



Urinary multi-mycotoxin exposure in the Portuguese population and associated sociodemographic determinants[☆]

Elias Maris^{a,b,c,1}, Sónia Namorado^{d,e,1,*}, An Chen^a, Roger Pero-Gascon^{a,f},
Marthe De Boevre^a, Sarah De Saeger^{a,g}, Maria João Silva^{e,h}, Paula Alvito^{i,j}

^a Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium

^b Laboratory of Molecular Bacteriology, Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium

^c Laboratory of Bioinformatics and (Eco-)Systems Biology, VIB Center for Microbiology, VIB, Leuven, Belgium

^d Department of Epidemiology, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

^e NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal

^f Department of Chemical Engineering and Analytical Chemistry, Institute for Research on Nutrition and Food Safety (INSA-UB), University of Barcelona, Spain

^g Department of Biotechnology and Food Technology, Faculty of Sciences, University of Johannesburg, South Africa

^h Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

ⁱ Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

^j Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal

ARTICLE INFO

Keywords:

Mycotoxins
Human biomonitoring
Chemical exposure
Co-exposure
Portugal
Sociodemographic characteristics
Free-deoxynivalenol
Tenuazonic acid
Urine
Exposome

ABSTRACT

European human biomonitoring (HBM) studies have increasingly adopted multi-mycotoxin approaches to capture complex exposure scenarios encountered in real-life diets. This nation-wide study provides the characterization, for the first time, of the mycotoxin profile of 295 urine samples collected from the Portuguese population in the continent and islands (archipelagos of Madeira and Azores), using an optimized and validated LC-MS/MS method for the characterization of 39 mycotoxins/metabolites. Nearly all study participants (99.3%) were exposed to at least one mycotoxin, with around half (54.2%) being co-exposed to only 2 detectable mycotoxins. Free-deoxynivalenol (free-DON, 84.7%) and tenuazonic acid (TeA, 95.6%) were the most prevalent mycotoxins. Citrinin (CIT, 11.5%), α -zearalenone (α -ZEL, 10.5%) and ochratoxin A (OTA, 9.5%) showed detection rates near 10%. Median concentrations (P50) for the two predominant mycotoxins were 0.79 μ g/L (0.72 μ g/g creatinine) for free-DON and 2.45 μ g/L (2.08 μ g/g creatinine) for TeA, with maximum values of 11.77 μ g/L (10.06 μ g/g creatinine) and 90.91 μ g/L (114.15 μ g/g creatinine), respectively. Sociodemographic patterns showed lower exposure in the Centre region and higher free-DON exposure in southern Portugal and the archipelagos. TeA exposure differed significantly by region and was lowest in the Lisbon Metropolitan Area and Algarve compared to the Centre reference region. Low educational levels and spring sampling correlated with higher exposure for both toxins, but only education levels showed statistical significance in the linear regression analyses. For both TeA and free-DON, no differences in exposure were observed by sex, occupational status, or degree of urbanization. Overall, these findings underscore the importance of continuous surveillance, particularly under the current climate change scenario, and highlight the relevance of integrating human biomonitoring into national food safety and public health strategies.

[☆] This article is part of a Special issue entitled: 'ICFC2025' published in Food Research International.

^{*} Corresponding author at: Department of Epidemiology, National Institute of Health Doutor Ricardo Jorge, Avenida Padre Curz, 1649-016 Lisbon, Portugal.

E-mail addresses: Elias.Maris@UGent.be (E. Maris), sonia.namorado@insa.min-saude.pt (S. Namorado), An.Chen@UGent.be (A. Chen), Roger.PeroGascon@UGent.be, rpero@ub.edu (R. Pero-Gascon), Marthe.DeBoevre@UGent.be (M. De Boevre), Sarah.DeSaeger@UGent.be (S. De Saeger), m.joao.silva@insa.min-saude.pt (M.J. Silva), Paula.Alvito@insa.min-saude.pt (P. Alvito).

¹ These authors contributed equally to the elaboration of this article as first authors.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by diverse fungal species that commonly contaminate food and feed products worldwide. Chronic dietary exposure to mycotoxins represents a significant public health concern due to its association with multiple adverse health outcomes, including immunosuppression, carcinogenicity, nephrotoxicity, hepatotoxicity, and endocrine disruption (Bennett & Klich, 2003; International Agency for Research on Cancer (IARC), 2012). Although regulatory authorities in Europe have established maximum levels for several mycotoxins in food to mitigate human exposure (European Commission, 2023, 2024), emerging evidence demonstrates that long-term, low-level exposure may pose substantial health risks, particularly when multiple mycotoxins co-occur in dietary matrices (De Ruyck et al., 2020; Alvito, Barcelo, De Meester, Rito and Suman, 2021; Karsauliya et al., 2022). Moreover, beyond these regulated compounds, non-regulated mycotoxins, including modified and emerging forms, are increasingly detected in diverse foodstuffs, representing an additional exposure pathway not currently captured by existing regulatory frameworks (Gruber-Dorninger et al., 2017; Nešić et al., 2023).

Human biomonitoring (HBM), which refers to the direct measurement of substances and/or their metabolites in biological specimens, provides an accurate assessment of internal mycotoxin exposure under real-life conditions (Heyndrickx et al., 2015; Martins et al., 2019; Vidal, Mengelers, et al., 2018). Advances over the past decade in sensitive and reliable analytical techniques, predominantly liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry (HRMS), have expanded HBM applications for detecting a broad spectrum of mycotoxin biomarkers in biological matrices (Arce-López et al., 2020; Delgado-Povedano et al., 2025). Urine has been the matrix of choice to measure mycotoxins in most HBM studies due to its non-invasive collection, ease of handling, and role as a principal excretion route (Gallardo-Ramos et al., 2024; Peris-Camarasa, Pardo, et al., 2025). Validated urinary biomarkers currently exist for several of the most relevant mycotoxins, including aflatoxins (AFM₁, aflatoxin-N₇-guanine), deoxynivalenol (DON and its glucuronides), ochratoxin A (OTA), fumonisins (FB₁), zearalenone (ZEN and its metabolites α -ZEL and β -ZEL), citrinin (CIT), T-2 and HT-2 toxins, and alternariol (AOH) (Vidal, Mengelers, et al., 2018). These biomarkers have been used to estimate exposure levels in various European populations and, in some cases, to compare these estimates with established health-based guidance values such as tolerable daily intakes (TDIs) (Alvito et al., 2022; Namorado et al., 2024). For example, EFSA reported that upper bound chronic dietary exposure of the sum of free-DON and its modified forms 3-Ac-DON, 15-Ac-DON and DON-3-glucoside, calculated from available occurrence data in food and feed, exceeded the group TDI of 1 $\mu\text{g}/\text{kg}$ bw per day for the infants, toddlers and other children and, in some cases, also for adolescents and adults (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017). Under the European Human Biomonitoring Initiative (HBM4EU), a Human Biomonitoring Guidance Value (HBM-GV) of 23 $\mu\text{g}/\text{L}$ was established for total-DON in 24 h urine (Alvito et al., 2022; Mengelers et al., 2024).

Recent European studies have increasingly adopted a multi-mycotoxin approach to better capture the complex exposure scenarios encountered in real-life diets. Several biomonitoring campaigns conducted over the past ten years have measured multiple mycotoxins in urine samples, revealing widespread exposure across populations, often with co-exposure to multiple compounds (De Ruyck et al., 2020; Heyndrickx et al., 2015; Peris-Camarasa, Coscollà, et al., 2025). In Portugal, emerging research has begun to characterize the occurrence of mycotoxin co-exposures in adults through human biomonitoring. One of the first comprehensive studies addressing multiple mycotoxins in the Portuguese population analysed 37 mycotoxin biomarkers in urine samples from 94 adult participants (mean age 48.4 ± 15.2 years) recruited as part of the National Food, Nutrition and Physical Activity

Survey (IAN-AF, 2015–2016) (Martins et al., 2019). In this study, exposure to six different mycotoxins - DON, ZEN, AOH, OTA, FB₁, and CIT - was confirmed through the detection and quantification of 12 urinary biomarkers of exposure, including both parent compounds and metabolites, in paired 24-h and first-morning urine samples (Martins et al., 2019). Notably, AOH was identified for the first time in urine samples from a European population. Associations between urinary mycotoxin biomarkers and consumption of specific food items were also observed (Martins et al., 2018; Martins, Assunção, et al., 2021). DON and its metabolites (DOM-1, DON-15-GlcA, and DON-3-GlcA) were among the most frequently detected biomarkers (Martins, Torres, et al., 2021).

Concerning general exposure to DON in the European population, urinary total-DON levels were measured in 1270 participants from six countries, including Portugal, as part of HBM4EU (Namorado et al., 2024). The HBM4EU Aligned Studies (Gilles et al., 2022; Govarts et al., 2023) employed post-harmonized questionnaire data and validated analytical methodologies, covering countries representing four geographical regions of Europe, thereby providing much-needed insights into the internal exposure of the European adult population to DON (Alvito et al., 2022; Namorado et al., 2024). This study aimed to characterize internal exposure levels in the adult European population and identify key determinants of exposure. The median (P50) and 95th percentile (P95) total-DON concentrations ranged between 0.41–10.16 $\mu\text{g}/\text{L}$ (0.39–9.05 $\mu\text{g}/\text{g}$ creatinine) and 3.25–46.58 $\mu\text{g}/\text{L}$ (2.12–33.50 $\mu\text{g}/\text{g}$ creatinine). Higher total-DON levels were observed among (i) male participants from France and Germany, (ii) samples collected during spring and summer, (iii) individuals with lower educational attainment, (iv) residents in rural areas, (v) unemployed individuals in France, Luxembourg and Portugal, and (vi) individuals with higher consumption of cereals and bread. Overall, 12.3% of the participants exceeded the HBM-GV of 23 $\mu\text{g}/\text{L}$ (Alvito et al., 2022; Mengelers et al., 2024), with country-specific exceedance rates ranging from 0.8% to 20.7%. Among the predictor variables considered, a lower educational level, residence in rural areas and higher consumption of cereals and bread were related to a higher exposure to total-DON.

Despite recent advances in exposure assessment, substantial knowledge gaps remain regarding internal exposure to a broad spectrum of mycotoxins, encompassing both regulated compounds such as DON and emerging mycotoxins such as tenuazonic acid (TeA), as well as the primary determinants driving population-level exposure variation (Visintin et al., 2023). Furthermore, analytical capacity remains a limiting factor. Improved and more sensitive methods are essential to expand the range of detectable mycotoxins and achieve lower limits of quantification, thereby enabling comprehensive population-level exposure mapping. The present study aimed to characterize, for the first time, the mycotoxin exposure profile of a national sample of Portuguese adults in early mid-life. Urine specimens were collected across all Portuguese regions, including the autonomous archipelagos of Madeira and the Azores, as part of the INSEF-ExpoQuim survey (2019–2020) (Gilles et al., 2022; Govarts et al., 2023), a cross-sectional study embedded within the Portuguese National Health Examination Survey (INSEF) (Nunes et al., 2019). Samples were analysed using an optimized and validated LC-MS/MS method capable of detecting 39 mycotoxins and/or their metabolites in urine. Furthermore, determinants of exposure, including sociodemographic characteristics and geographical location, were investigated to elucidate exposure pathways for chronic mycotoxin exposure.

2. Materials and methods

2.1. Study population and design

The present study used data and samples from the INSEF-ExpoQuim survey (Gilles et al., 2022; Govarts et al., 2023). For the INSEF-ExpoQuim survey, 863 potentially eligible INSEF participants (Nunes

et al., 2019) were selected and invited to participate. Inclusion criteria were: reside in Portugal in the 3 years prior to participation in the study, not institutionalized, aged between 28 and 39 years (in accordance with the HBM4EU framework that selected as target population young adults aged 25–39 years to ensure a relatively homogeneous group and comparability with age groups from other studies), with sufficient knowledge of Portuguese to be able to respond to the interview and having consented to be contacted for participation in future studies. The fieldwork was carried out between May 2019 and March 2020. The selected individuals received an invitation letter and were subsequently contacted by telephone to receive information on the study, schedule informed consent and sample collection, and schedule a telephone interview. First-morning urine samples were collected along with questionnaire data from 295 participants. Data were collected through computer-assisted telephone interviews using REDCap - Research Electronic Data Capture software. The study INSEF-ExpoQuim was approved by the Data Protection Officer and by the Ethics Committee for Health of the National Institute of Health Doutor Ricardo Jorge and by the Ethics Committees of the healthcare institutions involved in sample and data collection.

2.2. Data and samples

Data collected through questionnaires included information on sociodemographic characteristics, living conditions and residential history, habits/lifestyle, nutrition, health, occupation and specific information covering almost all exposure routes. First-morning urine ($N = 295$) was collected in a transparent container previously washed with a 10% nitric acid solution according to the HBM4EU guidelines and stored at $-20\text{ }^{\circ}\text{C}$ (Esteban López, 2018). Samples were thawed, shaken and homogenized before being aliquoted (500 μL) and stored at $-80\text{ }^{\circ}\text{C}$. The aliquots to be analysed were transported to the Centre of Excellence in Mycotoxicology and Public Health (CEMPH) laboratory at Ghent University frozen under dry ice before further multiple mycotoxin analysis and creatinine determination.

2.3. Chemicals and reagents

Mycotoxin standards 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), aflatoxin M₁ (AFM₁), beauvericin (BEAU), citrinin (CIT), cyclopiazonic acid (CPA), diacetoxyscirpenol (DAS), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3G), deepoxy-deoxynivalenol (DOM), enniatin A (ENNA), enniatin A₁ (ENNA₁), enniatin B (ENNB), enniatin B₁ (ENNB₁), fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), fumonisin B₃ (FB₃), fusarenon-X (FUS-X), fully hydrolyzed-fumonisin B₁ (HFB₁), neosolaniol (NEO), ochratoxin- α (OT α), ochratoxin A (OTA), roquefortin C (ROQ-C), sterigmatocystin (STERIG), T-2 toxin (T-2), zearalanone (ZAN), zearalenone (ZEN), ¹³C₁₇-Aflatoxin B₁ (¹³C₁₇-AFB₁), ¹³C₁₃-Citrinin (¹³C₁₃-CIT), ¹³C₁₅-Deoxynivalenol (¹³C₁₅-DON), ¹³C₃₄-Fumonisin B₁ (¹³C₃₄-FB₁), ¹³C₂₂-HT-2 toxin (¹³C₂₂-HT-2), and ¹³C₂₄-T-2 toxin (¹³C₂₄-T-2) were purchased from Fermentek (Jerusalem, Israel). Aflatoxin G₂ (AFG₂), alternariol (AOH), alternariol monomethyl ether (AME), HT-2 toxin (HT-2), nivalenol (NIV), tenuazonic acid (TeA), α -zearalanol (α -ZAL), β -zearalanol (β -ZAL), α -zearalenol (α -ZEL), β -zearalenol (β -ZEL), ¹³C₁₀-tenuazonic acid (¹³C₁₀-TeA), were purchased from Sigma Aldrich (St. Louis, MO, USA). To correct for sample preparation and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis variability, the ¹³C-labelled standards were fully labelled and used as internal standards when analysing urine samples.

Standards were combined into a mixture with varying concentrations, tailored to their linear range and biological relevance. Internal standards were either provided or dissolved in acetonitrile (ACN) solution. The exact concentrations of the standards were verified using certificates of analysis or adjusted based on calibration with reference

standards, ensuring precise and sensitive quantification. Ultrapure water was sourced from a purified-water system by Sartorius AG (Göttingen, Germany). Anhydrous magnesium sulfate (purity 98.6%) and sodium chloride (purity 100%) were purchased from VWR International (Radnor, PA, USA). Glacial acetic acid (LC-MS grade, purity 99.95%), formic acid (LC-MS grade, purity 99%), absolute acetonitrile (LC-MS grade), and methanol (LC-MS grade) were purchased from BioSolve (Valkenswaard, The Netherlands). Sodium hydroxide (purity >99%) and ammonium acetate (purity $\geq 98\%$) were sourced from Merck (Darmstadt, Germany). Picric acid (1.3% in H₂O, saturated) and creatinine powder (molecular weight = 113.12 g/mol) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Human biomonitoring of multiple mycotoxins in urine

2.4.1. Urine sample pretreatment

Sample pretreatment for multiple mycotoxin determination in urine was based on a QuEChERS approach (Quick, Easy, Cheap, Effective, Rugged, Safe). The sample preparation was refined based on the previously described protocols to enhance sensitivity and efficiency in quantification (Maris et al., 2025; Martins et al., 2019; Vidal, Claeys, et al., 2018). Initially, urine samples were acclimated at room temperature. Then, a volume of 450 μL from each sample was transferred into separate 15 mL Falcon tubes. To prepare a matrix-matched calibration curve, nine additional 15 mL Falcon tubes were labelled and filled with 450 μL of control urine. These control urine samples were obtained from CEMPH (Ghent University) laboratory personnel and pooled. Standard addition was applied in case a mycotoxin was present in the control sample. For calibration curve preparation, varying volumes of the mycotoxin standard mixture were added to seven tubes. Each sample and eight of the calibration tubes were mixed with 30 μL of the internal standard solution and 4 mL of an extraction solvent consisting of formic acid, acetonitrile, and water (3:52:45, v/v/v). Next, 900 mg of magnesium sulphate and 225 mg of sodium chloride were added to each tube, followed by vortexing for 2 min to facilitate extraction using an L46 vortex mixer from Labinco (Breda, Netherlands). The tubes were then placed on an overhead shaker from Agitelec (Paris, France) for 30 min and centrifuged at $4000 \times g$ for 6 min using a Multifuge 3 S-R centrifuge from Heraeus (Hanau, Germany). The supernatant was carefully transferred into glass tubes and dried under a gentle nitrogen flow at $40\text{ }^{\circ}\text{C}$ using a Turbovap LV system from Biotage (Uppsala, Sweden). The resulting dry residues were reconstituted in 100 μL of injection solvent (a 60:40 v/v mixture of mobile phase A and mobile phase B), vortexed for 2 min, and briefly centrifuged at $2100 \times g$. Particles were removed from the reconstituted solution by centrifugal filtration using a 0.22 μm PVDF Durapore® filter (Millipore, Cork, Ireland) and centrifugation at $9000 \times g$ for 5 min. The final filtrates were transferred into UPLC vials with insert, closed with a pre-cut septum cap, and gently tapped to remove any air bubbles, making them ready for analysis using LC-MS/MS.

2.4.2. Instrumentation

LC-MS/MS analysis was carried out at Ghent University (CEMPH) using a Waters UPLC® system coupled with a Quattro XEVO TQ-XS mass spectrometer (Waters, Manchester, UK). Data collection and processing were managed using MassLynx™ version 4.2 and TargetLynx® version 4.2 software (Waters, Manchester, UK). Chromatographic separation was achieved on a Waters Acquity UPLC I-Class system (Milford, MA, USA), utilizing an ACQUITY UPLC® HSS T3 column (2.1 $\times$ 100 mm, 1.8 μm) along with an ACQUITY UPLC® HSS T3 VanGuard pre-column (2.1 $\times$ 10 mm, 1.8 μm). Gradient elution involved mobile phase A, consisting of H₂O, methanol, and acetic acid (94:5:1, v/v/v), and mobile phase B, made up of H₂O, methanol, and acetic acid (2:97:1, v/v/v), both containing 5 mM ammonium acetate. The injection volume was set at 10 μL , and the autosampler temperature was maintained at $10\text{ }^{\circ}\text{C}$ throughout the analysis. The chromatographic separation was conducted over a

runtime of 18 min with a constant flow rate of 0.3 mL/min. The gradient elution started with 95% mobile phase A, which was gradually reduced to 35% A within the first 7 min. Between 7 and 11 min, phase A was further lowered to 25%. By minute 13, mobile phase B was increased to 99% and held constant for one minute. The gradient was then rapidly returned to the initial conditions of 95% A, with the system equilibrating until the end of the 18-min runtime. Multiple Reaction Monitoring (MRM) was used to ensure precise identification, with two specific transitions monitored per analyte and dwell time optimized for each. Both electrospray ionization in positive mode (ESI+) and negative mode (ESI-) were utilized. The capillary voltage was adjusted to 3.0 kV for ESI+ and 2.5 kV for ESI-. The source and desolvation temperatures were maintained at 120 °C and 500 °C, respectively. Cone gas flow was set at 150 L/h, desolvation gas flow at 800 L/h, collision gas flow at 0.15 mL/min, nebulizer pressure at 7 bar, with ion guide offsets of 3.0 and 0.3 for offset 1 and offset 2, respectively.

2.4.3. Method validation

The UHPLC-MS/MS method was validated for the determination of 39 mycotoxins and metabolites in urine according to European Medicine

Agency guidelines by the International Council for Harmonisation for bioanalytical method validation (ICH M10) (European Medicines Agency (EMA), 2022). Validation experiments included determination of the limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), within-run and between-run accuracy and precision assessed at two concentration levels (acceptance criteria: $\pm 15\%$ for QC levels and $\pm 20\%$ at the LLOQ), and matrix factor evaluation across individual lots of the biological matrix to ensure consistent analyte signal suppression or enhancement. Selectivity was confirmed by analysing blank matrix samples from multiple sources to demonstrate absence of interfering peaks at the retention times of the analyte and internal standard. Carry-over was assessed using blank injections following the highest calibration standard, verifying that the analyte response did not exceed 20% of the LLOQ and 5% for the internal standard. Method validation parameters are presented in Table 1. When mycotoxin concentrations in the sample exceeded the ULOQ, the sample was diluted and reanalysed to ensure accurate quantification within the validated calibration range.

Table 1

Validation parameters of the combined urine extraction and instrument analysis method.

	Detection capability	Quantification range		Recovery %		Intermediate precision RSD _{wt} %		Repeatability RSD _R %	
	LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)	X1	X2	X1	X2	X1	X2
3/15-ADON	0.25	0.46	50.00	100.9	103.3	14	7	15	6
AFB ₁	0.308	0.566	53.0	91.7	94.1	19	15	15	9
AFB ₂	0.012	0.022	4.70	94.6	103.0	16	4	16	4
AFG ₁	0.30	0.55	59.00	99.2	89.4	16	11	17	4
AFG ₂	0.017	0.032	3.45	97.4	104.7	16	10	16	9
AME	0.12	0.23	44.00	97.4	99.5	18	7	19	8
AOH	0.25	0.46	40.50	98.6	103.6	19	14	19	13
BEAU	0.0008	0.0015	0.49	94.2	99.0	10	15	12	14
CIT	0.012	0.022	9.55	98.2	100.6	8	4	8	4
CPA	0.12	0.22	50.00	104.7	103.7	14	13	13	10
DAS	0.0095	0.0174	0.50	99.9	99.1	14	3	16	2
DOM	0.15	0.27	50.00	92.7	103.8	20	14	16	14
Free-DON	0.11	0.23	44.50	101.5	104.7	15	13	17	10
DON-3G	0.12	0.23	48.50	94.1	96.7	17	11	17	9
ENN A	0.0011	0.0021	0.50	103.1	96.7	14	14	15	14
ENN A ₁	0.0011	0.0021	0.49	97.9	98.5	14	15	13	15
ENN B	0.0011	0.0021	0.49	98.3	100.6	14	9	12	9
ENN B ₁	0.0008	0.0015	0.50	97.5	99.0	11	11	11	12
FB1	0.15	0.28	5.95	111.0	102.7	7	9	8	9
FB2	0.081	0.15	8.20	110.9	103.9	3	6	3	6
FB3	0.22	0.40	12.45	110.5	103.3	5	8	5	8
FUSX	0.73	1.34	63.50	104.1	106.6	17	11	18	12
HFB1	0.13	0.23	50.00	94.5	99.4	17	13	17	11
HT2	0.029	0.054	27.00	97.4	99.1	7	2	7	2
NEO	0.072	0.132	65.50	99.4	104.4	7	10	7	10
NIV	1.30	2.40	52.50	101.3	103.3	18	9	19	10
OTA	0.013	0.023	6.30	99.3	101.0	12	15	14	15
OT-ALPHA	0.36	0.67	69.50	96.1	107.5	17	14	17	13
ROQ-C	0.27	0.49	100.00	92.7	89.2	18	14	17	9
STERIG	0.12	0.22	63.50	97.2	81.0	12	12	14	11
T2	0.0047	0.0087	0.50	98.7	100.1	15	5	13	4
TeA	0.78	1.43	50	115.3	91.8	15	8	8	6
α -ZAL	0.28	0.55	48.00	96.2	98.7	18	12	16	13
β -ZAL	0.42	0.77	52.50	97.7	101.2	13	13	14	13
ZAN	0.09	0.16	20.00	98.1	97.5	11	4	11	3
α -ZEL	0.27	0.50	44.50	101.0	99.2	19	15	17	14
β -ZEL	0.19	0.35	45.50	94.1	98.9	14	11	15	11
ZEN	0.06	0.12	77.50	97.4	98.5	5	6	5	6

3/15-ADON: 3- and 15-acetyl-deoxynivalenol; AFB₁: aflatoxin B₁; AFB₂: aflatoxin B₂; AFG₁: aflatoxin G₁; AFG₂: aflatoxin G₂; AME: alternariol monomethyl ether; AOH: alternariol; BEAU: beauvericin; CIT: citrinin; CPA: cyclopiazonic acid; DAS: diacetoxyscirpenol; DOM: deoxy-deoxynivalenol; Free-DON: deoxynivalenol; DON-3G: deoxynivalenol-3-glucoside; ENN A: enniatin A; ENN A₁: enniatin A₁; ENN B: enniatin B; ENN B₁: enniatin B₁; FB₁: fumonisin B₁; FB₂: fumonisin B₂; FB₃: fumonisin B₃; FUSX: fusarenol-X; HFB₁: fully hydrolyzed fumonisin B₁; HT2: HT-2 toxin; NEO: neosolaniol; NIV: nivalenol; OTA: ochratoxin A; OT- α : ochratoxin alpha; ROQ-C: roquefortin C; STERIG: sterigmatocystin; T2: T-2 toxin; TeA: tenuazonic acid; α -ZAL: alpha-zearalanol; β -ZAL: beta-zearalanol; ZAN: zearalanone; α -ZEL: alpha-zearalanol; β -ZEL: beta-zearalanol; ZEN: zearalenone; LOD: limit of detection; LLOQ: lower limit of quantification; ULOQ: upper limit of quantification; RSD_{wt}%, percentage relative standard deviation under intermediate precision conditions; RSD_R%, percentage relative standard deviation under repeatability conditions; X1: lowest concentration level of the calibration standard that can be reliably quantified using the validated method (i.e. LLOQ); X2: highest concentration level of the calibration standard that can be reliably quantified using the validated method (i.e. ULOQ).

2.4.4. Analysis of creatinine in urine

Measuring urinary creatinine levels is crucial for adjusting analyte concentrations, thereby ensuring accuracy and reliability of the results. Creatinine concentrations in urine were quantified at CEMPH, Ghent University, using an in-house developed and validated with reference material spectrophotometric method based on the Jaffe reaction (Maris et al., 2025). To prepare the picrate reagent, 3.05 g of NaOH was weighed and dissolved in 600 mL of ultrapure water, and then 300 mL of a 1.3% picric acid solution was added. For the calibration curve, 100.00 mg of creatinine was measured using an analytical balance and dissolved in 100 mL of ultrapure water using a volumetric flask. Calibration standards were prepared at the following concentrations: 0, 2, 5, 10, 20, 50, 100, and 200 µg creatinine/mL. In a 96-well plate, 100 µL of each calibration standard was pipetted into duplicate wells, while 100 µL of diluted urine samples (1:20, urine:ultrapure water) were added in triplicate. A volume of 200 µL of the picric acid reagent was then added to each well. The plate was incubated for 15 min on an orbital shaker, protected from exposure to light. Following incubation, absorbance was measured at 520 nm using a SpectraMax M5 spectrophotometer (Molecular Devices, San Jose, CA, USA). The creatinine quantification method was validated using inter-laboratory reference materials RV72 and RV73 (creatinine in urine) provided by the German external quality assessment scheme (G-EQUAS, Erlangen, Germany). Data analysis was carried out with SoftMax PRO 7 software (Molecular Devices, San Jose, CA, USA).

2.5. Statistical analysis

Left-censored values (<LOD or < LLOQ) of mycotoxins with a detection rate above 75% were substituted using multiple imputation. For values <LOD, the imputed values were drawn below the LOD, while for values <LLOQ, the imputed values were drawn from the interval between the LOD and the LLOQ. A truncated lognormal distribution was fitted to the data of each for the biomarkers using the observed values above the limits. Afterwards, random values were imputed for the estimated distribution below the limits. Imputations were performed on log-transformed values to better approximate normality and then back-transformed for analysis. For the mycotoxins with a detection rate between 5% and 10%, the quantifiable values were used to calculate descriptive statistics.

Descriptive statistics of free-DON and TeA levels (volume based and standardized for creatinine) (N, arithmetic mean (AM), geometric mean (GM) and 95% CI, percentiles P5, P10, P25, P50, P75, P90, P95 and maximum) were calculated. The normality of the distribution of free-DON and TeA levels was assessed using Kolmogorov-Smirnov (KS) normality tests. Exposure values for free-DON and TeA were stratified by sampling season, sex, educational level, degree of urbanization and occupational status (GM, P95 and their 95% confidence intervals were calculated). Differences between groups were assessed using Mann-Whitney and Kruskal-Wallis tests and multiple pairwise-comparisons using Wilcoxon rank sum tests with Bonferroni continuity correction. Multiple linear regression analyses were performed for log transformed imputed free-DON and TeA concentrations adjusted to creatinine as dependent variables and including all the sociodemographic variables as independent variables to adjust for their potential effect. The validity of the models was assessed, namely in respect to linearity of the data, normality of residuals, homogeneity of residuals variance and independence of residuals error terms. The beta coefficients of the multiple linear regression models were exponentiated to be presented as ratios of levels between the different categories.

A significance level of 0.05 was considered as statistically relevant for all the analysis. Statistical analysis was performed using the softwares SPSS v30, R v4.3.2 and R Studio 2024.12.1.

3. Results and discussion

3.1. Characterization of the study participants

A total of 295 participants were included in this study, and their characteristics are summarized in Table 2. The majority of the study participants were women (58%), compared with 42% men. Samples were collected throughout the year, with around half being collected during the summer (51.4%) and around one quarter in autumn (25.4%). The number of samples obtained during spring was lower than in the other seasons due to the interruption of the study fieldwork in March 2020 caused by the COVID-19 pandemic (study fieldwork started in May 2019 and was intended to continue through spring 2020). Educational level showed a skewed distribution toward higher qualifications: 20.0% of the participants reported low education (ISCED ≤2), 36.3% medium (ISCED 3–4), and 43.7% high (ISCED ≥5). This bias toward higher education is typical of voluntary biomonitoring studies, as individuals with greater educational background demonstrate higher awareness of environmental health issues and, as such, have higher participation rates in surveys (Morrens et al., 2017). In terms of the area of residence, the distribution of the participants was well balanced, with slightly higher proportions of participants living in rural areas (35.9%) and towns/suburbs (36.9%) compared with cities (27.1%). Most participants were employed (87.1%), as is to be expected for the age group (28–39 years) included in the study (OECD, 2019).

3.2. Occurrence of mycotoxins in urine

Human biomonitoring is a vital tool for assessing exposure to environmental chemicals, enabling the tracking of exposure trends over time and the identification of population groups at higher risk. The present study, conducted as part of the INSEF-ExpoQuim study (2019–2020) (Gilles et al., 2022; Govarts et al., 2023), represents the first HBM study in Portugal aimed to detect multiple mycotoxins in a national sample of

Table 2
Characterization of the study participants.

Variable	Category	N	%
Sex	Female	171	58.0
	Male	124	42.0
Age (years)	Mean (Min-Max)	34.6 (28–39)	
Sampling season	Winter	52	17.6
	Spring	16	5.4
	Summer	152	51.5
	Autumn	75	25.4
Educational level*	Low (ISCED 0–2)	59	20.0
	Medium (ISCED 3–4)	107	36.3
	High (ISCED ≥5)	129	43.7
Degree of urbanization	Cities	80	27.1
	Towns/suburbs	109	36.9
	Rural areas	106	35.9
NUTS2 region**	North (PT11)	46	15.6
	Algarve (PT15)	30	10.2
	Centre (PT16)	49	16.6
	Lisbon and Tagus Valley (PT17)	25	8.5
	Alentejo (PT18)	47	15.9
	Azores (PT20)	62	21.0
	Madeira (PT30)	36	12.2
Occupational status	Working	257	87.1
	Not working	20	6.8
	No information	18	6.1

* Classification according to the International Standard Classification of Education 2011 (ISCED 2011). ** Classification according to the classification from EUROSTAT (2003) (Regulation (EC) No 1059/2003 of the European Parliament and of the Council of 26 May 2003 on the Establishment of a Common Classification of Territorial Units for Statistics (NUTS), 2003 (European Parliament and Council of the European Union, 2003); UNESCO Institute for Statistics, International Standard Classification of Education - ISCED, 2011 (UNESCO Institute for Statistics, 2011)).

295 early mid-life adult participants from the Portuguese population, including the islands. Fig. 1 shows the detection frequencies and co-exposures for mycotoxins found in the whole set of first-morning urine samples analysed. Out of the 39 mycotoxin or metabolites analysed, free-DON and TeA were most frequently detected, with 84.7% and 95.6% positive samples, respectively. CIT (11.5%), α -ZEL (10.5%) and OTA (9.5%) showed detection rates near 10%. Detection frequencies between 1% and 5% were observed for β -ZAL (4.1%), α -ZAL (3.7%), ZEN (1.7%), β -ZEL (1.4%) and AFB₂ (1.4%). For AFB₁, DON-3G and ENNB the frequency of detection was 1%, while AFM₁, AFG₂, AME, DOM, ENNB₁, FUSX, OT α were found in 0.3–0.7% of the samples. The remaining assayed mycotoxin metabolites (3ADON + 15ADON, AFG₁, AFG₂, AOH, BEAU, CPA, DAS, ENNA, ENNA₁, FB₁, FB₂, FB₃, HFB₁, T₂, HT₂, NEO, NIV, ROQ-C, STERIG and ZAN) were not detected in any of the urine samples analysed. Table S-1 summarizes the distribution of co-exposures in the study population, showing how often each number of concurrent exposures occurred and the corresponding prevalence of detection. The number of co-exposures to mycotoxins in the Portuguese population ranged from 0 to 5 (Table S-2), with simultaneous exposures to two mycotoxins being the most common ($n = 160$, 54.2%). Among the study population, 160 individuals (Table S2) were exposed to two mycotoxins, of whom 146 were co-exposed exclusively to free-DON and TeA (Fig. 1, right panel). Overall, 242 out of 295 participants (82.0%) showed combined exposure to free-DON and TeA, although this co-exposure was not always limited to these two mycotoxins and could also include additional compounds. Notably, in 49.5% of participants, free-DON and TeA were the only mycotoxins detected. These findings highlight the importance of considering emerging mycotoxins, such as TeA, alongside regulated ones, such as free-DON, in exposure assessment frameworks. Recently, Guo et al. have demonstrated that DON and TeA co-occurrence exacerbates the disruption of overall metabolic balance, potentially impairing the normal function of cells by affecting energy production, protein synthesis, and cell proliferation (Guo et al., 2025). Overall, although human exposure levels estimated in this study for DON and TeA are below those associated with *in vitro* synergistic toxicity, potential health risks, particularly under repeated, long-term and combined exposure, cannot be excluded. These findings reinforce the importance of continued biomonitoring efforts and further toxicological studies to better characterize the combined effects of these mycotoxins on human health.

Our results are consistent with previous biomonitoring studies

conducted in European countries such as Belgium, Germany, Norway, and Portugal, where co-exposures to several mycotoxins were shown to frequently occur, often originating from diverse dietary sources and reflecting overlapping contamination profiles in staple foods (Heyndrickx et al., 2015; Martins et al., 2019; Schmidt, Cramer, et al., 2021; Wallin et al., 2015). Furthermore, the combined deleterious effects from co-exposure can lead to additive or synergistic effects that should be considered for their regulation (van den Brand et al., 2022; Zingales et al., 2024).

Table 3 presents exposure biomarkers concentrations expressed both as volume-weighted concentration ($\mu\text{g/L}$) and creatinine-adjusted values (μg biomarker/g creatinine) for mycotoxins with a detection frequency higher than 5%. Multiple imputation was used to handle left-censored data for the biomarkers with high detection frequencies. Although multiple imputation introduces additional uncertainty and relies on distributional assumptions, this strategy was used only for free-DON that had a detection frequency of 84.7% and for TeA that had a detection frequency of 95.6%. A sensitivity analysis using a simpler substitution method, namely the substitution by half the limit was performed and yielded comparable results, supporting the robustness of the results presented.

Median concentrations (P50) for the two predominant mycotoxins were 0.79 $\mu\text{g/L}$ (0.72 $\mu\text{g/g}$ creatinine) for free-DON and 2.45 $\mu\text{g/L}$ (2.08 $\mu\text{g/g}$) for TeA, with maximum values of 11.77 $\mu\text{g/L}$ (10.06 $\mu\text{g/g}$ creatinine) and 90.91 $\mu\text{g/L}$ (114.15 $\mu\text{g/g}$ creatinine), respectively.

Recent HBM studies in Europe (Table S-3) reported urinary free-DON concentrations of similar magnitude to the one observed in the present study, namely, a median of 0.49 $\mu\text{g/L}$ in a multicentre survey across four European countries (Netherlands, Belgium, Norway and Czech Republic) (De Ruyck et al., 2020) and 0.94 $\mu\text{g/L}$ (0.55 $\mu\text{g/g}$ creatinine) in an occupational survey in Norway (Martins et al., 2025). On the other hand, the 2015–2016 Portuguese biomonitoring survey, conducted as part of the National Food, Nutrition and Physical Activity Survey (IAN-AF), that has included 94 adult participants from northern Portugal, detected free-DON in 30% of first-morning urine samples with a median concentration of 0.38 $\mu\text{g/L}$ (0.48 $\mu\text{g/g}$ creatinine) (Martins et al., 2019). These prior Portuguese data and the current data were substantially lower than the median concentration of total-DON (including free-DON, 3- and 15-glucuronides, and DON-3G) in urine reported for the pooled European database during the exposure assessment of the European adult population conducted under the HBM4EU Aligned Studies, which

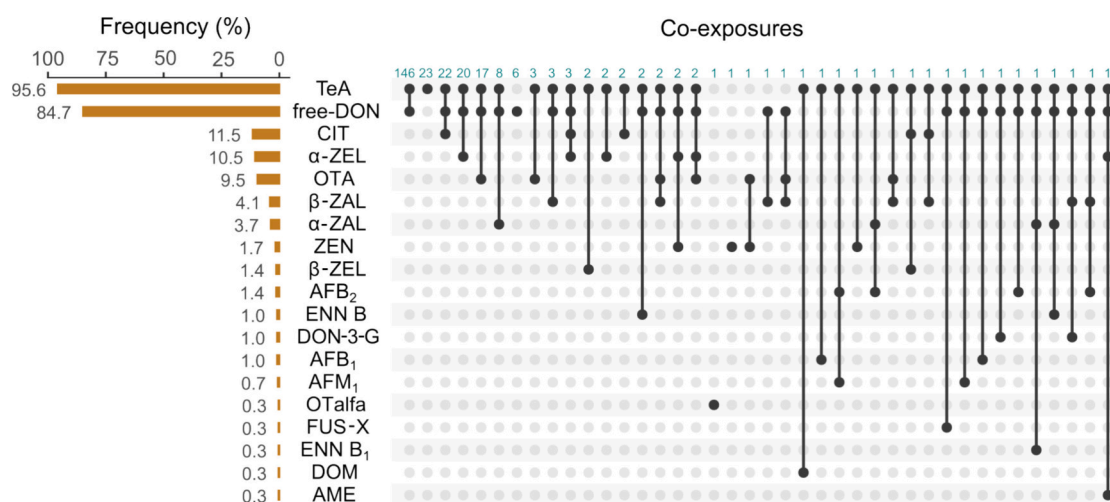


Fig. 1. Visualization of mycotoxin co-exposures in the study population ($N = 295$). The left panel displays the detection frequency (%) of individual mycotoxins, while the right panel (UpSet plot) shows co-occurrence patterns, ranked from the most to least common combinations; numbers indicate the corresponding counts (n). TeA: tenuazonic acid; DON: deoxynivalenol; CIT: citrinin; α -ZEL: alpha-zearalenol; OTA: ochratoxin A; β -ZAL: beta-zearalenol; α -ZAL: alpha-zearalanol; ZEN: zearalenone; β -ZEL: beta-zearalenol; AFB₂: aflatoxin B₂; ENNB: enniatin B; DON-3G: deoxynivalenol-3-glucoside; AFB₁: aflatoxin B₁; AFM₁: aflatoxin M₁; OT α : ochratoxin alpha; FUS-X: fusarenon-X; ENNB₁: enniatin B₁; DOM: deepoxy-deoxynivalenol; AME: alternariol monomethyl ether.

Table 3

Concentrations in µg/L and µg/g creatinine for mycotoxins detected in more than 5% of the urine samples (N = 295).

Mycotoxins in urine	% DF	% > LOQ	AM	GM	GM 95% CI	P5	P10	P25	P50	P75	P90	P95	Max
Free-DON (µg/L)	84.7	73.2	1.63	0.63	0.52–0.75	0.04	0.06	0.15	0.79	2.26	4.43	5.75	11.77
Free-DON (µg/g creatinine)	–	–	1.24	0.54	0.46–0.65	0.04	0.06	0.18	0.72	1.66	2.95	4.40	10.06
TeA (µg/L)	95.6	69.8	4.68	2.41	2.11–2.75	0.39	0.54	1.17	2.45	5.36	10.40	16.56	90.91
TeA (µg/g creatinine)	–	–	4.05	2.10	1.84–2.38	0.33	0.49	1.00	2.08	4.26	8.91	15.51	114.15
CIT (µg/L)	11.5	7.8	0.12*	0.04*	–	–	–	–	0.04*	–	–	–	1.59
CIT (µg/g creatinine)	–	–	0.09*	0.04*	–	–	–	–	0.04*	–	–	–	0.79
α-ZEL (µg/L)	10.5	10.2	11.9*	4.07*	–	–	–	–	3.78*	–	–	–	112.71
α-ZEL (µg/g creatinine)	–	–	12.8*	3.40*	–	–	–	–	3.32*	–	–	–	204.82
OTA (µg/L)	9.5	9.2	0.27*	0.12*	–	–	–	–	0.12*	–	–	–	2.66
OTA (µg/g creatinine)	–	–	0.17*	0.09*	–	–	–	–	0.08*	–	–	–	1.21

DON: deoxynivalenol; TeA: tenuazonic acid; CIT: citrinin; α-ZEL: alpha-zearalenol; OTA: ochratoxin A; DF: Detection frequency; LOQ: Limit of Quantification; AM: Arithmetic mean; GM: Geometric mean; 95% CI: 95% Confidence Interval; P5: percentile 5; P10: percentile 10; P25: percentile 25; P50: percentile 50; P75: percentile 75; P90: percentile 90; P95: percentile 95; Max – Maximum; *value was obtained using only positive samples as no imputation method was used due to the low frequency of detection.

was 5.97 µg/L, and the median concentrated reported for Portugal which was 7.99 µg/L (Namorado et al., 2024). Other studies reported total-DON concentrations of 5.10–6.90 µg/L in Norway (Eriksen et al., 2021), 12.00 µg/L in Sweden (Schmidt, Lindemann, et al., 2021), and 4.58 µg/L (5.05–6.07 µg/g creatinine) in Italy (De Santis et al., 2019). These markedly higher values are expected, as total-DON accounts for conjugated metabolites that are not captured when measuring free-DON alone. Therefore, comparisons between studies assessing free-DON and total-DON should be made cautiously, as they reflect different exposure metrics. According to the European Food Safety Authority, the estimated mean chronic dietary exposure to DON in adults is at levels that may pose a health concern, including immunotoxic and reprotoxic effects (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017). In vitro studies have generated some conflicting results as to its genotoxicity, but in vivo studies integrating the analysis of DNA and chromosome damage showed that DON is not genotoxic in rodents, following oral exposure (Le Hégarat et al., 2014). It is worth mentioning that DON is considered as not carcinogenic (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017; International Agency for Research on Cancer (IARC), 1993). However, studies indicate that the presence of DON in the diet can exacerbate the genotoxicity of gut microbiota, also raising questions about the synergistic effects between food contaminants and gut microbiota regarding intestinal carcinogenesis (Payros et al., 2017). These findings underscore the importance of continued research using standardized mycotoxin standards (parent and metabolites compounds) and analytical methods to ensure accurate monitoring, facilitate cross-study comparisons, and assess potential trends in exposure to multiple mycotoxins among the general population, as well as the associated health risks.

Concerning TeA, the median concentration observed in this study was slightly lower than in other reports (Table S-3), namely 3.50 µg/L in a multicentre survey across four European countries (De Ruyck et al., 2020) and 3.60 µg/L for the Sweden population (Schmidt, Lindemann, et al., 2021), the last including TeA and its epimer allo-TeA measured in dried urine spots. Variability in study parameters, including number of study participants, population groups, analytical methods and limits of quantification among others, likely contribute to these differences and hampers direct comparisons. The toxicokinetics and toxicological data available for TeA are still limited. After oral exposure, TeA seems to be rapidly absorbed, distributed and excreted in urine within 24 h, either in humans or in rodents (Asam et al., 2013; Puntischer et al., 2019; Visintin et al., 2025). In contrast with other *Alternaria* toxins, TeA has not evidenced endocrine disruptive, immunotoxic or genotoxic effects in vitro. However, its mutagenic potential is unclear, due to negative and positive results reported in mutation assays in bacteria and mammalian cells, respectively (Louro et al., 2023).

In conclusion, this work confirms ubiquitous DON exposure across European populations and highlights the emergence of *Alternaria*

toxins, underscoring their inclusion in routine monitoring.

In contrast to free-DON, TeA was included for the first time in a HBM survey conducted on the Portuguese population and was detected in 95.6% of the participants, with a median concentration of 2.45 µg/L (2.08 µg/g creatinine). Interestingly, AOH, another *Alternaria* toxin, was previously detected in 13% of the participants from the IAN-AF study, with a median value of 0.21 µg/L (0.18 µg/g creatinine), but was not detected in the current study. The EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a scientific opinion in 2011 on the risks posed by *Alternaria* presence in food to animal and public health (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011). More recently, EFSA published a scientific report on the dietary exposure assessment of *Alternaria* toxins in the European population (Arcella et al., 2016), concluding that the estimated chronic dietary exposure to the *Alternaria* toxins AOH, AME and TeA exceeds the relevant threshold of toxicological concern, highlighting the need for additional compound-specific toxicity data. As a result, the European Commission established an indicative value for monitoring *Alternaria* toxins in food (European Commission, 2022). Regulatory bodies use a threshold of toxicological concern (TTC) of 1500 ng/kg bw per day for risk assessment purposes.

CIT was the third most frequently detected mycotoxin, although it was only detected in 11.5% of the samples with a median concentration of 0.04 µg/L (0.04 µg/g creatinine) among positive samples. This detection frequency falls between previously reported values in Southern Europe. A multi-country European survey documented CIT detection in 14.4% of samples with a median concentration of 0.62 µg/L among positive samples (De Ruyck et al., 2020), approximately 15-fold higher than the value determined in the present study. Similarly, a recent Spanish biomonitoring study reported CIT detection in 20% of the samples with a mean concentration of 0.56 µg/L among positive samples (Peris-Camarasa, Coscollà, et al., 2025), also substantially exceeding Portuguese levels. The earlier 2015–2016 Portuguese IAN-AF survey reported lower CIT detection frequency (2%), although the median concentration among the positive samples (0.75 µg/L; 1.03 µg/g creatinine) (Martins et al., 2019) was also higher than in the present study (Table S-3). Notably, CIT prevalence in all Portuguese surveys remain substantially lower than in Northern and Central European populations (e.g. Germany, Belgium), where detection frequencies of 59–100% and median concentrations in the range 0.18–0.60 µg/L have been documented (Degen et al., 2023; Heyndrickx et al., 2015).

The fourth most detected mycotoxin was α-ZEL, that was detected in 10.5% of samples with a median concentration of 3.78 µg/L (3.32 µg/g creatinine) among positive samples. Detection frequency was similar to a multi-country European survey (9.0%), yet the median in the European study was 27.80 µg/L (De Ruyck et al., 2020), approximately 7-fold exceeding the present study. The 2015–2016 Portuguese IAN-AF survey reported lower α-ZEL detection frequency (5%), although the median

concentration (2.70 µg/L; 2.14 µg/g creatinine) (Martins et al., 2019) was remarkably similar to the present study (Table S-3), suggesting relatively stable α-ZEL exposure levels in Portuguese adults across the four-year period between the two studies.

OTA was detected in 9.5% of the samples, with a median concentration of 0.12 µg/L (0.08 µg/g creatinine) among positive samples. OTA detection frequency in the present study was substantially lower than reported in previous European biomonitoring investigations. A multi-country European survey documented a 25.5% detection frequency with a median concentration of 1.64 µg/L (De Ruyck et al., 2020), more than 13-fold higher than the present study. A HBM study in Sweden reported markedly higher OTA detection frequency (74%) despite lower median concentration (0.040 µg/L) (Schmidt, Lindemann, et al., 2021), indicating regional or dietary differences in OTA exposure determinants. The 2015–2016 Portuguese IAN-AF survey reported a higher OTA detection frequency (27%) compared to the present study (Martins et al., 2019). The median OTA concentration in the present study represents an approximately 17-fold higher exposure compared to the IAN-AF median (0.007 µg/L; 0.008 µg/g creatinine), suggesting that OTA exposure in Portuguese adults may be higher than initially suggested by the IAN-AF survey.

Nevertheless, comparisons of detection frequencies across studies should be interpreted with caution, as they may be influenced by

methodological differences, including analytical sensitivity, sampling strategies, and urine normalization approaches.

3.3. Sociodemographic determinants of free-DON and TeA exposure

Table 4 summarizes the distribution of free-DON concentrations in urine according to sociodemographic variables. Significantly higher GM values were observed among males compared with females (0.80 vs 0.52 µg/L, respectively, $p = 0.020$), although the statistical significance was lost after adjustment for creatinine (0.60 vs 0.51 µg/g creatinine, $p = 0.495$). Samples collected during spring exhibited notably higher GM values (0.96 µg/g creatinine, $p = 0.085$) compared with those obtained in winter, autumn and summer (0.40, 0.51 and 0.59 µg/g creatinine, respectively). Seasonal differences in creatinine-adjusted urinary free-DON concentrations were further explored using Dunn's post-hoc test following a Kruskal–Wallis test. A statistically significant difference was observed between spring and winter ($p = 0.021$). In addition, a borderline difference was noted between summer and winter ($p = 0.066$). No significant differences were detected for the remaining seasonal comparisons.

In terms of educational level, participants with higher education presented significantly lower GM levels (0.39 µg/g creatinine, $p = 0.002$) than those with medium or low education (0.72 and 0.68 µg/g

Table 4
Distribution of free-DON in urine in µg/L and µg/g creatinine per sociodemographic variables.

Variable	Category	Free-DON in urine (µg/L)				Free-DON in urine (µg/g creatinine)			
		n	GM [95%CI]	P95 [95%CI]	p	n	GM [95%CI]	P95 [95%CI]	p
Sampling season	Winter	52	0.49 [0.31–0.80]	4.95 [2.89–5.25]	0.276 ^a	52	0.40 [0.27–0.65]	2.88 [2.15–7.30]	0.085 ^a
	Spring	16	1.11 [0.49–2.44]	- [-]		16	0.96 [0.46–2.08]	- [-]	
	Summer	152	0.64 [0.50–0.89]	7.97 [5.45–10.51]		152	0.59 [0.47–0.79]	5.84 [4.14–6.51]	
	Autumn	75	0.62 [0.44–0.87]	4.34 [3.46–7.07]		75	0.51 [0.38–0.72]	3.11 [2.46–4.18]	
Sex	Female	171	0.52 [0.40–0.67]	5.35 [4.15–6.49]	0.020 ^b	171	0.51 [0.40–0.64]	3.70 [2.93–5.15]	0.495 ^b
	Male	124	0.80 [0.66–1.18]	7.94 [5.34–8.95]		124	0.60 [0.51–0.88]	5.69 [3.58–7.30]	
Educational level	Low (ISCED 0–2)	59	0.85 [0.54–1.34]	8.02 [6.76–8.50]	0.002 ^a	59	0.68 [0.44–1.06]	5.49 [4.39–6.10]	0.002 ^a
	Medium (ISCED 3–4)	107	0.83 [0.64–1.14]	6.84 [4.63–9.83]		107	0.72 [0.58–0.99]	4.80 [3.04–8.39]	
	High (ISCED ≥5)	129	0.43 [0.34–0.61]	5.34 [3.90–5.73]		129	0.39 [0.32–0.54]	3.23 [2.60–5.34]	
	Cities	80	0.73 [0.48–1.02]	7.07 [4.54–9.38]	0.552 ^a	80	0.58 [0.40–0.80]	4.14 [2.72–5.97]	0.776 ^a
Degree of urbanization	Towns/suburbs	109	0.58 [0.45–0.85]	5.83 [4.23–7.54]		109	0.50 [0.40–0.74]	5.49 [3.04–6.16]	
	Rural areas	106	0.60 [0.46–0.87]	6.60 [4.99–10.66]		106	0.57 [0.44–0.79]	4.43 [3.20–8.39]	
NUT2 region	North (PT11)	46	0.56 [0.35–0.85]	3.43 [2.63–3.58]	0.007 ^a	46	0.47 [0.31–0.72]	2.53 [1.51–2.75]	0.001 ^a
	Algarve (PT15)	30	0.98 [0.50–1.92]	8.80 [5.92–10.70]		30	0.76 [0.40–1.44]	6.82 [3.23–9.12]	
	Centre (PT16)	49	0.36 [0.23–0.55]	4.33 [2.49–5.50]		49	0.33 [0.22–0.49]	2.53 [1.66–2.83]	
	Lisbon and Tagus Valley (PT17)	25	0.54 [0.25–1.00]	5.08 [4.05–5.25]		25	0.40 [0.19–0.70]	2.89 [2.40–2.93]	
	Alentejo (PT18)	47	0.49 [0.33–0.90]	6.70 [4.23–11.11]		47	0.41 [0.30–0.76]	5.15 [2.80–6.51]	
	Azores (PT20)	62	0.95 [0.69–1.70]	9.12 [5.92–11.77]		62	0.86 [0.66–1.50]	6.32 [4.44–10.06]	
	Madeira (PT30)	36	0.80 [0.48–1.29]	6.13 [3.99–8.05]		36	0.79 [0.50–1.20]	5.85 [3.32–7.30]	
Occupational status	Working	257	0.63 [0.52–0.77]	5.92 [-]	0.798 ^a	257	0.55 [0.46–0.67]	4.45 [-]	0.600 ^a
	Not working	20	0.79 [0.42–1.52]	11.43 [4.80–11.77]		20	0.74 [0.41–1.34]	7.87 [2.81–8.13]	

DON: deoxynivalenol; GM: Geometric mean; 95% CI: 95% Confidence Interval; P95: percentile 95; ^a: Kruskal–Wallis Test; ^b: Mann–Whitney Test.

creatinine, respectively). Pairwise comparisons using Dunn's post-hoc test indicated significantly higher concentrations of free-DON in individuals with low education compared to high education ($p = 0.005$) and in individuals with medium education compared to high education ($p = 0.003$). No significant differences were detected for the remaining pairwise comparisons. The present findings agree with those reported by [Namorado et al. \(2024\)](#) in one of the few studies conducted in Portugal that evaluated the association between mycotoxin levels in urine and sociodemographic variables under the HBM4EU Aligned Studies ([Namorado et al., 2024](#)). This work reported associations between total-DON concentrations and the participants' level of education, showing that participants with lower educational attainment had higher total-DON levels than those with higher education, similarly to what was observed in the current study.

Concerning the degree of urbanization of the residence area, no significant differences were observed ($p = 0.776$).

Regional differences in creatinine-adjusted urinary free-DON concentrations were observed across Portuguese NUTS2 regions (Kruskal–Wallis $p = 0.001$), with participants from the southern region and the archipelagos exhibiting the highest exposure values, including Algarve (0.76 $\mu\text{g/g}$ creatinine), the Azores (0.86 $\mu\text{g/g}$ creatinine), and Madeira (0.79 $\mu\text{g/g}$ creatinine), respectively ([Fig. 2](#), left). Subsequent Dunn's post-hoc analyses identified several statistically significant pairwise differences, notably between Central Portugal and the Azores ($p < 0.001$), Central Portugal and Algarve ($p = 0.005$), Alentejo and the Azores ($p = 0.005$), Central Portugal and Madeira ($p = 0.010$), Northern Portugal and the Azores ($p = 0.017$), the Lisbon Metropolitan Area and the Azores ($p = 0.019$), and Alentejo and Algarve ($p = 0.041$). In addition, borderline differences were observed between Algarve and the Lisbon Metropolitan Area ($p = 0.069$), Northern Portugal and Algarve ($p = 0.086$), and Alentejo and Madeira ($p = 0.081$). Taken together, these findings indicate pronounced regional variability in free-DON exposure, with consistently higher levels observed in southern Portugal and the archipelagos compared with other mainland regions.

Concerning the occupational status of the participants, working individuals presented lower free-DON exposure than non-employed individuals (0.55 vs 0.74 $\mu\text{g/g}$ creatinine), although the difference was not statistically significant ($p = 0.600$).

[Table 5](#) presents the distribution of TeA concentrations in urine according to sociodemographic variables. Higher TeA levels were observed in men (2.34 vs 1.93 $\mu\text{g/g}$ creatinine), although the difference observed between both sexes was not statistically significant ($p = 0.147$). TeA concentrations were also higher in samples collected during spring and summer (2.56 and 2.38 $\mu\text{g/g}$ creatinine) compared with those from winter and autumn (1.69 and 1.80 $\mu\text{g/g}$ creatinine, $p = 0.073$).

Similarly to the analysis done for free-DON, pairwise comparisons showed that exposure to TeA in summer was significantly higher than that occurring in autumn ($p = 0.042$) and winter ($p = 0.048$). No statistically significant differences were observed between the values obtained in the other seasonal pairs.

Participants with higher education presented significantly lower GM concentrations (1.71 $\mu\text{g/g}$ creatinine, $p = 0.001$) than those with medium or low education (2.13 and 3.15 $\mu\text{g/g}$ creatinine, respectively). Pairwise comparisons using Dunn's post-hoc test indicated significantly higher concentrations in individuals with low education compared to high education ($p < 0.001$) and in individuals with low education compared to medium education ($p = 0.019$). No significant differences were detected for the remaining pairwise comparisons.

Concerning the degree of urbanization of the residence location, although higher TeA exposure was observed in individuals living in rural areas (2.50 $\mu\text{g/g}$ creatinine vs 1.78 and 1.99 $\mu\text{g/g}$ creatinine for cities and towns/suburbs, respectively), the differences observed did not present statistical significance ($p = 0.147$).

Regional differences in creatinine-adjusted urinary TeA concentrations were evaluated across Portuguese NUTS2 regions using the Kruskal–Wallis test, followed by Dunn's post-hoc comparisons. Significantly lower TeA levels were detected in the Lisbon Metropolitan Area (1.22 $\mu\text{g/g}$ creatinine) and in Northern Portugal (1.64 $\mu\text{g/g}$ creatinine), compared with several other regions. One hypothesis is that participants from the Lisbon area consume fewer TeA-contributing foods or obtain these products more often through large retailers with centralized sourcing and quality control, and quicker storage turnover, potentially reducing contamination. Pairwise analyses revealed statistically significant differences between the Lisbon Metropolitan Area and Central Portugal ($p = 0.003$), the Lisbon Metropolitan Area and Alentejo ($p = 0.008$), the Lisbon Metropolitan Area and the Azores ($p = 0.010$), and between Northern Portugal and Central Portugal ($p = 0.031$). In addition, borderline differences were observed between Northern Portugal and Alentejo ($p = 0.072$), the Lisbon Metropolitan Area and Madeira ($p = 0.073$), and Northern Portugal and the Azores ($p = 0.092$). [Fig. 2](#) (right) shows the spatial distribution of creatinine-adjusted TeA concentrations across Portuguese NUTS II regions.

Concerning the occupational status of the participants, although higher TeA levels were observed among non-working individuals, this difference did not reach statistical significance ($p = 0.518$).

Results from a multiple linear regression analysis for free-DON concentrations adjusted for creatinine are presented in [Table 6](#). No significant differences were observed by sex ($p = 0.117$) or age ($p = 0.509$). The sampling season was also not significantly associated with the outcome, although a borderline higher estimate was observed for

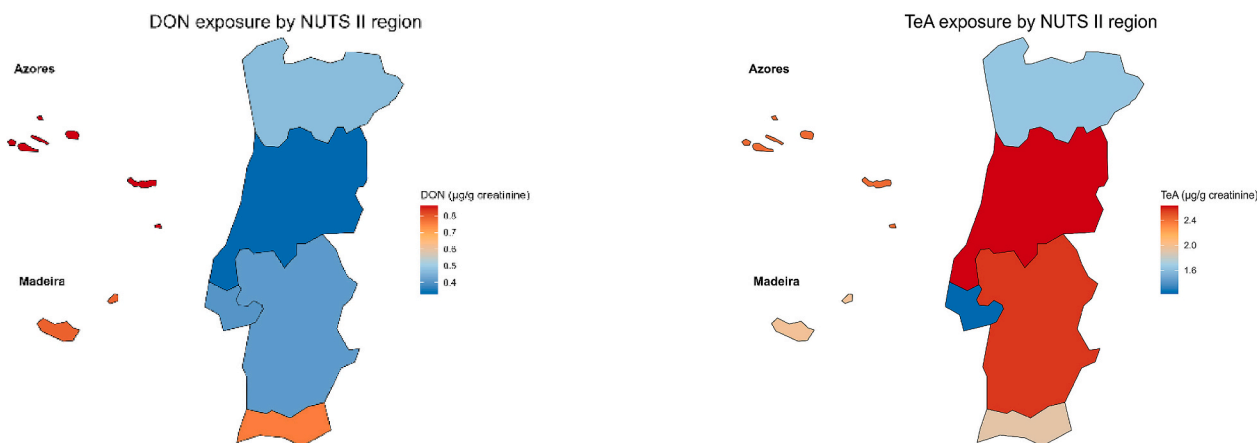


Fig. 2. Spatial distribution of urinary exposure across Portuguese NUTS II regions. The left panel shows regional geometric mean (GM) concentrations of free-deoxynivalenol (DON), and the right panel shows regional GM concentrations of tenuazonic acid (TeA) ($\mu\text{g/g}$ creatinine). Colours indicate increasing exposure levels. Mainland Portugal is shown together with the autonomous regions of the Azores and Madeira. NUTS II boundaries were obtained from Eurostat.

Table 5
Distribution of TeA in urine in µg/L and µg/g creatinine per sociodemographic variables.

Variable	Category	TeA in urine (µg/L)				TeA in urine (µg/g creatinine)			
		n	GM [95%CI]	P95 [95%CI]	p	n	GM [95%CI]	P95 [95%CI]	p
Sampling season	Winter	52	2.06 [1.60–3.15]	14.09 [7.59–32.27]	0.272 ^a	52	1.69 [1.34–2.60]	14.63 [6.34–16.45]	0.073 ^a
	Spring	16	2.94 [1.71–4.14]	- [-]		16	2.56 [1.55–3.67]	- [-]	
	Summer	152	2.62 [2.24–3.16]	14.12 [11.39–18.51]		152	2.38 [2.05–2.84]	13.70 [9.43–16.65]	
	Autumn	75	2.17 [1.56–3.06]	23.26 [17.68–51.24]		75	1.80 [1.33–2.55]	16.97 [11.69–76.03]	
Sex	Female	171	2.00 [1.66–2.40]	14.87 [11.58–18.17]	0.002 ^b	171	1.93 [1.62–2.32]	15.12 [10.43–16.67]	0.147 ^b
	Male	124	3.11 [2.77–4.06]	16.66 [12.21–32.27]		124	2.34 [2.10–3.06]	16.09 [8.03–16.47]	
Educational level	Low (ISCED 0–2)	59	3.90 [2.93–5.20]	22.72 [16.07–31.78]	<0.001 ^a	59	3.15 [2.39–4.14]	16.09 [14.33–17.20]	0.001 ^a
	Medium (ISCED 3–4)	107	2.46 [1.97–3.19]	20.71 [13.40–28.50]		107	2.13 [1.75–2.78]	16.25 [9.30–18.79]	
	High (ISCED ≥5)	129	1.90 [1.60–2.36]	12.77 [7.93–17.68]		129	1.71 [1.46–2.16]	10.94 [7.20–16.65]	
	Cities	80	2.24 [1.76–3.07]	16.99 [10.08–26.53]		80	1.78 [1.41–2.47]	15.50 [8.63–16.45]	
Degree of urbanization	Towns/suburbs	109	2.30 [1.90–2.97]	13.73 [9.54–40.18]	0.496 ^a	109	1.99 [1.69–2.59]	10.59 [7.09–30.62]	0.147 ^a
	Rural areas	106	2.67 [2.12–3.34]	19.89 [12.30–25.86]		106	2.50 [2.02–3.08]	16.43 [13.86–17.59]	
	North (PT11)	46	1.94 [1.26–3.32]	23.86 [17.51–34.47]		46	1.64 [1.12–2.77]	16.43 [12.11–18.84]	
NUT2 region	Algarve (PT15)	30	2.49 [1.67–3.30]	12.57 [9.94–13.46]	0.202 ^a	30	1.91 [1.32–2.52]	8.84 [7.08–9.99]	0.039 ^a
	Centre (PT16)	49	2.84 [2.29–3.53]	11.53 [7.90–10.20]		49	2.63 [2.12–3.27]	14.25 [6.22–17.73]	
	Lisbon and Tagus Valley (PT17)	25	1.64 [1.13–2.70]	14.07 [5.39–15.27]		25	1.22 [0.82–2.03]	11.08 [4.99–12.18]	
	Alentejo (PT18)	47	3.00 [2.10–4.05]	24.19 [8.33–90.91]		47	2.56 [1.84–3.49]	15.32 [7.72–114.15]	
	Azores (PT20)	62	2.67 [2.05–3.88]	17.99 [11.72–41.36]		62	2.42 [1.91–3.52]	16.82 [9.70–48.52]	
	Madeira (PT30)	36	2.03 [1.30–3.40]	21.10 [9.06–32.27]		36	1.98 [1.32–3.22]	15.75 [10.65–16.45]	
	Working	257	2.44 [2.11–2.81]	16.90 [13.41–20.20]		257	2.13 [1.85–2.45]	15.82 [10.76–16.47]	
	Not working	20	2.78 [1.72–4.50]	25.92 [8.79–26.81]		20	2.60 [1.65–4.09]	15.77 [11.63–15.98]	

TeA: tenuazonic acid; GM: Geometric mean; 95% CI: 95% Confidence Interval; P95: percentile 95; ^a: Kruskal-Wallis Test; ^b: Mann-Whitney Test.

spring ($\beta = 1.489$ [95%CI: 0.894–11.142]; $p = 0.074$) compared to winter, in accordance with the results from the univariate analysis. Educational level showed a significant association, with individuals with medium education presenting higher levels compared to the high education reference group ($\beta = 1.478$ [95%CI: 1.143–2.554]; $p = 0.009$), while no significant differences were observed for low education. Degree of urbanization and occupational status were not significantly associated with the free-DON concentrations. Regarding regional variation, significantly higher levels were observed among participants from the Azores ($\beta = 2.021$ [95%CI: 1.734–5.515]; $p < 0.001$) and Madeira ($\beta = 1.685$ [95%CI: 1.438–5.695]; $p = 0.003$) compared to the Centre reference region in accordance with the results from the univariate analysis, whereas no statistically significant differences were found for the remaining regions. Overall, results confirmed that the geographic region and the educational attainment were the primary sociodemographic predictors of the free-DON concentrations.

Results from the multiple linear regression analysis for the TeA concentrations adjusted to creatinine are presented in Table 7. No significant differences were observed according to sex ($p = 0.147$) or age ($p = 0.596$). Similarly, sampling season did not show a significant association, although higher estimates were observed for spring ($\beta = 1.345$ [95%CI: 0.727–4.897]; $p = 0.191$) compared to winter (reference category). Educational level emerged as one for the only significant

predictors of TeA concentrations, with individuals with low education showing significantly higher levels compared to the high education group ($\beta = 1.511$ [95%CI: 1.131–2.347]; $p = 0.009$), while no significant differences were found for medium education. No significant associations were identified for degree of urbanization or occupational status. Regarding regional differences, participants from the Algarve presented significantly lower levels compared to the Centre reference region ($\beta = 0.629$ [95%CI: 0.243–0.945]; $p = 0.034$), while a borderline association was observed for Lisbon and Tagus Valley ($\beta = 0.683$ [95%CI: 0.256–1.021]; $p = 0.057$). No significant differences were observed for the remaining regions.

4. Conclusions

The present results confirmed mycotoxins as part of the human exposome of the Portuguese population reinforcing the need for further studies regarding the characterization of the other determinants of exposure and the assessment of the risk associated with this exposure. Co-occurrence of several mycotoxins was observed in the analysed samples, pointing also to the need to assess the effects of combined exposure.

The observed concentrations of urinary free-DON and TeA were within the same order of magnitude as the concentrations reported in

Table 6

Adjusted multivariable regression model for sociodemographic variables associated with free-DON concentrations in urine adjusted for creatinine ($\mu\text{g/g}$ creatinine) ($n = 277$).

Variable	Category	Adjusted coef [95%CI]	p
Sex	Female	Ref	–
	Male	1.252 [0.927–1.962]	0.117
Age (years)	–	0.911 [0.929–1.038]	0.509
	Winter	Ref	–
Sampling season	Spring	1.489 [0.894–11.142]	0.074
	Summer	1.257 [0.695–2.619]	0.375
	Autumn	1.316 [0.800–2.839]	0.203
Educational level	Low	1.192 [0.817–2.144]	0.254
	Medium	1.478 [1.143–2.554]	0.009
	High	Ref	–
Degree of urbanization	Towns/suburbs	Ref	–
	Cities	1.236 [0.804–2.312]	0.249
	Rural areas	1.136 [0.768–1.838]	0.436
	North	1.251 [0.716–3.156]	0.281
	Algarve	1.235 [0.640–3.848]	0.323
NUTS2 region	Centre	Ref	–
	Lisbon and Tagus	1.163 [0.573–3.568]	0.441
	Valley	1.322 [0.896–3.050]	0.107
	Alentejo	2.021 [1.734–5.515]	<0.001
	Azores	1.685 [1.438–5.695]	0.003
Occupational Status	Madeira	Ref	–
	Working	1.119 [0.666–2.652]	0.418
	Not working		

Ref: reference category. $R^2 = 0.126$; $p = 0.003$.

other recent European studies. Exposure levels were higher among the individuals with lower educational background and during warm seasons (summer and spring).

The observed temporal variations in mycotoxin levels, warrant continued monitoring, particularly under climate change scenarios that might contribute to the increase in mycotoxins levels in the environment and subsequently to the increase in human exposure. Altogether, these findings highlight the importance of continued surveillance and the integration of HBM into national food safety and public health strategies to allow for a more comprehensive risk assessment and potential mitigation strategies.

CRedit authorship contribution statement

Elias Maris: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sónia Namorado:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review and editing. **An Chen:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Roger Pero-Gascon:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Marthe De Boevre:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Sarah De Saeger:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Maria João Silva:** Conceptualization, Funding acquisition, Writing – review & editing. **Paula Alvito:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Ethical statement

This study used data and samples collected in the INSEF-ExpoQuim survey that was conducted in accordance with the Declaration of Helsinki. The INSEF-ExpoQuim survey was approved by the Ethical Committees of the National Institute of Health Doutor Ricardo Jorge (12.02.2019), of the Regional Health Administrations of North (T1061; 02.04.2019), Center (63/2019; 24.06.2019), Lisbon and Tagus Valley

Table 7

Adjusted multivariable regression model for sociodemographic variables associated with TeA concentrations in urine adjusted for creatinine ($\mu\text{g/g}$ creatinine) ($n = 277$).

Variable	Category	Adjusted coef [95%CI]	p
Sex	Female	Ref	–
	Male	1.236 [0.928–1.636]	0.147
Age (years)	–	0.926 [0.948–1.031]	0.596
	Winter	Ref	–
	Spring	1.345 [0.727–4.897]	0.191
Sampling season	Summer	0.894 [0.543–1.481]	0.669
	Autumn	0.928 [0.570–1.485]	0.732
Educational level	Low	1.511 [1.131–2.347]	0.009
	Medium	1.157 [0.856–1.573]	0.336
	High	Ref	–
Degree of urbanization	Towns/suburbs	Ref	–
	Cities	1.114 [0.754–1.677]	0.563
	Rural areas	1.145 [0.824–1.593]	0.417
	North	0.759 [0.393–1.207]	0.192
	Algarve	0.629 [0.243–0.945]	0.034
NUTS2 region	Centre	Ref	–
	Lisbon and Tagus	0.683 [0.256–1.021]	0.057
	Valley	1.024 [0.650–1.640]	0.893
	Alentejo	0.968 [0.621–1.489]	0.860
	Azores	0.844 [0.461–1.305]	0.337
Occupational Status	Madeira	Ref	–
	Working	1.108 [0.719–2.044]	0.468
	Not working		

Ref: reference category. $R^2 = 0.093$; $p = 0.052$.

(027/CES/INV/2019; 06.08.2019), Alentejo (14/2019/CE; 15.05.2019) and Algarve (07/2019; 11.04.2019), of the Health Service of the Autonomous Region of Madeira (19/2019; 17.06.2019) and of the Hospital of Horta (19.03.2019).

Funding

The INSEF-ExpoQuim study was supported under the HBM4EU project that was co-funded by the European Union's research and innovation programme Horizon 2020 under grant agreement No. 733032 and by the participating countries through the participating organizations.

The present study was supported by INSA, Ghent University and by national funds through FCT – Fundação para a Ciência e a Tecnologia, I. P., under the projects CESAM - Centro de Estudos do Ambiente e do Mar, references UID/50017/2025 (doi.org/10.54499/UID/50017/2025) and LA/P/0094/2020 (doi.org/10.54499/LA/P/0094/2020), and CHRC – Comprehensive Health Research Center, reference UID/06291/2025 (<https://doi.org/10.54499/UID/06291/2025>).

Elias Maris is supported by an iBOF project from Flanders (grant number 01IB1320). An Chen is supported by an FWO (Research Foundation - Flanders) PhD Fellowship fundamental research (11A0725N). Roger Pero-Gascon is supported by an FWO Junior postdoctoral fellowship (12D4423N). Marthe De Boevre is supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 946192,

HUMYCO).

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.

Acknowledgements

The authors would like to thank the participants and the members of the fieldwork teams from the INSEF-ExpoQuim study. The authors would also like to thank the database managers that made the information available to HBM4EU, the laboratories who performed the analytical measurements and the HBM4EU project partners involved in WP7 and in WP9 for developing all the supporting materials and for organizing the QA/QC scheme under HBM4EU, respectively. The graphical abstract was created using BioRender.com.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2026.119820>.

Data availability

The authors do not have permission to share data.

References

- Alvito, P., Assunção, R. M., Bajard, L., Martins, C., Mengelers, M. J. B., Mol, H., ... Silva, M. J. (2022). Current advances, research needs and gaps in mycotoxins biomonitoring under the HBM4EU—Lessons learned and future trends. *Toxins*, 14 (12), 826. <https://doi.org/10.3390/TOXINS14120826>
- Alvito, P., Barcelo, J., De Meester, J., Rito, E., & Suman, M. (2021). Mitigation of mycotoxins during food processing: Sharing experience among Europe and South East Asia. *Science and Technology of Cereals, Oils and Foods*, 2021(6), 59–70. doi:10.16210/j.cnki.1007-7561.2021.06.004.en.
- Arcella, D., Eskola, M., & Gómez Ruiz, J. A. (2016). Dietary exposure assessment to Alternaria toxins in the European population. *EFSA Journal*, 14(12), Article e04654. <https://doi.org/10.2903/J.EFSA.2016.4654>
- Arce-López, B., Lizarraaga, E., Vettorazzi, A., & González-Peñas, E. (2020). Human biomonitoring of mycotoxins in blood, plasma and serum in recent years: A review. *Toxins*, 12(3), 147. <https://doi.org/10.3390/TOXINS12030147>
- Asam, S., Habler, K., & Rychlik, M. (2013). Determination of tenuazonic acid in human urine by means of a stable isotope dilution assay. *Analytical and Bioanalytical Chemistry* 2013 405:12, 405(12), 4149–4158. doi:<https://doi.org/10.1007/s00216-013-6793-5>.
- Bennett, J. W., & Klich, M. (2003). Mycotoxins. *In clinical microbiology reviews*, 16(3), 497–516. <https://doi.org/10.1128/CMR.16.3.497-516.2003>
- van den Brand, A. D., Bokkers, B. G. H., te Biesebeek, J. D., & Mengelers, M. J. B. (2022). Combined exposure to multiple mycotoxins: An example of using a tiered approach in a mixture risk assessment. *Toxins*, 14(5), 303. <https://doi.org/10.3390/toxins14050303>
- European Commission (2022). Commission Recommendation (EU) 2022/553 of 5 April 2022 on Monitoring the Presence of Alternaria Toxins in Food, Official Journal of the European Union, L 107, 06.04.2022, pp. 2–5.
- European Commission (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food. Official Journal of the European Union, L 119, 05.05.2023, pp. 103–157.
- European Commission (2024). Commission Regulation (EU) 2024/1038 of 9 April 2024 amending Regulation (EU) 2023/915 as regards maximum levels of T-2 and HT-2 toxins in food. Official Journal of the European Union, L, 10.04.2024, 2024/1038.
- De Ruyck, K., Huybrechts, I., Yang, S., Arcella, D., Claeys, L., Abbeduto, S., De Keyser, W., De Vries, J., Ocke, M., Ruprich, J., De Boevre, M., & De Saeger, S. (2020). Mycotoxin exposure assessments in a multi-center European validation study by 24-hour dietary recall and biological fluid sampling. *Environment International*, 137, Article 105539. <https://doi.org/10.1016/J.ENVINT.2020.105539>
- De Santis, B., Debegnach, F., Miano, B., Moretti, G., Sonogo, E., Chiaretti, A., Buonsenso, D., & Brera, C. (2019). Determination of deoxynivalenol biomarkers in Italian urine samples. *Toxins* 2019, Vol. 11, page 441, 11(8), 441. doi:<https://doi.org/10.3390/TOXINS11080441>.
- Degen, G. H., Reinders, J., Kraft, M., Völkel, W., Gerull, F., Burghardt, R., ... Fromme, H. (2023). Citrinin exposure in Germany: Urine biomarker analysis in children and adults. *Toxins*, 15(1). <https://doi.org/10.3390/toxins15010026>
- Delgado-Povedano, M. D. M., Maris, E., Kellner, N., Mulisa, G., Gámiz-Gracia, L., García-Campaña, A. M., ... Pero-Gascon, R. (2025). Liquid chromatography-tandem mass spectrometry for the determination of multiple mycotoxins in serum through suspect screening and targeted approaches: Advancing human mycotoxin biomonitoring. *Microchemical Journal*, 208, Article 112562. <https://doi.org/10.1016/J.MICROC.2024.112562>
- EFSA Panel on Contaminants in the Food Chain (CONTAM). (2011). Scientific opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food. *EFSA Journal*, 9(10). <https://doi.org/10.2903/j.efsa.2011.2407>
- EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., ... Edler, L. (2017). Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA Journal*, 15(9), Article e04718. <https://doi.org/10.2903/J.EFSA.2017.4718>
- Eriksen, G. S., Knutsen, H. K., Sandvik, M., & Brantsæter, A. L. (2021). Urinary deoxynivalenol as a biomarker of exposure in different age, life stage and dietary practice population groups. *Environment International*, 157, Article 106804. <https://doi.org/10.1016/j.envint.2021.106804>
- Esteban López, M. C. A. (2018). Annex 2.2.3 to D7.3 – SOP3: Procedure for obtaining human samples.
- European Medicines Agency (EMA). (2022). *ICH guideline M10 on bioanalytical method validation and study sample analysis*. EMA/CHMP/ICH/172948/2019.
- Gallardo-Ramos, J. A., Marín-Sáez, J., Sanchis, V., Gámiz-Gracia, L., García-Campaña, A. M., Hernández-Mesa, M., & Cano-Sancho, G. (2024). Simultaneous detection of mycotoxins and pesticides in human urine samples: A 24-h diet intervention study comparing conventional and organic diets in Spain. *Food and Chemical Toxicology*, 188, Article 114650. <https://doi.org/10.1016/J.FCT.2024.114650>
- Gilles, L., Govarts, E., Martin, L. R., Andersson, A. M., Appenzeller, B. M. R., Barbone, F., ... Schoeters, G. (2022). Harmonization of human biomonitoring studies in Europe: Characteristics of the HBM4EU-aligned studies participants. *International Journal of Environmental Research and Public Health*, 19(11). <https://doi.org/10.3390/ijerph19116787>
- Govarts, E., Gilles, L., Rodriguez Martin, L., Santonen, T., Apel, P., Alvito, P., ... Schoeters, G. (2023). Harmonized human biomonitoring in European children, teenagers and adults: EU-wide exposure data of 11 chemical substance groups from the HBM4EU aligned studies (2014–2021). *International Journal of Hygiene and Environmental Health*, 249. <https://doi.org/10.1016/j.ijheh.2023.114119>. Elsevier GmbH.
- Gruber-Dorninger, C., Novak, B., Nagl, V., & Berthiller, F. (2017). Emerging mycotoxins: Beyond traditionally determined Food Contaminants. *Journal of Agricultural and Food Chemistry*, 65(33), 7052–7070. <https://doi.org/10.1021/acs.jafc.6b03413>
- Guo, X., Wang, S., Liang, J., & Xu, M. (2025). Mitochondrial damage and associated combined toxicity induced by deoxynivalenol and Alternaria toxins co-exposure. *Food Chemistry*, 465. <https://doi.org/10.1016/j.foodchem.2024.142064>
- Heyndrickx, E., Sioen, I., Huybrechts, B., Callebaut, A., De Henaauw, S., & De Saeger, S. (2015). Human biomonitoring of multiple mycotoxins in the Belgian population: Results of the BIOMYCO study. *Environment International*, 84, 82–89. <https://doi.org/10.1016/j.envint.2015.06.011>
- International Agency for Research on Cancer (IARC). (1993). *Some naturally occurring substances: Food items and constituents, Heterocyclic Aromatic Amines and Mycotoxins*. International Agency for Research on Cancer (IARC). (2012). In J. I. Pitt, C. P. Wild, R. A. Baan, W. C. Gelderblom, J. D. Miller, R. T. Riley, & F. W. N (Eds.), *Improving Public Health Through Mycotoxin Control, IARC Scientific Publication N.158*.
- Karsauliya, K., Yahavi, C., Pandey, A., Bhatia, M., Sonker, A. K., Pandey, H., Sharma, M., & Singh, S. P. (2022 Oct). Co-occurrence of mycotoxins: A review on bioanalytical methods for simultaneous analysis in human biological samples, mixture toxicity and risk assessment strategies. *Toxicol.*, 30(218), 25–39. <https://doi.org/10.1016/j.toxicol.2022.08.016>
- Le Hégarat, L., Takakura, N., Simar, S., Nesslany, F., & Fessard, V. (2014). The in vivo genotoxicity studies on nivalenol and deoxynivalenol. *EFSA Supporting Publications*, 11(11), 697E. <https://doi.org/10.2903/sp.efsa.2014.en-697>
- Louro, H., Vettorazzi, A., López de Cerain, A., Spyropoulou, A., Solhaug, A., Straumfors, A., ... Marko, D. (2023). Hazard characterization of Alternaria toxins to identify data gaps and improve risk assessment for human health. *Archives of Toxicology*, 98(2), 425–469. <https://doi.org/10.1007/s00204-023-03636-8>, 2024.
- Maris, E., Ndiangamandla, P., Adelusi, O. A., Akinmoladun, O. F., Odukoya, J. O., Fagbohun, R. T., ... De Saeger, S. (2025). Nixtamalization of maize to reduce mycotoxin exposure: A human biomonitoring intervention study in Soweto, South Africa. *Toxins*, 17(11), Article 527. <https://doi.org/10.3390/TOXINS17110527>
- Martins, C., Assunção, R., Cunha, S. C., Fernandes, J. O., Jager, A., Petta, T., ... Alvito, P. (2018). Assessment of multiple mycotoxins in breakfast cereals available in the Portuguese market. *Food Chemistry*, 239, 132–140. <https://doi.org/10.1016/J.FOODCHEM.2017.06.088>
- Martins, C., Assunção, R., Nunes, C., Torres, D., & Alvito, P. (2021). Are data from mycotoxins' urinary biomarkers and Food surveys linked? A review underneath risk assessment. *Food Reviews International*, 37(4), 373–398. <https://doi.org/10.1080/87559129.2019.1709200>
- Martins, C., Torres, D., Lopes, C., Correia, D., Goios, A., Assunção, R., Alvito, P., Vidal, A., De Boevre, M., De Saeger, S., & Nunes, C. (2021). Deoxynivalenol exposure assessment through a modelling approach of food intake and biomonitoring data – A contribution to the risk assessment of an enteropathogenic mycotoxin. *Food Research International*, 140, Article 109863. <https://doi.org/10.1016/J.FOODRES.2020.109863>
- Martins, C., Vidal, A., De Boevre, M., De Saeger, S., Nunes, C., Torres, D., Goios, A., Lopes, C., Assunção, R., & Alvito, P. (2019). Exposure assessment of Portuguese population to multiple mycotoxins: The human biomonitoring approach.

- International Journal of Hygiene and Environmental Health*, 222(6), 913–925. <https://doi.org/10.1016/j.ijheh.2019.06.010>
- Martins, C., Viegas, C., Eriksen, E., Graff, P., Afanou, A. K., Straumfors, A., ... Viegas, S. (2025). Unraveling the occupational exposure to mycotoxins in a waste management setting: Results from a case study in Norway. *Frontiers in Public Health*, 13. <https://doi.org/10.3389/fpubh.2025.1536836>
- Mengeler, M. J. B., van den Brand, A. D., Zhao, S., Hoogenveen, R., & Ougier, E. (2024). Human biomonitoring guidance values for Deoxynivalenol derived under the European human biomonitoring initiative (HBM4EU). *Toxins*, 16(3). <https://doi.org/10.3390/TOXINS16030139>
- Morrens, B., Den Hond, E., Schoeters, G., Coertjens, D., Colles, A., Nawrot, T. S., Baeyens, W., De Henauw, S., Nelen, V., & Loots, I. (2017). Human biomonitoring from an environmental justice perspective: Supporting study participation of women of Turkish and Moroccan descent. *Environmental health* 2017 16:1, 16(1), 48-. doi:<https://doi.org/10.1186/S12940-017-0260-2>
- Namorado, S., Martins, C., Ogura, J., Assunção, R., Vasco, E., Appenzeller, B., I Halldorsson, T., Janasik, B., Kolossa-Gehring, M., Van Nieuwenhuysse, A., Ólafsdóttir, K., Rambaud, L., Riou, M., Silva, S., Wasowicz, W., Weber, T., Esteban-López, M., Castaño, A., Gilles, L., ... Alvito, P. (2024). Exposure assessment of the European adult population to deoxynivalenol – Results from the HBM4EU aligned studies. *Food Research International*, 198, 115281. doi:<https://doi.org/10.1016/j.foodres.2024.115281>
- Nešić, K., Habschied, K., & Mastanović, K. (2023). Modified mycotoxins and multitoxin contamination of Food and feed as major analytical challenges. *Toxins*, 15(8). <https://doi.org/10.3390/toxins15080511>
- Nunes, B., Barreto, M., Gil, A. P., Kislaya, I., Namorado, S., Antunes, L., ... Dias, C. M. (2019). The first Portuguese National Health Examination Survey (2015): Design, planning and implementation. *Journal of Public Health (Oxford, England)*, 41(3), 511–517. <https://doi.org/10.1093/pubmed/fdy150>
- OECD. (2019). *OECD economic surveys: Portugal 2019. OECD Economic Surveys: Portugal, OECD Economic Surveys: Portugal*. https://doi.org/10.1787/ECO_SURVEYS-PRT-2019-EN
- Payros, D., Dobrindt, U., Martin, P., Secher, T., Bracarense, A. P. F. L., Boury, M., ... Oswald, I. P. (2017). The Food contaminant Deoxynivalenol exacerbates the genotoxicity of gut microbiota. *MBio*, 8(2). <https://doi.org/10.1128/MBIO.00007-17>
- Peris-Camarasa, B., Coscollà, C., Dualde, P., & Pardo, O. (2025). Urinary biomonitoring of mycotoxins in Spanish adults: Predictors of exposure and health risk evaluation. *Toxics*, 13(10), Article 856. <https://doi.org/10.3390/toxics13100856>
- Peris-Camarasa, B., Pardo, O., Dualde, P., & Coscollà, C. (2025). Multi-mycotoxin determination in human urine by UHPLC-MS/MS: An environmentally friendly and high-throughput approach. *Journal of Chromatography A*, 1760, Article 466317. <https://doi.org/10.1016/J.CHROMA.2025.466317>
- Puntscher, H., Hankele, S., Tillmann, K., Attakpah, E., Braun, D., Kütt, M. L., ... Warth, B. (2019). First insights into Alternaria multi-toxin in vivo metabolism. *Toxicology Letters*, 301, 168–178. <https://doi.org/10.1016/j.toxlet.2018.10.006>
- Schmidt, J., Cramer, B., Turner, P. C., Stoltzfus, R. J., Humphrey, J. H., Smith, L. E., & Humpf, H. U. (2021). Determination of urinary mycotoxin biomarkers using a sensitive online solid phase extraction-UHPLC-MS/MS method. *Toxins*, 13(6), 418. <https://doi.org/10.3390/TOXINS13060418/S1>
- European Parliament and Council of the European Union (2003). Regulation (EC)No 1059/2003 of the European Parliament and of the council of 26 may 2003 on the establishment of a common classification of territorial units for statistics (NUTS). Official Journal of the European Union, L 154, 21.06.2003, pp. 1–41.
- Schmidt, J., Lindemann, V., Olsen, M., Cramer, B., & Humpf, H. U. (2021). Dried urine spots as sampling technique for multi-mycotoxin analysis in human urine. *Mycotoxin Research* 2021 37:2, 37(2), 129–140. doi:<https://doi.org/10.1007/S12550-021-00423-1>
- UNESCO Institute for Statistics, international standard classification of education - ISCED (2011).
- Vidal, A., Claeys, L., Mengelers, M., Vanhoorne, V., Vervaeke, C., Huybrechts, B., De Saeger, S., & De Boevre, M. (2018). Humans significantly metabolize and excrete the mycotoxin deoxynivalenol and its modified form deoxynivalenol-3-glucoside within 24 hours. *Scientific Reports*, 8(1), 5255. <https://doi.org/10.1038/s41598-018-23526-9>
- Vidal, A., Mengelers, M., Yang, S., De Saeger, S., & De Boevre, M. (2018). Mycotoxin biomarkers of exposure: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 17(5), 1127–1155. <https://doi.org/10.1111/1541-4337.12367>
- Visintin, L., García Nicolás, M., Vangeenderhuysen, P., Goessens, T., Alladio, E., Pomian, B., Vanhaecke, L., De Saeger, S., & De Boevre, M. (2023). Unraveling biomarkers of exposure for tenuazonic acid through urinary metabolomics. *Food and Chemical Toxicology*, 182, Article 114183. <https://doi.org/10.1016/J.FCT.2023.114183>
- Visintin, L., Lu, E. H., Lin, H. C., Bader, Y., Nguyen, T. N., Michailidis, T. M., De Saeger, S., Chiu, W. A., & De Boevre, M. (2025). Derivation of human toxicokinetic parameters and internal threshold of toxicological concern for tenuazonic acid through a human intervention trial and hierarchical Bayesian population modeling. *Journal of Exposure Science & Environmental Epidemiology* 2025 35:4, 35(4), 632–643. doi:<https://doi.org/10.1038/s41370-025-00746-6>
- Wallin, S., Gambacorta, L., Kotova, N., Warensjö Lemming, E., Nälsson, C., Solfrizzo, M., & Olsen, M. (2015). Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis. *Food and Chemical Toxicology*, 83, 133–139. <https://doi.org/10.1016/J.FCT.2015.05.023>
- Zingales, V., Esposito, M. R., Quagliata, M., Cimetta, E., & Ruiz, M. J. (2024). Cytotoxic effects induced by combined exposure to the mycotoxins sterigmatocystin, ochratoxin A and patulin on human tumour and healthy 3D spheroids. *Food and Chemical Toxicology*, 192, Article 114951. <https://doi.org/10.1016/j.fct.2024.114951>