperate semi-humid continental monsoon climate, with an annual average sunshine duration of 2607.5 hours and a sunshine rate of 59%. The annual average total solar radiation in the territory is 498 kJ / cm², which is a high radiation area and rich in light energy resources. The annual average temperature of the county is 10.7 °C to 14.2 °C. The coldest month is January with an average temperature of - 0.5 °C to 4.6 °C; the hottest month is July with an average temperature of 23.8°C to 26.9 °C. The average annual range is 27.4 °C to 29.6 °C. The average precipitation is 571.7mm, the precipitation season is obvious, and the interannual variation is large. Rice is one of the main food crops in S county [7].

2.2 Sampling and heavy metal analysis

2.2.1 Instruments and reagents
All measurements were conducted by using a ContrAA 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena, Jena, Germany) equipped with a xenon short-arc lamp, a high-resolution double-echelle monochromator, and a charge coupled device array detector. Transverse heating techniques were used for the graphite furnace atomizer. An MPE-60 autosampler was installed for liquid samples. High-purity Ar (99.999%) was applied as the protective and purge gas. A ZM200 ultra centrifugal grinding apparatus (Retsch, Haan, Germany) was used to grind the cigarette tobacco and butt samples. A BT-214D electronic balance (Sartorius, Gottingen, Germany) was employed to weigh the samples. An ETHOS A microwave digestion system (Milestone, Milan, Italy) was applied to the digestive pretreatment of the samples. The method was optimised based on the proper atomic lines for Cr (357.869 nm), Cd (228.802 nm), Pb (283.306 nm), and As (193.696 nm).

The certified reference material (CRM) for the chemical composition of Wheat flour (GBW(E)100493) and the standard solutions (1000 mg/L Cr, 1000 mg/L Cd, 1000 mg/L Pb, and 1000 mg/L As) were purchased from the National Research Centre for Certified Reference Materials of China (Beijing, China). The working standard solutions were prepared daily through a stepwise dilution of the standard stock solutions using 0.5% (v/v) HNO₃, NH₄H₂PO₄, Mg(NO₃)₂; and Pd(NO₃)₃ (Sigma-Aldrich, St. Louis, MO, USA) were used as chemical modifiers for the determination of the Cr, Cd, Pb, and As levels. The reagents were of analytical grade, and all solutions were prepared using deionized water (18.2 MΩ/cm) produced using a PureLab Prime system (PALL, Washington, NY, USA). All containers and glassware were cleaned by soaking in the 5 mol/L HNO₃ for at least 24 hours and rinsed three times with deionized water prior to use.

2.2.2 Sample preparation
A total of 81 surface upper agricultural soil layer samples (0-20cm) in 9 parts of local farmlands were collected in autumn 2019 and 81 corresponding rice grain samples were also collected at the same time. The classic grid sampling method was used for soil sampling: divide the study area, a 50 m × 50 m square, into 10 m × 10 m equal-sized squares (n=25), and the center of each small square is the sampling point. Each sampling point consists of 8-10 small samples in the range of 1 m to form a mixed sample. At each sampling point across all land uses, a soil sample was collected at a depth of 0-20 cm with a diameter of 5 cm. The soil samples were kept in coolers with ice packs at the time of...
sampling and then kept in cold storage (-20 °C) until pretreatment. Location, plant species, general topography, soil type, tillage, and current and previous crop were recorded for all samples.

The grain samples were taken from the same site where the soil samples were collected when the grain were suitable for harvest. A total of nine local crop production areas were randomly selected in this work. For instance, as shown in Fig. 1, the sampling sites uniformly distribute in and around one of the studied farmlands (9 soil samples and 9 rice samples). All the soil and grain samples were collected from local major agricultural regions like that.

Soil: Soil samples (n=81) were collected from the fields under cultivation of rice (n=81) during the harvesting season, 2019. Soil samples were air-dried, cleaned by removing visible traces of leaves and other waste materials, homogenised, sieved through mesh size 2mm and stored in clean and airtight polyethylene bags until further analysis.

Grains: grain samples were washed with distilled water, oven dried (80°C) to a constant weight, ground to fine powder and stored in airtight polyethylene bags at 4°C until further analysis.

Approximately 0.20 g of soil or cereal sample was weighed and added into the polytetrafluoroethylene (PTFE) digestion vessel with 7 mL of concentrated HNO₃ and 1 mL of hydrogen peroxide (H₂O₂). Subsequently, the samples were digested using a two-step temperature program. During the first step, the temperature was linearly increased to 190°C over 10 min; the maximum power of the rotating magnetron was 1000 W. During the second step, the temperature was maintained at 190°C for 30 min. After digestion and cooling, each solution was evaporated to approximately 2 mL and diluted with deionized water in a 50 mL volumetric flask for the GFAAS analysis. The results were reported as the average of three repeated measurements, and all digestions were conducted in triplicate.

2.2.3 Analysis conditions of heavy metals
The analytical working solutions included the following: 0, 4.0, 8.0, 12.0, 16.0 and 20.0 µg/L for Cr; 0, 2, 4, 6, 8 and 10 µg/L for Cd and As; and 0, 10.0, 20.0, 40.0, 60.0 and 80.0 µg/L for Pb. These solutions were prepared daily using appropriately diluted dilutions of the stock standard solutions. Next, 20 µL of the sample solution or the standard solution was transferred to GFAAS together with the modifiers. The optimized conditions and temperature programs were shown in Table 1. To verify the accuracy of the metal analysis, certified reference materials (GBW(E)100493 The chemical composition of Wheat flour), from the National Research Center for Standards in China, were used. Reagent blanks and standard reference materials were analyzed for quality assurance and quality control. The recoveries of the elements ranged from 98.29 to 100.6%.
Table 1 Optimized conditions for the determination of Cr, Cd, Pb and As levels by HR-CS GFAAS

| Soil | Cr | 110 | 1350 | 2350 | 2500 | Mg(NO₃)₂ |
|------|----|-----|------|------|------|----------|
|      | Cd | 110 | 700  | 1350 | 2500 | NH₄H₂PO₄ |
|      | Pb | 110 | 850  | 1500 | 2500 | NH₄H₂PO₄ |
|      | As | 155 | 850  | 2350 | 2500 | Pd(NO₃)₂ |

| Grain | Cr | 110 | 1350 | 2350 | 2500 | Mg(NO₃)₂ |
|-------|----|-----|------|------|------|----------|
|       | Cd | 110 | 700  | 1300 | 2500 | NH₄H₂PO₄ |
|       | Pb | 110 | 800  | 1500 | 2200 | NH₄H₂PO₄ |
|       | As | 155 | 800  | 2300 | 2500 | Pd(NO₃)₂ |

2.3 Estimation of carcinogenic risk

For humans, ingestion, inhalation, and dermal contact are the three main exposure pathways of heavy metals in soil. The models applied in this study are based on those developed by the USEPA [8]. The cancer risk (CR, unitless) and total cancer risk (CRt) were used to assess the carcinogenic risks of heavy metals through the consumption of rice grains by the residents, which were calculated using the following equations:

\[
CR = \frac{EF \times ED \times VI \times MC}{BW \times AT} \times CSF
\]

\[
CR_t = \sum CR_i
\]

Where EF is the exposure frequency; ED is exposure duration; VI is ingestion rate if grain crop (kg/day); MC is metal contents in the rice grains (mg/kg); BW is body weight for local adults (kg); AT is average time for carcinogens; and CSF represents the cancer slope factor (mg/kg/day), respectively.

For CR, values of \(<10^{-6}\) (unitless) denote absence of risk, while values of \(>10^{-4}\) denote significant risk.

The above equations were obtained from relevant USEPA documents [9-13].

2.4 Data quality control

Data quality control was performed to eliminate data contamination. We used the mean and standard deviation (SD) to detect outliers, which were identified as four times the SD [14].

3. RESULTS AND DISCUSSION

The data from statistic values (minimum, maximum, median, mean and SD) of the total concentrations of heavy metals in soil and grain samples were listed in Table 2.

Table 2 Concentration ranges and safety limits of heavy metals in soil and grains (mg/kg, fresh weight)

| Soil (n=81) | Cr     | Cd     | Pb     | As     |
|------------|--------|--------|--------|--------|
| Minimum    | 21.672 | 0.096  | 9.124  | 1.786  |
| Median     | 38.201 | 0.276  | 22.118 | 9.218  |
| Maximum    | 49.896 | 0.521  | 57.052 | 18.442 |
| **Mean**   | **36.443** | **0.126** | **28.407** | **13.723** |
| SD         | 7.803  | 0.209  | 9.192  | 4.425  |
| Safety limits | USDA  | 3000.0 | 85.0   | 420.0  | 75.0   |

"
The overall data (see Table 2) showed that the maximum concentrations of heavy metals in the soil samples were lower than the safety limits given by FAO/WHO and Chinese regulations. The maximum concentrations of these four heavy metals in the grain samples were lower than the safety limits given by USDA; while the mean contents of Cr and Pb in the grain samples were higher than the safety limits given by Chinese regulations (GB 2762-2017); and As levels in some grain samples were also higher than the maximum values given by China.

Carcinogenic risks of heavy metals in grains were calculated by Eqs. (1) and (2), adapted from USDOE [19] and USEPA [20,21]. The results were shown in Table 3. The carcinogenic risk values of Cr, Pb and As in grain exceeded the safety limit level of $10^{-4}$ and Cd exceeded the acceptable limit level of $10^{-6}$ [21]. The results indicated a considerable risk of inducing human cancer. In S county, the overall incidence rate of cancer is $254.27/10^3$, and the incidence rate of gastric cancer is $156.44/10^3$ [3]. The incidence rate of gastric cancer is $46.00\%$ of the total incidence rate, ranking first in all the cancer incidence rates of local residents. The total carcinogenic risk value of heavy metals in grains was $1.06/10^3$, which indicated that the content of heavy metals in grains is one of the factors that should be considered in the study of local high incidence of gastric cancer.

| Grain (Rice) | China EPA $^b$ | 250.0 | 0.4 | 100.0 | 30.0 |
|-------------|----------------|-------|-----|-------|------|
| Minimum     | 0.106          | 0.028 | 0.245| 0.018 |      |
| Median      | 2.372          | 0.051 | 0.494| 0.026 |      |
| Maximum     | 4.203          | 0.182 | 1.955| 0.241 |      |
| Mean        | **2.018**      | **0.052** | **0.380** | **0.172** |      |
| SD          | 0.963          | 0.036 | 0.443| 0.066 |      |
| Safety limits | FAO/WHO $^c$ | 5.00 | 0.30 | 5.00 | –    |
|             | China $^d$     | 1.00 | 0.20 | 0.20 | 0.20 |

$^a$ USDA (2000) [15]
$^b$ GB15618-2018 (5.5<pH≤6.5) [16]
$^c$ FAO/WHO (2017) [17].
$^d$ GB 2762-2017 [18].

Table 3 Carcinogenic risks to humans from heavy metals in rice grains

|       | Cr   | Cd   | Pb   | As    |
|-------|------|------|------|-------|
| CR    | 5.57E-04 | 2.19E-05 | 1.21E-04 | 3.65E-04 |
| CR Total | **1.06E-03** |   |   |       |

4. CONCLUSION
Agricultural soil and corresponding rice grains grown in S county, Hebei province were sampled to investigate the relationship between the risk of gastric cancer and heavy metal contamination. All contents of soil heavy metals fall within the safety limits of the USDA and China EPA. But the contents of three heavy metals (Cr, Pb and As) in grain were above the national food safety limits in China. The carcinogenic risk assessment of heavy metals in rice indicated that the content of heavy metals in grains may be related to the local high incidence of gastric cancer.

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