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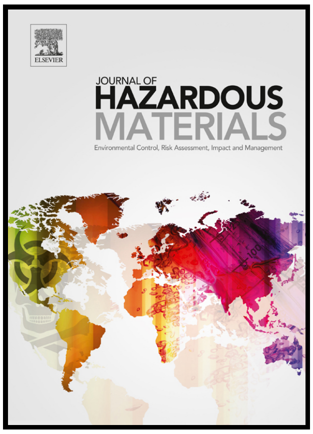

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PII: S0304-3894(24)01274-3

DOI: https://doi.org/10.1016/j.jhazmat.2024.134695

Reference: HAZMAT134695

To appear in: *Journal of Hazardous Materials*

Received date: 25 February 2024
Revised date: 2 May 2024
Accepted date: 21 May 2024

Please cite this article as: Zuoyin Zhu, Wenbo Guo, Haisheng Cheng, Hanke Zhao, Jie Wang, Mohamed F. Abdallah, Xinli Zhou, Hulong Lei, Weilong Tu, Hongyang Wang and Junhua Yang, Co-contamination and interactions of multiple mycotoxins and heavy metals in rice, maize, soybeans, and wheat flour marketed in Shanghai City, *Journal of Hazardous Materials,* (2024) doi:https://doi.org/10.1016/j.jhazmat.2024.134695

Co-contamination and interactions of multiple mycotoxins and heavy metals in rice, maize, soybeans, and wheat flour marketed in Shanghai City

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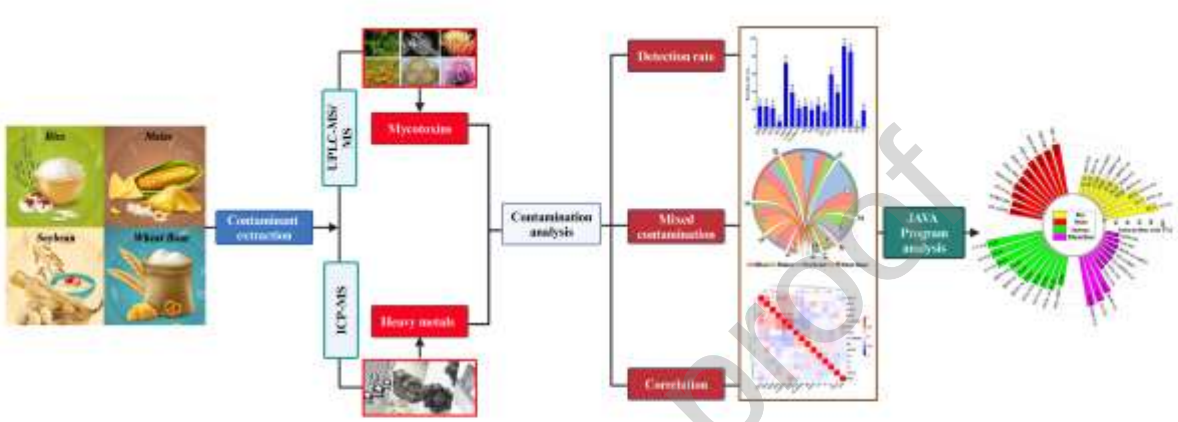
Abstract:

Mycotoxins and heavy metals extensively contaminate grains and grain products, posing severe health risks. This work implements validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) methods to quantify the concentration of 12 mycotoxins and five heavy metals in rice, maize, soybeans, and wheat flour samples marketed in Shanghai. The mixed contamination characteristics were analyzed using correlation cluster analysis and co-contamination index, and the probabilities of all cross combinations of contaminations were analyzed using a self-designed JAVA language program. The results showed that grains and grain products were frequently contaminated with both mycotoxins and heavy metals, mostly with deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), ochratoxin A (OTA), aflatoxins, fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), arsenic (As), chromium (Cr) and cadmium (Cd). All the samples (100%) were contaminated with two or more contaminants, and 77.3% of the samples were co-contaminated with more than four contaminants. In cereals and cereal products, the following combinations were closely associated: (FB3+3-ADON), (FB1+As), (FB1+FB2), (DON+FB1), (DON+Cd), (As+Cd), (DON+Cd+As), (FB1+FB2+As), and (DON+3-ADON+15-ADON). The results indicated that mycotoxins and heavy metals frequently co-occurred in Shanghai grains and grain products, and they provided primary data for safety assessments, early warnings, and regulatory measures on these contaminants to protect public health.

Graphical abstract



Keywords: grains and grain products, mycotoxins, heavy metals, combined contamination, interactivity analysis

1. Introduction

Contamination of food with toxic substances is a major global issue that poses severe threats to human health and worldwide economy [1]. The primary food contaminants include but are not limited to, mycotoxins and heavy metals [2]. Throughout the food chain, consumers are likely co-exposed to two or more categories of food contaminants, which may have combined toxic effects. Recently, an increasing number of studies have reported that, in particular, mycotoxins and heavy metals are the two most significant contributors to food contamination, and interactions between them may exacerbate their toxicities from their individual exposure [3-6]. Mycotoxins are toxic metabolites produced by toxigenic fungal species of the genera *Aspergillus, Penicillium,* and *Fusarium* during the growth, harvest, and storage of grains [7]. More than 400 mycotoxins have been detected worldwide in grains and other crops [8]. Among them, aflatoxins (AFBs), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEN), and fumonisins (FBs) are of global concern [9]. Human exposure to mycotoxins can result in a wide range of toxic effects, both acute and chronic, including nephrotoxicity, embryotoxicity, and even carcinogenic effects [10, 11]. For example, aflatoxin B1 (AFB1) is known to cause hepatotoxicity and is classified as a group I carcinogen [12]. Fumonisin B1 (FB1) suppresses the expression of many tumor suppressor genes in tumor cells through non-genotoxic mechanisms, including DNA methylation, histone modification, and microRNA [13, 14].

Heavy metals are metallic elements with a density greater than 5 g/cm^3 and include, among others, chromium (Cr), cadmium (Cd), arsenic (As), and lead (Pb). They are widely present in the environment and enter the food chain due to anthropological activities such as industrial emissions and agricultural fertilizers, thus becoming hazardous to human health [15]. Accumulation of heavy metals in the human body can cause a wide range of health disorders, including neuromuscular defects, nephrotoxicity, hepatotoxicity, neurotoxicity, and carcinogenic effects [16-20]. It has been reported that Cd induces various epigenetic changes in mammalian cells *in vivo* and *in vitro*, leading to pathogenic risks and multiple types of cancers [21]. According to the China Food Safety Situation Analysis Report in 2021, the problem of heavy metal contamination in China's grains has been brought under initial control; however, there are still lingering issues [22]. Assessments of heavy metals in grains and grain products sold in other countries, such as Iran, Peru, Egypt, and Saudi Arabia, have also reported concerning amounts of Cd and Pb in cereals and other products; often varying degrees of contamination exist [23-26]. Considering the increasing evidence of the detrimental effects of these contaminants, a better understanding of how the simultaneous contamination will affect their overall toxicity is critical to the regulatory policies and other stakeholders. However, while many studies explore heavy metal or mycotoxin contamination in various food and grain products, most focus on investigating the sole toxic effect. Literature surveys showed that even in reports that analyze multiple contaminants, each is usually studied individually, and few of them have examined the association or interactions between contaminants [27-29]. As a result, a significant knowledge gap exists regarding the co-contamination of multiple prevalent heavy metals and mycotoxins. Many aspects of simultaneous contamination, such as the correlation between contaminants and the optimal methodology for analyzing the probability of their co-existence, still need to be solved.

Shanghai is one of China's largest and most developed cities, and its dense population mainly consumes grain and oil foods on a massive scale. In recent years, growing awareness has led consumers to prefer healthy, nutritious, safe, and environmentally friendly foods. This increase in demand for green and contamination-free products is contrasted by the increasing severity and prevalence of grains and grain products contaminated with mycotoxins and heavy metals [30, 31]. Assessment and regulation of these contaminants are therefore crucial to consumer health and to keep up with market trends towards safety and quality.

The present study implemented validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) to analyze four typical grains and grain products (rice, maize, soybeans, and wheat flour) marketed in 16 districts of Shanghai. We assessed the contamination with 12 mycotoxins (AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), DON, 3-ADON, 15-ADON, FB1, FB2, FB3, OTA, and ZEN) and five heavy metals (Cr, As, Cd, mercury (Hg), and Pb), mainly those for which the Codex Alimentarius Commission (CAC), the European Union, the U.S.A, or China have set clear limits. Furthermore, the study analyzes the condition and characteristics of the multi-mycotoxin and heavy metal co-contamination in rice, maize, soybean, and wheat flour from Shanghai City using correlation cluster analysis and co-contamination index. In addition, interaction analysis was performed on the multi-contaminants found in grains and grain products to identify the co-contamination and assess their co-existence probabilities using a self-designed JAVA language program. In the end, the study provides data for the assessment, early warnings, and regulatory measures related to mycotoxins and heavy metal contamination, which are essential to ensuring the quality and safety of grains and grain products and protecting public health, as well as providing innovation for the establishment of national standards involving multiple contaminants.

2. Materials and methods

2.1 Chemicals and Instruments

Twelve mycotoxin standards: AFB1, AFB2, AFG1, AFG2, DON, 3-ADON, 15-ADON, FB1, FB2, FB3, OTA, and ZEN were purchased from Pribolab (Qingdao, China), which all had a purity > 98%. The high-purity solvents (HPLC grade) of methanol (MeOH), acetonitrile (ACN), formic acid (HCOOH), and ammonium acetate (CH_3COONH_4) were purchased from Merck (Merck, Darmstadt, Germany). Anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), nitric acid (HNO_3), and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich (Sigma-Aldrich, MO, USA).

A UPLC XEVO TQ-S ultra-performance liquid chromatography-tandem mass system (Waters, MA, USA) was used for the UPLC-MS/MS analysis. An ICP-MS spectrometer (model ICAP RQ, Thermo Fisher Scientific, MA, USA) was used to measure the digested. Sample digestion was conducted using a MARS6 microwave digestion system (CEM, NC, USA). Extract concentration was determined using the HSC-24B Nitrogen Blowing Instrument (Troody Analytical Instrument Co., Ltd, Shanghai, China). The sample solution was centrifuged using a 5804R high-speed centrifuge (Eppendorf Corp., Germany). A SK8210LHC ultrasonic cleaning device (Kudos Ultrasonic Instrument Co., Ltd, Shanghai, China) was used for supersonic-assisted extraction. Samples were weighed on an AL104 balance (Mettler Toledo, NY, USA) and ground using a BJ-800A Food Grinder (Hangzhou, China). A Milli-Q ultrapure water instrument (Millipore, MA, USA) was used to purify the water in all solution preparations and the ultra-high-performance liquid phase mobile phase.

2.2 Sample collection and extraction

A total of 300 samples consisting of rice (n = 82), maize (n = 50), soybeans (n = 78), and wheat flour (n = 90) were collected from over 100 supermarkets and farmers' markets located in 16 districts of Shanghai during September 2021 and September 2022. **Figure 1** depicts the sampling sites for grain and grain products marketed in 16 districts of Shanghai City, China (the map was drawn using Adobe Photoshop CC-Adobe Inc, USA, CA). The collected samples can be traced back to 232 manufacturers and 219 sample origins. To ensure sample representativeness, various samples of the same batch were randomly selected at each site. The information of each sample was documented, including the name and address of the site, date of sampling, the personnel obtaining the sample and their signature, the name of the sample, sample origin, production date and batch number of the sample, the status of the sample such as any visual observations (whether the packaging is broken, moldy, or spoiled), the number of samples collected, and the sampling environment (including temperature and humidity). Each sample was at least 500 g, placed in a sealed bag to be transported to the laboratory under refrigeration (between 0 and 4 °C), and then divided into four equal portions using the cone quadrature method. One part was randomly selected for contamination testing, and the remaining parts were frozen and stored at -20 °C. All samples were collected, divided, prepared, and stored separately to avoid cross-contamination.

Samples were mixed thoroughly and ground to fine powder using a grinding mill. Extraction and analysis of 12 mycotoxins was done as described before in the literature [32, 33], with some modifications. Briefly, 2.00 g of the homogenized sample was weighed and placed in a 50 mL falcon tube. After that, 1.6 mL of ultrapure H_2O was added, and the mixture was vortexed for 5 min. Then, 8.4 mL of ACN containing 1% HCOOH was added, and the mixture was vortexed for 10 min and sonicated for 60 min. Subsequently, 2.0 g of anhydrous $MgSO_4$ and 0.5 g of NaCl were added, and the mixture was vigorously shaken for 30 s, sonicated for 10 min, and centrifuged at 5000 rpm for 5 min. From the mixture, 5 mL of the supernatant were transferred into a new tube and evaporated under a gentle stream of nitrogen gas (Troody Analytical Instrument Co., Ltd, Shanghai, China) at 40 °C. The residue was re-dissolved in 1 mL of 5 mmol/L aqueous CH_3COONH_4-ACN (80:20, *V/V*) solution, vortexed for 1 min, and centrifuged at 12000 rpm for 15 min. The mixture was filtered through a 0.22 μm membrane (Sigma-Aldrich, Shanghai) before the UPLC-MS/MS analysis.

For heavy metals, a pretreatment (digestion procedure) was conducted using a microwave oven. In a digestion tank, a 0.20 g homogeneous sample for heavy metal digestion was added to 6 mL HNO_3 and incubated for 1 h. Afterward, 1 mL of H_2O_2 was added, and the mixture was placed into a microwave digester for digestion. The heating procedure used for the digestion was as shown in **Table S1**. After the digestion, the temperature was reduced to 70 °C, the digestion tank deflated in the fume hood, and the acid rushing device was replaced, after which the acid was driven at 1200 W for 26 min to 1-2 mL of the digested liquid. Finally, the digestion tank was washed with ultrapure water 3~4 times after cooling, the digestion liquid was transferred to a centrifuge tube, and the volume was fixed to 25 mL. The solution was filtered through a 0.45 μm filter membrane before the ICP-MS analysis.

2.3 UPLC-MS/MS and ICP-MS parameters

Chromatographic separation of the targeted mycotoxins was performed on an Agilent Poroshell 120 EC-C_{18} column (100 mm×3.0 mm, 2.5 mm) obtained from Waters (Waters, MA, USA). The mobile phase A consisted of pure ACN, and the mobile phase B was H_2O with 0.1% HCOOH to detect three FBs and 5 mmol/L CH_3COONH_4 in H_2O for the remaining mycotoxins. The gradient elution program was as follows: 0-3 min, 10% A; 3-5 min, 70%-90% A; 5-6 min, 90% A; 6-6.1 min, 90%-10% A; and 6.1-8 min, 10% A. The flow rate was 0.4 mL/min, the injection volume was 3 μL, and the column temperature was 40 °C. Electrospray ionization source (electron spray ionization, ESI) positive and negative ion modes were used for simultaneous scanning, and the atomization gas and auxiliary gas were high-purity nitrogen (99.99%). The collision gas was high-purity argon, and the capillary interfacial voltages were 2.5 kV (ESI^+) and 1.5 kV (ESI^-). The atomization temperature was 500 °C, and the source temperature was 150 °C. The gas flow rate was 7.0 bar for both nebulized and desolventizing gas at 1000 L/h. Quantification of the target compounds was performed under multiple reaction monitoring (MRM) mode. Other mass spectrometry parameters are detailed in **Table S2**. Heavy metal analysis was performed on an ICAP RQ (Thermo Fisher Scientific, MA, USA) with an Octopole Reaction System Inductively Coupled Plasma Mass Spectrometer. The condition of the ICP-MS analysis was as follows: the flow rate of the nebulizer was set at 1.1 L/min, the temperature of the nebulizer chamber at 2.7 ℃, the sampling depth at 5 mm, the plasma power supply at 1550 V, and the flow rate of the auxiliary gas at 0.8 L/min.

Method validation and quality control were performed according to the National Manual for Risk Monitoring of Food Contaminants and Harmful Factors (CFSA, 2020) and the European Commission decision (2002/657/EC) guidelines [34, 35]. The two methods were validated for rates of recovery, limits of detection (LOD), limits of quantification (LOQ), repeatability (RSD_r), and reproducibility (RSD_R). The matrix effect was evaluated through the slope ratio of the matrix-matched calibration curve to the solvent standard calibration curve. The LOD and LOQ were designated as the concentration with a signal-to-noise (*S/N*) ratio of 3 and 10, respectively. Recoveries were also evaluated through spiking in blank samples at low, middle, and high concentration levels. Among them, mycotoxin concentrations were five ng/mL, 50 ng/mL, and 100 ng/mL, while heavy metal concentrations were 0.1 mg/kg, 1 mg/kg, and 10 mg/kg.

2.4 Evaluation of mycotoxins and heavy metals in samples

The Chinese Maximum Residue Limits (MRLs) for mycotoxins and metals in grains and grain products were used as a reference in the current work [36, 37]. In the absence of regulations for mycotoxins and heavy metals in China, the limits issued by the Codex Alimentarius Commission (CAC), the European Union, and the U.S. were used. **Table S3** summarizes the standard limits for mycotoxins and heavy metals in grains and grain products.

2.5 Interaction rate calculation

A self-designed JAVA language program was used to perform interaction analysis on the co-contamination of the studied contaminants. The program was applied with a computer software copyright (**Supplementary Material 1**), the language of the computer program is shown in **Supplementary Material 2**, and the interaction rate was calculated through the formula:

$$R = \frac{x \cdot C_i^j}{n} \times 100$$

Where R is the interaction rate; x is the frequency of occurrence of the combination; n is the total number of samples; C is the number of combinations; i is the total number of contaminants investigated; and j is the number of contaminants interacted.

2.6 Statistical analysis and data visualization

The contamination levels and indexes of multi-mycotoxin and heavy metal contamination levels in grains and grain products were performed using Origin 2021 (OriginLab, MA, USA). Mycotoxin and heavy metal correlation cluster analysis was conducted using OmicStudio (https://www.omicstudio.cn/tool). The data were analyzed using a one-way ANOVA test followed by Duncan's multiple comparisons to identify differences between mean values using SPSS 16.0 (SPSS Inc., IL, USA), with $P<0.05$ indicating significant differences. In case the level of contaminants in the sample was lower than the LOD, it was considered as “not detected” and replaced by 1/2 LOD when calculating the mean value of the contaminants [38].

3. Results

3.1. Validation of the analytical methods

The LOD and LOQ values for the developed method are shown in **Table S4.** As for UPLC-MS/MS analysis, the matrix effect was evaluated through the slope ratio of the matrix-matched calibration curve to the solvent standard calibration curve. The results showed that the slope ratio ranged from 0.31 to 3.6 (**Table S5**), demonstrating a matrix effect. Therefore, the matrix-matched multi-external standards were used for qualitative and quantitative analysis. The results from the validation step verified the feasibility of the developed method. The standard curves for 12 mycotoxins showed excellent linearity (coefficient $R^2 \geq 0.992$ for all standard curves). The LODs ranged from 0.05 to 4.0 μg/kg for the 12 mycotoxins analyzed. Other validation results of the developed UPLC-MS/MS method are shown in **Table S6**. The average recoveries at three spiked levels ranged from 82.2% to 119.7% in the four matrices (rice, maize, soybean, and wheat flour). The RSD_r and RSD_R ranged between 0.8% and 9.7%, respectively. For ICP-MS analysis, the LODs ranged from 0.001 to 0.02 mg/kg, and the R^2 prepared with standard solutions for Pb, Cd, Cr, As, and Hg were ≥ 0.994. The average recoveries at three spiked levels ranged from 82.6% to 119.5% in the four matrices (rice, maize, soybean, and wheat flour). The RSD_r and RSD_R were between 0.9% and 9.5%, respectively. All the contaminations of spiked recovery rate were between

80~120%, and the RSD_r and RSD_R were all < 10%.

3.2. Occurrence of mycotoxins and heavy metals in grains and grain products

A general overview of the contamination rate of the 17 targeted contaminants is depicted in **Figure 2A.** As shown, mycotoxins and heavy metals were detected in all samples (grains and grain products) with different extents. Of the 12 mycotoxins, the detection rate of DON (72.33%) was the highest, followed by OTA (59.33%), 3-ADON (39.00%), and FB3 (24.67%), AFB1 and FB1 (23.67% for each), AFB2 (23.33%), AFG1 and 15-ADON (21.67% for each), FB2 (19.67%), ZEN (18.00%), and AFG2 (6.33%). For heavy metals, As had the highest detection rate (91.33%), while for Cd, Cr, and Pb, the contamination rates were 85.00%, 39.00%, and 18.67%, respectively. Hg minimally contaminated the samples, as detected in only 2 (0.67%) samples. Within the rice samples (n = 82), OTA was the most common detected mycotoxin (73.17%), followed by DON (48.78%), and the remaining detection rates for other mycotoxins ranged from 1.22% to 43.90%. Among the five heavy metals analyzed, As had the highest detection rate (100.00 %) in rice. The detection rates for the other heavy metals were as follows: Cd at 90.24%, Cr at 26.83%, Pb at 12.20%, and Hg at 1.22% (**Figure 2B**). For maize samples (n = 50), FB1 (98.00%) was the most detected mycotoxin, followed by DON and FB2 (80.00% for each), OTA (68.00%), and the remaining mycotoxin detection rates ranged from 4.00% to 58.00%, while 15-ADON was not detected. On the other hand, As had the highest detection rate (88.00%), and the rest were in the order of Cd (30.00%), Pb (10.00%), and Cr (6.00%), while Hg was not detected (**Figure 2C**). In the soybean samples (n = 78), DON (75.64%) had the highest detection rate, followed by OTA, which was detected in 66.67% of the samples. Other mycotoxins were detected in a range between 1.28% to 46.15%. The detection rate of Cd among the five heavy metals was the highest (98.72%), and the remaining were in the order of Cr > As > Pb > Hg, with detection rates of 94.87%, 93.59%, 20.51%, and 1.28%, respectively (**Figure 2D**). In the wheat flour samples (*n* = 90), DON was at the highest detected rate of all mycotoxins (86.67%), while the remaining mycotoxins were detected in a range between 3.33% and 35.56%. Among the heavy metals, Cd was found at the highest rate of 98.89%, and the rest was in the order of As > Pb > Cr, with detection rates of 83.33%, 26.67%, and 21.11%, respectively. Similar to maize, Hg was not detected in wheat flour samples (**Figure 2E**).

When comparing the detection rates for each mycotoxin across the four categories of grain and grain products, it could be seen that AFB1 was found at a higher rate in soybeans (42.31%) than in other matrices. For other members of AFs (AFB2, AFG1, and AFG2), they were all found at a higher rate in maize than in the other three groups. DON was most frequently detected in wheat flour (86.67%), followed by maize (80.00%) and soybeans (75.64%). Regarding FB1 and FB2, they were most frequently detected in maize, with detection rates of 98.00% and 80.00%, respectively. ZEN had an overall low detection rate but was more frequently found in rice samples at a detection rate of 34.15%. OTA was detected at relatively higher rates in rice, maize, and soybeans (66.67% to 73.17%) but less frequently in wheat flour (35.56%). Furthermore, the highest detection rate for Cr was found in soybeans samples (94.87%), which was higher than the detection rates in maize (88.87%) and rice and wheat flour (20-30%). The overall detection rate of As was high (91.33%) in each grain and grain product category (83.33% to 100.00%). For Cd, the detection rate was high in rice, soybean, and wheat flour (90.24% to 98.89%) but at a lower rate in maize

(30.00%). For Pb, this heavy metal was detected at low rates in all four groups of samples, ranging from 10.00% to 26.67%, while Hg was detected in a few rice and soybean samples and was not detected in maize and wheat flour samples (**Figure 2B-2E**).

In most samples, at least four to eight different contaminants were found. The average and range values of each contaminant are listed in **Table 1**. The average levels of the different mycotoxins (AFB1, AFB2, AFG1, 3-ADON, FB3, and ZEN) in rice were in a range of 0.05 and 0.55 μg/kg. The average values of DON and 15-ADON were 6.35 μg/kg and 9.75 μg/kg, respectively, with the contamination level of DON in the range of 5.00-24.67 μg/kg. In addition, OTA contamination averaged at 1.14 μg/kg with a range of 0.15-41.52 μg/kg. The average values of heavy metals Cr, As, Cd, and Pb in rice were from 0.018 to 0.117 mg/kg. In maize, the contamination level of DON was 162.37 μg/kg on average with a range of 10.23-2091.52 μg/kg, and the average level of 3-ADON was 2.58 μg/kg. Furthermore, the contamination levels of FB1, FB2, and FB3 were 106.97, 19.71, and 16.08 μg/kg, respectively, while the contamination levels of the remaining six mycotoxins were below 0.46 μg/kg with a range of 0.06-3.93 μg/kg. Additionally, the contamination level of Pb was 0.058 mg/kg on average and ranged from 0.051-1.342 mg/kg in maize, while the average contamination levels of the remaining three heavy metals were below 0.030 mg/kg. In soybean, the contamination average level of AFB1 was 0.30 μg/kg and ranged from 0.08-2.81 μg/kg. However, the contamination levels of the remaining three AFs (AFB2, AFG1, and AFG2) were below 0.07 μg/kg. The average level of DON was 28.09 μg/kg, and that of its derivatives 3-ADON and 15-ADON ranged from 0.37-890.99 μg/kg. The average contamination levels of FB1, FB2, and FB3 were 3.88, 1.95, and 2.66 μg/kg, and the contamination range of the three mycotoxins was 0.40-281.34 μg/kg. The contamination level of ZEN was 32.37 μg/kg on average and ranged from 0.41-1505.48 μg/kg, while OTA averaged 0.56 μg/kg and had a range of 0.13-9.05 μg/kg. Cr had an average contamination level of 0.279 mg/kg with a range of 0.071-1.634 mg/kg, while the contamination levels of the remaining three heavy metals were below 0.025 mg/kg. In wheat flour, the contamination level of DON was 86.49 μg/kg on average, with a range of 10.79-698.84 μg/kg, while for3-ADON was 31.31 μg/kg on average, with a range of 0.35-1890.20 μg/kg, and for 15-ADON was 51.97 μg/kg with a range of 25.76-534.56 μg/kg. The contamination level of FB1 was 1.17 μg/kg on average, with a range of 0.96-78.72 μg/kg. However, the remaining mycotoxins were detected at levels lower than their LOD values. For the four heavy metals, the average contamination levels were below 0.051 mg/kg.

There were no significant differences in the average contamination levels of AFB1, AFG1, AFG2, 3-ADON, and OTA among 300 grains and grain products (**Table 1**). On the other hand, the contamination levels of AFB2, DON, 15-ADON, FB1, FB2, FB3, and ZEN were significantly different among grains and grain products ($P<0.05$). For heavy metals, Cr levels in soybean and As levels in rice were significantly different from the other three cereals. Furthermore, Cd contamination levels were different across all four grains and grain products, and Pb contamination levels in rice and maize were significantly different ($P<0.05$) than in soybean and wheat flour (**Table 1**).

Comparing the MRLs standards for mycotoxins and metals issued by the CAC, EU, US, and China with the current results showed that several samples were contaminated beyond the acceptable levels (**Table 2**). The detected levels of OTA in two of the 82 rice samples

(2.44%) were higher than MRLs standards, exceeding by 6.43 and 41.52 μg/kg. In one rice sample (1.22%), the As content was 0.23 mg/kg. In maize, two samples (4.0%) contained DON exceeding the standard limits as their contamination levels were 1631.25 and 2091.52 μg/kg. There were also two maize samples (4.0%) in which Pb content exceeded the MRLs; the excess amounts were 0.89 and 1.34 mg/kg. In soybean, one sample (1.28%) contained OTA levels exceeding the standard limits by 9.05 μg/kg, and four samples (5.13%) were contaminated with ZEN in a range of 78.82-1505.48 μg/kg beyond the MRLs. Two soybean samples (2.56%) contained Cr exceeding the MRLs by 1.05 and 1.63 μg/kg, and in one sample (1.28%), Pb exceeded the standard limits by 0.36 mg/kg. AFs and FBs were not found in excessively high levels in grain or wheat flour samples, and none of the 17 contaminants exceeded their corresponding MRLs in wheat flour.

3.3. Combined contamination of mycotoxins and heavy metals in grains and grain products

The current study found that mycotoxins and metals are widespread in Shanghai's rice, maize, soybean, and wheat flour. All 300 samples investigated were contaminated with at least two contaminants, 88.6% of the samples were co-contaminated with at least four contaminants, 59.0% with more than six, and 17.4% of the samples were contaminated with more than eight contaminants. As shown in **Figure 3**, around 81.3% of the total number of the samples were co-contaminated with 4-8 contaminants, which can be further divided into 55 samples affected by five contaminants (18.3%), 61 samples by six contaminants (20.3%), 64 samples by seven contaminants (21.3%), and eight contaminants co-contaminating 30 samples (10.0%). A small number of samples were affected by fewer or more contaminants:

samples with only two contaminants accounted for 2.7% of the total, 14 samples (4.7%) were contaminated with nine contaminants, and 8 (2.7%) were contaminated with ten contaminants (**Figure 3A and 3B**). Of the samples contaminated with four to six contaminants, the most significant proportion were wheat flour samples, while in those contaminated with seven contaminants, the most common grain was soybean. Samples contaminated with eight or nine contaminants were mainly maize. Also, all soybean samples contained more than five contaminants, and all wheat flour samples had fewer than nine contaminants (**Figure 3A**).

In 79.2% of rice samples, there were more than three contaminants, while in 46.2% of the samples, there were more than five, and in 9.8% of the samples, there were more than seven contaminants. The most frequent number of contaminants co-contaminating rice was seven, accounting for 22.0% of samples (**Figure 3C**). Almost all maize samples were co-contaminated with more than three contaminants, 78.0% of samples were affected by more than five contaminants, and 50.0% by more than seven contaminants. Maize samples were most commonly co-contaminated with eight contaminants, accounting for 30.0% of the samples (**Figure 3D).** Almost all soybean samples were co-contaminated with more than three contaminants, 88.4% of the samples contained over five co-contaminants, and 21.8% were contaminated with more than seven contaminants. The most frequent number of co-contaminants found in soybeans was seven, which accounted for 43.6% of the samples (**Figure 3E**). The total number of contaminants in all wheat flour samples was below nine. Those affected by more than three contaminants accounted for 81.1% of all wheat flour samples, those affected by more than five contaminants accounted for 62.2%, and those affected by more than seven contaminants accounted for 11.1%. Wheat flour samples contained five (27.8%) contaminants, accounting for a more significant portion than any other co-contaminant (**Figure 3F**).

3.4. Correlation and cluster analysis of mycotoxins and heavy metals in grains and grain products

Correlations between the 17 contaminants and similarities in contaminated levels were analyzed using correlation clustering analysis and heat mapping. Correlation coefficients with an absolute value between 0.1 and 0.3 represent a weak correlation between the contaminants, whereas those between 0.3 and 0.5 indicate a moderate correlation. When the absolute values of correlation coefficients are greater than 0.5, it is considered a strong correlation between them [39, 40]. In rice, significant moderate correlations were found between AFG1 and 3-ADON, DON, and ZEN. Also, significant moderate correlations were found between DON and 3-ADON or FB3, and between FB2 and FB3 ($P < 0.01$). Moreover, between 3-ADON and FB3, there were significant strong correlations ($P < 0.01$). Cluster analysis results showed similar levels in AFB1, AFG1, FB3, DON, 3-ADON, and ZEN contamination, in OTA, 15-ADON, and Pb contamination, in AFB2, AFG2, FB2, Cd, As, and Cr contamination (**Figure 4A**). In maize, significant moderate correlations were found between FB1 and AFB2, FB2, FB3, between DON and FB3, between FB2 and AFB1, between Pb and Cd, AFG1, and between OTA and Cd ($P < 0.05$). Also, the results showed similar levels of OTA, ZEN, DON, and 3-ADON contamination, in AFB1, AFG1, AFG2, FB2, As, and Cr contamination in FB1, FB3, AFB2, Cd, and Pb contamination (**Figure 4B**). In soybeans, there were significant moderate correlations between ZEN and AFB1, FB2 and Cr, between AFB1 and AFB2, FB3, AFG1 and As, between As and Cr, between FB3 and

DON, AFG1, and between FB1 and FB2, 3-ADON ($P < 0.01$). Also, between 3-ADON and AFB1, 15-ADON, and DON, between DON and AFB1, 15-ADON, and between 15-ADON and AFB1, there were significant and strong correlations ($P < 0.01$). The results of cluster analysis showed similar levels of contamination in FB1, FB2, FB3, ZEN, 3-ADON, and AFG1, and similar levels of contamination in AFB1, AFB2, DON, and 15-ADON, in OTA, AFG2, As, Cr, Pb, and Cd (**Figure 4C**). In wheat flour, significant moderate correlations were found between OTA and AFB2, 15-ADON, between AFG1 and 3-ADON, and between DON and Cd ($P < 0.01$), while significant and strong correlations were found between FB1 and FB2, and between Pb and Cr ($P < 0.01$). The results of cluster analysis showed that between DON, 15-ADON, Pb, Cd, and Cr, between FB1, FB2, FB3, ZEN, AFB1, and As, between AFB2, AFG1, AFG2, OTA, and 3-ADON also had similar levels of contamination (**Figure 4D**).

3.5. Interaction analysis of mycotoxins and heavy metals in cereal foods

A self-designed JAVA language program was used to analyze all the co-combinations and the interaction rates of each targeted 17 contaminants in rice, maize, soybeans, and wheat flour. A total of 8082 combinations were present in the 300 samples tested. Of the two contaminants pairs, the overall highest interaction rate was for Cr+Cd found in soybeans (94.87%). In rice, the highest rate was for As+Cd (90.24%), followed by OTA+As (73.17%). In maize, FB1+As had the highest interaction rate (86.00%), and FB1+FB2 had an interaction rate of 80.00%. In soybeans, in addition to Cr+Cd, the interaction probabilities of Cd+As (93.59%) and Cr+As (91.03%) were also high, and the likelihood of fungal-heavy metal interaction between DON and Cd (74.36%) was also notable. In wheat flour samples, the interaction rate for DON+Cd was the highest at 85.56%, followed by As+Cd (82.22%) and DON+As (73.33%) (**Figure 5A**). For three contaminant combinations, the highest interaction rate was for Cr+As+Cd (91.03%), also found in soybean samples. In rice samples, OTA+As+Cd had the highest interaction rate (68.29%), followed by DON+As+Cd (46.34%). In maize, FB1+FB2+As had the highest (76.00%), followed by DON+FB1+As (68.00%). In soybeans, following Cr+As+Cd, the next highest interaction rate (71.79%) was for DON+Cr+Cd, and the interaction rate between DON and heavy metals was more elevated in soybeans than in other products. In wheat flour, DON+As+Cd had the highest interaction rate of 72.22%, but the interaction rates of all other combinations were less than 31.11% (**Figure 5B**). The highest interaction rate of four contaminant combinations was found in the DON+Cr+Cd+As combination in soybean samples (69.23%). In rice, DON+OTA+As+Cd had the highest interaction rate at 36.59%. In maize, the highest interaction rate was for DON+FB1+FB2+As (62.00%), followed by FB1+FB2+OTA+As (54.00%). Aside from the overall highest rate found for DON+Cr+As+Cd in soybeans, OTA+Cr+As+Cd had the second-highest rate at 60.26%. The highest interaction rate found for combinations of four contaminants in wheat flour was DON+OTA+As+Cd at 27.78% (**Figure 5C**). The highest interaction rate of all combinations of five contaminants was 46.00%, found for DON+FB1+FB2+OTA+As in maize. In rice, the interaction rate for OTA+DON+3-ADON+As+Cd was highest (20.70%), and that of the highest was for DON+OTA+Cr+As+Cd (44.87%) in soybeans. The highest interaction rate was for DON+Pb+Cr+As+Cd (15.56%) found in wheat flour samples (**Figure 5D**). The interaction rates for 6-8 contaminant combinations in all samples were at most 29.49%, which was for

AFB1+DON+15-ADON+Cr+As+Cd, the probability of interaction rates decreased with the increase in the number of contaminants (**Figure 5E and 5F**). The interaction rates for combinations with more than nine contaminants were lower than 3.66%. The frequencies of occurrence and interaction rates of all combinations are listed in Supplementary Material 3.

4. Discussion

Various factors underlie the type and concentrations of contaminants in cereals. For example, fungi can have climatic preferences, and thus, geographic differences can directly affect them. Also, local soil composition and different agricultural practices can affect exposure to heavy metals, and inadequate processing or storage can promote fungal growth or introduce new contaminants to products [41]. Consequentially, mycotoxins and heavy metals can contaminate cereal products in all stages of production, from growth and harvest to processing, transportation, and storage, posing a significant health risk to animal and human health [42]. Of the 12 mycotoxins and five heavy metals analyzed in this study, those mainly present in rice, maize, soybean, and wheat flour products from Shanghai included aflatoxins, *Fusarium* toxins, ochratoxins, fumonisins, and heavy metals, especially Cr, As, Cd, and Pb. While overall contamination levels were relatively low, the patterns in the contaminants could be drawn according to the grain or grain product type. Notably, the co-contamination of samples with multiple toxins and heavy metals was more serious, depending on the sample type. The survey results showed that grains and grain products in Shanghai were susceptible to heavy metals and mycotoxin contamination, and the 300 samples (100%) co-contaminated mycotoxins and heavy metals to various extents. The mycotoxin DON was detected most frequently in 72.23% of all samples; the detection rate and average level of DON contamination were similar to a previous survey of grains and grain products from 17 European countries, including Switzerland, Seville, and Poland [43]. Interestingly, the range of contamination found for DON in cereals from Shanghai was lower than that in cereals from European countries, which could reflect the influence of climatic conditions or geographic differences on fungal growth and toxin production [44]. On the other hand, As was the most prevalent heavy metal, with a detection rate of 91.33%. Elevated As contamination rates may be attributed to the product's origins or agricultural practices, such as using As-contaminated groundwater for irrigation or applying As-rich fertilizers and pesticides [45].

In rice, the most concerning mycotoxin was OTA, with a contamination rate of 73.17% and an average concentration of 1.136 μg/kg. While the concentrations of OTA were in line with previous reports in rice samples from China and Vietnam (0.85 μg/kg on average and range 0.29-1.63 μg/kg), this study does report a high detection rate [46, 47]. As and Cd were the most prevalent in heavy metals, with detection rates of 100% and 90.24% and contamination levels of 0.117 and 0.018 mg/kg, respectively. One sample of rice was found to have As levels exceeding regulatory limits. Such levels of As contamination have been previously reported in white rice samples from Portuguese and Spanish markets (0.17 mg/kg) [48] and in various rice brands in Turkey (0.098 mg/kg) [49] and could be considered an intermediate level of contamination. Cd contamination in this survey was comparable in terms of its level in rice from some municipalities in Fujian of China, which averaged 0.064 mg/mg [50]. The information indicated that the contamination of OTA, As, and Cd in the rice sample of Shanghai provides a potential threat to the food from rice production.

In maize samples, the mycotoxin with the highest detection rate was FB1 (98.00%), with an average contamination level of 106.97 μg/kg. FBs were known to be one of the most prevalent mycotoxins in maize [51]. Liu *et al.* investigated 249 maize samples from eight provinces in China and found FB1 in 66.7% of the samples with an average concentration of 817 μg/kg [52]. On the other hand, contaminations of heavy metals in maize were mainly lower than the LOQ values, except Pb was found in 30% of maize samples. These findings were inconsistent with a piece of previous information that examined heavy metal contamination from the Shanxi province of China, which reported Pb as one of the most prevalent heavy metal contaminants [53]. However, Wang *et al.* also found Cd contamination to be more toxic than other heavy metals, whereas Cd was not detected in quantifiable amounts in the maize sample of their study, which might be related to the difference in product sources.

Of the mycotoxins analyzed in this study, DON was the most frequently detected in soybean samples (75.64%), with an average contamination level of 28.09 μg/kg. This was in line with findings by Pleadin *et al.* [54], which investigated unprocessed soybeans and detected DON contamination in 32-56% of the samples with a range concentration of 28-205 μg/kg from 2014 to 2015 in Croatian. Moreover, DON has been detected in 63% of soybean products, such as Canadian infant soy-based cereals [55]. The high detection rate of DON but the low level of contamination might be due to the lower susceptibility of soybeans to the toxigenic Fusarium species during crop reproductive stages [56]. Another reason was that most of the soybean samples were collected from farmers' markets or bulk soybeans sold in markets, which also caused DON exposure during storage. It is worth noting that of the four groups investigated here, soybeans had the highest detection rate for AFB1 at 42.31% and an average concentration of 0.30 μg/kg. Similarly, a survey of AFB1 residues in Brazilian commercial soybeans and their products showed that AFB1 was detected in 43.3% of the samples, with an average contamination level of 0.50 μg/kg [57]. Of the heavy metals, the detected rates of Cd and Cr contamination were high in the soybean samples. The Cd content in the Iranian (0.15 mg/kg) and Argentinean (0.04 mg/kg) soybean mineral surveys was higher than in the present study [58, 59]. In other published studies, higher values, especially of Cr, with 0.60 and 1.75 mg/kg, were found [60, 61]. It was evident that DON, AFB1, and heavy metal contaminations in soybeans have always existed and deserve more attention.

In wheat flour samples, DON and its derivatives 3-ADON and 15-ADON were the most prevalent contaminants. DON has been previously found to be the dominant mycotoxin in wheat flour products on the Hungarian market [62], with a detection rate of 87% and an average contamination level of 169 μg/kg. Plant infection with *Fusarium* head blight can directly affect DON levels in wheat flour [63], and DON is currently the most contaminated mycotoxin in wheat flour all over the world [64]. Among the heavy metals, the detection rate of Cd was the highest at 98.89%. Cd can quickly accumulate in the soil and be absorbed through the roots of wheat, and can then be transferred to the wheat tissues, resulting in the contamination of wheat flour [65]. The contamination level of Cd in wheat flour in the Shanxi Province, China, was 0.02 mg/kg, slightly higher than that in this study [66]. Mounting evidence suggested that DON and its derivatives and Cd contamination contributed to the safety risk of wheat flour production in Shanghai, which should pay enough attention to the regulators.

Our results further indicated that mycotoxin and heavy metal contaminations were prevalent in grains from Shanghai. When checking results on detection rates, contamination levels, and proportion of samples contaminated beyond regulatory limits, it could be seen that soybean products were the most prevalent contaminated, whereas contamination level in wheat flour was relatively light. The most prevalent mycotoxin in wheat flour and soybean samples was DON. FBs and OTA were the most prevalent in maize and rice samples, respectively. Overall, mycotoxins had the lowest detection rates in rice. As and Cd were the primary heavy metals contaminating rice, maize, and wheat flour, whereas, in soybeans, Cr was also a prevalent contaminant in addition to As and Cd. The factors that have resulted in these differences remain to be investigated, but it can be speculated that the environmental conditions such as temperature or moisture that affect the growth of toxin-producing fungi, the differences in geography or climatic conditions, and the types of cereals all played an important role in determining which mycotoxins were present to what extent [7]. As for heavy metals, exposure to contaminated soil and water was the primary way for food crops to be contaminated with heavy metals, and crops grown in areas with mineral mining and industrial activity were more likely to be contaminated with heavy metals [67]. Therefore, variations in heavy-metal prevalence could reflect the region of production.

Because food crops were often infested with multiple fungi, each producing multiple mycotoxins, co-contamination of several mycotoxins at lower doses was more common than that of a single mycotoxin with higher concentrations. Moreover, combining mycotoxins and heavy metals could induce enhanced toxicity [68]. In this study, all 300 samples contained two or more contaminants, and 88.6% contained more than four types of contaminants. Correlation clustering analysis showed weak correlations among the majority combinations with high interaction rates. However, in rice, between 3-ADON and FB3, there were significant and strong correlations, with an interaction rate of 28.05%. Serious co-contamination was found in rice samples. Similarly, reports on cereals commercially available in Greece showed that 30% of samples were contaminated with AFBs, DON, OTA, and ZEN at the same time [69]. In maize, almost all samples were co-contaminated with more than three contaminants, and the combinations with high interaction rates were FB1+As, FB1+FB2, DON+FB1, and FB1+FB2+As. Co-contamination of a few mycotoxins has often been reported in maize, the information showed that samples from 2018 in Michigan, USA, were contaminated with at least six, with FB1, FB2, and FB3 having strong correlations [70]. Recent evidence suggested that 82% of maize sampled from five Chinese provinces were contaminated with at least two mycotoxins [28]. All soybean samples were co-contaminated with at least four contaminants, and soybeans were more contaminated with heavy metals. The combination of DON and Cd is particularly notable since their co-occurrence and combined toxicity have been previously reported [3]. Furthermore, there was a strong correlation between any two combinations in DON, 3-ADON, 15-ADON, and AFB2. In some European countries, the co-occurrence of DON with its acetylated forms (3-ADON, 15-ADON) and ZEN was found in feed ingredients [71]. It is known that 3-ADON and 15-ADON are the biosynthetic precursors of DON, and when DON is produced by different *Fusarium* species, 3-ADON, and 15-ADON tend to accompany the production of DON [72]. In wheat flour samples, the probability of being affected by more than three contaminants was 81.1%. Interaction rates of DON+Cd, As+Cd, and DON+Cd+As were higher than 70%, while significant and strong correlations were found between Pb and Cr. Similarly, fungal toxins and heavy metals were concurrently present in organic wheat with DON, Cd, and Pb in Belgium [73]. DON is frequently reported in wheat flour products, and it has been shown to positively correlate with six *Fusarium* mycotoxins (deoxynivalenol-3-glucoside (D-3-G), 3-A-DON, 15-A-DON, and ZEN) [74], and to be present in all mycotoxin-contaminated wheat flour samples in Brazillian [75]. Comprehensive analyses of contamination levels, correlations, and interactions showed that FB3+3-ADON, FB1+As, FB1+FB2, DON+FB1, DON+Cd, As+Cd, DON+Cd+As, FB1+FB2+As, and DON+3-ADON+15-ADON were more closely related in cereals and cereal products.

The co-occurrence of mycotoxins and heavy metals in grain and grain products from Shanghai demonstrated the need to monitor these toxins better and raise concerns about the risks of their coexistence in products. While the prevalence of co-contamination may not be surprising, many sources of contamination, such as industrial discharge, soil contamination, or fungal growth, often involve multiple contaminants, which is a prominent problem in food safety. When combining different contaminants, toxic effects are not just additive but could be synergistic and exacerbate individual toxicity. When toxins have different target organs, they could also introduce additional complexities in their impact, making the health issues more difficult to predict or mitigate. However, mycotoxins are organic sources, and heavy metals are inorganic ones, the correlation and how they might affect each other requires further research.

5. Conclusion

Mycotoxins and heavy metals widely contaminate grains and grain products, jeopardizing the quality and safety of grains, causing substantial economic losses, and posing serious health hazards to animals and humans. This study investigated mycotoxin and heavy metal contamination in cereal foods and explored the correlation and interactions between 17 contaminants. All the data demonstrated that cereal foods sold in Shanghai were frequently contaminated with heavy metals and mycotoxins, mostly commonly by DON and its derivatives, OTA, AFs, FBs, As, Cr, and Cd. Our findings illustrated the frequent co-occurrence of mycotoxins and heavy metals in cereal products from Shanghai. Not only do our results raise concerns regarding the risks that co-contamination might pose to consumer health, demonstrating the need to monitor these toxins holistically and further study their interactive effects, but they also inform regulatory policies and future research on the toxin combinations most urgently requiring attention. Furthermore, the correlation analyses identify combinations with high interaction rates to provide direction for future investigations on their joint toxicity and enable the establishment of relevant combined standards.

Environmental implication

Mycotoxins and heavy metals extensively contaminate grains and grain products, posing severe health risks. In this study, interaction analysis was performed on the multi-contaminants found in grains and grain products to identify co-contamination and assess their probabilities of co-existence using a self-designed JAVA language program. The study provides data for the assessment, early warnings, and regulatory measures related to mycotoxins and heavy metal contamination. Furthermore, the joint analyses demonstrated the importance of monitoring co-contamination and identifying the possible combinations with higher interaction rates to perform future investigations, as well as providing innovation for establishing national standards involving multiple contaminants.

CRediT authorship contribution statement

Zuoyin Zhu and Wenbo Guo: Writing the original draft, Software, Methodology, Formal analysis, Data curation, and Conceptualization. **Haisheng Cheng:** Writing-review & editing, Methodology. **Hanke Zhao, Jie Wang, and Mohamed F. Abdallah:** Writing-review & editing. **Xinli Zhou, Hulong Lei, and Weilong Tu:** Methodology, Investigation. **Junhua Yang and Hongyang Wang:** Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence this study.

Acknowledgments

This work was supported by the Social Development Science and Technology Research Projects from the Science and Technology Commission of Shanghai Municipality (No. 21DZ1201300) and STI 2030-Major Projects (Grant No. 2023ZD04046).

Appendix A. Supplementary material

Table S1 Heat-up procedure for microwave disintegrator

Table S2 Mass spectrometry parameters for the detection of the tested 12 mycotoxins

Table S3 Regulatory limits of mycotoxins (μg/kg) and heavy metals (mg/kg) in grain and grain products

Table S4 Limits of detection and quantification for the targeted mycotoxins (μg/kg) and heavy metals (mg/kg) and linear equations

Table S5 Matrix effects of the 12 mycotoxins in different sample

Table S6 Method performance characteristics of the 17 contaminations analyzed in grains and grain products.

Data Availability

Data will be made available on request.

Graphic abstract

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Figures

Figure 1 Selected sampling sites for grain and grain products marketed in 16 districts of Shanghai City.

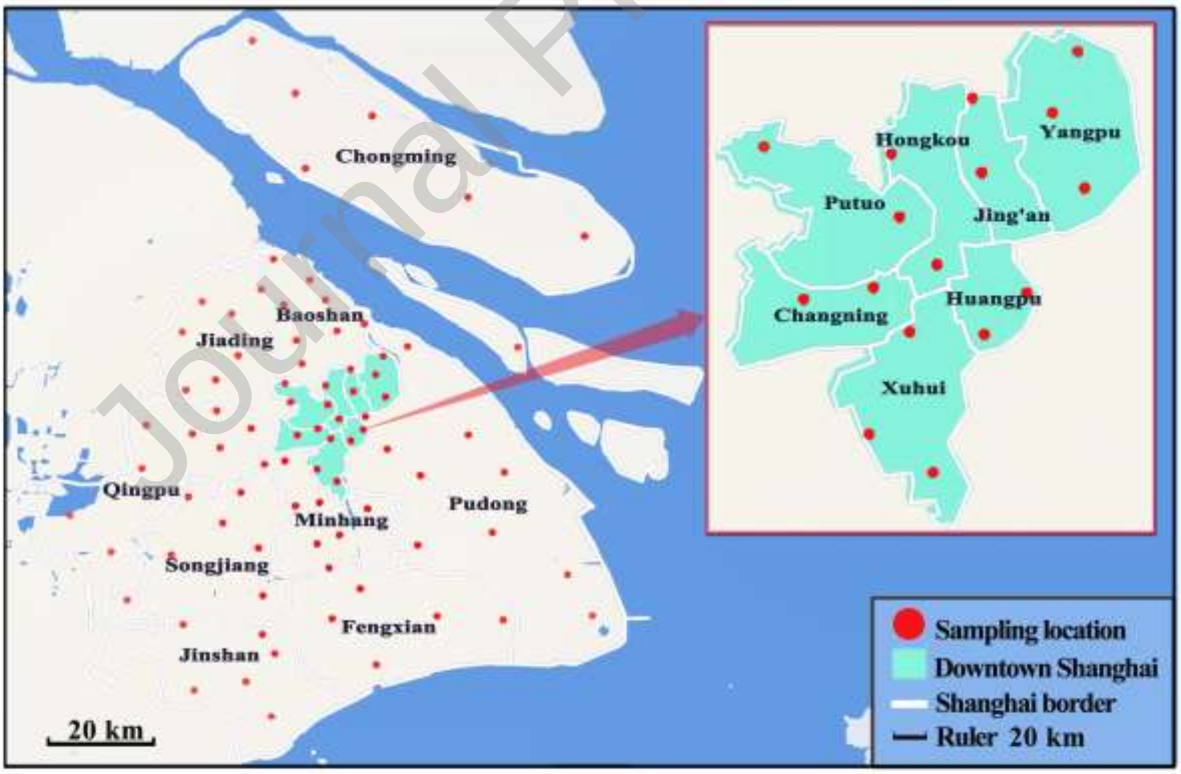


Figure 2 The detection rates of the targeted 17 contaminants in 300 grain and grain product samples. (A), detection rates in rice (B), detection rates in maize (C), detection rates in soybean (D), and detection rates in wheat flour (E). The colors of the bars indicate the types of grain. Rice, maize, soybean, and wheat flour correspond to blue, green, pink, red, and yellow, respectively.

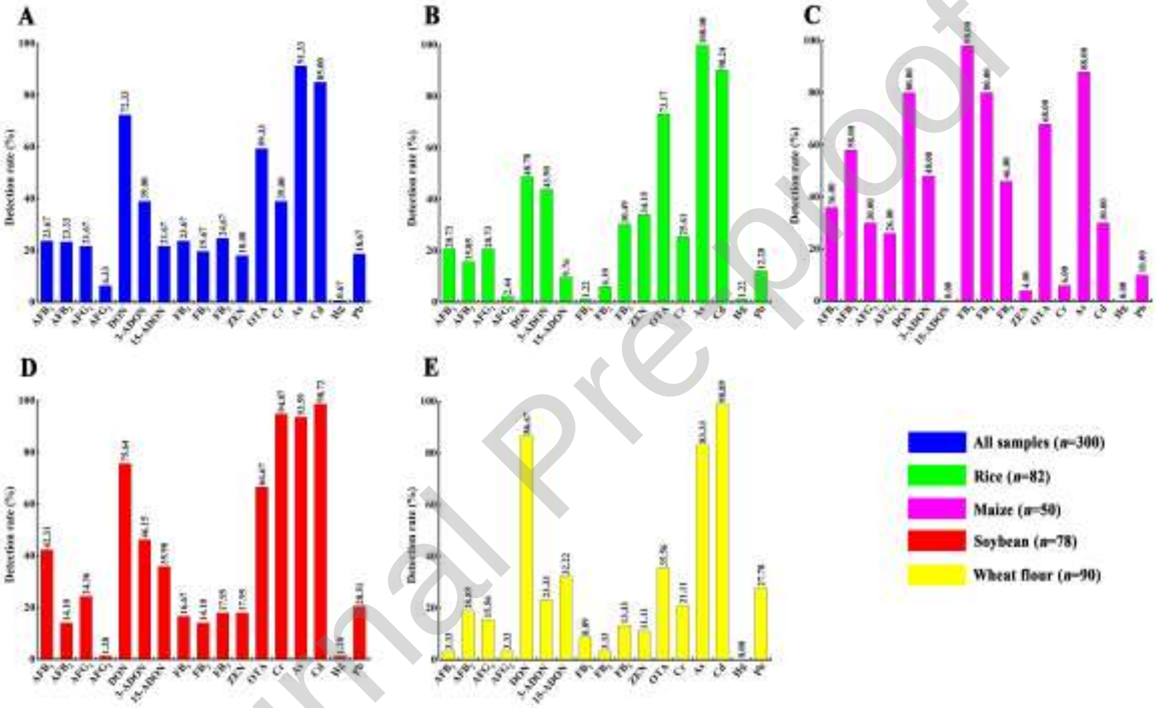


Figure 3 Distribution of samples based on the number of co-contaminants and proportions by grain and grain product type. (A and B) the number of co-contaminants and their proportions in all samples; (C) rice; (D) maize; (D) soybean; (E) wheat flour samples. Values in white circles represent the number of contaminants detected in the samples.

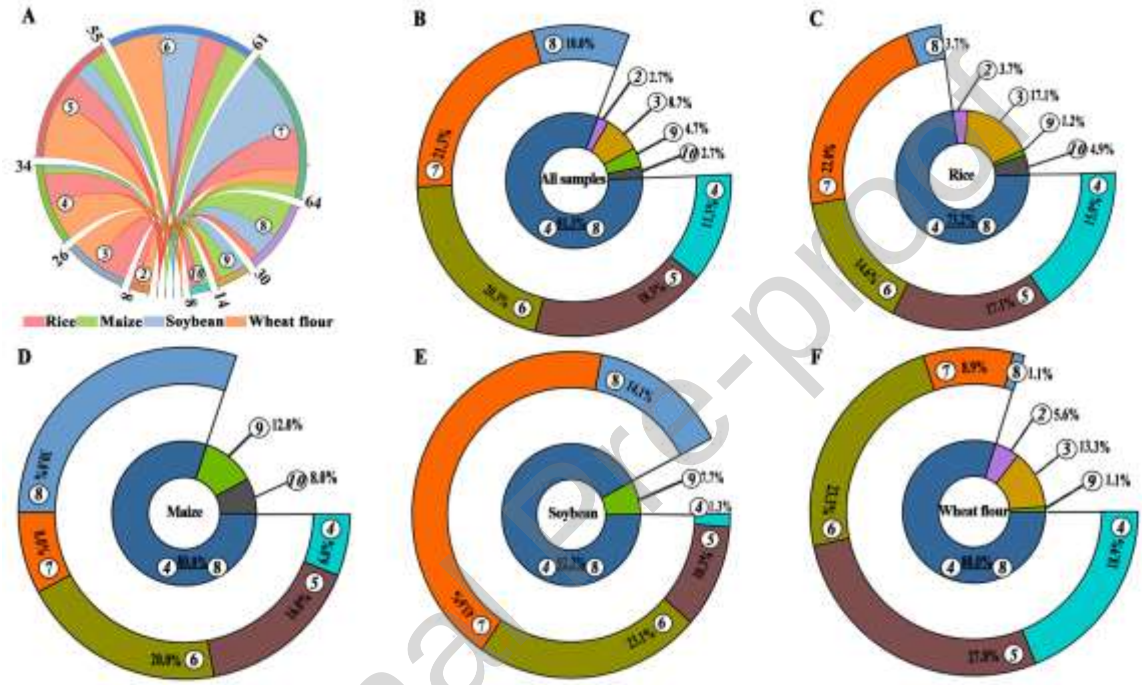


Figure 4 Heatmap of the correlation cluster analyses of the 17 contaminants in rice (A), maize (B), soybean (C), and wheat flour (D). The "*" and "**" represented the significance levels of $P < 0.05$ and $P < 0.01$, respectively.

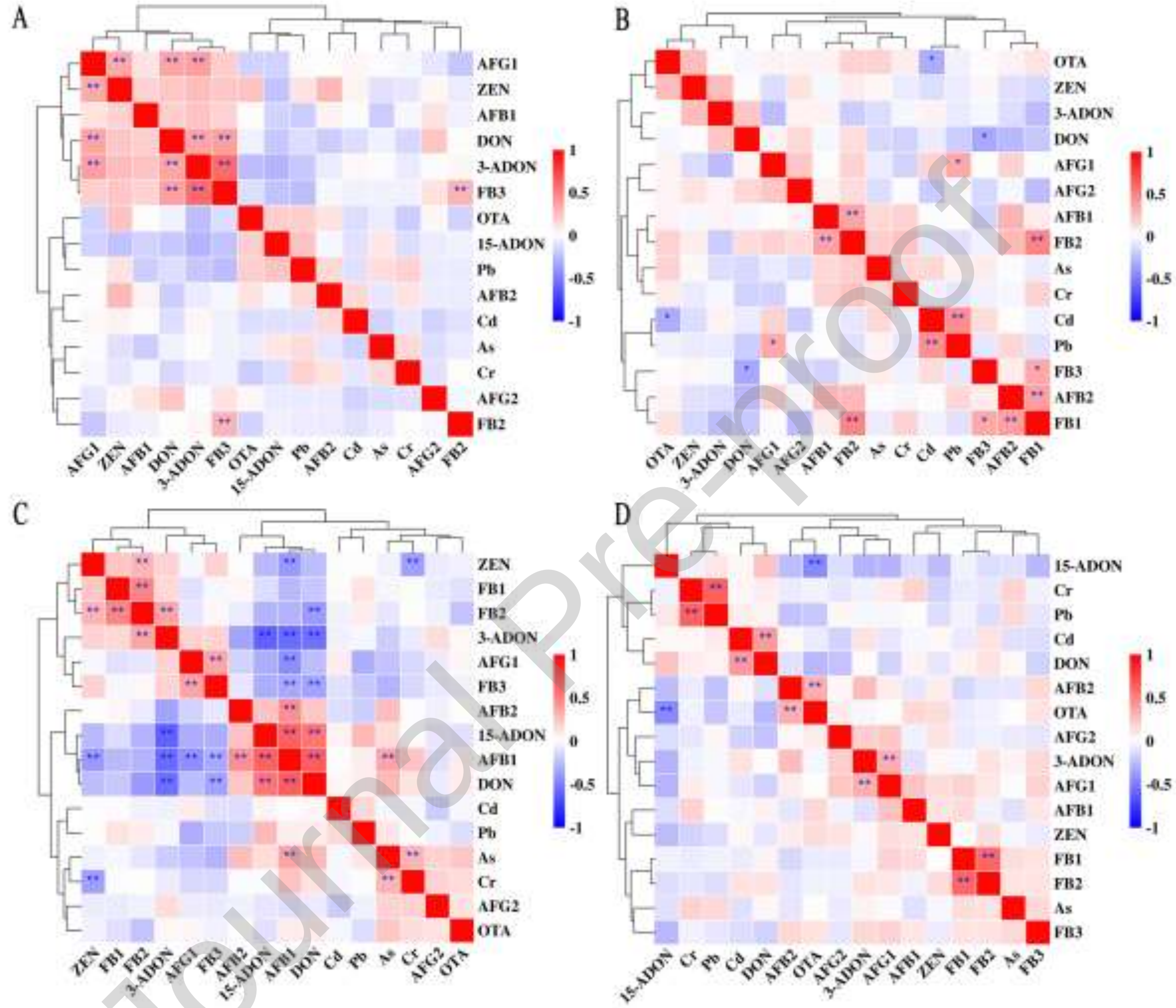
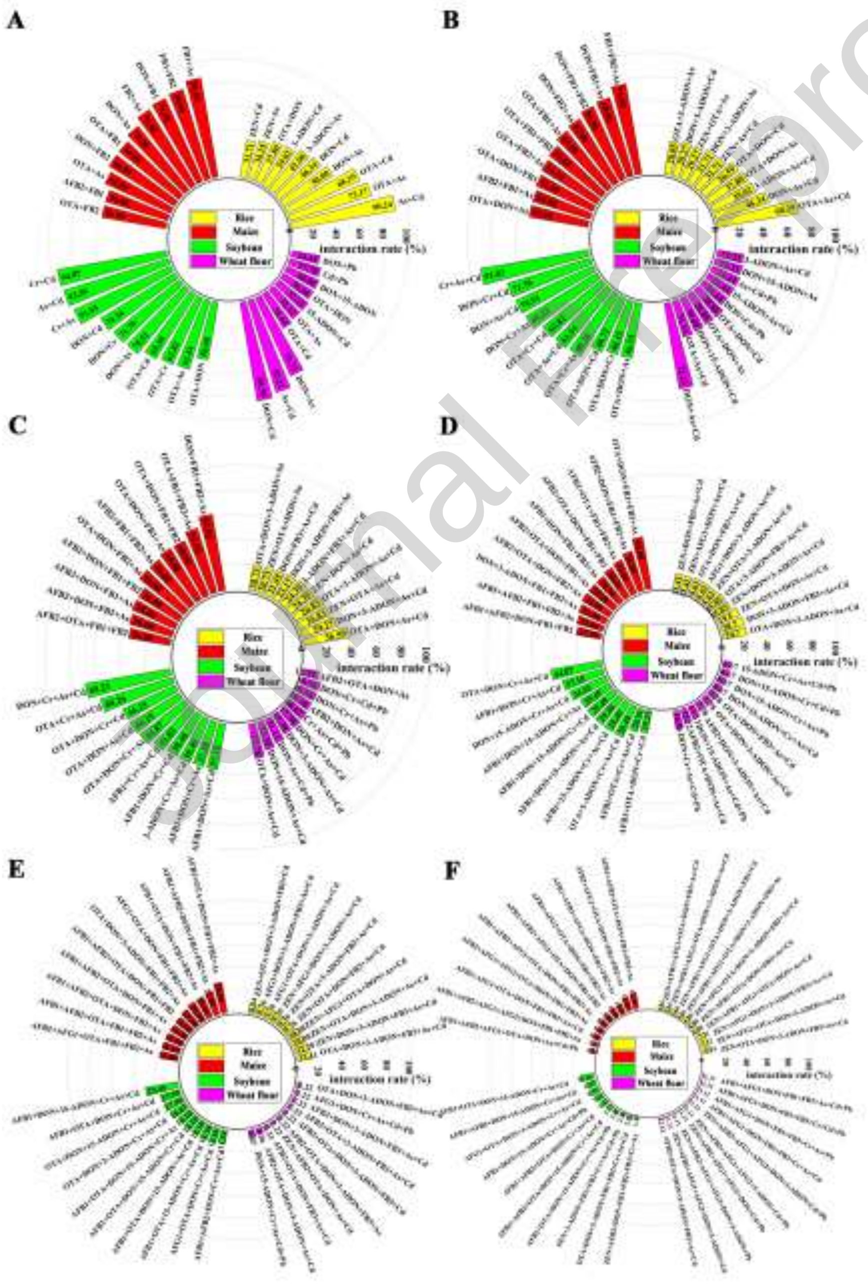


Figure 5 Co-occurrence, combinations, and interaction rates of 17 contaminants in rice, maize, soybeans, and wheat flour. (A), (B), (C), (D), and (E) are combinations of 2, 3, 4, 5, and 6 contaminants with interaction rates greater than or equal to 10%, respectively. (F) illustrates 7 or 8 contaminants in co-occurrence with interaction rates greater than or equal to 5%. The colors of the bars indicate the type of grain in which the rates are found with rice (yellow), maize (red), soybean (green), and wheat flour (purple).



Tables

Table 1 Contamination levels of the 17 tested contaminants in rice, maize, soybeans, and wheat flour samples.

Contaminants		Rice		Maize		Soybean		Wheat flour	
		Average	Range	Average	Range	Average	Range	Average	Range
Mycotoxins (μg/kg)	AFB1	0.05 [a]	0.08~0.36	0.08 [a]	0.06~1.04	0.30 [a]	0.08~2.81	-	-
	AFB2	0.06 [a]	0.092~0.59	0.26 [a]	0.08~1.84	0.07 [b]	0.10~0.97	0.05[a]	0.07~0.46
	AFG1	0.05 [a]	0.07~0.54	0.05 [a]	0.08~0.23	0.05 [a]	0.08~0.17	-	-
	AFG2	-	-	0.05	0.06~0.26	-	-	-	-
	DON	6.35 [a]	5.00~24.67	162.37 [c]	10.23~2091.52	28.09[a]	8.70~196.02	86.49[b]	10.79~698.84
	3-ADON	0.35 [a]	0.34~0.96	2.58 [a]	0.36~40.19	0.95 [a]	0.37~19.60	31.31 [a]	0.35~1890.20
	15-ADON	9.75 [a]	6.08~251.70	-	-	73.87 [b]	0.89~890.99	50.97 [b]	25.76~534.56
	FB1	-	-	106.97 [a]	4.18~446.92	3.88 [b]	0.40~281.34	1.17 [b]	0.96~78.72
	FB2	-	-	19.71 [a]	3.53~76.13	1.95[b]	0.66~123.74	-	-
	FB3	0.55 [b]	0.86~2.65	16.08 [a]	1.63~142.96	2.66 [b]	0.78~168.33	-	-
	ZEN	0.75 [a]	0.26~9.76	-	-	32.37[b]	0.41~1505	-	-

							.48		
	OTA	1.14^{a}	0.15~41.52	0.46^{a}	0.13~3.93	0.56^{a}	0.13~9.05	-	-
Heavy metals (mg/kg)	Cr	0.053^{b}	0.065~0.809	0.030^{b}	0.102~0.120	0.279^{a}	0.071~1.634	0.051^{b}	0.016~0.316
	As	0.117^{a}	0.064~0.232	0.006^{b}	0.004~0.024	0.011^{b}	0.004~0.130	0.010^{b}	0.005~0.087
	Cd	0.018^{b}	0.003~0.088	0.002^{c}	0.004~0.010	0.023^{a}	0.006~0.084	0.015^{b}	0.006~0.031
	Hg	-	-	-	-	-	-	-	-
	Pb	0.018^{a}	0.041~0.198	0.058^{b}	0.051~1.342	0.025^{ab}	0.006~0.376	0.025^{ab}	0.046~0.097

"-" indicates that the detected value was below the corresponding LOD. Different letters in the same row indicate significant differences at 0.05 level (P<0.05).

Contaminants		Rice (n = 82)		Maize (n = 50)		Soybean (n = 78)		Wheat flour (n = 90)	
		Sample count	Rate (%)	Sample count	Rate (%)	Sample count	Rate (%)	Sample count	Rate (%)
Mycotoxins	ZEN	-	-	-	-	4	5.13	-	-
	AFB1	-	-	-	-	-	-	-	-
	Total AFs	-	-	-	-	-	-	-	-
	OTA	2	2.44	-	-	1	1.28	-	-
	DON	-	-	2	4.00	-	-	-	-
	3FBs	-	-	-	-	-	-	-	-
Heavy	Cr	-	-	-	-	2	2.56	-	-

metals	As	1	1.22	-	-	-	-	-	-
	Cd	-	-	-	-	-	-	-	-
	Hg	-	-	-	-	-	-	-	-
	Pb	-	-	2	4.00	1	1.28	-	-

Table 2 Samples with contamination exceeding the MRLs of the 17 targeted contaminants in rice, maize, soybeans, and wheat flour samples.

"-" indicates that no sample of the corresponding contaminant and sample group was contaminated beyond MRLs standards.

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.

Highlights

- The frequency of co-contamination with mycotoxins and heavy metals is relatively high in rice, maize, soybeans, and wheat flour in Shanghai city.
- All samples of grains and grain products contained two or more contaminants, and 88.6% of which contained more than four kinds of contaminants.
- The intimate interactions of multiple contaminants were presented in the binary and

ternary combinations between mycotoxins and heavy metals.