Edible Quality and Safety Evaluation of *Zanthoxylum* from Western China

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**Abstract.** In order to investigate the edible safety of Chinese prickly ash (*Zanthoxylum*), the contents of four inorganic contaminants (lead, cadmium, mercury, and arsenic) and two pesticide residues (permethrin and cypermethrin) in seventeen prickly ash samples were detected, which gathered from the main plant region of Western China. In addition, potential risks due to the contaminants which exposure to it had been evaluated. Estimated daily intakes (EDIs) and hazard quotient (HQ) of the contaminants were also analyzed. The data showed that EDIs of inorganic contaminants were lower than the acceptable daily intake (ADI), and their HQ values, ranging from 2.65% (As) to 19.05% (Pb), were considerably <1. In addition, HQ values for permethrin and cypermethrin were determined to be 1.83% and 2.09%, respectively. These findings demonstrated a high level of edible quality and safety of *Zanthoxylum* from western China.

**Keywords:** *Zanthoxylum*; inorganic contaminants; pyrethroids; safety evaluation.

1. Introduction

*Zanthoxylum* (Prickly ash) is an important industrial woody plant of rutaceae. It includes more than 200 kinds of prickly ash distributed around the world[4]. Its fruit is a famous spicery in Chinese cooking culture, offering a unique numb taste especially in Sichuan cuisine. In the past decade, the planting area of Chinese prickly ash has exceeded 500,000 ha and the output has exceeded 100,000 tons, with increased agricultural inputs such as fertilizers and pesticides. *Zanthoxylum* is rich in nutrients, such as volatile oil[5], numb-taste composition[3], phenols[4], etc. Many researches have studied *Zanthoxylum* in terms of its organic compositions, such as volatile oil[5], numb-taste composition[3] and so on. Currently, most processed products of *Zanthoxylum* are food products. In addition to condiment oil, prickly ash powder, essential oil and other traditional food ingredients in Sichuan hot pot, highly processed products such as prickly ash sauce, have gradually entered the market. Furthermore, other highly processed products in medicine and commodity of *Zanthoxylum* become more abundant, with one example being prickly ash essential oil soap[6]. Nevertheless, few researches studied its edible quality and safety, especially focus on potential contaminations and this quality thus merits further research.

Inorganic contaminants, e.g., Pb, Cd, Hg, As, etc., in food can arise through many ways, such as contaminated soil, irrigation water, industrial emissions, and harvesting process[7]. In agriculture, farmers use pesticides (e.g., pyrethroid, organophosphorous, etc.) to obtain high yield and quality of agricultural products[8]. Insecticides, and herbicides are widely used especially in developing countries, including China[9]. This can lead to contamination of pesticides in food. Consumption of pesticide-contaminated food can cause great harm to human health[10], leading to various symptoms, such as,
headache, nausea, declined reproductive function, endocrine system disorders, etc. [11]. Therefore, whether or not the amount of contaminants in food exceed the maximum allowable concentrations set by the relevant institutions has raised public and scientific concerns [12]. A few studies have reported the detection of pesticide residues in other condiments, such as white and black pepper [13]. Furthermore, there is no definitive standard of maximum allowable concentrations of pesticide residue in *Zanthoxylum* domestically or worldwide.

The aims of this work are to determinate the presence and content of inorganic contaminants and pesticide residues in two varieties *Zanthoxylum bungeanum* Maxim. and *Zanthoxylum armatum* DC. of *Zanthoxylum* with 17 accessions gathered from main planting districts in China, such as Jinyang, Hanyuan in Sichuan province and Hancheng, Fengxian in Shannxi province; and to comprehensively evaluate their edible safety. The results will provide new information regarding the edible quality of *Zanthoxylum*.

2. Materials and Methods

2.1. Sample Collection and Preparation
Seventeen samples of *Zanthoxylum* were collected from seventeen districts in the region of Western China (Table 1). The samples were crushed and homogenized before passing through a 40-mesh screen. The resultant powder samples were dried at 60 °C for 48 h for further analysis.

2.2. Standards and Reagents
Stock standard solutions of Pb, Cd, Hg, As (1000 μg/mL each), and pyrethroid pesticides (permethrin and cypermethrin in n-hexane, 100 μg/mL each) were purchased from Beijing Tanmo science and technology of quality control Co., Ltd. (Beijing, China). These solutions were of a purity range of 99.71% to 99.99%, and were stored in a freezer at -18 °C prior to use. The reagents were purchased from Chengdu Jere Technology Co., Ltd. (Chengdu, China). Deionized water was purified by a Milli-Q water purification system (Millipore, Milli-Q, USA).

2.3. Analytical Methods

2.3.1. Determination of Contaminations. The determination of Pb and Cd was carried out according to the National Food Safety Standard of China: determination of lead (cadmium) in foods [14, 15]. The quantification of Pb and Cd was conducted using a flame atomic absorption spectrometer (FAAS) equipped with an air-C2H2 flame and a polarizer for Zeeman background correction (Z-2000, Japan). FAAS was operated with the instrument recommended procedure. The digestion and determination of Hg and As were conducted according to the National Food Safety Standard of China: Determination of mercury (arsenic) in foods [16, 17]. The quantification of Hg and As, was carried out using an AFS-3000 (Beijing Haiguang Instrument Co., Ltd., Beijing, China) and operated with the instrument recommended procedure. The Determination of pyrethroids in *Zanthoxylum* were carried out according to the Agricultural Standards of China [18].

2.3.2. Comparison content of Contaminants with MRLs. Maximum Residue Limits (MRLs) are not the toxicological limits ensuring that food is safe for consumption. The obtained data were compared with the Chinese established MRLs, according to the Safety Standard of *Zanthoxylum* concentration limits for Pb, Cd, Hg, and As [19]. There are no standard MRLs for permethrin and cypermethrin in *Zanthoxylum*. Therefore, MRLs for permethrin and cypermethrin were compared to MRLs of malathion in *Zanthoxylum* [19].

2.3.3. Risk Evaluation of Intake Contaminants. MRLs represent the highest concentration of contaminants allowed in food, they however lacks food consumption information, which is an essential data for food safe assessment. Eestimated daily intakes (EDI) is used to accurately identify whether or not the food is sufficiently safe for consumption [20]. In addition, it is possible to compare the exposure levels to the chronic ADI levels [21]. The dietary exposure was analyzed based on an integration of the
analytical data concerning the contaminants of interest (obtained from this study) and the food consumption data [22]. The computing method of EDIs, hazard quotient (HQ) and hazard index (HI) were referred to [9]. The commodity can be deemed safe when the value of HI is lower than 1. Conversely, food may contain high health hazard or consumer is not be adequately protected when the value of HI exceed 1 [23].

3. Results and Discussion

3.1. Quality Assurance of Contaminants Quantitative Determination
DL (defensive lineman) of Pb, Cd, Hg, and As were 0.0180, 0.0021, 0.000375, and 0.000167 mg·kg⁻¹, respectively. These values were obtained with correlation coefficients, \( R^2 \geq 0.999 \). In addition, LODs (Limit of detection) of permethrin and cypermethrin were 0.016 and 0.011 mg·kg⁻¹, respectively. The precision of results were described in terms of relative standard deviation (RSD) [24]. RSDs were between 0.3% and 5.5% for the quantification of inorganic contaminants, between 2.12% and 6.0% for the quantification of pesticides.

3.2. The Content Level of Contaminants in Zanthoxylum
Concentrations of Pb and As in all analyzed samples were within the safety range (<MRLs), and all four inorganic contaminants were detected in almost all samples (Table 1). Concentrations of Cd and Hg were above MRLs in more than half of samples. As indicated by mean values, Pb was the highest, while Hg was the lowest, in consistent with the previously reported data [25]. Moreover, Zanthoxylum belongs to Rutaceae, is the perennial plants that are enriched with harmful elements. The sources of heavy metals, especially Cd and Hg should be focus and controlled, the measured content of which were above MRLs. Two types of pyrethroids found in analyzed samples exceeded MRLs; cypermethrin was the majority (found in 94.11% of samples). Half of samples (50%) contained permethrin of concentration lower than the LOD. Concentration range of the detected permethrin were from under LOD to 0.6126 mg·kg⁻¹ (Table 1). Mean values, ranges, and total number of samples containing four inorganic contaminants and two pesticides above MRLs are presented in Table 2.

3.3. Evaluation of Risk Based on Amount of Intake Contaminants
Food and Agriculture Organization and World Health Organization (FAO/WHO) and Joint Expert Committee on Food Additive have recommended that the Provisional Tolerated Weekly Intake (PTWI) of Pb, Cd, Hg, and As are 0.025, 0.007, 0.005, and 0.015 mg·kg⁻¹bw, respectively [26]. In the present study, ADI of Pb, Cd, Hg and As were calculated for adults with a mean body weight of 60 kg. The ADI of pesticides are in reference to the standards established by Joint Meeting of Pesticide Residues (JMPR) [21]. A list of ADI values used for the assessment of chronic exposure as well as the contribution of inorganic contaminants and pyrethroids to the calculated total EDI and HI are tabulated in Table 3. For inorganic contaminants, HQ values ranged from 2.65% (for As) to 19.05% (for Pb). HQ values for permethrin and cypermethrin were 1.83% and 2.09%, respectively. HI values of lower than 1 also show that these inorganic contaminants as well as pyrethroids are at the safe levels. Because Zanthoxylum can easily suffer from pest attack, farmers often spray pesticides on the plant to prevent such attack. Unfortunately, MRLs for permethrin and cypermethrin in prickly ash are currently not available. According to the data in this work, permethrin and cypermethrin were detected in most analyzed samples. It concluded that MRLs for permethrin and cypermethrin in Zanthoxylum should urgently be investigated and made available to ensure food safety of consumers.
Table 1. Mean content of the monitoring contaminants (Pb, Cd, Hg, As, permethrin and cypermethrin) in samples

| No. | Samples       | Plant district | Pb (mg·kg⁻¹) | Cd (mg·kg⁻¹) | Hg (mg·kg⁻¹) | As (mg·kg⁻¹) | Pesticides (mg·kg⁻¹) |
|-----|---------------|----------------|--------------|--------------|--------------|--------------|-----------------------|
| 1   | Hancheng      | Hancheng of Shanxi province | 1.537±0.01188 | 0.0448±0.0752 | 0.0034 | 0.0123±0.0006 |
| 2   | Fengxian      | Fengxian of Shanxi province | 0.504±0.1200 | 0.0524±0.0333 | 0.0003 | 0.0655±0.0712 |
| 3   | Moxian        | Moxian of Sichuan province | 0.019±0.0035 | 0.0007±0.0006 | 0.0003 | 0.0336±0.0030 |
| 4   | Zhaotong      | Zhaotong of Yunnan province | 0.086±0.0869 | 0.0378±0.0934 | 0.0003 | 0.627±0.0217 |
| 5   | Yuexi Guangjiao | Yuexi of Sichuan province | 0.016±0.0496 | 0.019±0.0695 | 0.0036 | 0.773±0.3065 |
| 6   | Henan         | Jujuan of Henan province | 1.744±0.1355 | 0.0299±0.0627 | 0.0003 | 0.782±0.3062 |
| 7   | Jiulong Huajiao | Jiulong of Sichuan province | 0.785±0.0288 | 0.0200±0.0315 | 0.0003 | 0.595±0.0328 |
| 8   | Wudu          | Majie town of Wudu of Gansu province | 0.0356±0.0813 | 0.0304±0.0577 | 0.0003 | 0.463±0.1601 |
| 9   | Wudu          | Majie town of Wudu of Gansu province | 0.124±0.0410 | 0.0361±0.0475 | 0.0003 | 0.347±0.0476 |
| 10  | Wudu          | Majie town of Wudu of Gansu province | 0.0369±0.0369 | 0.0394±0.0948 | 0.0003 | 0.427±0.0172 |
| 11  | Wudu          | Majie town of Wudu of Gansu province | 0.030±0.0131 | 0.0359±0.0852 | 0.0003 | 0.385±0.0172 |
| 12  | Jinyang       | Jinyang of Sichuan province | 0.981±0.1031 | 0.0359±0.0852 | 0.0003 | 0.427±0.0172 |
| 13  | Hanyuan       | Yidong town of Hanyuan province | 1.264±0.1181 | 0.0406±0.0490 | 0.0003 | 0.285±0.0172 |
| 14  | Hanyuan       | Sanjiao town of Hanyuan province | 0.923±0.0750 | 0.0409±0.0693 | 0.0003 | 0.385±0.0564 |
| 15  | Hanyuan       | Sanjiao town of Hanyuan province | 0.559±0.0633 | 0.0400±0.0922 | 0.0003 | 0.323±0.0172 |
| 16  | Santai        | Santai of Mianyang of Sichuan province | 0.846±0.1063 | 0.0443±0.0749 | 0.0003 | 0.498±0.0178 |
| 17  | Santai        | Santai of Mianyang of Sichuan province | 0.576±0.0967 | 0.0420±0.0793 | 0.0003 | 0.391±0.0172 |

Table 2. 4 inorganic contaminants and 2 pyrethroids detected in samples from Western China.

| The monitoring contaminants | MRL (mg·kg⁻¹) | Mean value (mg·kg⁻¹) | Range (min-max) | No.of samples > MRL | No.of samples < DL |
|-----------------------------|---------------|----------------------|-----------------|---------------------|-------------------|
| Inorganic contaminants      |               |                      |                 |                     |                   |
| Pb                          | 1.86          | 0.8504               | 0.0-1.7440      | 0                   | 1(5.88%)          |
| Cd                          | 0.05          | 0.0768               | 0.00125-0.1355  | 11(64.71%)          | 0                 |
| Hg                          | 0.03          | 0.0380               | 0.00192-0.0524  | 14(82.35%)          | 0                 |
| As                          | 0.30          | 0.0712               | 0.0076-0.1619   | 0                   | 0                 |

| Pyrethroids                 |               |                      |                 |                     |                   |
| Permethrin                  | 8.00          | 0.1147               | 0.0-0.6126      | 0                   | 7(50%)            |
| Cypermethrin                | 8.00          | 0.5136               | 0.0-0.8786      | 0                   | 1(5.88%)          |
Table 3. EDI of the monitoring contaminants (Pb, Cd, Hg, As, permethrin and cypermethrin) in all samples and risk assessment.

| The monitoring contaminants | ADI (μg kg⁻¹ bw day⁻¹) | EDI (μg kg⁻¹ bw day⁻¹) | HQ(%) |
|-----------------------------|-------------------------|------------------------|-------|
| **Inorganic contaminants**  |                         |                        |       |
| Pb                          | 3.571 a                 | 0.6803                 | 19.05 |
| Cd                          | 1 a                     | 0.0614                 | 6.14  |
| Hg                          | 0.7142 a                | 0.0304                 | 4.25  |
| As                          | 2.1428 a                | 0.0569                 | 2.65  |
| Total EDI                   | 0.8290μg·kg⁻¹·bw day⁻¹  |                        | 32.09%|
| Hazard index (HI)           |                         |                        |       |
| **Pyrethroids**             |                         |                        |       |
| Permethrin                  | 50                      | 0.0917                 | 1.83  |
| Cypermethrin                | 20                      | 0.4108                 | 2.09  |
| Total EDI                   | 0.5025μg·kg⁻¹·bw day⁻¹  |                        | 3.92% |
| Hazard index (HI)           |                         |                        |       |

a PTWI/7

3.4 Estimation Intake and Risk of the Monitoring Contaminants Based on Samples

Contributions of Zanthoxylum samples to the total EDIs and HQs were calculated, and the data are shown in Table 4. HQ values for inorganic contaminants were from 7.46% for sample No. 3 (Maoxian Dahongpao) to 55.6% for sample No. 6 (Henan Dahongpao). For pyrethroids, pesticide residues were not detected in sample No. 14 (Hanyuan Zhenglujiao), and their HQ values were between 0 to 4.31%. In the present work, all samples had HQ of lower than 1, a safe level of Zanthoxylum that the presence of these inorganic contaminants and pesticides is indicated.

It is worth noting that the dietary intakes of inorganic contaminants and pyrethroids estimated in the present study only showed possible exposures from Zanthoxylum but did not imply any other condiments, such as, black pepper, salt, amomum, and so on. In turn, they cannot be considered as ‘total’ dietary exposures for inorganic contaminants (pyrethroids) because fruits, vegetables, or grains were not considered. Moreover, other factors, such as the dietary or digestion process, were neglected. Chinese prickly ash seed is generally used as spice rather than direct consumption. Other uses of Chinese prickly ash can be in the form of powder or oil. Thus, HQ values presented in this study can be the overestimates of actual exposures to inorganic contaminants and pyrethroids.

The aim of this paper is to measure the content of four inorganic contaminants (including Pb, Cd, Hg, and As) and two pyrethroids (permethrin and cypermethrin) in Chinese prickly ash, as well as to assess the edible safety of Chinese prickly ash. The results show that nearly all samples contained inorganic contaminants. As indicated by mean values, Pb was the highest, while Hg was the lowest, in consistent with the previously reported data [25]. It is known that heavy metals have impacts on the human body once they enter the food chain [27]. Polluted water and soil produce a greater effect on the content of heavy metals in crops [28]. Moreover, Chinese prickly ash seed is generally used as spice rather than direct consumption. Other uses of Chinese prickly ash can be in the form of powder or oil. Thus, HQ values presented in this study can be the overestimates of actual exposures to inorganic contaminants and pyrethroids.

In addition to a few other research that have studied pesticide residuals in Chinese prickly ash, the measured contents of two pyrethroids presented in this work were lower than the reference MRLs. Additionally, more than half of samples were positive for such pesticides. Although the HQ values of Chinese prickly ash were low, the residual pesticides in Chinese prickly ash should not be ignored. This study only present two common pyrethroid pesticides, the assessment of edible safe is therefore partial. Potential exposure to other hazards may also include organophosphorus and carbamate pesticide residuals, and so on. Nowadays, consumers advocating pollution-free food urge that use of pesticides should be reduced, while use of a more biological pest control should be implemented.

It is worth noting that the dietary intakes of inorganic contaminants and pyrethroids estimated in the present study only showed possible exposures from Zanthoxylum but did not imply any other
condiments, such as, black pepper, salt, amomum, and so on. In turn, they cannot be considered as ‘total’ dietary exposures for inorganic contaminants (pyrethroids) because fruits, vegetables, or grains were not considered. Moreover, other factors, such as the dietary or digestion process, were neglected [29]. *Zanthoxylum* fruit is generally used as spice rather than direct consumption. Other products of *Zanthoxylum* can be in the form of powder or oil. Thus, HQ values presented in this study can be the overestimates of actual exposures to inorganic contaminants and pyrethroids.

**Table 4.** DI and risk assessment for each samples.

| No. | 4 Inorganic contaminants | 2 Pyrethroids |
|-----|--------------------------|---------------|
|     | EDIa (μg·kg⁻¹·bw·day⁻¹) | HQ (%)        |
| 1   | 1.4206                   | 51.76         |
| 2   | 0.5677                   | 28.00         |
| 3   | 0.0864                   | 7.46          |
| 4   | 0.8624                   | 33.93         |
| 5   | 0.7634                   | 26.99         |
| 6   | 1.5776                   | 55.60         |
| 7   | 0.6731                   | 22.41         |
| 8   | 1.0056                   | 20.03         |
| 9   | 1.0844                   | 36.67         |
| 10  | 0.4880                   | 20.73         |
| 11  | 0.9987                   | 33.39         |
| 12  | 0.9641                   | 37.42         |
| 13  | 1.1773                   | 44.14         |
| 14  | 0.8865                   | 33.84         |
| 15  | 0.6036                   | 25.50         |
| 16  | 0.5872                   | 35.21         |
| 17  | 0.6352                   | 28.30         |
|     | 0.0098                   | 51.76         |
|     | 0.5811                   | 28.00         |
|     | 0.5300                   | 7.46          |
|     | 0.5139                   | 33.93         |
|     | 0.8630                   | 26.99         |
|     | 0.6258                   | 55.60         |
|     | 0.7211                   | 22.41         |
|     | 0.4992                   | 20.03         |
|     | 0.2777                   | 36.67         |
|     | 0.3420                   | 20.73         |
|     | 1.0637                   | 33.39         |
|     | 0.6683                   | 37.42         |
|     | 0.2283                   | 44.14         |
|     | 0.00                   | 33.84         |
|     | 0.2764                   | 25.50         |
|     | 1.0995                   | 35.21         |
|     | 0.4533                   | 28.30         |

aEDI of each sample was calculated as the sum of the EDI for 4 inorganic contaminants in that sample.
bEDI of each sample was calculated as the sum of the EDI for 2 pyrethroids in that sample.

**4. Conclusion**

The work demonstrated that although Pb, Cd, Hg, and As were detected in almost all *Zanthoxylum* samples in Western China with high frequencies, their EDIs values were lower than ADIs, and HI values were well below 1. Such values are adequately safe for consumers, not at the hazard levels that can cause harm to health. Nonetheless, the risk due to contaminated food should be monitored, and the pollution of soils and irrigating water should be regulated so that inorganic contaminants or pesticide residues in food (including *Zanthoxylum*) remain at the safe levels. Based on the results for pesticides determination, the MRLs for permethrin and cypermethrin in *Zanthoxylum* should be urgently pay more attention in food security.

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