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Multi-mycotoxin biomonitoring in Italian adults: Revealing the connection between diet habits and mycotoxin exposure

Octavian Augustin Mihalache [a,*,1], Cristiana Mignogna [b,1], Raquel Torrijos [a,c], José F. Rinaldi de Alvarenga [b], Elisa Monica [b], Maria Sole Morandini [b], Cristina Del Burgo-Gutiérrez [b], Marialaura Bonaccio [d], Emilia Ruggiero [d], Daniele Del Rio [b], Alice Rosi [b], Pedro Mena [b,2], Chiara Dall'Asta [a,*,2]

[a] Department of Food and Drug, University of Parma, Viale delle Scienze 27/A, 43124 Parma, Italy
[b] Human Nutrition Unit, Department of Food and Drug, University of Parma, Via Volturno 39, 43125 Parma, Italy
[c] Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Ave. Vicent Andrés Estellés s/n 46100, Burjassot, Valencia, Spain
[d] Department of Epidemiology and Prevention, IRCCS NEUROMED, Via dell'Elettronica, 86077 Pozzilli, Isernia, Italy

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ABSTRACT

This study investigated the relationship between food consumption patterns and mycotoxin exposure. We analyzed 300 spot urine samples from Italian adults for 24 mycotoxins and their metabolites using enzymatic hydrolysis followed by ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-ESI-MS/MS). Target mycotoxins were aflatoxins (AFs), fumonisins B1 and B2 (FB1, FB2), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), ochratoxin A (OTA), ochratoxin-alpha (OT-alpha), zearalenone (ZEN), zearalanone (ZAN), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), T-2/HT-2 toxin, deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM-1), enniatins B, B1, A, A1 (ENNs), and beauvericin (BEA). Participants were scored according to three dietary patterns: the Mediterranean Diet Score, the Plant-based Diet Index, and the general Pro-vegetarian Food Pattern. For each index, low- and high-adherence groups were defined based on score distribution. Emerging mycotoxins, such as *Alternaria* toxins, ENNs, and BEA showed the highest prevalence (>90%), while DON (34%), OTA (33%), and ZEN (25%) had lower prevalence, with DON showing the highest mean concentration (7.10 μg/g creatinine). Cereals, nuts, legumes, fruits, and vegetables were significantly associated with increased mycotoxin exposure, especially AFs, OTA, ENNs, and DON. Estimated daily intakes for DON were negligible, whereas some individuals classified as high adherers to one or more of these dietary patterns approached or exceeded the safe threshold for ZEN. Exposure to OTA represented a potential health risk across all individuals. These findings support periodic updating of EU maximum-level frameworks for contaminants in food and related exposure and risk assessments so that increasingly consumed plant-based raw materials and composite foods are better represented in monitoring and risk characterization. Such updates would support a safe and effective transition toward sustainable diets in line with public health goals.

1. Introduction

To support health and environmental sustainability and achieve the UN Sustainable Development Goals (SDGs) and Paris Agreement, consumption trends have shifted toward alternative diets mainly based on plant-based foods (Bunge et al., 2022; Willett et al., 2019). While the health and environmental benefits of new dietary patterns have been widely explored, there is scant data on how a shift in dietary choices may lead to a shift in exposure to contaminants. The Food and Agriculture Organization (FAO) acknowledges the health and environmental benefits of plant-based diets, but it also highlights that the shift may lead to higher exposure levels to chemicals like mycotoxins (FAO & WHO, 2019). Mycotoxins are among the most relevant natural chemical contaminants of plant-derived foods. They are toxic secondary metabolites

* Corresponding authors.
E-mail addresses: augustinoctavian.mihalache@unipr.it (O.A. Mihalache), chiara.dallasta@unipr.it (C. Dall'Asta).
[1] These authors contributed equally to the present work.
[2] These authors are co-senior authors.

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produced by fungi and can enter the food chain through crop infection before or after harvest, occurring particularly in cereals, dried fruits, nuts, spices, and other plant-derived commodities (EFSA, 2026; WHO, 2013). Exposure to mycotoxins can pose a significant burden due to their wide spectrum of toxic effects, including carcinogenicity, genotoxicity, cytotoxicity, immune modulation, and estrogenic effects (WHO, 2013).

For many foodborne chemical contaminants, diet is a major exposure pathway, although its relative contribution depends on the contaminant, exposure sources, lifestyle, environment, and individual characteristics. With the growing popularity of plant-based meat alternatives and vegetarian diets, several studies have reported a potential increase in exposure to mycotoxins through plant-derived foods (Fleury et al., 2017; Mihalache et al., 2024; Penczynski et al., 2022). Similarly, adherence to healthy dietary patterns measured through tools such as the alternate Mediterranean diet and alternate Healthy Eating Index is associated with exposure to environmental chemicals (Yu et al., 2024). These findings underscore the need for regulatory frameworks and food safety monitoring systems to evolve in tandem with shifting dietary trends, ensuring that efforts to promote healthier and more sustainable diets are complemented by robust strategies to minimize unintended chemical exposures. Therefore, it is crucial to understand how the dietary shifts toward more plant-based food reflect the shift in exposure to mycotoxins.

Traditionally, dietary exposure assessments have mainly relied on food consumption data and mycotoxin occurrence data in foods, whereas human biomonitoring (HBM) provides a complementary approach for assessing internal exposure to contaminants from multiple sources and pathways (Angerer et al., 2007; De Ruyck et al., 2020; Gilles et al., 2021).

Urine is one of the most commonly used biological matrices, as it can contain mycotoxin metabolites or the parent compounds. The use of HBM provides insights into both aggregated and internal exposure, considering various sources and routes of exposure, and inter-individual differences in factors such as diet, metabolism, and lifestyle (Angerer et al., 2007). By measuring the concentrations of chemicals in the human body, HBM complements external exposure assessments and provides essential data for health risk evaluations (Santonen et al., 2023). Highlighting the association between food consumption patterns and urinary concentration of mycotoxins might reveal which patterns may lead to potentially increased exposure levels. These findings may aid in developing targeted monitoring programs for chemical exposure related to specific food groups, which are linked to healthy and sustainable dietary patterns, and help promote a healthy diet while minimizing exposure to natural toxins.

This cross-sectional study investigated associations between food consumption patterns and urinary mycotoxin biomarker concentrations in Italian adults, as measured by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Mycotoxin daily intakes were estimated by reverse dosimetry, and risk characterization was performed based on urinary biomarker concentrations.

2. Materials and methods

2.1. Study population

Data and biological samples were collected at baseline from volunteers participating in an acute intervention study. The study was approved by the Ethics Committee of the Area Vasta Emilia Nord region (Italy, 1352/2020/SPER/UNIPR OPCT) and registered on ClinicalTrials.gov (NCT05414084).

Three hundred subjects, aged 19–73 years, were enrolled; 57% of the volunteers were women, with a median body weight of 66.5 kg (IQR, 57.8–75.2) and a median body mass index (BMI) of 23 kg/m^2 (IQR, 21.5–25.3).

Urinary creatinine concentrations ranged from 26.49 to 484.23 mg/dL (0.265 – 4.842 g/L), with a median of 124.73 mg/dL (1.247 g/L). Using the commonly applied spot-urine suitability interval of 0.3 – 3.0 g/L creatinine (Cocker et al., 2011), 288 of 300 samples (96.0%) were within the conventional range, while 2 samples were below and 10 were above this interval. These thresholds were used to describe urine dilution/concentration status rather than as an exclusion criterion.

2.2. Urine collection and creatinine analysis

Overnight fasting (>8h) spot urines were self-collected in wide-mouth specimen containers, aliquoted and frozen at −80 °C until analysis. Creatinine assessment was performed on an AU5800 Beckman Coulter AU analyser (Beckman Coulter, Brea, CA, USA) using creatinine reagent OSR6178, in accordance with standardized hospital routine procedures.

2.3. Chemicals and reagents

Acetonitrile (MeCN), methanol (MeOH), ultrapure water, formic acid (FA), and acetic acid were obtained from VWR Chemicals (VWR International, Milan, Italy), while the ammonium acetate and anhydrous magnesium sulfate (MgSO4) were supplied by Sigma-Aldrich (Merk Life Science S.r.L., Milan, Italy).

Analytical standards of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) were obtained from Fermentek.Ltsd (Jerusalem, Israel). The mycotoxins ochratoxin A (OTA), ochratoxin alpha (OTα) zearalenone (ZEN), zearalanone (ZAN), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), fumonisin B1 (FB1), fumonisin B2 (FB2), HT-2, T-2, deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM), enniatin B (ENNB), enniatin B1 (ENNB1), enniatin A (ENNA), enniatin A1 (ENNA1), beauvericin (BEA), alternariol (AOH), alternariol monomethyl ether (AME), and tentoxin (TEN) were purchased from Biopure (Romer Labs Division Holding Gmbh, Getzersdorf, Austria). For those mycotoxins provided as dried powders, the appropriate solvent was added according to the manufacturer's instructions and prepared at a concentration of 1000 μg/L. Then, a working solution of 1 μg/L was prepared combining all mycotoxins in MeCN, except FB1 and FB2, which were prepared separately in MeCN: H2O (50:50, v/v) due to their instability in pure acetonitrile (Sulyok et al., 2020). These solutions were used for preparing the subsequent matrix-matched calibration curves used for quantification and stored at −20 °C.

2.4. Sample preparation

Urine samples were thawed and centrifuged for 5 min on a 5830 R centrifuge (Eppendorf, Hamburg, Germany) at 10,000 rpm at 4 °C to eliminate solid particles. Then, the deconjugation of glucuronide and sulfate metabolites was performed using β-glucuronidase from *Helix pomatia* (100,000 units/mL) (Merck, Milan, Italy) (Marín-Sáez et al., 2024). For this, 0.5 mL of the urine samples were treated for 15 h at 52 °C with 100 μL of the enzyme, resulting in a final concentration of 5000 units/mL. β-Glucuronidase from *Helix pomatia* was selected because of the multi-analyte scope of the method and its broad deconjugation capacity, as also applied in recent urinary multi-mycotoxin biomonitoring methods. We acknowledge that T-2/HT-2 toxins represent a specific case, since hydrolysis efficiency and stability of their conjugates may depend on the enzyme source (Welsch and Humpf, 2012). Therefore, T-2/HT-2 results were interpreted descriptively and were not used for reverse-dosimetry-based risk characterization.

After enzymatic treatment, a salt-assisted liquid–liquid extraction (SALLE) procedure was performed. Briefly, the hydrolyzed samples were centrifuged for 5 min at 10,000 rpm and 4 °C, and the supernatants were transferred to 15 mL Falcon tubes. Then, 0.5 mL H_2O and 1 mL of MeCN containing 1% FA (v/v) were added, and the mixture was horizontally shaken for 20 min at 230 S/min in an HS 501 horizontal shaker (Ika-

Werke, Staufen im Breisgau, Germany). Next, 0.5 g of anhydrous $MgSO_4$ was added, and the mixture was vortexed for 1 min.

The mixture obtained was centrifuged for 10 min at 10,000 rpm at 4 °C, and 0.6 mL of the supernatant was transferred to a 4 mL glass vial and dried with N_2. Prior to the analysis, dried extracts were resuspended in 150 μL of MeOH:H_2O, 75:25 (v/v).

2.5. UHPLC-ESI-MS/MS analysis of mycotoxins

A UHPLC Dionex Ultimate 3000 coupled to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA) and equipped with an electrospray ionization source (ESI) was used. For chromatographic separation, an XSelect® HSS T3 column (2.1 x 150 mm, 2.5 μm) equipped with its respective precolumn (Waters, Wexford, Ireland) was used. Mobile phases were ultrapure water with 0.2% acetic acid (v/v) and 5 mM ammonium acetate (eluent A) and MeOH with 0.2% acetic acid (v/v) (eluent B). To achieve mycotoxin separation, a multi-step elution gradient was configured as follows: 0 min, 5% B; 8 min, 90% B; 12 min, 5% B, 18 min, 5% B. No column cleaning was performed inside the gradient. Specifically, this method does not achieve a 100% organic phase, as it is not required to elute mycotoxins, and no carry-over or increase in system pressure was observed. The flow rate was set at 0.4 mL/min. The column temperature was set at 40 °C, and the injection volume selected was 3 μL.

Mass spectrometry analyses were conducted using both positive (ESI +) and negative (ESI-) ionization modes, depending on the mycotoxin being studied. AFB_1, AFB2, AFG1, AFG2, TEN, FB1, FB2, HT-2, T-2, OTA, BEA, ENNB, ENNB1, ENNA, ENNA1, and DOM operated in ESI+, whereas AOH, AME, ZEN, α-ZEL, β-ZEL, ZAN, and OTα operated in ESI-. The mycotoxins were analyzed under selected reaction monitoring (SRM) acquisition mode. Optimized MS/MS parameters for the mycotoxins are described in Supplementary Material 1. The spray voltage was set at + 3.5 kV, and the capillary and vaporizer temperatures were programmed at 270 °C and 200 °C, respectively. The sheath gas and the auxiliary gas (N_2) were set at 50 and 5 arbitrary units, respectively. Argon (Ar) was used as the collision gas. Chromaleon and Xcalibur Roadmap Software (version 2.2; Thermo Fisher Scientific Inc.) were used for instrument control, data acquisition, and processing.

2.6. Method characterization

The method was characterized in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision (repeatability and reproducibility), and matrix effect based on the performance criteria stipulated in European Commission Regulation 2021/808/EC (EC, 2021), 2006/401/EC (EC, 2006), SANTE/12089/2016, and SANTE/11312/2021. A blank urine sample, previously verified to be free of the target mycotoxins in the study, was used for method characterization.

For linearity evaluation, nine-point calibration curves were constructed by spiking the blank urine extracts with concentrations ranging from 0.1 to 100 μg/L and injecting them in triplicate ($n = 3$). The LODs and LOQs were expressed as the minimum analyte concentration with a signal-to-noise ratio of 3 and 10, respectively.

The accuracy of the method was evaluated through recovery studies of mycotoxins spiking five independent samples ($n = 5$) at three concentration levels, covering from the LOQ (level 1), 2 times the LOQ (level 2), and 5 times the LOQ (level 3). Specific information regarding the spiking levels can be found in Supplementary Material 1. The intra-day precision (repeatability) was measured on the same day for the three spiking levels ($n = 5$), while the inter-day precision (reproducibility) was measured over three non-consecutive days ($n = 9$). The results were expressed as relative standard deviation (%RSD).

The matrix effect was evaluated by comparing the slope of the matrix-matched calibration curve with that of the calibration curve prepared in solvent, and the results were expressed as signal suppression/enhancement (SSE%). A SSE > 100% indicates signal enhancement, whereas a SSE < 100% means signal suppression.

2.7. Estimated daily intakes and risk characterization

For HBM-based intake reconstruction, the treatment of urinary biomarker concentrations below the limit of detection (LOD) or limit of quantification (LOQ) is an important source of uncertainty. Left-censored urinary concentrations were therefore treated using the substitution approach. For the dietary exposure and risk characterization, an upper-bound (UB) scenario was applied, in which concentrations below the LOD were replaced by the LOD and concentrations below the LOQ were replaced by the LOQ (EFSA, 2010). This approach was used to obtain conservative intake estimates from the urinary biomarker data before reverse-dosimetry calculation.

Reverse-dosimetry-based intake reconstruction was limited to total DON, total ZEN-related biomarkers, and OTA. These mycotoxins were selected because they combined sufficient detection and quantification frequency in the study population with the availability of human urinary excretion data suitable for intake reconstruction within the applied modelling framework. For the remaining mycotoxins, either the proportion of left-censored data was too high and/or sufficiently established human toxicokinetic parameters for urinary reverse dosimetry were not available.

For the DON reverse-dosimetry calculation we used the urinary excretion estimate reported by Turner et al. (2010), together with an assumed adult daily urinary volume of 1.5 L/day. We acknowledge that more recent human intervention studies have further refined DON toxicokinetics and support urinary recovery values in the same general range, while also indicating that 24-h urine is the preferred matrix for DON intake reconstruction (Vidal et al., 2018; Mengelers et al., 2019) while recent reviews emphasize the broader uncertainty associated with urinary mycotoxin biomarkers and reverse-dosimetry approaches (Cramer et al., 2025). Because the present study relied on spot urine samples, the original volume-based framework was retained for consistency, and the resulting EDI estimates were interpreted at the population level.

Urinary biomarker concentrations were expressed both as volumetric concentrations (μg/L) and as creatinine-adjusted concentrations (μg/g creatinine). Creatinine-adjusted concentrations were used for descriptive biomonitoring comparisons across spot urine samples, as they reduce variability related to urine dilution. However, reverse-dosimetry-based intake reconstruction was performed using volumetric urinary concentrations (μg/L), because the applied equation is based on daily urine volume. Estimated daily intakes (EDI) for total deoxynivalenol (DON), zearalenol (ZEN), and ochratoxin A (OTA) were calculated using Equation (1):

$$\mathrm{EDI}\,(\mu g/kg\,bw/day) = \frac{C \times V \times 100}{BW \times UE} \quad (1)$$

where C = urinary concentration of mycotoxin (μg/L), V = mean volume of daily urine production for adults (1.5 L), BW = body weight reported by the volunteers, UE = urinary excretion rates in percentage: 72% for total DON (DON + DOM-1) (Turner et al., 2010), 36.8% for total ZEN (ZEN + ZAN + α-ZEL + β-ZEL) (Gambacorta et al., 2013), and 2.5% for OTA (Degen, 2016; Studer-Rohr et al., 2000).

For DON, EFSA established a tolerable daily intake (TDI) of 1 μg/kg bw/day derived from studies indicating a reduced body weight gain in mice (EFSA, 2017). Meanwhile for ZEN, EFSA set a TDI of 0.25 μg/kg bw/day based on its oestrogenic effects, and this value was subsequently considered appropriate as a group TDI for ZEN and its modified forms (EFSA, 2011; EFSA, 2016). In the present study, risk characterization was based on total ZEN-related urinary biomarkers, including ZEN, ZAN, α-ZEL, and β-ZEL. Because α-ZEL and β-ZEL were detected only in a small number of samples, their contribution did not affect the

reconstructed ZEN-related exposure estimates.

To assess whether the EDI to mycotoxins poses a potential health risk, we compared the EDI with health-based guidance values (HBGV) using the hazard quotient (HQ) (Equation (2). When the HQ > 1, it indicates a non-tolerable exposure with potential adverse health effects (EFSA, 2019).

$$HQ = \frac{EDI}{HBGV}(x) \quad (2)$$

OTA has been classified by IARC as possibly carcinogenic to humans (Group 2B) (IARC, 2019), and EFSA considered that uncertainties regarding the mode of action for kidney carcinogenicity make it inappropriate to establish a health-based guidance value. Therefore, the margin of exposure (MOE) approach was applied.

The MOE is used to assess potential health risks for the presence of chemical contaminants in food and feed. The MOE is represented by the ratio of the benchmark dose lower limit (BMDL) of the mycotoxin and the estimated exposure of the consumer to the mycotoxin (Equation (3):

$$MOE = \frac{BMDL10}{EDI} \quad (3)$$

The $BMDL_{10}$ for OTA is 14.5 μg/kg bw/day for neoplastic effects and 4.73 μg/kg bw/day for non-neoplastic effects (EFSA, 2020). For the characterization of non-neoplastic and neoplastic effects, the exposure indicates a potential health risk when the MOE is lower than 200 and 10,000, respectively, while a MOE > 200 or > 10,000 reflects a negligible level of risk (EFSA, 2023).

2.8. Dietary assessment and dietary pattern classification

Dietary data were used to describe habitual food-group consumption and to derive dietary-pattern indices for association analyses with urinary mycotoxin biomarkers.

Dietary habits during the year preceding enrolment were evaluated through a 188-item, semi-quantitative food frequency questionnaire (European Prospective Investigation into Cancer and Nutrition (EPIC) Food-Frequency Questionnaire (FFQ)) (Pisani, 1997; Pala et al., 2003). The quali-quantitative analysis of nutritional intake was carried out by using the NAF software that estimated the daily intake of macro and micronutrients and food items (Pala et al., 2003). Food items were also classified into food groups, as reported in the scientific dossier "Linee Guida per una Sana Alimentazione Italiana" (CREA, 2018), as 1) cereals and derivatives, tubers, 2) fruit and vegetables, 3) nuts and seeds, 4) meat, fish and eggs, 5) legumes, 6) milk and derivatives, 7) dressing fats and sauces. In addition, 5 other groups were constituted: 8) coffee, tea and herbal tea, 9) alcoholic drinks (wine, beer, spirits), 10) soft drinks (fruit and vegetable juices, carbonated/soft/isotonic drinks), 11) sweets, consisting of cakes, pastries, biscuits, chocolate, sweeteners, ice cream, 12) miscellaneous and savoury snacks, among them pizza, bouillon, soups and unclassified. All the groups were expressed as grams per day (g/day).

Some dietary indices for the evaluation of intake patterns were derived by "a priori" methods. Briefly, adherence to the Mediterranean diet was assessed by applying the Mediterranean Diet Score (MDS) developed by Trichopoulou et al (2003). Each of the 9-food components considered was assigned 0 or 1 point based on the sex-specific median as the population consumption cutoff. The Plant-based Diet Index (PDI) measures adherence to a plant-based dietary pattern considering 18 food groups, both plant- and animal-based (Satija et al., 2016). The groups were divided into quintiles based on consumption, and each quintile was assigned a score ranging from 1 to 5. For plant food components, the highest quintile received the highest score, while a consumption lower than the first quintile received only 1 point. In contrast, the animal food groups were assigned an inverse scoring system, where the higher the consumption, the lower the points. The general Pro-Vegetarian Food Pattern (gPVG) was computed based on 12 components, 7 of which were positively scored and 5 negatively scored, adjusted for total energy intake using the residual method (Oncina-Cánovas et al., 2022).

The three dietary-pattern indices were applied independently to all participants and were not intended to define mutually exclusive dietary groups. Accordingly, the same participant could be classified as having high adherence to more than one dietary pattern. For the PDI and gPVG, low– and high–adherence categories were defined separately for each index based on the corresponding score distributions using tertiles of consumption. By contrast, the Mediterranean Diet Score (MDS; range 0–9) was categorized into three adherence levels based on score distribution: low (0–3), intermediate (4–5), and high (6–9). The resulting group sizes were: MDS, low adherence n = 89 and high adherence n = 84; gPVG, low adherence n = 113 and high adherence n = 100; and PDI, low adherence n = 112 and high adherence n = 105. No separate animal-based control group was defined.

2.9. Statistical analysis

Data normality was assessed using the Shapiro-Wilk test, and urinary concentration data were not normally distributed ($p < 0.05$). To check whether mycotoxin urinary concentrations are related to food consumption patterns, Spearman correlation was applied. Additionally, Mann-Whitney comparisons between the urinary concentration of each mycotoxin were applied for: a) low consumers (first tertile, T1) versus high consumers (third tertile, T3), b) low versus high adherence to a Mediterranean, plant-based, or pro-vegetarian diet. General linear models were used to examine the association between mycotoxin concentration levels (dependent variable) and food consumption in the form of food groups (independent variable), while controlling for sex, age, and BMI. All the analyses were considered significant at $p < 0.05$.

The statistical analyses and illustrations were realized using SPSS Statistics 26 (IBM Software Group, Chicago, IL), Tableau Software 2020.1 (Salesforce, Seattle, WA), and R Statistical Software (v 4.5.2; R Core Team 2021).

3. Results

3.1. Method characterization

The performance characteristics, including linearity, LODs, LOQs, recoveries, intra-day and inter-day precision, and matrix effects, are summarized in Table 1. All the mycotoxins exhibited good linearity, with a coefficient of determination (R^2) > 0.9901 over the studied range. The LODs and LOQs of the tested mycotoxins were determined using matrix-matched solutions and ranged from 0.02 μg/L to 1.50 μg/L and 0.06 μg/L to 5.00 μg/L, respectively.

Satisfactory recoveries were obtained for the three fortification levels tested, ranging from 70.2% to 103.9%. Moreover, intra-day precision (repeatability, $\%RSD_r$) and inter-day precision (reproducibility, $\%RSD_R$) were < 16.9% and < 19.9%, respectively, thus fulfilling the recommendations for the determination of contaminants (EC, 2006). Regarding matrix effect, a high ion suppression (%SSE < 80%) was generally observed in most mycotoxins, except for FB_1, where ion enhancement was evident (139.9%). For this reason, matrix-matched calibration curves were used.

Overall, the findings demonstrate the suitability of the proposed SALLE method for the extraction and determination of 24 mycotoxins in urine samples.

3.2. Mycotoxin detection frequency and co-exposure

Creatinine-adjusted concentrations are presented in the main text to describe biomonitoring results from spot urine samples, whereas unadjusted volumetric concentrations (μg/L), used for reverse-dosimetry-based EDI calculations, are provided in Supplementary Material 2.

When comparing with previous biomonitoring studies, creatinine-

Table 1
Performance characteristics of the proposed LC-MS/MS method in urine.

Mycotoxin	Linearity (R^2)	LOD (μg/L)	LOQ (μg/L)	Recovery (%) ($n = 5$)			Intra-day Precision (%RSD_r) ($n = 5$)			Inter-day Precision (%RSD_R) ($n = 9$)			SSE (%)
				Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	
AFB_1	0.9930	0.03	0.10	97.1	93.2	89.3	8.9	3.3	3.5	7.0	10.3	11.5	46.7
AFB_2	0.9953	0.05	0.17	99.0	91.2	80.2	7.0	6.4	3.4	9.0	14.9	6.1	33.5
AFG_1	0.9901	0.03	0.10	92.8	94.5	91.8	8.9	7.2	4.2	11.2	11.5	15.4	42.4
AFG_2	0.9909	0.50	1.67	89.7	90.5	90.6	7.8	4.3	8.0	13.1	18.7	11.2	52.7
AOH	0.9906	1.07	3.57	103.9	105.6	94.6	10.1	8.5	7.4	10.1	7.5	9.5	21.3
AME	0.9922	0.02	0.06	96.1	90.0	84.0	12.5	2.3	0.4	15.4	15.4	16.5	41.2
TEN	0.9917	0.02	0.06	96.8	99.5	87.0	11.6	4.9	3.4	10.1	7.0	17.2	53.1
FB_1	0.9951	0.30	1.00	70.8	70.2	82.6	6.4	1.8	5.7	6.5	2.8	13.1	139.9
FB_2	0.9965	0.30	1.00	92.7	77.0	75.6	6.2	0.8	4.3	9.3	12.8	12.7	74.3
HT-2	0.9914	0.05	0.17	94.7	89.5	100.9	4.5	5.0	8.9	10.9	13.4	8.8	40.4
T-2	0.9955	0.04	0.14	72.7	89.6	93.9	0.1	5.1	9.2	10.6	11.0	7.4	48.4
OTA	0.9961	0.10	0.33	82.2	86.0	81.9	9.8	7.9	1.6	6.6	19.8	16.6	55.0
ZEN	0.9918	0.02	0.06	100.2	87.0	81.5	7.6	9.0	4.1	13.1	13.6	15.8	48.3
BEA	0.9914	0.04	0.13	106.7	79.4	86.3	2.6	4.8	4.0	8.4	18.0	15.5	61.3
DON	0.9938	1.50	5.00	97.2	94.5	80.9	5.5	7.4	3.4	12.7	9.0	18.6	17.1
ENNB	0.9934	0.04	0.13	101.6	85.8	86.0	9.4	4.9	5.1	10.7	14.7	12.6	50.6
$ENNB_1$	0.9970	0.04	0.13	98.0	86.2	84.8	8.9	3.1	2.6	6.9	13.4	16.7	34.2
ENNA	0.9979	0.03	0.10	101.7	91.1	88.3	2.8	7.5	2.0	6.4	16.3	17.9	59.4
$ENNA_1$	0.9977	0.04	0.13	87.4	98.8	93.2	15.4	11.0	9.9	18.5	9.0	15.8	44.7
α-ZEL	0.9984	0.25	0.83	90.9	81.3	78.5	7.1	9.4	1.8	10.6	8.9	4.5	97.5
β-ZEL	0.9976	0.04	0.13	101.4	99.0	103.2	16.9	11.8	9.1	16.6	11.6	14.5	30.4
ZAN	0.9968	0.08	0.25	92.4	87.3	94.1	14.8	11.0	6.3	18.7	12.6	18.3	36.9
DOM-1	0.9953	0.04	0.13	98.1	90.8	88.1	12.2	11.4	8.9	11.1	11.3	17.3	33.3
OT-α	0.9970	0.05	0.17	97.9	88.7	79.9	7.5	5.6	8.6	11.3	8.9	16.7	84.9

adjusted values were compared with studies reporting μg/g creatinine, whereas unadjusted volumetric values from Supplementary Material 2 were used for comparisons with studies reporting μg/L.

Mycotoxin detection frequency was assessed in urine samples from 300 individuals, and the concentrations were reported creatinine-adjusted (Table 2) or unadjusted (Supplementary Material 2). Aflatoxins (AFs) were present in up to 38 (12.6%) of the participants. Mean creatinine-adjusted concentrations for AFs were between 0.31 and 5.87 μg/g creatinine with maximum concentrations reaching 8.06 μg/g creatinine.

Alternaria toxins had a high prevalence, with AME being found in 284 (94.6%) samples. Mean creatinine-adjusted concentrations varied from 0.65 μg AME/g creatinine to 4.36 μg AOH/g creatinine.

Fumonisins (FUMs) showed the lowest detection frequency, with FB1 detected in 9 participants (3.0%) and FB2 detected in 4 participants (1.3%). Mean creatinine-adjusted concentrations were 3.70 μg/g creatinine for FB1 and 1.90 μg/g creatinine for FB2, with maximum concentrations of 8.35 and 4.43 μg/g creatinine, respectively.

HT-2 and T-2 toxin had a particular pattern, with 221 (73.6%) individuals being exposed to HT-2 in comparison with only 92 (30.6%) being exposed to T-2 toxin. Mean concentrations were 1.93 μg/g for HT-2 and 0.51 μg/g for T-2.

OTA, a carcinogenic and genotoxic mycotoxin, and its main metabolite OT-alpha, which is less toxic, were detected in 99 (33%) and 60 (20%) individuals, respectively. Moreover, mean concentration values ranged from 0.92 to 1.27 μg/g creatinine.

While ZEN was present in 75 (25%) individuals, its metabolites (α-ZEL, β-ZEL, ZAN) were less prevalent (4 – 22 samples; 2.7 – 7.3%) and the mean concentrations were between 0.50 and 1.82 μg/g creatinine.

The emerging mycotoxins ENNs and BEA were among the most prevalent, with an occurrence of up to 288 samples (96%) and low mean concentrations between 0.61 – 0.76 μg/g creatinine.

DON and its metabolite DOM-1 were detected in 90 (34%) and 64 (21.3%) individuals, respectively. Mean concentrations were between 3.54 and 7.10 μg/g creatinine, with some individuals being exposed to levels as high as 60.47 μg/g creatinine DON.

We found at least two mycotoxins per individual, with one participant being exposed to 23 mycotoxins (Fig. 1). In this study, more than 50% of participants were simultaneously exposed to mixtures of 6 to 10 different mycotoxins, indicating a high burden of co-exposure through their diet. The most frequent combinations included mycotoxins from different fungi such as *Aspergillus, Fusarium,* and *Alternaria*, indicating the complex nature of dietary exposure. The most frequent family-level pattern was *Alternaria* toxins + ENNs/BEA + trichothecenes, observed in 109 participants (36.6%), followed by *Alternaria* toxins + ENNs/BEA + OTA + trichothecenes in 48 participants (16.1%).

3.3. Associations between urinary mycotoxin concentrations and food consumption patterns

Coffee and tea consumption showed weak but significant positive correlations only with selected biomarkers, namely TEN ($\rho = 0.140$), tDON ($\rho = 0.123$), and OTA ($\rho = 0.198$) ($p < 0.05$; Fig. 2). Nuts and seeds were more broadly correlated with urinary mycotoxin biomarkers, including AFs (AFB1, AFB2, AFG1, and AFG2; $\rho = 0.200–0.237$), *Alternaria* toxins (AME, AOH, and TEN; $\rho = 0.183–0.246$), fumonisins (FB1 and FB2; $\rho = 0.176–0.183$), tDON ($\rho = 0.205$), tZEN ($\rho = 0.211$), OTA ($\rho = 0.237$), and ENNs (ENNA, ENNA1, ENNB, and ENNB1; $\rho = 0.160–0.236$). No significant correlations with NS were observed for BEA, HT-2, or T-2.

Fruits, vegetables, and legumes had a weak correlation with AFs, ENNs, *Alternaria* toxins, tZEN, tDON, and OTA ($\rho = 0.11 – 0.29$; $p < 0.05$).

The only significant correlations with cereals and tubers were observed for tDON and OTA ($\rho = 0.12 – 0.19$; $p < 0.05$).

Some food groups, such as meat, fish, eggs, and soft drinks, were negatively correlated with mycotoxin exposure (ρ ranging from −0.16 to −0.11; $p < 0.05$), as it was expected since these foods are not relevant contributors to mycotoxin exposure.

Alcoholic beverages were also weakly negatively correlated with selected biomarkers when analyzed as an aggregated food group (ρ ranging from −0.19 to −0.12; $p < 0.05$. However, alcoholic beverages were not disaggregated into specific categories such as beer, wine, or spirits. Therefore, these findings should not be interpreted as beverage-specific associations, since individual alcoholic beverages may differ in their contribution to mycotoxin exposure.

To further assess the association between mycotoxin exposure and food consumption patterns, regression analyses were performed using

Table 2
Detection frequency and creatinine-adjusted urinary concentrations of mycotoxins in urine samples.

Mycotoxin	Detection frequency, n (%)	Concentration, μg/g creatinine			
		Mean ± STD	Median (IQR)	Min	Max
AFB1	9 (3.0%)	0.31 ± 0.21	0.10 (0.06 – 0.56)	0.02	1.08
AFB2	15 (5.0%)	1.15 ± 0.84	0.79 (0.34 – 1.31)	0.16	5.85
AFG1	38 (12.6%)	1.60 ± 0.60	1.10 (0.64 – 1.94)	0.18	6.09
AFG2	9 (3.0%)	5.87 ± 1.97	5.86 (4.78 –6.97)	1.11	8.06
AOH	39 (13.0%)	4.36 ± 2.34	2.55 (2.30 – 5.96)	1.99	9.43
AME	284 (94.6%)	0.65 ± 0.39	0.54 (0.37 – 0.82)	0.15	2.50
TEN	130 (43.3%)	1.03 ± 0.70	0.87 (0.23 – 0.63)	0.29	5.79
FB1	9 (3.0%)	3.70 ± 2.60	2.46 (1.61 – 5.85)	1.18	8.35
FB2	4 (1.3%)	1.90 ± 1.19	1.15 (0.98 – 2.06)	0.87	4.43
HT-2	221 (73.6%)	1.93 ± 1.12	1.56 (1.05 – 2.58)	0.26	6.92
T-2	92 (30.6%)	0.51 ± 0.30	0.38 (0.23 – 0.63)	0.07	4.30
OTA	99 (33%)	0.92 ± 0.50	0.76 (0.47 – 1.19)	0.21	2.86
ZEN	75 (25.0)	0.50 ± 0.31	0.40 (0.27 – 0.62)	0.16	1.52
ENNB	272 (90.6%)	0.76 ± 0.57	0.59 (0.42 – 0.90)	0.14	12.02
ENNB1	282 (94.0%)	0.73 ± 0.63	0.57 (0.39 – 0.87)	0.14	9.13
ENNA	125 (41.6%)	0.68 ± 0.48	0.53 (0.33 – 0.82)	0.11	6.63
ENNA1	186 (62.0%)	0.72 ± 0.65	0.59 (0.39 – 0.86)	0.14	6.19
BEA	288 (96.0)	0.61 ± 0.49	0.42 (0.27 – 0.78)	0.07	5.09
DON	90 (34.0%)	7.10 ± 3.51	4.97 (3.79 – 8.02)	1.58	60.47
alpha-ZEL	8 (2.7%)	1.47 ± 0.70	1.47 (1.20 – 1.74)	0.93	2.01
beta-ZEL	4 (1.3%)	1.82 ± 0.62	1.87 (1.45 – 2.23)	1.07	2.48
ZAN	22 (7.3%)	0.80 ± 0.42	0.68 (0.48 – 1.07)	0.35	1.67
DOM-1	64 (21.3%)	3.54 ± 1.53	2.22 (1.50 – 4.35)	0.61	22.74
OT-alpha	60 (20.0%)	1.27 ± 0.86	0.74 (0.50 – 1.35)	0.14	9.54

mycotoxin excretion as the outcome, and food group consumption tertiles as predictors. Specifically, high consumption (third tertile consumers) was compared with low consumption (first tertile consumers) (Fig. 3). Cereals and tubers were significant predictors of exposure to AFs, OTA, tDON, TEN, and tZEN ($\beta = 0.09 – 0.55$; $p < 0.05$). Likewise, a higher consumption of fruits, vegetables, and legumes would also lead to a higher exposure to AFs, *Alternaria* toxins, ENNs, OTA, and tZEN ($\beta = 0.03 – 0.61$; $p < 0.05$).

Some associations showed a small effect size despite being statistically significant, indicating a modest increase in AFs levels with the increased intake of fruits and vegetables. However, other mycotoxins like TEN and OTA were strongly associated with increased consumption of plant-based foods. Of note, an increased consumption of milk and cheese was associated with increased exposure to BEA and tZEN ($\beta = 0.17$; $p < 0.05$).

Sample sizes for the low- and high-adherence groups differed slightly across dietary-pattern indices. For MDS, the low- and high-adherence groups included 89 and 84 participants, respectively; for PDI, 112 and 105 participants; and for gPVG, 113 and 100 participants. Significant differences in urinary mycotoxin concentrations were observed when comparing individuals with low versus high adherence to the three dietary patterns considered: Mediterranean (MDS), Pro-vegetarian (gPVG), and Plant-Based Diet (PDI) (Fig. 4).

The concentration levels of eight mycotoxins were significantly higher ($p < 0.05$) among individuals with a high adherence to a MDS, gPVG, and PDI diet compared to those with a low adherence. When comparing within individual food groups (Supplementary Material 3), only tDON, OTA, and TEN levels were higher in individuals with low versus high consumption of cereals and tubers ($p < 0.05$). While for the consumption of legumes, fruits, and vegetables, up to 11 mycotoxins were significantly higher in the high consumption groups ($p < 0.05$) (Supplementary Material 3).

We also noticed that specific diet mycotoxins such as HT-2 and T-2 toxins were significantly higher only in the MDS adherence group. Meanwhile, the urinary concentrations of tZEN were significantly higher in the high adherence groups across all three dietary patterns. BEA levels were also significantly higher in consumers with high adherence to gPVG and PDI, but not in those with high adherence to MDS.

3.4. Estimated daily intake and risk characterization

Since there are differences in mycotoxin levels when comparing low versus high adherence to sustainable diets, we wanted to investigate whether the estimated daily intake of mycotoxins indicates potential health risks based on adherence to the diets (Figs. 5–7).

Fig. 5 presents EDIs for tDON, tZEN, and OTA for individuals with low/high adherence to a MDS, PDI, and a gPVG diet in the upper bound scenario. The mean EDIs of tDON across all three dietary patterns were between 0.13 and 0.17 μg/kg bw/day for low and high adherence groups, with the highest EDIs being 0.74 and 0.84 μg/kg bw/day. Nonetheless, even among high-adherence consumers, the highest EDIs represented no more than 84% of the TDI (HQ = 0.84), indicating that current exposure levels are within established safety margins (Fig. 6).

For tZEN, the mean EDIs were between 0.12 and 0.16 μg/kg bw/day, considering the low and high adherence groups, respectively. For some consumers with a high adherence to one of the dietary patterns from this study, the EDIs levels reached 126 – 143% of the TDI for ZEN (HQ = 1.26 – 1.43), indicating potential health concerns. Among participants classified in the low- and high-adherence groups, OTA mean EDIs were between 0.16 and 0.19 μg/kg bw/day, respectively. The maximum EDIs in these groups were 2.39 and 2.90 μg/kg bw/day, respectively. Within the same low- and high-adherence groups, the MOE for non-neoplastic effects was below 200 for all participants except two, indicating potential health risks regardless of dietary-pattern adherence (Fig. 7).

Similarly, the MOE for neoplastic effects was between 5 and 860 (< 10,000) indicating potential health risks.

4. Discussion

4.1. Analytical method performance in relation to previous urinary multi-mycotoxin methods

Method sensitivity was compared with previous urinary multi-mycotoxin biomonitoring methods. Compared with De Ruyck et al. (2020), lower or comparable LOQs were obtained for selected biomarkers such as AME, ZEN, β-ZEL, DOM-1, and OT-α, whereas higher LOQs were observed for DON, AOH, OTA, FB1/FB2, and T-2/HT-2. In comparison with the recent SALLE-based multi-analyte urine method of Marín-Sáez et al. (2024), comparable or lower LOQs were obtained for several shared mycotoxins, including DON, DOM-1, OTA, OT-α, ZEN, ZAN, α-ZEL, β-ZEL, and T-2/HT-2, while LOQs were slightly higher for BEA and some ENNs. These differences are expected given the different target panels, extraction procedures, matrix effects, and instrumental platforms.

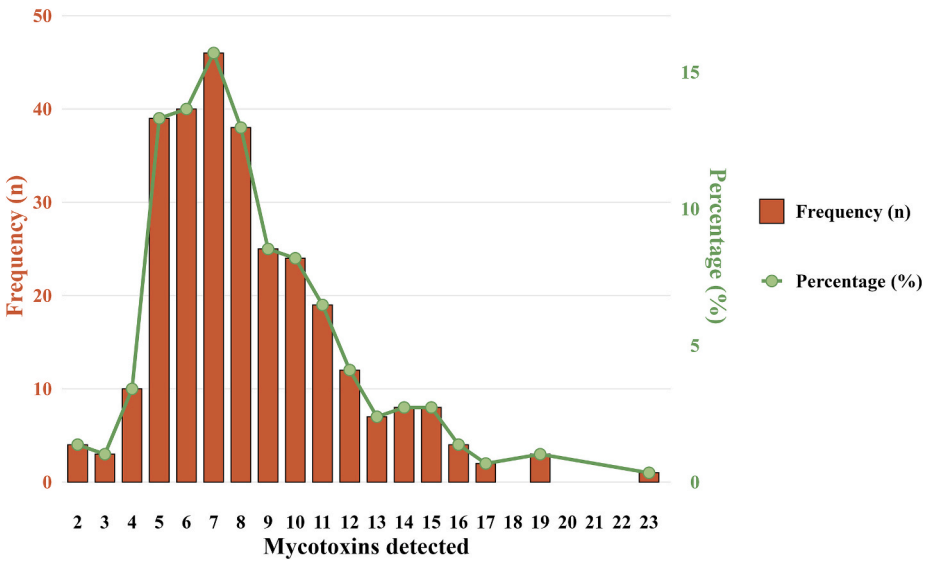

Fig. 1. Frequency and percentage of mixtures of mycotoxins in urine samples of 300 individuals.

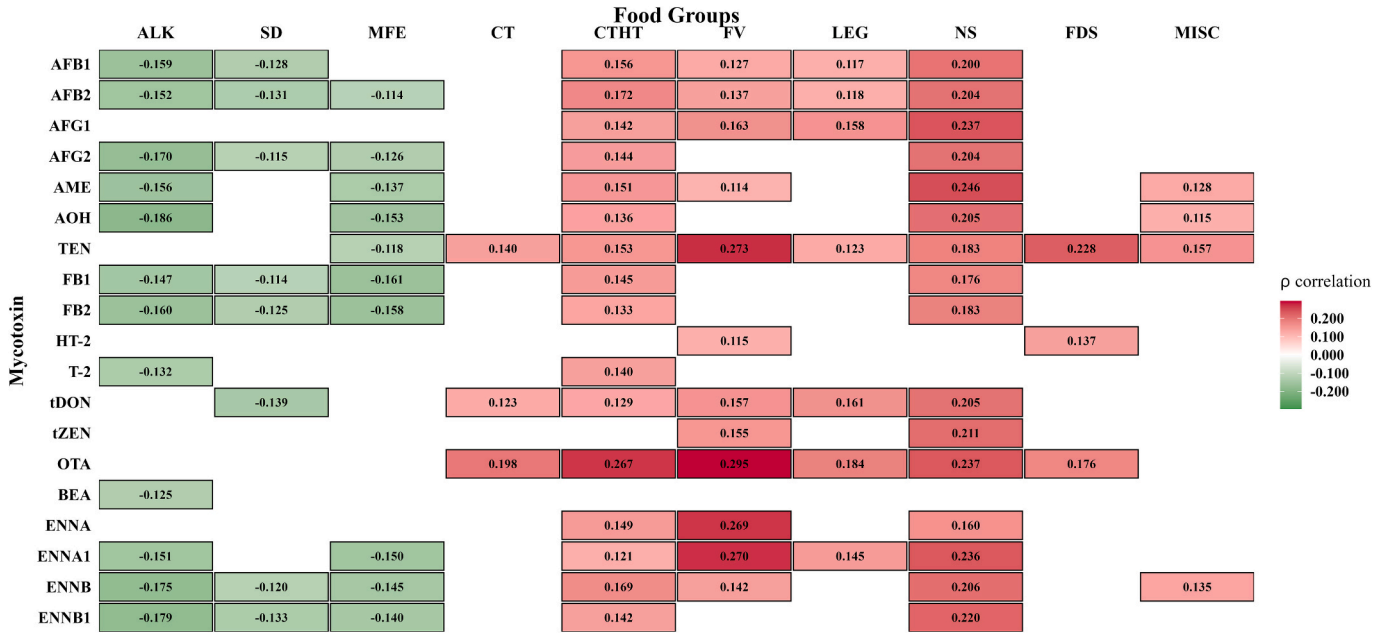

Fig. 2. Spearman's correlation between mycotoxin levels and food groups consumption; all presented values are significant at $p < 0.05$; ALK = alcoholic drinks; SD = soft drinks; MFE = meat, fish, eggs; CT = cereals and tubers; CTHT = coffee, tea, and herbal tea; FV = fruits and vegetables; LEG = legumes; NS = nuts and seeds; FDS = fats, dressings, and sauces; MISC = miscellaneous; tZEN = total ZEN; tDON = total DON.

4.2. Comparison with previous human biomonitoring studies

The present study provides human biomonitoring data on a broad panel of urinary mycotoxin biomarkers in Italian adults. Overall, the detected exposure profile was consistent with previous biomonitoring studies showing frequent exposure to DON, OTA, ZEN-related compounds, *Alternaria* toxins, and emerging Fusarium mycotoxins, although differences were observed across mycotoxin families (De Ruyck et al., 2020; Gerding et al., 2014; Gilles et al., 2021; Marín-Sáez et al. 2024). These differences may reflect geographic variability in food contamination, dietary habits, population characteristics, analytical sensitivity, and enzymatic hydrolysis among others.

Aflatoxins were detected at relatively low frequency in the present study. Charusalaipong et al. (2024) reported mean AFB1 levels of 1.16 μg/g in British children, while at the European level, urinary AFs are up to 4.7 μg/L (De Ruyck et al., 2020), which are higher than the maximum values from our study (0.68 μg/L) (Supplementary Material 2). Aflatoxin biomarker interpretation should also consider that AFM1, a validated urinary biomarker of AFB1 exposure, was not included in the analytical panel. Therefore, although AFB1 was detected in 9 participants, the present aflatoxin data should be interpreted as targeted urinary biomarker findings rather than as a comprehensive assessment of AFB1 internal exposure. Future multi-mycotoxin biomonitoring studies may benefit from including AFM1.

Alternaria toxins showed a contrasting profile, with AME being one of the most frequently detected biomarkers in the present study. However, compared with results across multiple European countries (0.02–22.4 μg/L) (De Ruyck et al., 2020), the unadjusted urinary concentrations of AOH and AME in our study were lower, ranging from 0.59 to 3.73 μg/L. This suggests that, although Alternaria-related biomarkers were highly

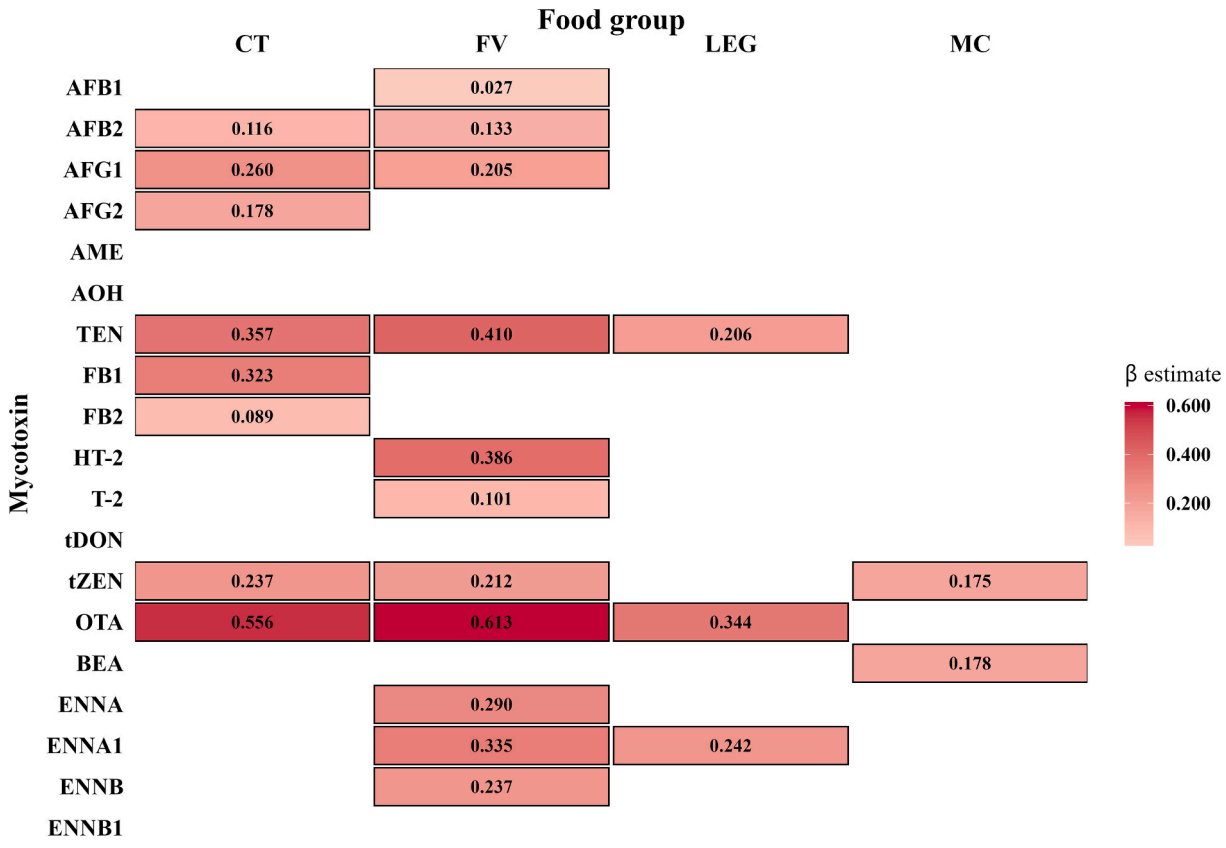

Fig. 3. Regression modelling adjusted for sex, age, and BMI showing the association between mycotoxin exposure and high consumption (tertile 3 – T3) of specific food groups using as reference tertile 1 (T1) group with low consumption; estimates represent beta coefficients; CT = cereals and tubers; FV = fruits and vegetables; LEG = legumes; MC = milk and cheese; tZEN = total ZEN; tDON = total DON.

prevalent in our population, their urinary concentration levels were generally modest compared with some previously reported European values.

Fumonisins were rarely detected in the present study, but the detected concentrations were not negligible. Previous studies indicate lower FUMs mean levels of 0.13 – 0.16 μg/g creatinine in Sweden (Wallin et al., 2015), while data from previous biomonitoring projects revealed mean values that ranged widely between 0.005 (Germany), 0.06 (Italy), and 2.5 μg/L (Portugal) (De Santis et al., 2021). These results indicate a lower exposure compared to our mean unadjusted FUMs values of 1.95 – 4.10 μg/L.

For type A trichothecenes, HT-2 was more frequently detected than T-2 in the present study, but mean concentrations were lower than those reported in Spain and the UK. In Spain and the UK, mean urinary concentrations were 10 times higher at 14.3 μg/g (Rodríguez-Carrasco et al., 2014) and 15.48 μg/g (Charusalaipong et al., 2024), suggesting a higher exposure in those populations compared to our participants.

OTA and OT-α were detected in approximately one-third and one-fifth of participants, respectively. Lower mean levels have been reported in Belgium (0.03 μg/g) (Heyndrickx et al., 2015) and for British children (0.09 to 0.15 μg/g) (Charusalaipong et al., 2024; Gratz et al., 2020), while in Sweden and Spain OTA urinary levels were similar to ours (Gallardo-Ramos et al., 2024; Wallin et al., 2015).

ZEN and its related metabolites were detected less frequently than *Alternaria* toxins and ENNs/BEA. Current research indicates modest mean levels of ZEN and its metabolites in Sweden, ranging from 0.13 to 0.19 μg/g (Wallin et al., 2015), and in Spain, at 1.10 μg/g creatinine (Gallardo-Ramos et al., 2024).

ENNs and BEA were among the most frequently detected biomarkers in the present study, although mean concentrations were relatively low. These concentrations were lower than those reported in Spain, where ENNB median levels were 2.42 μg/g (IQR = 0.007 – 3.97) (Gallardo-Ramos et al., 2024). ENNB1 values in Europe range from 0.84 to 17.9 μg/L (De Ruyck et al., 2020), which are higher than our findings (0.67 – 5.32 μg/L), indicating variability in occurrence based on country.

DON and DOM-1 were detected in 34.0% and 21.3% of participants, respectively. The current total DON mean values in Europe range between 3.08 and 12.15 μg/g creatinine, with maximum values of 21.58 – 189.90 μg/g creatinine (Namorado et al., 2024). Previous Italian studies found mean DON values of up to 12.24 and 16.10 μg/g creatinine for vegetarians (De Santis et al., 2019). DOM-1 was reported in 9% of UK children at 0.21 μg/g creatinine (Gratz et al., 2020) as well as in Spanish adults (96%; 8.9 μg/g creatinine) (Vidal et al., 2016) and Portuguese adults (41%; 7.81 μg/g creatinine) (Martins et al., 2019). It should be taken into consideration that DOM-1 is a minor de-epoxidation product of DON formed by the intestinal microbiota primarily in the colon (Sun et al., 2022). Because the composition of the gut microbiota differs among individuals, DOM-1 conversion may vary widely among populations.

4.3. *Multi-mycotoxin co-exposure and mixture interpretation*

As the co-occurrence of mycotoxins has been widely reported in foods (Karsauliya et al., 2022; Smith et al., 2016), it is not surprising that the participants in this study were exposed to different mycotoxin mixtures. The family-level analysis showed that co-exposure occurred mainly across mycotoxin families rather than within a single family, with frequent combined detection of biomarkers related to *Alternaria* toxins, ENNs/BEA, and trichothecenes.

However, it is important to note that the presence of multiple mycotoxins in urine may reflect not only co-exposure, but also differences in absorption, distribution, metabolism, and excretion kinetics. Given

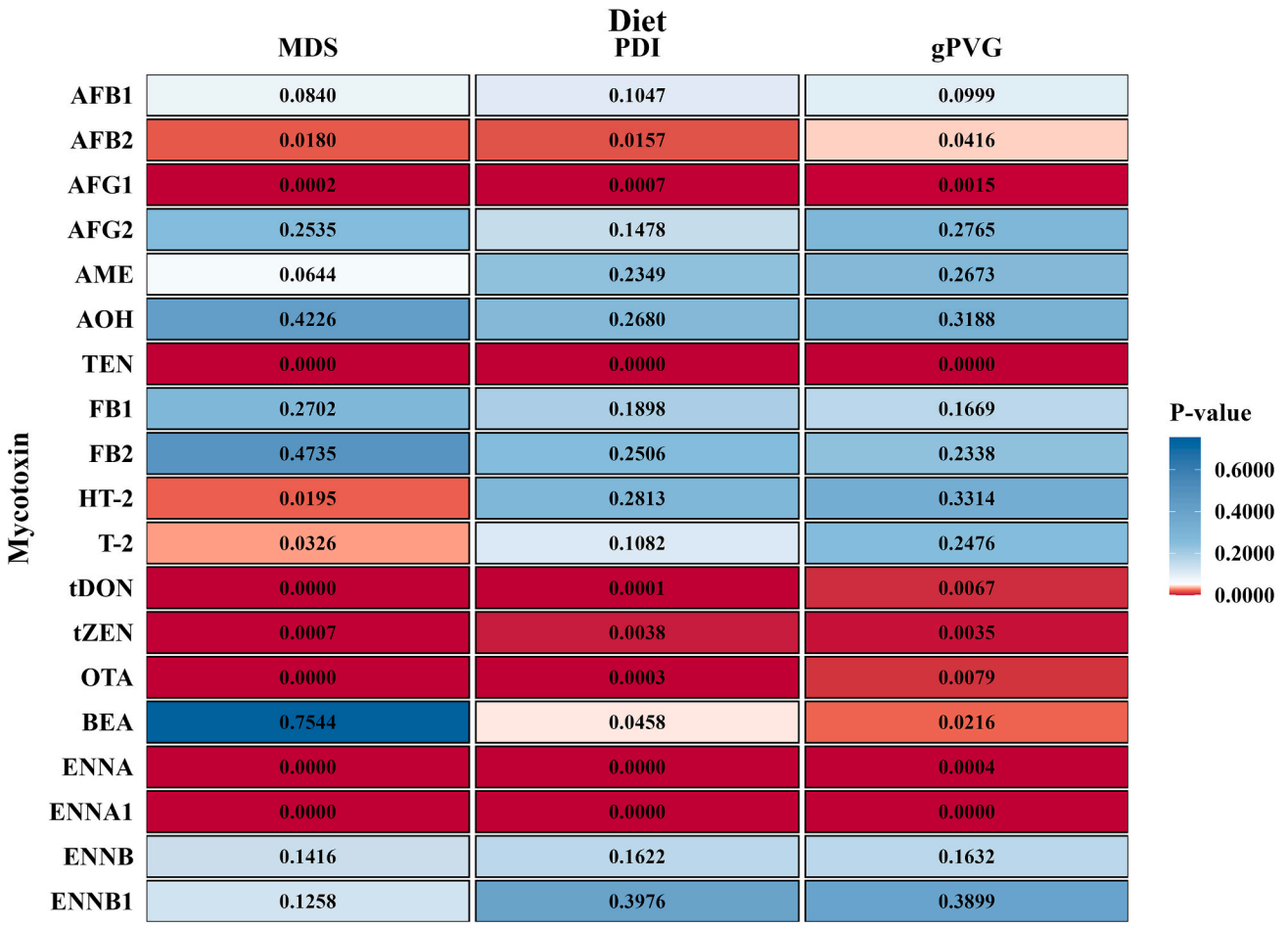

Fig. 4. Mann–Whitney comparison for low (T1) versus high (T3) adherence to a Mediterranean Diet (MDS), Plant-based diet (PDI), and Pro-vegetarian diet (gPVG); red = significant differences $p < 0.05$; blue = non-significant differences $p > 0.05$; tDON = total DON; tZEN = total ZEN. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

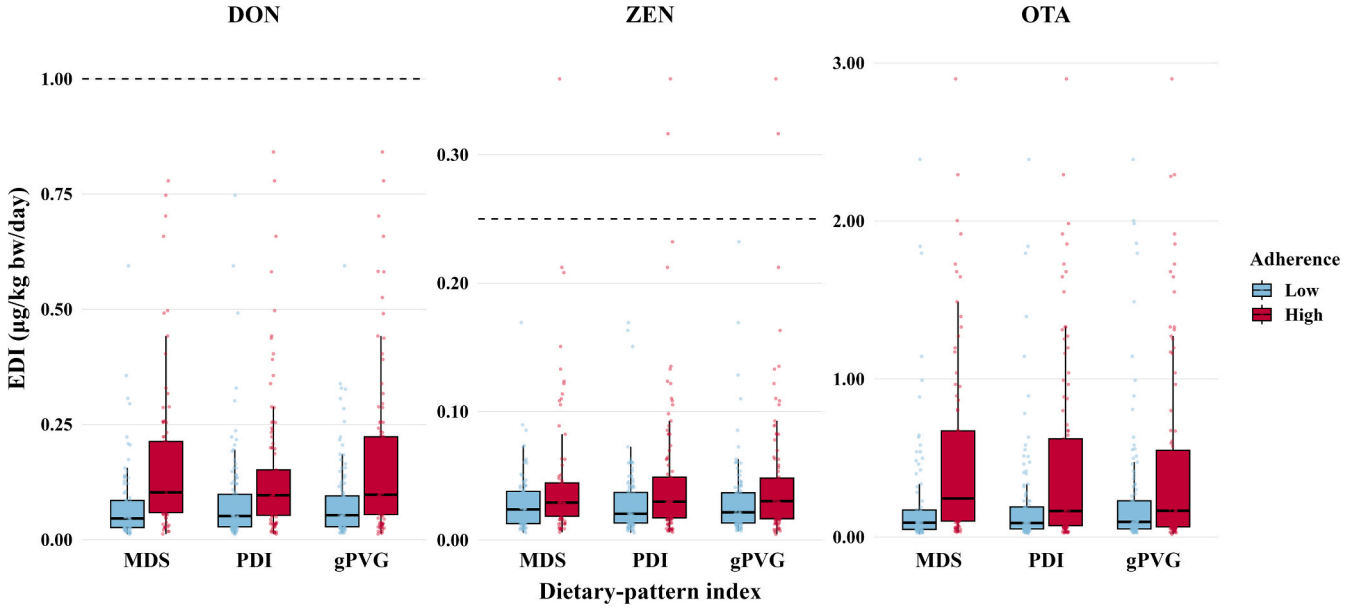

Fig. 5. EDI of mycotoxins for consumers with a low/high adherence to a Mediterranean Diet (MDS), Plant-based diet (PDI), and Pro-vegetarian diet (gPVG) in the upper bound scenario.

their varying half-lives, co-detection in urine may occur even when mycotoxins are ingested at different times, making it a cumulative

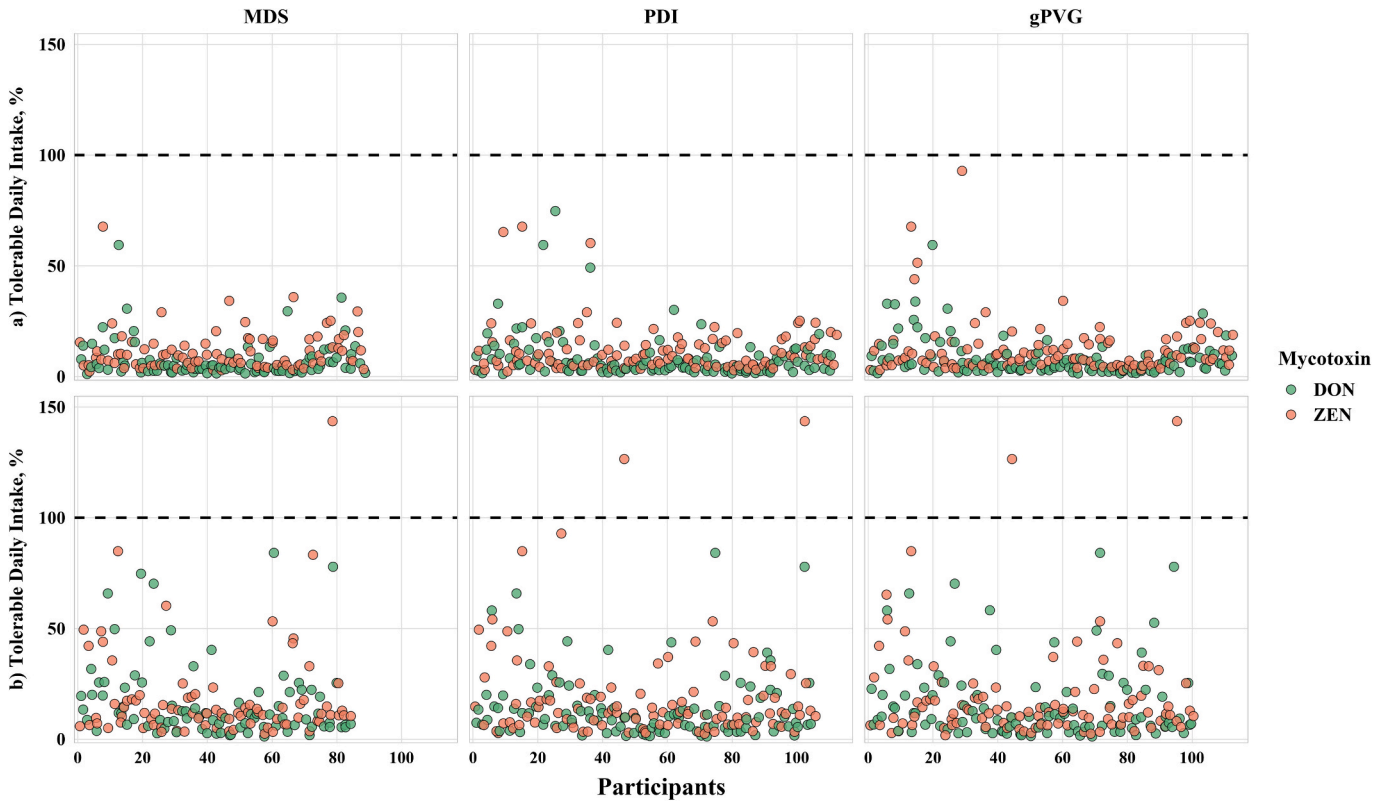

Fig. 6. Percentage of the tolerable daily intake (TDI) for DON and ZEN considering only a) low adherence and b) high adherence to a MDS, PDI, and gPVG diet in the upper bound scenario.

marker of recent exposure (Cramer et al., 2025).

Our findings highlight consumers' exposure to both regulated and emerging mycotoxins with potential health concerns related to additive or synergistic toxic effects (Alassane-Kpembi et al., 2017; Vejdovszky et al., 2017; Wang et al., 2020). Given the complexity of the mixtures, risk assessment strategies should increasingly focus on cumulative exposure rather than single-compound evaluations.

4.4. Dietary contributors to urinary mycotoxin biomarkers

The associations observed between urinary mycotoxin biomarkers and food group consumption support the role of plant-based foods as potential contributors to mycotoxin exposure. Coffee and tea were associated only with selected biomarkers, namely TEN, tDON, and OTA, while nuts and seeds were more broadly associated with AFs, *Alternaria* toxins, FUMs, tDON, tZEN, OTA, and ENNs. These food groups are relevant contributors to mycotoxin exposure in our population, aligning partially with findings from Sweden and Germany, where urinary DON and serum ENNB and OTA were associated with coffee, peanuts, and cereal intake (Warensjö Lemming et al., 2020; Penczynski et al., 2022).

Fruits, vegetables, and legumes were also weakly correlated with AFs, ENNs, *Alternaria* toxins, tZEN, tDON, and OTA, supporting previous findings from Spain and Germany (Gallardo-Ramos et al., 2024; Penczynski et al., 2022).

The associations observed for cereals and tubers are in line with multiple studies from the UK, Germany, and Sweden, where DON, OTA, and HT-2/T-2 were significantly correlated with cereal intake (Charusalaipong et al., 2024; Penczynski et al., 2022; Warensjö Lemming et al., 2020). However, other reports from West Africa, South Asia, and Italy indicate no association between OTA, aflatoxin biomarkers, and intake of cereals (wheat, maize, rice), legumes, or peanuts (Ali et al., 2017; Das Trisha et al., 2024; Ferri et al., 2017). These discrepancies likely reflect differences in dietary assessment methods, food contamination profiles, analytical approaches, and population-specific consumption patterns.

The weak inverse associations observed for meat, fish, eggs, and soft drinks might also reflect a dietary substitution effect, where higher energy intake from these categories coincides with lower consumption of mycotoxin-prone plant-based foods. This does not imply that such foods are protective but rather highlights how dietary patterns can influence exposure indirectly.

Regression analyses further supported the role of plant-based foods such as cereals, nuts, legumes, fruits, and vegetables as potential dietary contributors to mycotoxin exposure, in agreement with previous studies (Gallardo-Ramos et al., 2024; Penczynski et al., 2022). The association between milk and cheese and increased exposure to BEA and tZEN may reflect indirect dietary pattern effects or possible contamination routes, aligning with recent occurrence studies in dairy products (Ghanati et al., 2024; Rodríguez-Cañás et al., 2023).

The comparison of low and high adherence to MDS, gPVG, and PDI indicated that several mycotoxins were higher among participants with greater adherence to healthy and sustainable dietary patterns. This overlap indicates a potential common dietary exposure profile across all three diets due to common food groups such as cereals, legumes, nuts, fruits, and vegetables (Cardamone et al., 2024; Oncina-Cánovas et al., 2022; Satija et al., 2016).

HT-2 and T-2 toxins were significantly higher only in the MDS high-adherence group. This is expected since trichothecenes are associated with cereals (Mousavi Khaneghah et al., 2019), which are a major food group of the Mediterranean Diet (Cardamone et al., 2024). Meanwhile, tZEN concentrations were significantly higher in the high-adherence groups across all three dietary patterns. ZEN and its metabolites are known to occur in cereals (Mousavi Khaneghah et al., 2019) and recent studies indicate their presence in legume-based foods as well (Mihalache et al., 2024). BEA levels were also significantly higher in consumers with a high adherence to pro-vegetarian and plant-based diets, indicating a

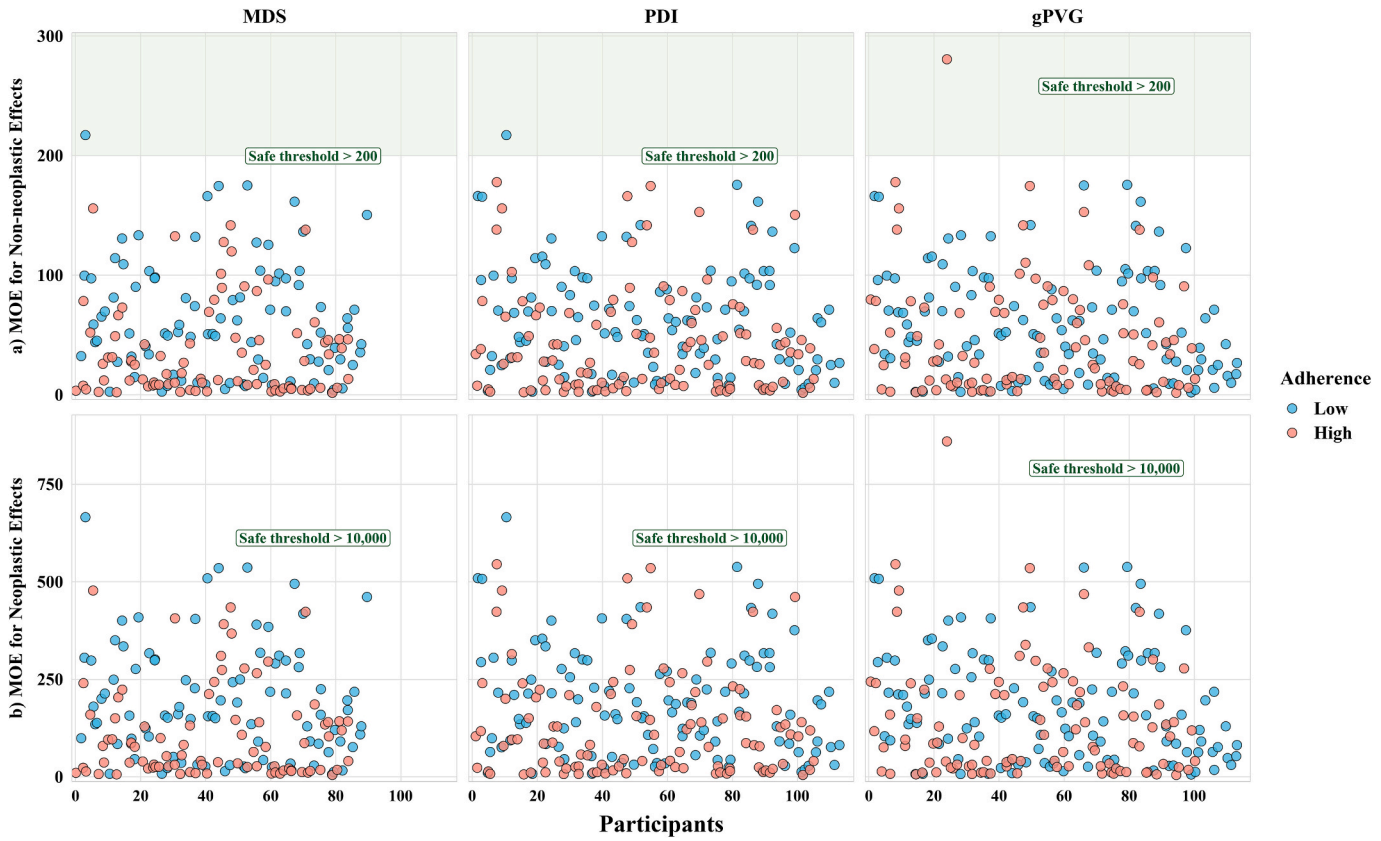

Fig. 7. Risk characterization for OTA considering only low adherence (blue) and high adherence (red) to a MDS, PDI, and gPVG diet in the upper bound scenario for a) non-neoplastic effects – safe threshold > 200, b) neoplastic effects – safe threshold > 10,000. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

source of exposure more closely linked to these dietary patterns than to the Mediterranean diet.

Our findings reinforce the role of plant-based foods such as cereals, nuts, legumes, fruits, and vegetables as important sources of mycotoxin exposure. Together, these results indicate that higher adherence to healthy and sustainable dietary patterns may be associated with increased exposure to some mycotoxins, emphasizing the need for food safety authorities and risk managers to strengthen monitoring and mitigation strategies while preserving the well-established nutritional and environmental benefits of these diets.

4.5. Intake reconstruction and risk characterization

For DON, the EDIs from the present study remained below the TDI in both low and high adherence groups. Previous Italian biomonitoring studies reported similar DON EDI of 0.29 – 1.17 μg/kg bw/day with 6 – 7% participants above the TDI (De Santis et al., 2019; Solfrizzo et al., 2014). While in Spain and Germany DON was below the TDI (Carballo et al., 2021; Penczynski et al., 2022), other studies across Europe reported DON urinary exposure levels above the TDI for 1 – 33% of participants (Martins et al., 2019; Mitropoulou et al., 2018; Warth et al., 2012). Overall, at the European level, biomonitoring data indicates that chronic dietary exposure to DON may represent a risk for about 12% of the population (EFSA, 2017; Namorado et al., 2024).

For tZEN, the mean EDI was higher in the high-adherence groups, and some individuals approached or exceeded the TDI. In Spain, total ZEN exposure was between 3.66 – 4.46 μg/kg bw/day, reaching 1784% of the TDI (Carballo et al., 2021), while in the UK, ZEN EDI for children was between 0.02 – 0.70 μg/kg bw/day (278% of the TDI) (Charusalaipong et al., 2024; Gratz et al., 2020). These findings indicate that ZEN-related exposure may be relevant for specific subgroups and dietary patterns.

For OTA, the MOE values observed in the present study indicated potential health concern for both non-neoplastic and neoplastic endpoints, irrespective of dietary-pattern adherence. This is in line with previous Italian results where the mean OTA EDI was 0.14 μg/kg bw/day with maximum levels of 2.04 μg/kg bw/day (Solfrizzo et al., 2014). However, lower exposure levels were reported in Spain, the UK, and Belgium (Carballo et al., 2021; Charusalaipong et al., 2024; Gratz et al., 2020; Heyndrickx et al., 2015). At the European level, the mean OTA exposure is lower than estimated in our study, ranging from 0.0006 to 0.0017 μg/kg bw/day (EFSA, 2020).

4.6. Food safety implications for dietary transitions

Currently, there is an increased adoption of plant-based diets related to the rising trends of flexitarianism, vegetarianism, and veganism (Venter de Villiers et al., 2024; Zhu et al., 2025). The transition is centered around health and environmental concerns (Cramer et al., 2017; Willett et al., 2019). Sustainable diets focus on the primary consumption of foods derived from plants such as whole grains, fruits, vegetables, legumes, and nuts (FAO & WHO, 2019). However, some key food groups of the alternative patterns may include items with a high contribution to exposure to mycotoxins, which can have potential health implications if not properly monitored.

Food safety implications for food derived from plants depend on the pre/post-harvest conditions, handling of products post-processing and at the retail level, and the implementation of appropriate food safety management actions (FAO, 2022). Plant-based products can be unprocessed or minimally processed staples such as cereals, legumes, and nuts, which are known to be susceptible to mycotoxin contamination and are well monitored, and more complex foods such as processed plant-based

meat alternatives (i.e., plant-based burgers). The latter often contain multiple ingredients from diverse sources, potentially increasing the range of mycotoxin exposure if not carefully controlled (FAO, 2022).

Mycotoxins can be present in raw ingredients such as cereals (wheat, oats), nuts (peanuts, almonds), and legumes (soy, chickpeas), and may get carried over to the end products (FAO, 2022). The food safety aspects of plant-based food differ from those of animal-based food; therefore, any dietary shifts require careful consideration for food safety management actions. While the EU regulatory framework for mycotoxins is well-established for individual crops such as cereals and nuts, there is currently a lack of specific regulations addressing formulated plant-based products, including meat and milk analogues (Mihalache, 2024). As the consumption of these alternative protein sources increases, updated regulatory frameworks are needed to ensure safety and minimize mycotoxin dietary exposure.

To support genuine, healthier, and more sustainable diets, policy-makers should adapt the existing regulatory framework to new dietary patterns and products. Further studies on chemical exposure based on dietary trends and monitoring contaminants in plant-based foods are required. While the current dietary transitions represent an opportunity to reduce environmental impact and improve human health, they can also disrupt agri-food systems by increasing exposure to natural toxins, with significant public health and regulatory implications. Even though our study does not include a comparison to non-plant-based dietary patterns, the detection of multiple mycotoxins across diverse consumption patterns of plant-based foods highlights the importance of adapting regulatory frameworks and monitoring strategies to keep pace with dietary transitions.

5. Conclusions

This Italian biomonitoring study provides important insights that can contribute to the development of national and European programs on mycotoxins to support consumer protection from the expected increased exposure due to modern dietary transitions.

The most prevalent mycotoxins were ENNs, BEA, and *Alternaria* toxins, followed by trichothecenes type A and B, OTA, and ZEN. Mean creatinine-adjusted urinary concentrations were as low as 0.31 μg/g creatinine AFB1 and as high as 7.10 μg/g creatinine DON, with mixtures of at least two and up to twenty-three mycotoxins per individual. Our findings revealed a higher exposure to mycotoxins among consumers classified with a high adherence to a sustainable diet and which had an increased consumption of plant-based foods. Mean estimated daily intakes for DON were within safe levels, while some individuals with a high adherence approached and exceeded the TDI for ZEN. Exposure to OTA was associated with potential health risks, regardless of dietary adherence. This indicates the need for continued monitoring and a higher priority for risk management measures.

As dietary patterns shift toward more plant-based and sustainable models, it is essential that food safety monitoring evolves accordingly. Aligning food safety regulations and risk assessments with current consumption trends is essential, especially given the widespread association of mycotoxins with emerging dietary trends. In particular, EU mycotoxin monitoring priorities, maximum-level frameworks, and dietary exposure/risk assessments should be periodically updated to better reflect current consumption patterns, including the increasing intake of cereals, legumes, nuts, oilseeds, and composite plant-based products. Updated regulatory frameworks will ensure a genuine, safe transition toward sustainable and health-promoting diets, supporting public health and future food policies.

CRediT authorship contribution statement

Octavian Augustin Mihalache: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Conceptualization. **Cristiana Mignogna:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Raquel Torrijos:** Writing – review & editing, Methodology, Investigation, Formal analysis. **José F. Rinaldi de Alvarenga:** Writing – review & editing, Investigation, Formal analysis. **Elisa Monica:** Writing – review & editing, Investigation. **Maria Sole Morandini:** Writing – review & editing, Investigation. **Cristina Del Burgo-Gutiérrez:** Writing – review & editing, Investigation. **Marialaura Bonaccio:** Writing – review & editing, Investigation. **Emilia Ruggiero:** Writing – review & editing, Investigation. **Daniele Del Rio:** Writing – review & editing, Supervision, Investigation. **Alice Rosi:** Writing – review & editing, Investigation. **Pedro Mena:** Writing – review & editing, Supervision, Resources, Investigation. **Chiara Dall'Asta:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary material containing UHPLC–MS/MS analytical parameters, spiking levels used for method validation, descriptive statistics of urinary mycotoxin concentrations, additional dietary association analyses, and family-level urinary mycotoxin co-detection patterns. Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110372.

Data availability

Data will be made available on request.

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