Journal Pre-proof

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PII: S0269-7491(22)00674-1

DOI: https://doi.org/10.1016/j.envpol.2022.119460

Reference: ENPO 119460

To appear in: *Environmental Pollution*

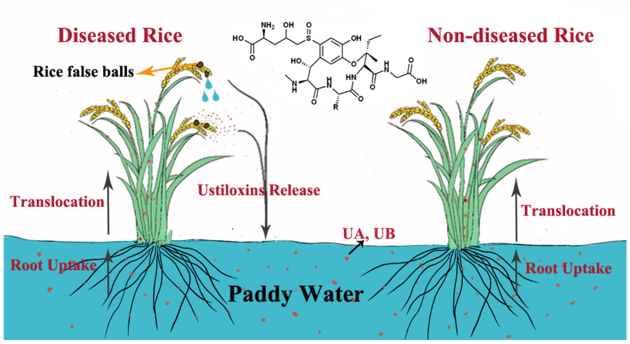
Received Date: 26 October 2021

Revised Date: 24 April 2022

Accepted Date: 8 May 2022

Please cite this article as: Sun, Q., Qian, Z., Liu, H., Zhang, Y., Yi, Xun'., Kong, R., Cheng, S., Man, J., Zheng, L., Huang, J., Su, G., Letcher, R.J., Giesy, J.P., Liu, C., Occurrence and translocation of ustiloxins in rice false smut-occurred paddy fields, Hubei, China, *Environmental Pollution* (2022), doi: https://doi.org/10.1016/j.envpol.2022.119460.

Qian Sun: Investigation, Data Curation, Writing-Original Draft, Methodology, Software, Formal analysis. **Zhisong Qian:** Investigation, Data Curation, Methodology. **Hao Liu:** Investigation, Validation, Formal analysis. **Yongkang Zhang:** Investigation, Data Curation. **Xun'e Yi:** Investigation, Data Curation. **Ren Kong:** Investigation, Data Curation. **Shiyang Cheng:** Methodology. **Jianguo Man:** Writing-Review & Editing. **Lu Zheng:** Writing-Review & Editing. **Junbin Huang:** Writing-Review & Editing. **Guanyong Su:** Writing-Review & Editing. **Robert J. Letcher:** Writing-Review & Editing. **John P. Giesy:** Writing-Review & Editing. **Chunsheng Liu:** Resources, Writing-Review & Editing, Supervision, Project administration, Funding acquisition.



Diseased Rice
Non-diseased Rice
Rice false balls
Translocation
Ustiloxins Release
Translocation
UA, UB
Root Uptake
Root Uptake
Paddy Water

Occurrence and translocation of ustiloxins in rice false smut-occurred paddy fields, Hubei, China

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Abstract: Ustiloxin A (UA) and ustiloxin B (UB), two major mycotoxins produced by the pathogen of rice false smut (RFS) during rice cultivation, have attracted increasing attentions due to their potential health risks. However, limited data are available about their occurrence and fate in paddy fields and contamination profiles in rice. In this study, a field study was performed to investigate the occurrence and translocation of UA and UB in RFS-occurred paddies. For the first time to our knowledge, we reported a ubiquitous occurrence of the two ustiloxins in the paddy water (range: 0.01 – 3.46 μg/L for UA and <0.02 – 1.15 μg/L for UB) and brown rice (range: 0.09 – 154.08 μg/kg for UA and <0.09 – 23.57 μg/kg for UB). A significant positive correlation was observed between ustiloxin levels in paddy water and brown rice (r_s = 0.48-0.79, $p < 0.01$). The occurrence of ustiloxin uptake in water-rice system was also evidenced by the rice exposure experiment, suggesting paddy water might be an important source for ustiloxin accumulation in rice. These results suggested that the contamination of ustiloxins in rice might occur widely, which was supported by the significantly high detection frequencies of UA (96.6 %) and UB (62.4 %) in polished rice (149 samples) from Hubei Province, China. The total concentrations of ustiloxins in the polished rice samples collected from Hubei Province ranged from < 20.7 ng/kg (LOD) to 55.1 μg/kg (dry weight). Further studies are needed to evaluate the potential risks of ustiloxin exposure in the environment and humans.

Keyword: rice false smut, ustiloxins, uptake, paddy water, rice

1. Introduction

Rice false smut (RFS), one of the most destructive rice fungal diseases, has been reported in more than 40 countries in Asia, North America and the Middle East (Sun et al., 2020; Chen et al., 2020). Apart from causing massive rice yield losses, the RFS pathogen can produce abundant mycotoxins (e.g., ustiloxins and ustilaginoidins) (Kioso et al., 1992; Lu et al., 2015; Lin et al., 2018; Wang et al., 2021), which might lead to contamination of the environment and affect human health. Ustiloxins, especially ustiloxin A (UA) and ustiloxin B (UB), which are the two dominate ustiloxins (Table S1) (Wang et al., 2016; Fu et al., 2015a, 2017; Hu et al., 2015, 2018), are highly produced during the progress of RFS occurrence. Serious ustiloxin contaminations in rice straws and grains collected from RFS-occurred paddies have been reported (Miyazaki et al., 2009; Hu et al., 2018). Furthermore, several studies have demonstrated that the ingested ustiloxin residues can be absorbed by gastrointestinal tract and might cause potential toxicity risks to liver, kidney and reproductive system of biota and human (Sun et al., 2021, 2022; Nakamura et al., 1994; Wang et al., 2017). Therefore, the environmental behaviors of ustiloxins in paddy fields need to be investigated urgently to evaluate the negative effects of RFS on food safety and human health.

Ustiloxins are highly hydrophilic (log K_{ow}: 0.06 - 1.6 (https://hmdb.ca)), and are liable to enter surrounding environmental matrices via rain or atmospheric deposition (Ladhalakshmi et al., 2012; Qiu et al., 2019). For example, the contamination of ustiloxins was found to be ubiquitous in the water of RFS-occurred paddies (Enshi City, Hubei Province, China), with concentrations up to 2.82 μg/L (Cheng et al., 2019). Rice plants, including non-infected plants, are supposed to be widely exposed to ustiloxins in RFS-occurred regions. More importantly, the high levels of ustiloxins have been detected in polished rice samples (Fu et al., 2015b; Sun et al., 2022), implying that rice plants might have capability to absorb ustiloxins from the environment. Residual ustiloxins might be taken up and accumulated in rice plants, which subsequently result in ubiquitous contamination of rice grains.

However, no studies have been performed to investigate the uptake, bioaccumulation and translocation of ustiloxins in rice plants.

The objectives of this study were to investigate the uptake and accumulation of ustiloxins by rice plants and to evaluate ustiloxin contamination in polished rice. Therefore, a field study was conducted to elucidate the distribution traits of UA and UB in RFS-occurred paddies. Simultaneously, the root uptake and translocation of UA in rice plants were verified by water-exposure experiment. Finally, the occurrence of UA and UB in polished rice across Hubei Province (China) was examined to investigate the contamination of ustiloxins in food chain. To our knowledge, this study for the first time revealed the root uptake and accumulation of ustiloxins by rice grown in RFS-occurred regions.

2. Materials and Methods

2.1 Sampling of surface waters and rice grains in paddy fields

Field studies were performed in two villages (Qingshan: 29°43’ 1” N, 109°11’ 57” E and Laozhai: 29°36’ 5” N, 109°8’ 43” E) of Xianfeng County, Enshi City, Hubei Province, China, where the rice false smut disease has been frequently observed in the past years. The first sampling was conducted in the middle of September, 2019. Paddy surface water samples were collected from 11 paddy fields (Sites S1-11, Table S2) at the dough grain stage of rice when discernible rice false smut balls had been generated. Three surface water samples (each included five subsamples) were carefully collected at three different sites for each rice paddy, including the opposite ends and the center of each paddy (Liu et al., 2012; Yashimura et al., 2016). Approximately 500 mL water from each sampling site was filtered through a 0.45 μm filter *in situ* and stored in pre-cleaned polypropylene bottles. Simultaneously, the numbers of rice false smut balls on panicles surrounding each sampling site (n = 100) were counted for grading the disease severity of RFS, following the Standard of Resistance Evaluation of Rice against False Smut, which was developed by the team of “Control of Rice Fase Smut” specialists in 2011. The water samples were temporarily stored in a sampling case with dry ice after collection until transferred to our laboratory and then kept in refrigerator (-20 ℃). The second sampling was conducted in early October of 2019, when most of rice crops were at the mature stage. The same 11 paddy fields were selected. Three surface water samples were obtained from three different locations of each paddy field and the numbers of rice false smut balls on panicles were counted surrounding each sampling site (n = 100) as described above. Five diseased panicle samples (with at least one false smut ball in each panicle) and five non-diseased panicle samples (without obvious disease symptoms in panicle) were collected separately with polypropylene bags around each water sampling site. The rice false smut balls on diseased panicles samples were counted and removed. All panicle samples were sealed in polypropylene seal bag and temporarily stored in sampling case with dry ice after collection. Once arrived at our laboratory, the rice grains were carefully separated from panicles and washed with ultrapure water (three times) to remove the fungus powder on the surface of rice husk. Then, the rice grains were freeze-dried (-45 ℃) and hulled to obtain corresponding brown rice for further analysis.

2.2 Rice exposure experiment

Rice exposure experiment was carried out at Huazhong Agriculture University, Wuhan, Hubei Province, China. The rice seedlings (Wanxian 98) were transferred to plastic boxes (26 cm × 18 cm × 28 cm) containing 8 kg paddy soil and 4 L water. Four seedlings were planted in each box, and cultivated under ordinary conditions until the eighth stage of panicle development (Chen et al., 2021; Wang et al., 2018). Then, the rice crops were transferred into a phytotron facility and grown in UA-containing water (0, 2.0, 10.0 μg/L). The contents of UA in water were monitored and adjusted every day to maintain a relatively stable exposure level. The rice crops were cultivated at about 25 ℃ and 85 % humidity. After 30 days, the rice samples (grains and straws) were collected and freeze-dried for further analysis.

2.3 Collection of rice samples in Hubei Province

One hundred and forty-nine polished rice samples with clear original information were collected from 13 cities of Hubei Province, China, from October to December 2019. All the rice samples collected were provided by the parents of the college students from Hubei Province in our school, and those rice samples were produced in Hubei Province. Upon arrival at our laboratory, these samples were stored in freezer (-20 ℃) until UA and UB analysis.

2.4 Ustiloxin analyses

Ustiloxins were extracted and purified following our previously published method with minor modifications (Sun et al., 2021, 2022). The lyophilized rice grains and straw samples were ground and sieved. About 0.5 g milled rice or straw samples were extracted with pure water, and the extracts were purified by passing through a WAX cartridge. The unfrozen paddy water was extracted by dichloromethane followed by SPE purification. The details for extraction procedures are described in the Supporting Information text.

The analyses of UA and UB were performed on a Waters ACQUITY UPLC® H-Plus Class system (UHPLC) coupled to Waters® XevosTM TQ-XS mass spectrometer (TQ-XS/MS) (Milford, MA, USA). More details of instrumental analysis and quality assurance/quality control were provided in Supporting Information text and Table S3.

2.5 Statistic analyses

All statistical analyses were performed with SPSS 22.0 (SPSS Inc., Chicago, IL, USA). For the calculation of the geometric value, a value equal to LOD (below detection limit) divided square root of 2 was assigned to those samples when the concentrations of UA or UB were <LOD. As data collected from fields were not normally distributed (verified by using Kolmogorov–Smirnov test), spearman's rank correlation coefficients were used to determine the associations of UA or UB in paddy waters and brown rice collected from farmers' paddy fields. The figures in this study were drawn using GraphPad Prism 8.

3. Results and Discussion

3.1 Distribution Characteristics of Ustiloxins in Surface Waters and Rice from RFS-occurred Paddy Fields

The descriptive statistics of ustiloxins distribution in the surface waters of RFS-occurred paddies from Xianfeng County (Enshi City, Hubei Province, China) were summarized in Table 1. UA and UB were ubiquitous in the water samples both at the dough grain stage and the mature stage of rice, indicating that ustiloxin contamination was widespread in surface waters throughout the ripening phase of rice. Among those water samples, the total concentrations of UA and UB (∑ustiloxins) ranged from 0.01 to 0.44 μg/L (median of 0.05 μg/L) at the dough grain stage and 0.02 to 4.03 μg/L (median value of 0.37 μg/L) at the mature stage. The levels of UA (range: 0.01-3.46 μg/L) were comparable with the reported values of 0.26 and 2.82 μg/L in paddy waters (Cheng et al., 2019). Generally, the residual of UB was significantly lower than UA (UB/UA=0.23) in this study, which was consistent with previous studies in which the ratios of UB/UA produced by *Ustilaginoidea virens* were in the range of 0.09 – 0.57 (Wang et al., 2016; Hu et al., 2018; Fu et al., 2017). Overall, these results clearly indicated that ustiloxin contamination was ubiquitous in the surface water of RFS-occurred paddies, which would cause extensive exposure of pre-harvested rice plants throughout the ripening phase.

In order to further investigate the distribution characteristics of ustiloxins in RFS-occurred regions, the occurrence of ustiloxins in rice grain samples at the mature stage, collected from diseased panicles (possessing at least one rice false smut ball per panicle) and non-diseased panicles (without obvious rice false smut ball in the panicle), was evaluated and the summary statistics were listed in Table 1. UA and UB were detectable in 100% of brown rice samples from diseased panicles (n = 33), with UA concentrations in the ranges of 2.61 - 151.08 μg/kg dw (median of 34.12 μg/kg dw, geometric mean (GM) of 22.91 μg/kg dw) and UB concentrations of 0.43-23.57 μg/kg dw (median of 7.31 μg/kg dw, GM of 4.66 μg/kg dw), suggesting that the contamination of ustiloxins was broadly occurred in rice plants subjected to RFS. Similarly, high levels of UA and UB were also detected in the brown rice collected from RFS-occurred paddy fields of Zhejiang (18.1-152.5 μg/kg for UA, 2.7-88.7 μg/kg for UB) (Cao et al., 2016) and Beijing (6.4-170 μg/kg for UA) (Fu et al., 2015b). Relatively higher concentrations of ustiloxins were detected in rice grains from diseased panicles (3.04-199.84 mg/kg for total UA and UB) (Fu et al., 2017; Hu et al., 2018; Ji et al., 2012), which might be influenced by the fungus powder residues on the surface of rice husk. However, it was important to note that UA and UB were detected frequently in rice grains collected from non-diseased panicles (n = 33), even though with substantially lesser concentrations (geometric means of 0.69 and 0.13 μg/kg dw) than those of diseased panicles from the same sampling sites. Previous studies demonstrated that rice plant had the ability to take up organic pollutants from surroundings through root uptake or foliar absorption (Zhao, et al., 2020, Fan et al., 2020). For example, various hydrophilic contaminants, such as microcystin, per-/poly-fluoroalkyl substances and pesticides, could be taken up by rice roots from surrounding soil or water and then translocated to above-ground parts of rice plants (Corbel et al., 2014; Yamazaki et al., 2019; Ge et al., 2017). The findings above suggested that ustiloxins could be accumulated and translocated in non-infected rice plants. To our knowledge, for the first time this study demonstrated the occurrence of UA and UB contamination in non-diseased rice plants, suggesting that ustiloxin pollution in rice might be largely underestimated. The serious ustiloxin contamination in brown rice observed above highlighted the need of further studies to better understand the pathways of ustiloxin transfer from RFS balls to rice.

3.2 Correlations and Pathways of Ustiloxins Accumulation in Rice

Generally, the concentrations of UA and UB in paddy waters showed increasing trends with the RFS disease severity of corresponding sampling sites. Positive correlations (r_s = 0.380 – 0.523, p = 0.029 – 0.002) were identified between the UA levels in water and the average numbers of RFS balls per 100 panicles around sampling sites at the dough rice stage (Fig. 1a) and mature stage (Fig. 1b). Similarly, the levels of UB in paddy waters were positively correlated with the numbers of RFS balls at the two sampling time points (Fig. 1c and 1d). Previous studies demonstrated that ustiloxins were generated by *Ustilaginoidea virens* prior to RFS ball's formation and mainly dominated in the RFS balls with contents ranged from 331.5 – 1926.7 μg/g at different maturity stages (Hu et al., 2020; Wang et al., 2016). During the maturity of RFS balls, ustiloxins were likely to be substantially released from RFS balls through air currents or rain splash, which would subsequently lead to serious ustiloxin contamination in the water of RFS-occurred fields. This result demonstrated that RFS balls might be the dominant source of ustiloxins entering into the paddy water in RFS-occurred fields. It was worth noting that the soil types and water properties, such as the organic matter content in soil and pH value in water, have been proposed to affect the environmental behaviors of various organic chemicals (Miller et al., 2016). More studies should be carried out to investigate the temporal variation in the translocation, distribution and degradation of ustiloxins in paddy fields.

Consistent with the variation pattern of ustiloxins in paddy water mentioned above, ustiloxins levels in rice grains showed obviously increasing trend with the disease severity of corresponding sampling sites. Specifically, the elevated levels of ustiloxins in rice grains were observed from the sampling sites with higher contents of ustiloxins in water. More importantly, for diseased and non-diseased panicles, significantly positive linear relationships ($p < 0.01$, Fig. 2a and 2b) were obtained between UA concentrations in brown rice and in paddy water of corresponding sampling sites. Meanwhile, similar trends were observed for UB ($p < 0.01$, Fig. 2c and 2d). Moreover, the average ratios of UB/UA in grains of diseased panicles (0.22) and non-diseased panicles (0.25) were consistent with those in paddy water (0.23). The results indicated that the contaminated paddy water might be a predominated source of ustiloxin accumulation in rice grains. It was reported that the root uptake of organic contaminants was associated with their physicochemical characteristics, such as water solubility and octanol/water partition coefficients (Dettenmaier et al., 2009; Collins et al., 2006). It has been documented that ustiloxins were ionized organic pollutants with relatively low log K_{ow} (-1.6 and -1.5) (Table S1), suggesting that root uptake of ustiloxins in rice might occur during the progression of the RFS generation. Interestingly, a stronger correlation was observed between ustiloxin levels in paddy waters and in rice of non-diseased panicles compared with ustiloxin levels in diseased panicles, which suggested that ustiloxin levels in diseased panicles might simultaneously be affected via other uptake processes. Plants can absorb organic chemicals from the surrounding environment, such as air, soil and water (Hou et al., 2021). More studies should be carried out to investigate the uptake kinetics and uptake mechanism of ustiloxins by rice plants in RFS-occurred fields.

To further confirm the root uptake and accumulation of ustiloxins in rice plants, an exposure experiment was conducted under laboratory conditions in which rice at the eighth stage of panicle development was grown in water containing 0, 2.0 or 10.0 UA μg /L (Fig. S1). UA could be detected in both straws and brown rice after exposure for 30 days (Fig. 3a and 3b). Meanwhile, during the exposure period, UA was gradually accumulated in rice grains in a dose-depended manner (Figure S2). UA levels in rice grains here were comparable with those rice grains in non-diseased panicles in field study above. Those results confirmed the hypothesis above that UA could be taken up by roots and translocated acropetally within above-ground parts of rice plants during ripening phase. Therefore, the prevailing UA in rice grains observed in RFS-occurred fields might primarily attribute to the widespread contamination of UA in paddy waters. The bioconcentration factor (BCF, the ratio of UA concentrations in brown rice to corresponding surface waters, (μg/kg $_{grain}$)/(μg/L $_{water}$)) in this exposure were calculated to be in the range of 0.30 – 0.41 L/kg, which was comparable with some hydrophilic microcystin (log K_{ow} -1.2 ~ -1.54, BCF 0.53) (Cao et al., 2018). However, the BCFs of UA were 5-6 times lower than the factor of grains (geometric mean: 1.95, median: 1.48) from non-diseased panicles in fields, indicating that there might be an existence of other pathways for UA accumulation in rice plants, such as foliar uptake, in field condition. Although an exposure experiment of UB was not carried out, which was mainly restricted by the limited availability of pure UB, the uptake of UB in water-rice plant system was predictable due to the very similar structure with UA (Table S1) (Li et al., 2008). Overall, the field study and laboratory experiment demonstrated that ustiloxins could be taken up by the roots and accumulated in grains in water-rice system of RFS-occurred regions, which is likely a contributing factor to widespread contamination of rice grains even in non-diseased panicles in RFS-occurred regions.

3.3 Geographic Distribution of Ustiloxins in Polished Rice from Hubei Province

To further explore the occurrence of ustiloxins, the concentrations of UA and UB in 149 polished rice samples from Hubei Province (China) were analyzed. Consistent with the results from the field survey of this study, ustiloxins were prevalent in polished rice. UA was found in approximately 96% of the polished rice samples, with concentrations in the range of <11.6 ng/kg dw (LOD) – 54.79 μg/kg dw. In contrast, the levels and detection frequencies of UB (62.4%) found in those samples were generally lower than those of UA, which ranged from <9.1 ng/kg (LOD) to 2.76 μg/kg dw with a GM value of 0.06 μg/kg dw. The serious ustiloxin contamination observed in polished rice was supposed to be a result of the extremely high RFS incidence in Hubei Province annually (about 40%) (Lu et al., 2018; Zhang et al., 2006). Descriptive statistics of the concentrations of UA and UB in polished rice of 13 cities were shown in Fig S3 and summarized in Table S4 and S5. Among the 13 cities, the detection frequencies of UA were greater than 85 %, and higher abundances of UA were found in Yichang (GM: 2.76 μg/kg dw), Wuhan (GM: 1.75) and Xianning (GM: 1.38). With the exception of Enshi Tujia and Miao Autonomous Prefecture (93.0 %), Tianmen (100 %) and Xianning (81.8 %), much lower detection frequencies of UB (below 70 %) were found in the rest of the 13 cities, which might be closely linked with the fact that the contents of UB were generally lower (2-11 folds) in RFS balls than that of UA (Wang et al., 2016; Hu et al., 2018; Fu et al., 2017). Meanwhile, significant differences could be found between the spatial distribution of UA and UB, which might be due to the fact that the genetic diversity of pathogens and the ratios of UA/UB in RFS balls varied substantially in different places (Abbas et al., 2014). This result demonstrated the ubiquitous contamination of ustiloxins in rice, which might be the consequence of ustiloxin uptake and translocation in paddy fields as described above.

The geographical distribution of ∑ustiloxins in rice is shown in Fig. 4. A remarkable degree of spatial variation of ∑ustiloxins concentrations was found, with GM concentrations ranging from 0.37 to 2.79 μg/kg dw. Relatively higher levels of ∑ustiloxins were found in Yichang (2.79 μg/kg dw), Xianning (1.92) and Wuhan (1.66). The measured ustiloxin levels in rice from Hubei Province (GM range of 0.37 - 2.79 μg/kg dw) were approximately 40 times lower than those found in peeled rice from a supermarket of Heilongjiang Province (GM: 34.05 μg/kg dw) (Fu et al., 2015b), but were comparable with those found in brown rice from non-diseased panicles in the present study (GM: 0.82 μg/kg dw). Taken together, the results from this study suggested the contamination of ustiloxins might occur widely in rice planting countries, and widespread ustiloxin exposure in humans might be occurring. Although the toxicity of ustiloxins toward humans remains unknown, animal studies showed that exposure to ustiloxins was linked to obviously pathogenic changes (Nakamura et al., 1994; Wang et al., 2017). Furthermore, it was reported that relatively lower micromolar concentrations of ustiloxins were cytotoxic towards various eukaryotes (the half maximal inhibitory concentration (IC_{50}): 0.39-17.05 μM) (Joullie et al., 2011; Koiso et al., 1994; Li, et al., 2006). Further investigations are needed to elucidate the contamination profiles of ustiloxins in global rice and their potential threatens to environment and human health.

4. Conclusion

This study, for the first time, provided evidence that ustiloxins could be taken up by roots of rice plants from contaminated paddy waters in RFS-occurred paddy fields. Besides, the results indicated a ubiquitous occurrence of ustiloxins in polished rice collected from Hubei Province (China). Although sample sizes were limited, our findings highlighted a possibility that numerous people, particularly South and South-East Asian, were likely to be suffering from ustiloxin exposure via the diet. Considering that RFS is now categorized as one of the most devastating diseases of rice, further efforts are urgently needed to evaluate the contamination of ustiloxins in rice worldwide.

Conflict of interest

All of the authors declare no competing financial interests.

Acknowledgments

This work was supported by National Key R&D Program of China (2019YFD1100501) and International Cooperation Project of Hubei Province (2021EHB030).

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Figure legends

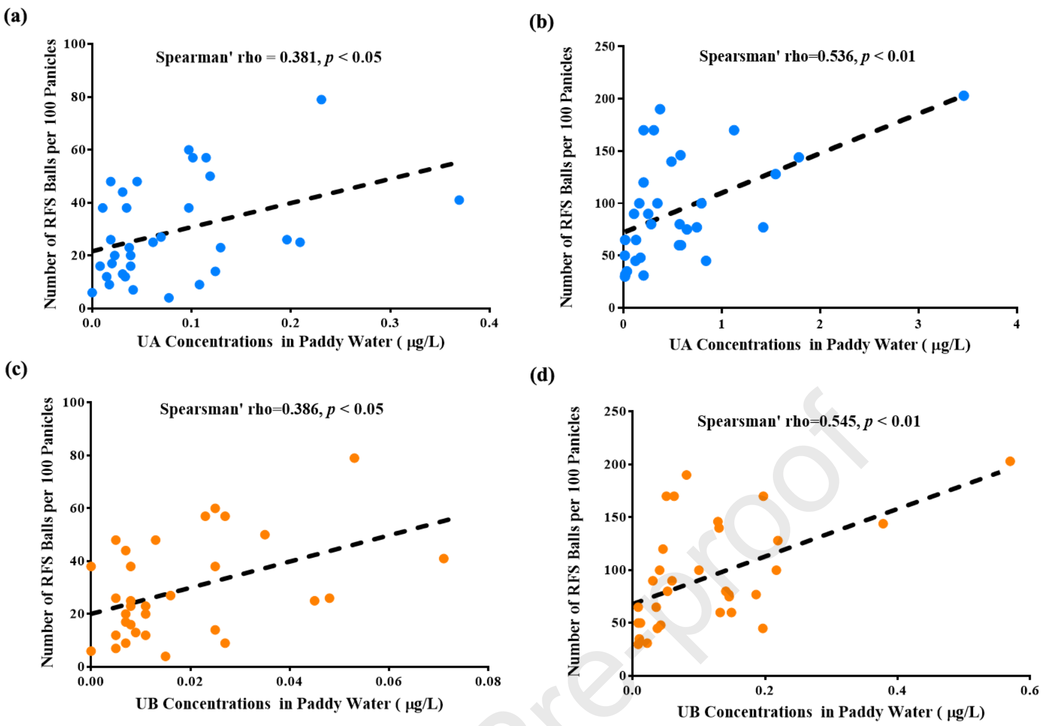
Figure 1. Correlation between numbers of RFS balls per 100 panicles and concentrations of UA in paddy waters at the dough grain stage of rice (a) and at the mature stage of rice (b); Correlation between numbers of RFS balls per 100 panicles and concentrations of UB in paddy waters at the dough grain stage of rice (c) and at the mature stage of rice (d). Each symbol denotes the result of an independent sampling site (n = 33).

Figure 2. (a) Association of concentrations of UA in brown rice collected from diseased panicles (with detectible rice false ball) and concentrations of UA in surrounding paddy waters (n = 33); (b) Correlation of UA residues in brown rice collected from non-diseased panicles (without detectible rice false ball) and concentrations of UA in surrounding paddy waters (n = 33); (c) Association of concentrations of UB in brown rice collected from diseased panicles (with detectible rice false ball) and concentrations of UB in surrounding paddy waters (n = 33); (d) Correlation of UB residues in brown rice collected from non-diseased panicles (without detectible rice false ball) and concentrations of UB in surrounding paddy waters (n = 33). Each symbol denotes the result of an independent sampling site.

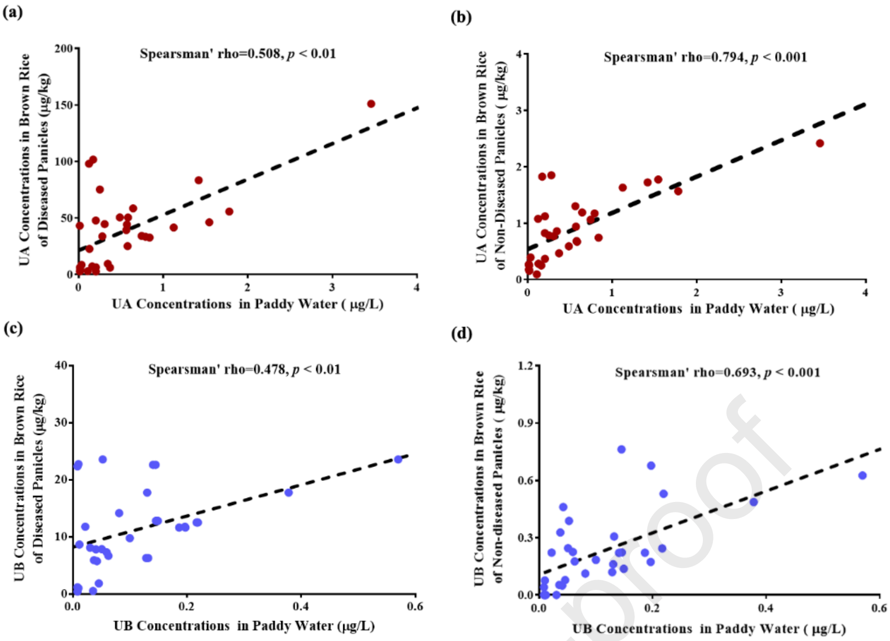
Figure 3. (a) A schematic setup of rice crops exposed to UA. (b) Concentrations of UA in brown rice and stalks of rice after exposure to 0, 2.0 or 10.0 μg/L UA for 30 days. ND means concentration below the detection limit. Means were averaged from three replicates and error bars indicate SEM.

Figure 4. Concentration distribution of ∑ustiloxins (geometric concentration of UA and UB) in polished rice from 13 cities of Hubei Province, China.

Fig. 1


(a)
Spearman' rho = 0.381, p < 0.05
Number of RFS Balls per 100 Panicles
UA Concentrations in Paddy Water (μg/L)
(b)
Spearsman' rho=0.536, p < 0.01
Number of RFS Balls per 100 Panicles
UA Concentrations in Paddy Water (μg/L)
(c)
Spearsman' rho=0.386, p < 0.05
Number of RFS Balls per 100 Panicles
UB Concentrations in Paddy Water (μg/L)
(d)
Spearsman' rho=0.545, p < 0.01
Number of RFS Balls per 100 Panicles
UB Concentrations in Paddy Water (μg/L)

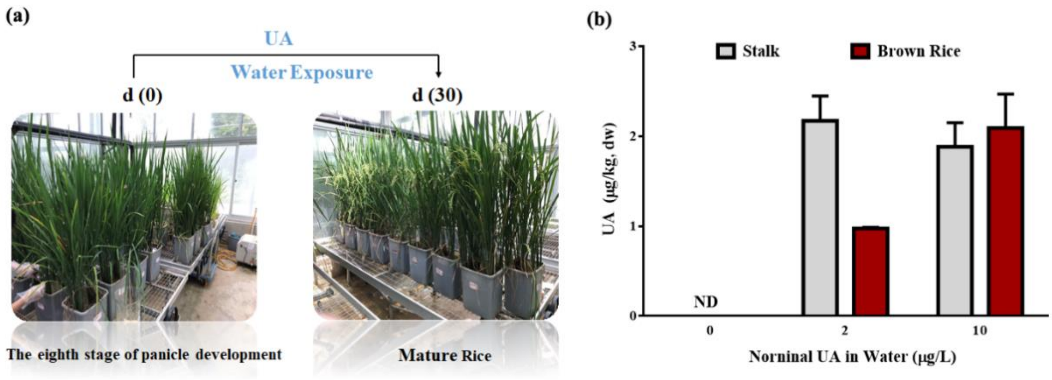
510 **Fig. 2**



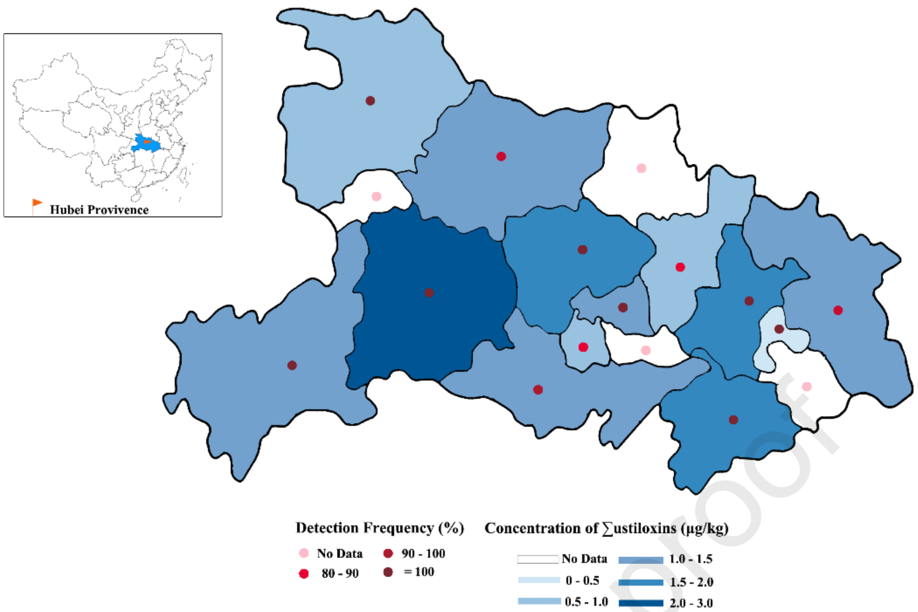
511

512

Fig. 3


(a)
UA
Water Exposure
d (0)
d (30)
The eighth stage of panicle development
Mature Rice
(b)
Stalk
Brown Rice
UA (μg/kg, dw)
ND
0
1
2
3
0
2
10
Norninal UA in Water (μg/L)

516 **Fig. 4**


Hubei Provivence
Detection Frequency (%)
No Data
90 - 100
80 - 90
= 100
Concentration of ∑ustiloxins (μg/kg)
No Data
1.0 - 1.5
0 - 0.5
1.5 - 2.0
0.5 - 1.0
2.0 - 3.0

517

Table 1. Descriptive statistics of UA and UB in rice grains samples and surface waters from RFS-occurred paddies in Enshi City, Hubei Provence, China.

Samples [a]		Surface Waters (μg/L)		Rice Grains (μg/kg dw)	
		Dough Grain Stage	**Mature Grain Stage**	**Diseased Panicles**	**Non-diseased Panicles**
UA	DF [b] (%)	100	100	100	100
	GM [c]	0.05	0.25	22.91	0.69
	Median	0.04	0.31	34.12	0.78
	Ranges	0.01-0.37	0.02-3.46	2.61-154.08	0.09-2.82
UB	DF [b] (%)	93.9	100	100	84.8
	GM [c]	0.01	0.07	4.66	0.13
	Median	0.01	0.06	7.31	0.18
	Ranges	<0.02 - 0.07	0.02-1.15	0.43-23.57	<0.09 - 0.82

[a] n = 33; [b] detection frequency; [c] geometric mean.

Highlights:

(1) Ustiloxins were widespread in surface waters of RFS-occurred paddies.
(2) Root uptake of ustiloxins from surface water widely occurred in paddy fields.
(3) Ustiloxins showed high detection frequency in rice plants due to root uptake.
(4) Ustiloxins contamination was ubiquitous in polished rice of Hubei province.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supporting Information

Occurrence and translocation of ustiloxins in rice false smut-occurred paddy fields, Hubei, China

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Contents

Text S1

Reagents

Ustiloxin A (UA) and ustiloxins B (UB) were isolated and purified from rice false smut balls according to a previous work (Sun et al., 2021), and the purity was > 95 %. Methanol (MeOH), acetonitrile (ACN), dichloromethane (DCM), formic acid (FA), ammonium acetate (AA) and ammonium hydroxide (NH_4OH) (LC-MS grade) were obtained from Honeywell (Seelze, Germany). CCl_3COOH was purchased from Aladdin Corporation (Shanghai, China). Ultrapure water was from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Oasis HLB (60 mg, 3 cc) and CNW BOUD WAX (200 mg, 3 cc) SPE cartridges were obtained from Waters (Wexford, Ireland) and Anpel laboratory Technologies (Shanghai, China), respectively.

Text S2

Rice and straws extraction. The lyophilized rice grains were milled to obtain brown rice. 500 mg of grounded polished/brown rice (150 μm) or 300 mg grounded straws (150 μm) and 10 mL pure water were added into a centrifuge tube, and the mixture was shaken vigorously for 10 min using vertical oscillator and then ultrasonic for another 10 min. Subsequently, the mixture was centrifuged at 7000 rpm for 10 min, and the aquatic solution was collected. Then, the remaining residues were extracted twice, and the combined supernatant was extracted with 25 mL dichloromethane. The upper layer was collected and loaded on WAX cartridge (CNW, 200 mg, 3 mL) after acidized with formic acid (pH = 5.0). After the cartridge was washed with 2 mL water (pH = 5.0) and 2 mL MeOH, the residues of ustiloxins were eluted with 2 mL 1 % NH_4OH-MeOH. The elutes were evaporated to dryness with nitrogen stream, and reconstituted in 200 μL 5 % MeOH-water for LC-MS/MS analysis.

Paddy water extraction. Briefly, 4 mL unfrozen paddy water was added into a 10 mL polypropylene tube containing 4 mL DCM. The mixture was shaken vigorously and centrifuged at 7000 rpm for 10 min. Subsequently, the supernatants were collected and acidified with 0.4 mL ammonium acetate solutions (500 mM, 5 % FA). Then, the aquatic solution was purified with a HLB cartridge (Waters, 60 mg, 3 mL). The cartridge was preconditioned with 3 mL MeOH and 3 mL of 50 mM ammonium acetate solution containing 0.5% FA, and was washed with 2 mL 0.5 % FA solution after loaded. Finally, the target analytes were eluted with 2 mL water-MeOH mixed solution (V:V = 5:95). The eluent was concentrated with nitrogen stream and then reconstituted in 200 μL 5 % MeOH-water. The solution was filtered (0.22 μm) for LC-MS/MS analysis.

Text S3

LC-MS/MS analysis. Analysis of UA and UB were carried out by Waters ACQUITY UPLC® H-Plus Class system (UHPLC) coupled to Waters® XevosTM TQ-XS mass spectrometer (TQ-XS/MS) (Milford, MA, USA) with electrospray ionization source in positive model (ESI (+)). The separation was accomplished using Acquity UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm, Waters) column with water (0.01% formic acid, A) and MeOH (B) as mobile phase. Gradient condition with a flow state of 0.3 mL/min was used for separation: 0-0.5 min, 5 % B; 0.5-5 min, 95 % B (linear); 5-7 min, 95% B; 7-7.2 min, 5 % B (linear); 7.2-9 min, 5% B. The injection volume was 2 μL and the column temperature was set at 40 ℃. Transitions of *m/z* 674.30 > 209.00 and *m/z* 646.25 > 181.10 were used for quantitative analysis of UA and UB, respectively. While, transitions of *m/z* 674.30 > 187.00 and *m/z* 646.25 > 187.00 were used for qualification analysis of UA and UB, respectively. The extraction recovery, matrix effect and LOD (limit of detection) and LOQ (limit of quantification) of proposed methods in this study for the analysis of UA and UB were shown in Table S3.

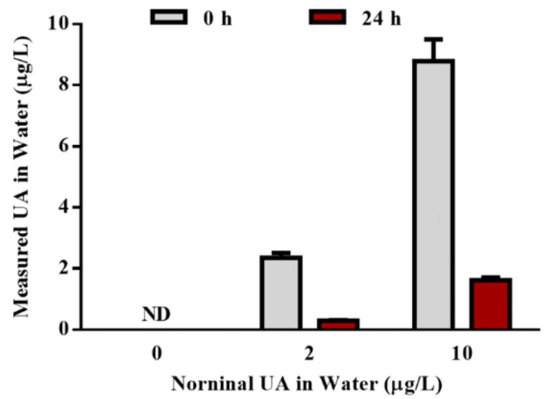


Fig. S1. Concentrations of UA in surface waters measured at two time points in rice exposure experiment. ND means concentration below the detection limit. Means were averaged from three replicates and the error bars indicated SEM.

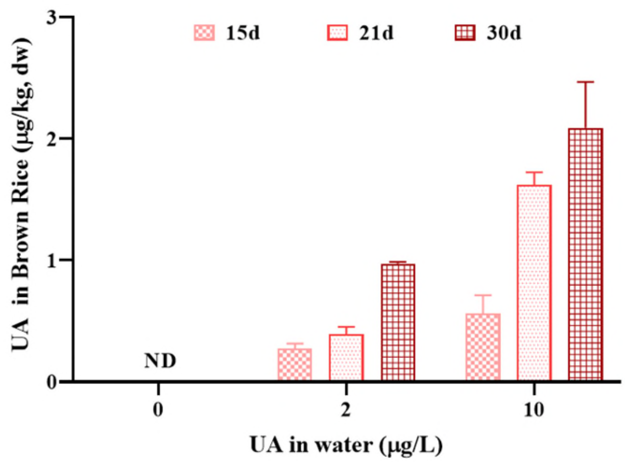


Fig. S2. Concentrations of UA in brown rice of rice plants after exposure to UA (0, 2 and 10 µg/L).

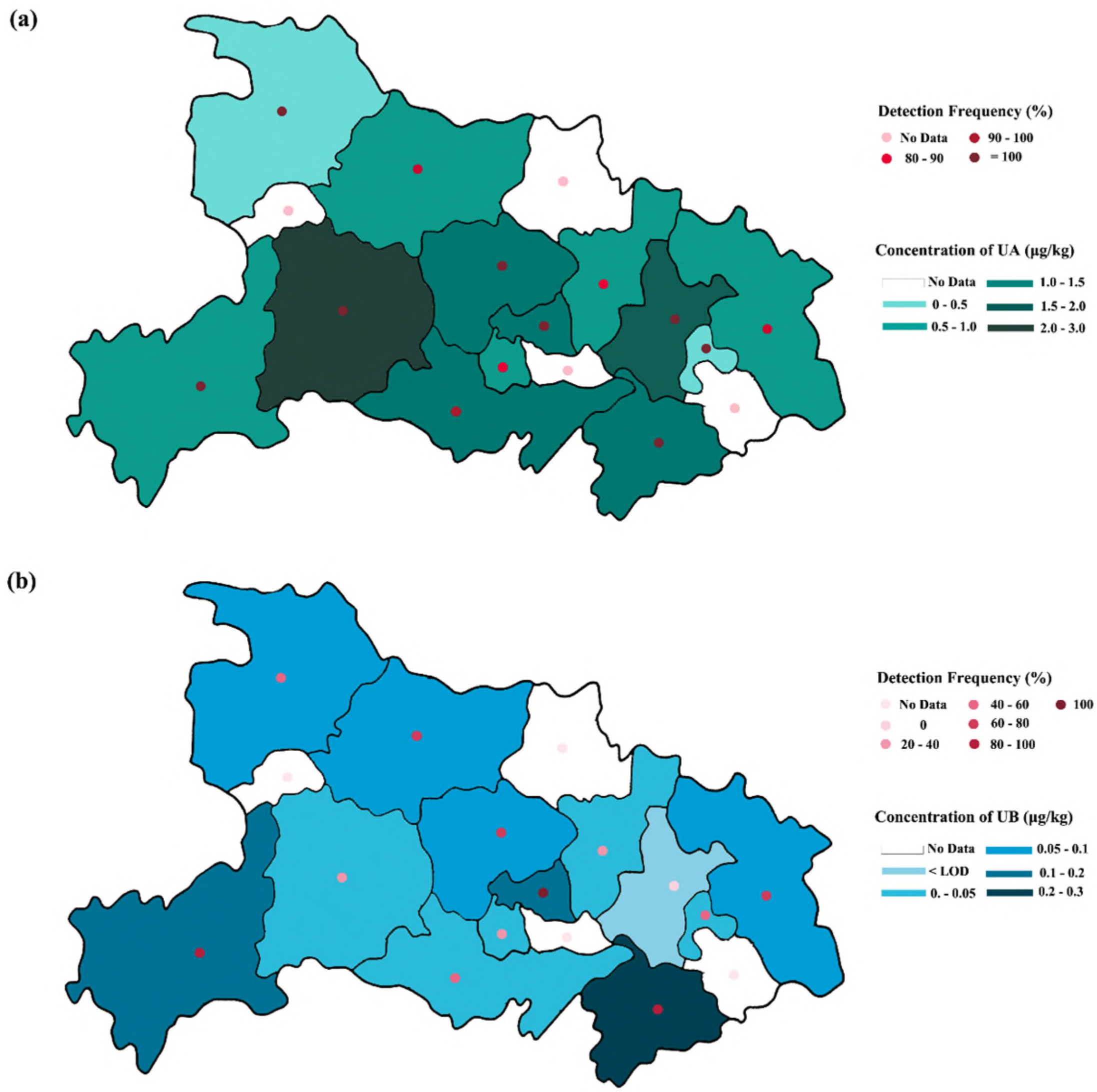


Fig. S3. Spatial distribution of UA (a) and UB (b) in rice across Hubei province. The colors of dots represent detection frequency of UA and UB in samples of rice. Colors of province or city show geometric concentrations of UA and UB in polished rice.

Table S1. Physico-chemical properties of usliloxin A and ustiloxin B.

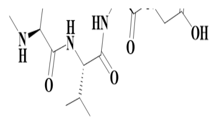
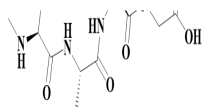
Compound	Structure	Water Solubility (g/L)	log K_{ow}	pKa	
				Acidic	Basic
Ustiloxin A		4.01	-1.6	1.05	8.8
Ustiloxin B		1.10	-1.5	1.05	8.85

Table S2. Information of the sampling sites.

No.	Location	Longitude	Latitude	Date and time	
				The first time	The second time
S1	Qingshan Village	109° 11' 37"	29° 43' 22"	2019.9.18 │ 9:05	2019.10.08 │ 8:23
S2	Qingshan Village	109° 11' 42"	29° 43' 3"	2019.9.18 │ 10:53	2019.10.08 │ 9:53
S3	Qingshan Village	109° 11' 45"	29° 43' 37"	2019.9.18 │ 14:35	2019.10.08 │ 13:33
S4	Qingshan Village	109° 11' 51"	29° 43' 41"	2019.9.18 │ 15:21	2019.10.08 │ 15:03
S5	Qingshan Village	109° 11' 57"	29° 43' 1"	2019.9.18 │ 16:05	2019.10.08 │ 16:43
S6	Laozhai Village	109° 8' 43"	29° 36' 5"	2019.9.19 │ 8:12	2019.10.09 │ 8:03
S7	Laozhai Village	109° 8' 45"	29° 36' 9"	2019.9.19 │ 9:43	2019.10.09 │ 9:28
S8	Laozhai Village	109° 8' 49"	29° 36' 12"	2019.9.19 │ 11:12	2019.10.09 │ 10:41
S9	Laozhai Village	109° 8' 51"	29° 36' 14"	2019.9.19 │ 14:02	2019.10.09 │ 14:03
S10	Laozhai Village	109° 8' 53"	29° 36' 23"	2019.9.19 │ 15:25	2019.10.09 │ 15:28
S11	Laozhai Village	109° 8' 32"	29° 36' 36"	2019.9.19 │ 16:49	2019.10.09 │ 16:51

Table S3. Method limits of quantification (LOQ), limit of detection (LOQ), extract recoveries and matrix effects of UA and UB in various matrixes (n = 3).

Compounds	Samples	Recovery ± SD %	Matrix Effect ± SD %	LOD [a]	LOQ
UA	Paddy Water	85.5 ± 3.5	91.5 ± 8.5	1.2	3.8
	Rice	89.3 ± 8.6	101.8 ± 0.4	11.6	38.9
	Straw	80.7 ± 2.1	80.8 ± 1.9	192.1	640.4
UB	Paddy Water	80.5 ± 2.1	92.0 ± 6.3	1.5	5.0
	Rice	85.1 ± 6.6	98.3 ± 1.2	9.1	30.3
	Straw	81.3 ± 3.2	86.5 ± 3.1	161.2	537.3

[a] ng/L (ng/kg)

Table S4. Descriptive statistics of UA residues (μg/kg dw) in 149 polished rice samples collected from Hubei provinces, China.

City	Sample Number	Detection Frequency (%)	Mean	Median	Geomean	Maximum
Enshi Tujia and Miao Autonomous Prefecture	43	100	1.70	0.69	0.69	9.67
Ezhou	2	100	0.66	0.66	0.33	1.23
Huanggang	21	95.2	1.37	0.90	0.86	7.10
Jingmen	6	100	1.64	1.71	1.36	2.53
Jingzhou	23	95.2	2.37	1.34	1.10	14.12
Qianjiang	8	87.5	1.05	0.69	0.55	2.10
Shiyan	2	100	0.45	0.45	0.43	0.60
Tianmen	2	100	1.07	1.07	1.06	1.22
Wuhan	2	100	1.75	1.75	1.75	1.87
Xianning	14	100	2.14	1.31	1.38	7.74
Xiangyang	13	92.3	5.19	0.98	0.87	54.79
Xiaogan	9	88.9	1.76	0.83	0.56	8.81
Yichang	4	100	3.33	3.36	2.76	5.39

Table S5. Descriptive statistics of UB residues (μg/kg dw) in 149 polished rice samples collected from Hubei provinces, China.

City	Sample Number	Detection Frequency (%)	Mean	Median	Geomean	Maximum
Enshi Tujia and Miao Autonomous Prefecture	43	93.0	2.06	0.35	0.16	2.76
Ezhou	2	50	0.09	0.09	0.04	0.18
Huanggang	21	61.9	0.17	0.09	0.06	0.81
Jingmen	6	66.7	0.20	0.18	0.08	0.55
Jingzhou	23	47.9	0.20	<LOD	0.03	2.01
Qianjiang	8	25.0	0.03	<LOD	0.01	0.20
Shiyan	2	50	0.43	0.43	0.08	0.85
Tianmen	2	100	0.25	0.25	0.24	0.31
Wuhan	2	0	<LOD	<LOD	<LOD	<LOD
Xianning	14	81.8	1.64	0.09	0.10	7.74
Xiangyang	13	61.5	0.14	0.15	0.06	0.45
Xiaogan	9	22.2	0.09	<LOD	0.02	0.54
Yichang	4	25.0	0.05	<LOD	0.02	0.17

Reference

1. Sun, Q., Liu, H., Zhang, Y.et al., **2021**. Detection of ustiloxin A in urine by ultra-high-performance liquid chromatography-tandem mass spectrometry coupled with two-step solid-phase extraction. *J. Chromatogr. B* 1181, 122916. https://doi.org/10.1016/j.jchromb.2021.122916