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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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Urinary analysis reveals high *Alternaria* mycotoxins exposure in the general population from Beijing, China

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ARTICLE INFO

Article history:
Received 8 July 2021
Revised 9 August 2021
Accepted 13 August 2021
Available online 11 January 2022

Keywords:
Alternaria mycotoxin
Biomonitoring
UPLC-MS/MS
Exposure assessment
Adults

ABSTRACT

Alternaria mycotoxins are of concern due to its adverse health effect, they affect various cereal crops and grain-based food along with modified forms that contribute to overall exposure. This study aimed to determine the frequency and level of exposure to *Alternaria* mycotoxins (tenuazonic acid, TeA; alternariol, AOH; alternariol monomethyl ether, AME; tentoxin, TEN; and altenuene, ALT) in human urine from Beijing adults. A total of 2212 urine samples were collected and analyzed for five mycotoxins using LC–ESI–MS/MS. More than 98% of the samples had at least one *Alternaria* mycotoxin detected. Among the mycotoxins, AME had the highest detection rate (96.0%), followed by TeA (70.5%). The calculated average daily intake values of AME (12.5 ng/kg b.w.) was 5 times the TTC value (2.5 ng/kg b.w.) set by the EFSA, indicating the potential health risks associated with mycotoxins. Immediate attention and subsequent actions should be taken to identify the sources of mycotoxins and the corresponding exposure pathways to humans in the investigated regions.

Introduction

Mycotoxins are secondary metabolites produced by fungi that can cause teratogenicity, immunosuppression and carcinogenicity in humans (Eskola et al., 2020) and animals (Pietsch et al., 2020). Human exposure to mycotoxins occurs directly through the intake of contaminated agricultural products (cereals, fruits, vegetables, etc.) or indirectly through the consumption of animal products (milk, eggs, etc.) and fish that were fed contaminated material (Eskola et al., 2020; Gruber-Dorninger et al., 2019; Pietsch et al., 2020; Saha Turna et al., 2021). The continuous development of LC-MS/MS methods makes it possible to analyze an almost non-exhaustive list of mycotoxins in a variety of food and feed matrices (Puntscher et al., 2018). These multi-mycotoxin screenings revealed a high prevalence of so called “emerging” mycotoxins (lack of comprehensive data available on both toxicological effects and occurrence), such as the *Fusarium* mycotoxins and *Alternaria* mycotoxins.

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https://doi.org/10.1016/j.jes.2021.08.025

The tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN) and altenuene (ALT) are the most frequently encountered *Alternaria* mycotoxins (Arcella et al., 2016; Gruber-Dorninger et al., 2016). Due to its stability during storage and processing (Ozcelik et al., 1990), a high level of exposure in humans is expected. Xu et al. (2016) confirmed high *Alternaria* mycotoxin contamination of China wheat samples harvested in 2015, 95% of the wheat samples were positive for more than one type of *Alternaria* mycotoxins (TeA was ranged from 6.0 μg/kg to 3330.7 μg/kg, TEN was ranged from 0.4 μg/kg to 258.6 μg/kg, AOH was ranged from 1.3 μg/kg to 74.4 μg/kg, and AME was ranged from 0.3 μg/kg to 54.8 μg/kg). Qiao et al. (2018) determined a high contamination of fresh cherry with *Alternaria* mycotoxins, with the highest concentration of TeA was 236.58 μg/kg. Contamination of processed fruits and vegetables with *Alternaria* mycotoxins has also been reported Zhao et al. (2014). Of the analyzed samples, TeA was detected in all tomato ketchup and tomato juice samples with concentrations ranged from 7.4 μg/kg to 1787 μg/kg. Since high levels of *Alternaria* mycotoxins were detected in grains, fruits, and their products, it is expected that the potential exposure of humans is reflected by the presence of appropriate urinary biomarkers. There is limited information on *Alternaria* metabolism and exposure in humans. (Hövelmann et al., 2016) reported the urinary TeA concentrations in individuals from German with a range of 0.16-44.4 ng/mL or 0.07-63.8 ng/mg creatinine.

TeA is known to pose acute toxicity and induce embryonal death in microgram doses and cause severe dysplasia in mice by inhibiting of the release of newly formed proteins from ribosomes (Davis et al., 1977; Smith et al., 1968). AOH and AME exert genotoxic and mutagenic effects on mammalian cell cultures. AOH forms ROS and interacts with DNA topoisomerase, causing single and double DNA strand breaks (Fehr et al., 2010; Tiemann et al., 2009; Wollenhaupt et al., 2008). Similarly, AME is also mutagenic and causes DNA strand breaks and cell cycle arrest (Solhaug et al., 2012). The dibenzo-α-pyrones AOH, AME and ALT structurally resemble estradiol. Recently, AOH was found to have weak estrogenic activity and was proved to act as a full agonist of the androgen receptor (Hessel-Pras et al., 2019; Schoevers et al., 2019). A major detoxification route for *Alternaria* mycotoxins in humans and animals is via glucuronidation, through the specific uridine-diphosphate glucuronosyltransferases for *Alternaria* mycotoxins have not yet been identified. A significant increase in the concentration of AOH in the β-glucuronidase/arylsulfatase treated group compared with nonhydrolyzed group has been identified by our previous study (Qiao et al., 2020).

In view of the potential for toxicity, the European Food Safety Authority (EFSA) used the threshold of toxicological concern (TTC) to assess the relative level of concern for dietary exposure; these values were determined to be 2.5 ng/kg bodyweight per day of AME and AOH, and at 1500 ng/kg bodyweight per day for TeA and TEN (Battilani et al., 2009). The traditional method to assess exposure to *Alternaria* mycotoxins is based on representative food contamination data combined with food consumption data (Arcella et al., 2016; Arce-López et al., 2020; CONTAM et al., 2011; Hickert et al., 2016; Zhao et al., 2015). This indirect approach is generally considered to be less reliable due to the exclusion of direct contact and inhalation exposures, as well as the nonuniform distributions of mycotoxins in foods, individual variations in toxicokinetics and bioavailability, and inaccurate estimations of food consumption on the basis of populations (Fan et al., 2019). Similar to all mycotoxins, the distribution of *Alternaria* mycotoxins in food exhibits high heterogeneity, and is thus subject to poor food sampling representativeness. The inaccuracy in food consumption measurement and the multiple food sources of *Alternaria* mycotoxins in diets contribute to the difficulty for reliable exposure assessment. In the human body, absorbed mycotoxins are primarily excreted into the urine as free mycotoxins and metabolites. These chemicals have been widely used as biomarkers for the estimation of human exposure to mycotoxins. With the development of analytical methods of mycotoxin biomarkers, monitoring mycotoxin biomarkers has gained increased acceptance for exposure assessment in recent years (Ali et al., 2020; Arce-López et al., 2020; Huang et al., 2021; Zhang et al., 2020).

China is a country that mainly has a grain-based dietary pattern, which makes Chinese residents at relatively high risk to *Alternaria* mycotoxins exposure. The results of dietary exposure assessment indicated high exposure levels in some Chinese populations (Xu et al., 2016; Zhao et al., 2014). However, to date, few biomonitoring studies have been conducted in China, and there are very limited data on the levels of *Alternaria* mycotoxins biomarkers in Chinese people. To fill this data gap, we conducted a survey in Beijing, China. The aim of this study was to investigate the levels of *Alternaria* mycotoxins in urine and estimate the participants' dietary *Alternaria* mycotoxins exposure as well as corresponding health risks.

1. Materials and methods

1.1. Chemicals and reagents

Ethyl acetate of LC gradient grade purity was from Dikma Technologies Inc. (Lake Forest, CA). LC-MS-grade methanol for the mobile phase was purchased from Honeywell International Inc. (Seelze, Germany). TeA and (acetyl-$^{13}C_2$)-tenuazonic acid (50 μg/mL in acetonitrile) were obtained from Sigma-Aldrich (Saint Louis, MO). AOH, AME, ALT, TEN and $^2H_3$-tentoxin were purchased from Toronto Research Chemicals (Toronto, Canada). LC-MS-grade ammonium bicarbonate and acetic acid were obtained from Sigma-Aldrich. Concentrated hydrochloric acid and sodium acetate were purchased from Sinopharm Chemical Reagent (Shanghai, China). The β-glucuronidase/aryl-sulfatase mixture was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Ultrapure water was obtained using an in-house Milli-Q Ultra-pure water system (Millipore, Bedford, MA).

1.2. Study population

Study subjects comprised individuals living in Beijing who were recruited in 2017 as part of the Beijing Population Health Cohort study subjects. The research was approved by the Ethical Review Committee of the Center for Disease Control and Prevention (CDC) [No. 2017D (6)], and informed written consent was collected from each participant. The participants

in this study had been living in Beijing for at least 2 years, and the age of the participants ranged from 17 to 75 years. We excluded individuals with previous medical records, who lacked urine samples and insufficient *Alternaria* mycotoxins measurements, and the had a BMI over 35 kg/m^2. A total of 2212 (1022 male and 1190 female) participants were included in the analysis. Body weight and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively, with the participants wearing light indoor clothing and no shoes. BMI was calculated as weight in kilograms divided by the square of height in meters.

1.3. Sample collection

The first morning urine sample (~10 mL) was collected from each participant in brown glass tubes.

To normalize analyte concentrations to ng/mg creatinine (Brien et al., 2016), the urinary creatinine levels of each sample were measured using the Jaffe method (Siemens Healthcare Diagnostics, Eshborn, Germany). Measurements were carried out with an automatic biochemistry analyzer (Hitachi, 7600-010, Tokyo, Japan), and the remaining samples were stored at −20°C and thawed directly before sample preparation.

1.4. Sample preparation and analysis

Details of the sample preparation methods used for *Alternaria* mycotoxins were described in our previous work (Qiao et al., 2020). In brief, a 1 mL aliquot of urine was fortified with labeled internal standards (100 μg/L, 50 μL) and adjusted with 1 mL of sodium acetate buffer to keep the pH between 5.2 to 5.3. The sample was then incubated overnight at 37°C with 10 μL of a β-glucuronidase/aryl-sulfatase mixture. Hydrochloric acid was used to terminate the enzymatic hydrolysis, and then the sample was extracted and purified with ethyl acetate. *Alternaria* mycotoxins were separated and quantified by ultra-performance liquid chromatography tandem triple-quadrupole mass spectrometry (LCMS-8060, Shimadzu, Japan) equipped with a 100 mm × 2.1 mm i.d., 1.7 μm, BEH C18 column (Waters, Milford, MA). Solutions Ver.5.85 software was used for data acquisition and analysis.

1.5. Quality assurance and quality control

All samples were analyzed using standard laboratory quality assurance and quality control protocols. With every batch of 60 samples, procedural blanks ($n = 3$) were analyzed to monitor contamination arising from laboratory materials and solvents used in sample extraction, and six samples were randomly selected for duplicate analysis. The results showed a coefficient variation of < 20% among the measured values for all target compounds. Methanol and a midpoint calibration check standard were injected after every 10 samples as a check for instrumental drift in sensitivity and carry-over of analytes between samples.

1.6. Estimated dietary exposure of mycotoxins

Probable daily intake (PDI, ng/kg bodyweight) of *Alternaria* mycotoxin was calculated from the measured urinary concentration according to the literature using the following formula

$$\text{PDI (ng/kg body weight)} = C \times V \times 100/(W \times E)$$

where C (ng/L) is the urinary *Alternaria* mycotoxin concentration, V is the mean volume of daily urine production (1.8 L/day) (Hövelmann et al., 2016), W (kg) is body weight, and E is mean urinary excretion rate (in percent) (90 for TeA ((Asam et al., 2013; Hövelmann et al., 2016), 7 for AME (Pollock et al., 1982)).

For the other mycotoxins, the relationships between the concentrations in urine and the exposure levels remain unclear with no well-established excretion rate; consequently, was not calculated.

Table 1 – Baseline characteristics of the participants in the study.

Characteristics	Male	Female	All
Subjects (n)	1022	1190	2212
Age (years)			
Mean ± SD	45.9 ± 14.2	45.7 ± 14.0	45.8 ± 14.1
Range	18–74	17–75	17–75
BMI			
Mean ± SD	25.8 ± 3.5	24.2 ± 3.4	25.0 ± 3.5
Range	14.8–34.9	15.40–35.0	14.8–35.0

1.7. Data analysis

For data analysis, concentrations below the LOQ were assigned a value equal to the LOQ divided by 2 (LOQ/2), and concentrations below the LOD were assigned a value equal to the LOD divided by 2 (LOD/2). Descriptive analysis of the study population characteristics and *Alternaria* mycotoxins exposures were conducted according to study outcomes. Differences between groups were identified by one-way ANOVA with the Tukey test, using SPSS (version 21.0). A value of $p < 0.05$ was considered significant. Medians and interquartile range (IQR) values were calculated for all continuous variables.

2. Results and discussions

2.1. Overview of demographic data of participants

The demographic data of the participants are summarized in Table 1. Among the 2212 participants, 46.2% were male and 53.8% were female. The mean age was 45.8 years, and the average BMI was 25.0 kg/m^2. There was no significant difference in age or BMI between males and females ($p > 0.05$).

2.2. Presence of mycotoxins in urine samples

The presence of 5 mycotoxins detected in urine samples from 2212 participants is listed in Table 2. In 2184 out of 2212 participants, one or more mycotoxins were detected. AME (incidence of 96.0%), was the most abundant mycotoxin in urinary samples; its concentration ranged from <LOQ to 2.022 μg/L (0 to 1.8036 μg/g creatinine). TeA was detected in 70.5% of the urine

Table 2 – Natural occurrence of *Alternaria* mycotoxins in human urine samples from Beijing.

Toxins	Not detected	<LOQ	>LOQ	Detection rate	Mean (SD) (μg/L)	Range (μg/L)
TeA	652(29.5%)	10(0.5%)	1550(70.1%)	1560(70.5%)	5.950 (17.764)	<LOQ-176.825
AME	89(4.0%)	24(1.1%)	2099(94.9%)	2123(96.0%)	0.0650 (0.1162)	<LOQ-2.0220
TEN	1689(76.4%)	145(6.6%)	378(17.1%)	523(23.6%)	0.006 (0.024)	<LOQ-0.810
AOH	1967(88.9%)	16(0.7%)	229(10.4%)	245(11.1%)	0.193 (1.234)	<LOQ-32.287
ALT	2204(99.6%)	1(0%)	7(0.3%)	8(0.4%)	0.020 (0.065)	<LOQ-2.910

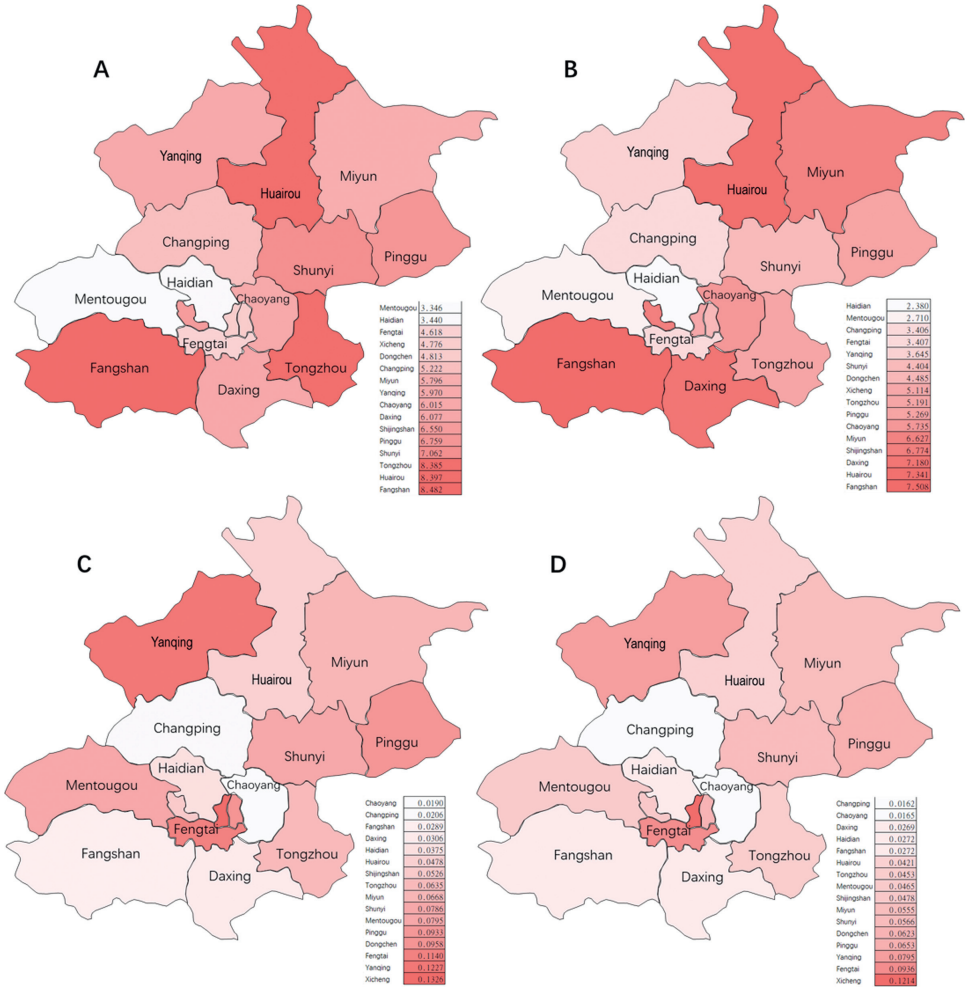

Fig. 1 – Distance distribution of the average concentration of the main *Alternaria* mycotoxins in the Beijing population (A is TeA in μg/L, B is TeA in μg/g creatinine, C is AME in μg/L, D is AME in μg/g creatinine).

samples, with a concentration ranging from <LOQ to 176.825 μg/L (0 to 347.134 μg/g creatinine). TEN, AOH and ALT were detected in 23.6%, 11.1% and 0.4% of the urine samples, respectively. There have been no reports on multiple *Alternaria* mycotoxins in urine, and only a few studies have reported on TeA. The incidence of TeA in the present research was lower than that in a previous study in Germany (100%) reported by Asam et al., 2013; however, there were a limited number of participants in their study ($n = 6$). In another German study (Hövelmann et al., 2016), TeA was also detected in all of the samples ($n = 48$) and quantifiable in 97.9% of these samples in a range of 0.16–44.4 μg/L (average = 6.58 μg/L) or 0.07–63.8 μg/g creatinine (average = 8.13 μg/g creatinine). The average concentration (5.95 μg/L and detection rate of TeA in our study were lower than those in Germany, while the maximum detected concentration in urine samples was much higher.

Approximately 47.5% of the positive samples contained two mycotoxins, AME and TeA were the most frequent combination detected in the urine samples, and a significant linear correlation in concentrations was observed between AME and TEN (adjusted R square = 0.208, $p < 0.01$). Although no correlations between the concentrations of *Alternaria* mycotoxins in urine samples has been reported, Zhao et al. (2015) and Xu et al. (2016) found similar results, with a significant lin-

Table 3 – Average mean and selected percentiles of TeA concentrations in urine (in μg/L in the first row and in μg/g creatinine in the second row) for the Beijing population.

	Average mean	Selected percentile (95% CI)				N
	(95% CI)	50th	75th	90th	95th	
Total	5.950(5.329-6.578)	1.337(1.161-1.478)	5.323(4.772-5.806)	14.883(12.781-16.855)	26.031(22.091-28.935)	2212
	5.004(4.337-5.759)	0.933(0.836-1.067)	3.911(3.497-4.348)	10.913(9.767-12.001)	20.268(17.721-24.016)	
Age group						
17-29 years	7.922(6.262-9.799)	1.881(1.365-2.685)	7.269(5.740-9.977)	22.126(16.324-27.906)	39.505(27.415-49.000)	336
	5.077(3.879-6.448)	1.104(0.808-1.475)	4.289(3.289-5.972)	13.009(10.727-15.473)	23.158(15.026-35.874)	
30-39 years	6.635(5.290-8.128)	1.421(1.140-1.840)	5.607(4.533-6.533)	16.163(12.526-20.529)	26.725(21.155-31.459)	505
	5.220(3.761-7.237)	0.931(0.734-1.217)	3.777(3.083-4.921)	10.034(7.630-11.810)	18.256(11.967-28.516)	
40-49 years	6.405(5.016-7.975)	1.477(1.081-1.993)	5.442(4.356-6.860)	16.055(11.054-21.543)	28.504(21.949-34.522)	460
	5.408(4.005-7.189)	0.988(0.775-1.293)	4.130(3.065-5.062)	11.583(8.124-19.206)	23.759(19.783-32.366)	426
50-59 years	4.288(3.506-5.129)	1.003(0.856-1.423)	4.927(3.781-6.000)	12.324(10.403-15.468)	19.085(15.498-21.890)	
	4.392(3.204-5.822)	0.882(0.711-1.108)	3.720(3.060-4.420)	8.772(7.328-12.803)	16.153(12.952-19.638)	
60 years and older	4.900(3.764-6.105)	0.978(0.702-1.303)	3.992(3.299-4.981)	11.155(9.369-13.418)	21.358(14.792-30.564)	485
	4.884(3.679-6.294)	0.837(0.596-1.178)	3.773(2.948-4.681)	10.561(8.300-14.384)	21.167(15.531-29.557)	
Gender						
Male	5.901(5.100-6.772)	1.542(1.261-1.817)	5.562(4.762-6.104)	14.729(11.901-18.069)	26.491(21.749-31.325)	1022
	4.124(3.434-4.895)	0.955(0.810-1.188)	3.472(3.039-4.043)	8.889(7.581-10.900)	18.417(12.885-23.488)	
Female	5.993(5.173-6.953)	1.170(0.978-1.368)	5.274(4.491-5.857)	15.203(12.499-18.420)	25.302(21.678-29.343)	1190
	5.760(4.719-6.822)	0.918(0.782-1.093)	4.232(3.778-4.900)	12.185(10.744-14.580)	22.084(9.089-29.288)	
Area						
Urban	4.954(4.058-6.004)	1.012(0.829-1.306)	4.624(3.842-5.398)	11.270(10.198-13.823)	21.865(15.704-26.910)	774
	4.364(3.293-5.784)	0.792(0.600-0.953)	3.459(2.966-4.232)	8.793(7.336-10.744)	13.934(11.883-17.695)	
Suburb	6.339(5.435-7.424)	1.504(1.290-1.863)	5.787(5.086-6.618)	16.269(12.892-19.358)	26.435(21.287-32.340)	907
	5.111(4.180-6.124)	1.028(0.852-1.243)	3.986(3.379-4.564)	11.845(9.935-15.079)	22.223(19.086-29.537)	
Exurban	6.738(5.594-8.137)	1.440(1.127-2.094)	5.395(4.650-7.356)	18.901(14.877-22.558)	31.055(23.116-42.503)	531
	5.756(4.456-7.339)	1.068(0.839-1.362)	4.424(3.562-5.419)	12.619(9.775-18.050)	26.953(19.653-33.210)	
Education						
Lower	5.567(4.867-6.285)	1.275(1.049-1.456)	5.031(4.359-5.606)	13.369(11.892-14.958)	22.052(19.368-26.803)	1494
	4.950(4.103-5.848)	0.908(0.788-1.092)	3.728(3.221-4.179)	10.423(9.095-11.630)	19.785(15.718-23.662)	
Higher	6.747(5.665-7.973)	1.436(1.177-1.862)	5.989(5.103-6.975)	20.365(15.371-22.822)	29.064(26.155-36.350)	718
	5.117(4.222-6.147)	0.962(0.802-1.237)	4.432(3.571-5.277)	11.817(9.752-14.612)	22.550(16.399-30.766)	

ear correlation in the toxin concentration of wheat samples. Unexpectedly, after correction with creatinine, the linear correlation was no longer significant. In addition, 25.9% of the positive samples contained three or more mycotoxins. This finding provides evidence that human exposure to *Alternaria* mycotoxins from diet is a serious matter in China.

2.3. *Geographical distribution of* Alternaria *mycotoxins in Beijing*

Urine samples were collected from 16 regions that covered all areas in Beijing. The concentrations of the main *Alternaria* mycotoxins detedted in urine from different regions are shown in Fig. 1.

Geographically, among the 16 districts, Fangshan District had the highest average level of TeA (8.482 μg/L), followed by Huairou District and Yanqing District, where the average level exceeded 8.0 μg/L. Although there were differences in the average urine TeA level of the populations in the 16 regions, after dividing the Beijing area into urban (Dongcheng, Xicheng, Chaoyang, Fengtai, Shijingshan and Haidian), suburban (Daxing, Tongzhou, Shunyi, Changping, Mentougou and Fangshan), and exurban (Huairou, Pinggu, Miyun and Yanqing) areas, through one-way analysis of variance, no significant difference ($p > 0.05$) was found in the TeA concentration between rural and urban areas in Beijing China (Table 3).

The highest average level (0.1326 μg/L, 0.1214 μg/g creatinine) of AME was from Xicheng, and the lowest average concentration (0.019 μg/L) of AME was from Chaoyang. The concentration of AME in the population from suburban areas was significantly lower than that from urban and exurban areas ($p < 0.05$), as shown in Table 4 and Fig. 1. Unfortunately, at present, there are no reports on the geographical distribution in Beijing, and we are unable to speculate on the source of the higher AME in the suburban population.

2.4. *Concentrations and distribution profiles of the main* Alternaria *mycotoxins in the general population*

Since the detection rates of TeA and AME both exceeded 75%, we focused on analyzing TeA and AME. As shown in Table 3, the value of 7.922 μg/L in the 17-29 years group was higher than that in the 50-59 years and over 60 years groups, but there were no significant differences among age groups ($p > 0.05$). Similarly, there was no significant difference between the sexes ($p > 0.05$). The average level of TeA in the highly educated group was higher than that in the less educated group, but it did not reach a significant level.

table 4 – Average mean and selected percentiles of AME concentrations in urine (in μg/L in the first row and in μg/g creatinine in the second row) for the Beijing population.

	Average mean	Selected percentile (95% CI)				N
	(95% CI)	50th	75th	90th	95th	
Total	0.0650(0.0604-0.0701)	0.0340(0.0310-0.0358)	0.0730(0.0680-0.0771)	0.1470(0.1375-0.1565)	0.2160(0.1936-0.2473)	2212
	0.0499(0.0461-0.0542)	0.0227(0.0212-0.0240)	0.0545(0.0504-0.0589)	0.1167(0.1091-0.1257)	0.1769(0.1596-0.2026)	
Age group						
17-29 years	0.0571(0.0458-0.0715)	0.0320(0.0265-0.0370)	0.0635(0.0549-0.0719)	0.1191(0.0905-0.1415)	0.1782(0.1381-0.2805)	336
	0.0350(0.0281-0.0435)	0.0168(0.0143-0.0207)	0.0380(0.0327-0.0468)	0.0767(0.0594-0.1110)	0.1319(0.1009-0.1686)	
30-39 years	0.0444(0.0392-0.0501)	0.0262(0.0235-0.0295)	0.0528(0.0442-0.0575)	0.0995(0.0840-0.1188)	0.1518(0.1230-0.1950)	505
	0.0319(0.0279-0.0362)	0.0154(0.0140-0.0175)	0.0369(0.0305-0.0441)	0.0730(0.0647-0.0887)	0.1175(0.0961-0.1711)	460
40-49 years	0.0614(0.0539-0.0706)	0.0344(0.0293-0.0410)	0.0749(0.0675-0.0820)	0.1444(0.1157-0.1611)	0.2155(0.1784-0.2654)	
	0.0425(0.0380-0.0484)	0.0238(0.0205-0.0277)	0.0504(0.0451-0.0618)	0.1084(0.0980-0.1221)	0.1392(0.1233-0.1782)	
50-59 years	0.0772(0.0624-0.0953)	0.0358(0.0290-0.0400)	0.0816(0.0691-0.0958)	0.1595(0.1371-0.1795)	0.2392(0.1818-0.3365)	426
	0.0633(0.0516-0.0770)	0.0250(0.0221-0.0302)	0.0708(0.0574-0.0804)	0.1412(0.1147-0.1739)	0.2068(0.1752-0.2884)	
60 years and older	0.0848(0.0754-0.0951)	0.0440(0.0390-0.0500)	0.1035(0.0898-0.1215)	0.1959(0.1726-0.2368)	0.2883(0.2511-0.3980)	485
	0.0744(0.0640-0.0856)	0.0362(0.0323-0.0425)	0.0817(0.0749-0.0969)	0.1617(0.1385-0.2199)	0.2988(0.2407-0.3747)	
Gender						
Male	0.0557(0.0492-0.0635)	0.0285(0.0260-0.0308)	0.0596(0.0545-0.0650)	0.1250(0.1027-0.1401)	0.1789(0.1580-0.2153)	1022
	0.0367(0.0327-0.0413)	0.0172(0.0152-0.0188)	0.0371(0.0337-0.0423)	0.0848(0.0735-0.0974)	0.1332(0.1143-0.1618)	
Female	0.0730(0.0665-0.0802)	0.0387(0.0360-0.0420)	0.0845(0.0773-0.0915)	0.1664(0.1486-0.1850)	0.2517(0.2135-0.2825)	1190
	0.0613(0.0550-0.0682)	0.0301(0.0276-0.0335)	0.0720(0.0635-0.0768)	0.1377(0.1237-0.1528)	0.2079(0.1797-0.2494)	
Area						
Urban	0.0766(0.0693-0.0847)	0.0435(0.0399-0.0486)	0.0987(0.0858-0.1130)	0.1785(0.1580-0.2002)	0.2516(0.2201-0.2805)	774
	0.0623(0.0546-0.0709)	0.0304(0.0270-0.0347)	0.0760(0.0696-0.0861)	0.1397(0.1268-0.1644)	0.2220(0.1831-0.2637)	
Suburb	0.0454(0.0394-0.0529)	0.0220(0.0200-0.0235)	0.0460(0.0420-0.0500)	0.0957(0.0824-0.1026)	0.1530(0.1275-0.1848)	907
	0.0334(0.0292-0.0381)	0.0150(0.0134-0.0164)	0.0335(0.0297-0.0382)	0.0745(0.0619-0.0837)	0.1205(0.1014-0.1405)	
Exurban	0.0818(0.0698-0.0956)	0.0450(0.0420-0.0498)	0.0840(0.0764-0.0945)	0.1627(0.1371-0.1881)	0.2621(0.2044-0.3660)	531
	0.0602(0.0525-0.0688)	0.0312(0.0281-0.0343)	0.0670(0.0600-0.0750)	0.1377(0.1167-0.1500)	0.1960(0.1591-0.2632)	
Education						
Lower	0.0668(0.0611-0.0727)	0.0340(0.0310-0.0361)	0.0751(0.0685-0.0805)	0.1528(0.1405-0.1648)	0.2278(0.1961-0.2536)	1494
	0.0531(0.0477-0.0586)	0.0231(0.0214-0.0249)	0.0574(0.0514-0.0623)	0.1252(0.1140-0.1363)	0.1953(0.1650-0.2237)	
Higher	0.0614(0.0535-0.0701)	0.0335(0.0303-0.0369)	0.0671(0.0605-0.0765)	0.1328(0.1146-0.1575)	0.2051(0.1736-0.2244)	718
	0.0434(0.0381-0.0495)	0.0220(0.0187-0.0244)	0.0493(0.0446-0.0558)	0.1004(0.0893-0.1133)	0.1592(0.1238-0.1788)	

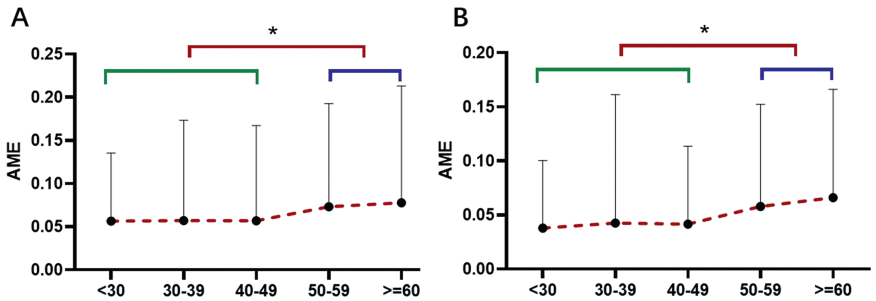

Fig. 2 – Average concentration of AME in different age groups. The results are expressed as the mean ± SD. Bars with superscript * differ significantly ($p > 0.05$) (A in μg/L and B in μg/g creatinine).

As shown in Table 4, the concentration of AME also did not differ by education level ($p > 0.05$), but the AME concentration in female urine was significantly higher than that in male urine, and there were significant differences in each age groups. The older age group was much higher than the younger group, the 60 years and older group had the highest concentration of AME in urine (Fig. 2). The body fat rate of women is generally higher than that of men, and the accumulation effect is stronger than that of men (Blaak et al., 2001). Similar to environmental permanent pollutants, the less polar the substance is, the more obvious the accumulation effect is; furthermore, the concentration in elderly people is higher than that in young people, and the concentration in women is higher than that in men (Jackson et al., 2017). AME is the least polar *Alternaria* mycotoxin. Therefore, it can be inferred that AME has an accumulation effect.

2.5. *Dietary intake of* Alternaria *mycotoxins*

After the participants' dietary intake of *Alternaria* mycotoxins was calculated using the above formula (Section 2.6), violin chart was used to show its distribution, as shown in Fig. 3. The maximum exposure of TeA was 6229.2 ng/kg b.w., and the quartiles of exposure within TeA were 0.5, 38.2, and 154.0

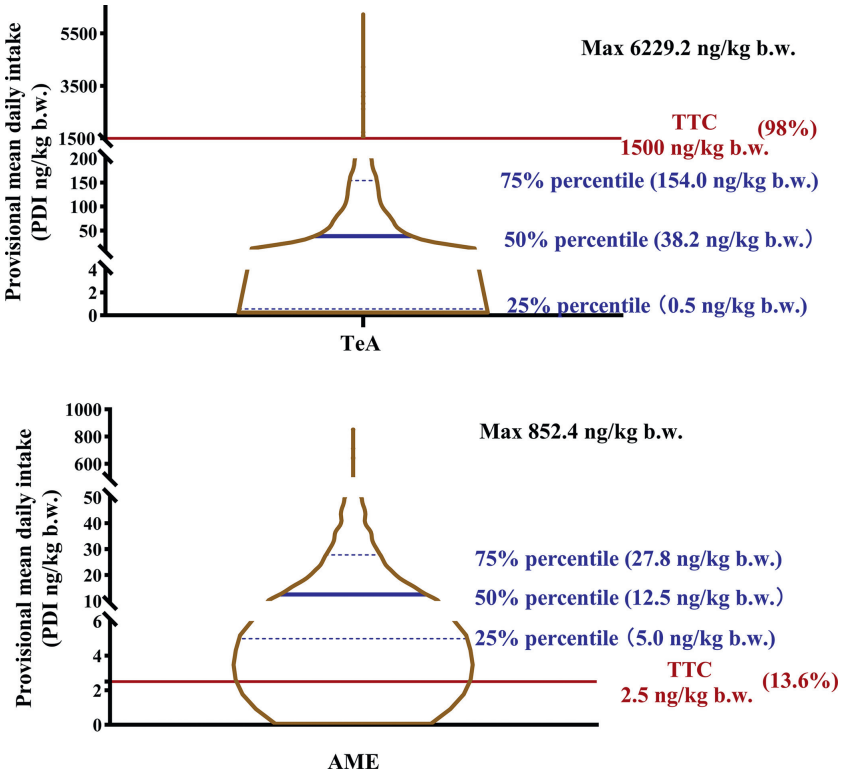

Fig. 3 – Distribution of dietary intake of the main Alternaria mycotoxins (red solid line, TTC value; blue solid line, median value; upper dashed line, 75th percent value; lower dashed line, 25th percent value).

ng/kg b.w.. As there are no specific international regulations for any of the Alternaria mycotoxins, we compared the exposure level with the TTC value established by the EFSA to assess the risk. The exposure level in the population under the 98% quantile was lower than the TTC value. Yannick et al. (Hickert et al., 2016) found that one of the participants ($n = 48$) slightly exceeded the TTC value. The average level of exposure to TeA for the Chinese population (38.2 ng/kg b.w.) was similar to that for the German population (183 ng/kg b.w.), which was much lower than the TTC value (1500 ng/kg b.w.). Additionally, the percentage of those that exceeded the TTC value (2%) was also similar. However, due to the differences in dietary patterns between Chinese and Europeans, the use of TTC values to evaluate Chinese exposure risks still needs to be explored.

The maximum exposure of AME was 852.4 ng/kg b.w.. The quartiles of exposure within AME were 5.0, 12.5, and 27.8 ng/kg b.w., and only 13.6% of the population had an exposure level lower than the TTC value. The average exposure level of AME was 12.5 ng/kg b.w., which was 5 times higher than the TTC value.

The European Food Safety Authority (EFSA) issued scientific opinions on the risks of *Alternaria* mycotoxins in feed and food to public and animal health in 2011 and 2016 (CONTAM et al., 2011; Hickert et al., 2016). In the 2011 EFSA scientific opinion on chronic dietary exposure to *Alternaria* mycotoxins in adults, the median AME values ranged from 1.6 to 3.6 ng/kg b.w. per day, the median TeA values ranged from 49 to 97 ng/kg b.w. per day. In the 2016 dietary exposure, the median values of AME ranged from 1.4 to 7.6 ng/kg b.w. per day, and the median values of TeA ranged from 86 to 178 ng/kg b.w. per day. The reported mean values of TeA were higher than those found in Beijing adults, and the mean values of AME were lower. A similar result was found when calculating the average daily exposure to *Alternaria* mycotoxins in Germany. Chronic exposure was calculated based on the mean consumption data. AME (6.67 ng/kg b.w. per day) was lower than those in Beijing adults, and TeA (450 ng/kg b.w. per day) was higher than that of our Beijing cohorts (Hickert et al., 2016). The observed ranges in exposure levels for various countries indicate high or low *Alternaria* exposures, which are likely a result of differences in the *Alternaria* contamination of major crops and differences in food habits of the populations studied. In future studies, this conjecture can be verified through the China Total Diet Study.

3. Conclusion

This study reported for the first time that five *Alternaria* mycotoxins were present in urine samples from residents of Beijing, China. The presence of a wide variety of *Alternaria* mycotoxin in urine samples indicated that individuals living in Beijing could be exposed to *Alternaria*. The calculated daily intake values of selected mycotoxins were even greater than the TTC values, indicating the potential health risks associated with mycotoxins. Immediate attention and subsequent actions should be taken to identify the sources of mycotoxins and the corresponding exposure pathways to humans in the investigated regions.

Acknowledgments

The authors gratefully acknowledge all the volunteers who collaborated with this study. This research was supported by the National Key Research and Development Program of China (Nos. 2017YFC1600500, 2019YFF0216303), the Research Special Found for Municipal Medical Public Welfare institute (No. 2017-BJYJ-15), and the Research Project of the National Institute of Metrology (Nos. AKY1934, 31-AKYZZ2039).

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