Supporting Information Document

Prevalence and Health Risk Evaluations of Mycotoxins in Drinking Water Sources in Nigeria

Daniel T. Koko[1,2], Moses O. Alfred[1,2], Nathaniel B. Bolujoko[1,2], Damilare Olorunnisola[1,2], Oluwaferanmi B. Otitoju[1,2], Olumuyiwa O. Ogunlaja[1,3], Friday Okonofua[4,5,6], Akhere Omonkua[5], Titus A. M. Msagati[7], Martins O. Omorogie[1,2], Aemere Ogunlaja[1,8], Olumide D. Olukanni[1,9], Emmanuel I. Unuabonah[1,2*]

[1]*African Centre of Excellence for Water and Environmental Research (ACEWATER), Redeemer's University, P.M.B 230, Ede, 232101, Osun State, Nigeria*

[2]*Department of Chemical Sciences, Faculty of Natural Sciences, Redeemer's University, P.M.B 230, Ede, Osun State, Nigeria*

[3]*Department of Chemical Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria*

[4]*Women's Health and Action Research Centre (WHARC), Km 11 Benin-Lagos Expressway, Igue-Iheya, Benin City, Edo State, Nigeria*

[5]*Centre of Excellence in Reproductive Health Innovation, University of Benin, Benin City, Nigeria*

[6]*Department of Obstetrics and Gynaecology, University of Benin and University of Benin Teaching Hospital, Benin City, Nigeria*

[7]Institute for *Nanotechnology and Water Sustainability, College of Science, Engineering and Technology, University of South Africa, South Africa, The Science Campus, 1709, Roodepoort, Johannesburg, South Africa*

[8]*Department of Biological Sciences, Faculty of Natural Sciences, Redeemer's University, P.M.B 230, Ede, Osun State, Nigeria*

[9]*Department of Biochemistry, Faculty of Basic Medical Sciences, Redeemer's University, Ede, Nigeria*

***Corresponding Author**

Unuabonah E. I. unuabonahe@run.edu.ng

S1: Quality Assurance and Quality Control (QA/QC)

Blank samples were prepared using UHP Millipore water and were used to check for possible contamination and interferences from solvents and materials used in the extraction process. This was done with every batch of samples extracted. The concentration obtained from the blank samples was corrected by being subtracted from the known concentration of the environmental samples. After each batch analysis, methanol (as blank) was injected to check for discrepancies in instrumental response and carry-over of the analytes of interest from prior injections. Quantification of analytes was carried out using the mycotoxin standards, and a standard calibration curve was obtained by analyzing aqueous solutions containing the analytes of interest ranging from a concentration of 1 to 1000 µg/L.

The instrument limit of detection (LOD) and limit of quantification (LOQ) were evaluated at three and ten times the signal-to-noise ratio respectively using the standard deviation of 8-point calibration intercepts divided by the slope. The LOD of the targeted mycotoxins obtained lies between 0.16 to 0.62 µg/L, while the LOQ ranged from 0.48 to 1.84 µg/L, and the coefficient of linearity (R^2) of calibration curves was obtained to be 0.9999 for DON, 0.9986 for OTA and 0.9999 for ZEN which are presented in **Table 1 (main manuscript)**.

S2: Recovery and Precision Method

The recovery of the samples was carried out by spiking 200 mL of the samples with each of the three mycotoxins (DON, OTA and ZEN) prior to SPE procedure in order to acquire concentration of 50, 500 and 1000 µg L^{-1} for each of them. Samples were prepared as duplicates, and the absolute method recovery was determined for all the compounds (Mata et al., 2015). For instrument precision, the three concentrations were considered as spiked concentrations of each mycotoxin repeated five times, carrying out assays (n = 5). The % recovery was calculated using the provided

equation for all three mycotoxins, while precision was evaluated and presented as the relative standard deviation.

Recovery of target analytes was achieved by repeating the described method for Millipore water and Millipore water spiked with 50 µg/L of the analytes. The percentage recovery (%R) of the mycotoxins was calculated using the equation:

$$\%R = \frac{n_{spiked} - n_{unspiked}}{real\ concentration} \times 100 \quad \text{(S1)}$$

Where n_{spiked} is the concentration (µg/L) of mycotoxin obtained for spiked millipore water, $n_{unspiked}$ is the concentration (µg/L) of mycotoxin in Millipore water (0 µg/L), and real concentration is the concentration of mycotoxin (µg/L) used for spiking the Millipore water.

S3: Instrumental Analysis

A Dionex Ultimate 3000 UHPLC system (Dionex Softron GmbH, Dornierstr. 4, Bayerm, Germany), equipped with a binary pump, an online degasser, a column oven and an autosampler, fitted with a reversed-phase C18 analytical column of 100 mm x 2.1 mm x 1.7 mm (Acquity UPLC® BEH, Waters, Ireland) was used to separate the analytes. The column was kept at a constant 35^{o}C temperature. The volume of the injected sample was 5 µL. Water and methanol with 0.1% formic acid served as the mobile phases A and B, respectively. The optimized mobile phase gradient elution was programmed as follows: the starting mobile phase composition (2% B) was held constant for 1 min, followed by a linear gradient from 2% B to 100% B for 9 min, then 100% B was maintained for 2 min, and finally dropped back to 2% B within 12.1 min, and kept constant at 2% B for 2 min. The total elution time was 14 min, with a flow rate of 0.3 mL/min. The Dionex ultimate 3000 ultrahigh performance liquid chromatography (UHPLC) (Thermo Scientific,

Massachusetts, United States) coupled to a high-resolution quadrupole-time-of-flight mass spectrometer (Impact II) system (Bruker Daltonics GmbH Fahrenheitstr. 4, Bremen, Germany), was used for the separation and the detection of analytes. The electrospray ionization source was operated in both positive and negative ion modes. The high-resolution mass spectra were recorded at an ionization potential of 50 eV. The software Bruker Compass Data Analysis 4.3 was used to process the captured data.

S4: Risk Assessment (RQ)

The RQ was determined using the following mathematical equations as follows:

$$RQ = \frac{MEC}{PNEC} \quad \text{(S2)}$$

where RQ is the risk quotient; PNEC is the predicted no-effect concentration of compounds (µg/L) and MEC is the measured environmental concentration of the compounds in water samples (µg/L). The PNEC is calculated for both acute and chronic tests using the EC_{50}/LC_{50} and NOEC respectively, divided by an assessment factor (AF) (Otitoju et al., 2023)

$PNEC_{acute}$ = (EC_{50}/LC_{50} of three acute toxicity tests)/AF_{acute} (S3)

where EC_{50}/LC_{50} is the median effect/lethal concentration.

The toxicity data (LC_{50} or EC_{50}) used in the current study for ecological risk assessment of DON, OTA, and ZEN in water samples were obtained from existing literature for vertebrates (Fish). The EC_{50} values are as follows: DON is 0.20 mg/L (200 µg/L) (Eagles et al., 2021), OTA is 0.067 mg/L (67 µg/L) (Tschirren et al., 2018), and ZEN is 950 µg/L (Muthulakshmi et al., 2018) for zebra fish in its embryonic stage.

To estimate the public health threat posed by the presence of these mycotoxins in water at the levels detected, we used the Exposure model (Sirot et al., 2013) which is calculated as the probable daily intake (PDI):

$$PDI_{i,j} = \frac{\sum_{k=1}^{n} C_{i,k}}{BW_i} x\, L_{k,j} \quad \text{(S4)}$$

Where $PDI_{i,j}$ is the probable daily intake of contaminant *j* for the subject *i*, $C_{i,k}$ daily consumption level of water *k* by the subject *i* (k = 1 to N), $L_{i,j}$ level of contaminant *j* in the water *k*, BW_i is the body weight of the subject *i*. Mean concentrations of mycotoxins were used for calculations while the average volume of water ingested as well as the body weights for the population groups: infants (1 litre, 10 kg), teenagers (1.586 litres, 56.8 kg), adult men (3.7 litres, 80 kg), and adult women (2.7 litres, 80 kg) obtained from Faizan and Rouster (Lorusso et al., 2019; Faizan and Rouster, 2020) were inputed into **equation S4**.

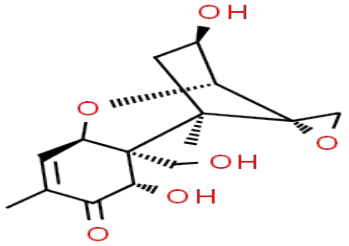
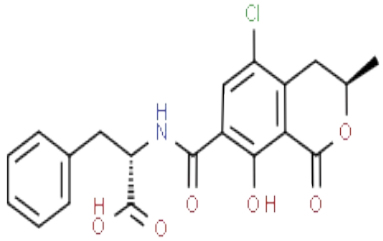
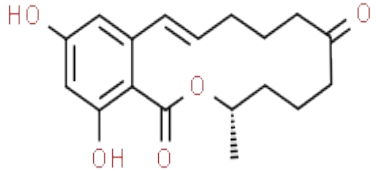
S5: Statistical Analysis

A total of 95 water samples and six variables (DON, OTA, ZEN, pH, Electrical Conductivity, and Total Dissolved Solids) were used. Statistical significance was set at a *p*-value of 0.05 or 0.01 (2-tailed). Data obtained from parametric Pearson's correlation are shown in **Fig. S6A-S6L)**. Multivariate statistical analysis was carried out using the IBM Statistical Package for the Social Sciences (SPSS) Statistics v21 principal component analysis (PCA) software, which was used to extract factors for establishing associations among some organic contaminants. The PCA is one of the universally used multivariate statistical methods of analysis in ecological assessment (Adesanya et al., 2020). It is widely adopted to establish the relationship between diverse

ecological variables and/or the total variability of a data set (Awolusi et al., 2018). Principal component analysis (PCA) is a multivariate method that evaluates data whose observations are explained by several inter-correlated quantitative dependent variables. The aim is to use statistical data to denote a set of novel orthogonal variables called principal components, and to demonstrate the arrangement of resemblance between the observations and the variables as points in spot maps (Bolujoko et al., 2022). The current study adopted PCA to establish a relationship between the targeted mycotoxin (DON, OTA, and ZEN) concentrations and some evaluated physicochemical parameters (pH, EC, and TDS) for groundwater, surface water, sachet water and bottled water samples across the three States (Osun, Oyo, and Lagos) in Southwestern Nigeria.

Data was subjected to the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Barlett Sphericity tests, was used. The preliminary results of the Kaiser-Meyer-Olkin (KMO) test (≥ 0.5) and Bartlett sphericity tests ($p < 0.001$) indicated that the raw data qualify for structure detection, and this further validates the results of the PCA. Correlation among the concentration of mycotoxins, pH, EC and TDS was evaluated with parametric Pearson's correlation in other to validate the results from PCA.

Table S1: Showing the structure and some physical properties of the mycotoxins(Schenzel et al., 2012)

Compound	CAS	Half-life in water (d)	Molecular Weight (g/mol)	pKa	Log K_{ow}	Water Solubility (mg/L)	Chemical Formula	Log K_{oc}	Chemical Structure
Deoxynivalenol	51481-10-8	38	296.3	11.9	0.1	5.18×10^{-4}	$C_{15}H_{20}O_6$	<0.7	
Ochratoxin A	17924-92-4		404	4.2* 7.09†	4.74	0.99	$C_{20}H_{18}ClNO_6$	2.63	
Zearalenone	17924-92-4	38	318.4	7.6	3.6	31.76	$C_{18}H_{22}O_5$	3.4	

https://www.chemspider.com/Chemical-Structure. * = pKa_1; † = pKa_2

Table S2: Samples distribution across the States

State	Type of Water Source				Total
	Groundwater	Surface water	Bottled water	Sachet water	total
Osun	12	11	05	05	33
Oyo	12	11	04	07	34
Lagos	10	10	04	04	28
Total	34	32	13	16	95

For groundwater samples, three samples were collected at each sampling location and made into composites across the three States. However, for surface water, three samples were collected from all the sampling sites across the three States in Nigeria without making them into composites. Five hundred millilitres (500 mL) of GW and SW samples were collected into amber glass bottles pre-washed with methanol and ultrapure water while for BTW and STW samples, they were 750 ml and 500 ml respectively were collected, being standard volumes for commercially packaged water in Nigeria. These samples were all preserved in an ice box (at approximately 4 oC) and transported to the laboratory. Extraction of the mycotoxins was done within 48 h of sample collection.

Table S3: Mean Mycotoxin Concentrations ± Standard Error of Mean (µg/L) in Drinking Water Sources

	GW	SW	BTW	STW	∑McTs
OSUN					
DON	6.06 ± 1.10	12.96 ± 2.23	2.02 ± 0.24	9.30 ± 2.26	30.33
OTA	1.93 ± 0.29	1.73 ± 0.30	1.47 ± 0.28	2.93 ± 0.79	8.06
ZEN	4.83 ± 0.86	5.84 ± 1.11	1.21 ± 0.24	14.96 ± 4.46	26.84
∑McTs	12.82	20.53	4.71	27.19	
OYO					
DON	4.54 ± 1.15	3.58 ± 0.71	1.90 ± 0.35	1.67 ± 0.37	11.69
OTA	1.49 ± 0.23	0.97 ± 0.25	1.53 ± 0.31	1.24 ± 0.40	5.23
ZEN	2.28 ± 0.52	3.02 ± 0.74	1.42 ± 0.30	8.59 ± 3.86	15.31
∑McTs	8.31	7.57	4.85	11.5	
LAGOS					
DON	6.06 ± 1.02	3.47 ± 1.22	2.32 ± 1.02	4.59 ± 0.59	16.43
OTA	2.11 ± 0.24	1.77 ± 0.68	0.75 ± 0.31	3.01 ± 1.50	7.64
ZEN	4.29 ± 0.72	1.84 ± 0.62	1.94 ± 1.08	10.56 ± 2.84	18.64
∑McTs	12.47	7.08	5.00	18.16	

GW = Groundwater; SW = Surface water; Bottled water; STW = Sachet water;
∑ McTs= summation of mycotoxins

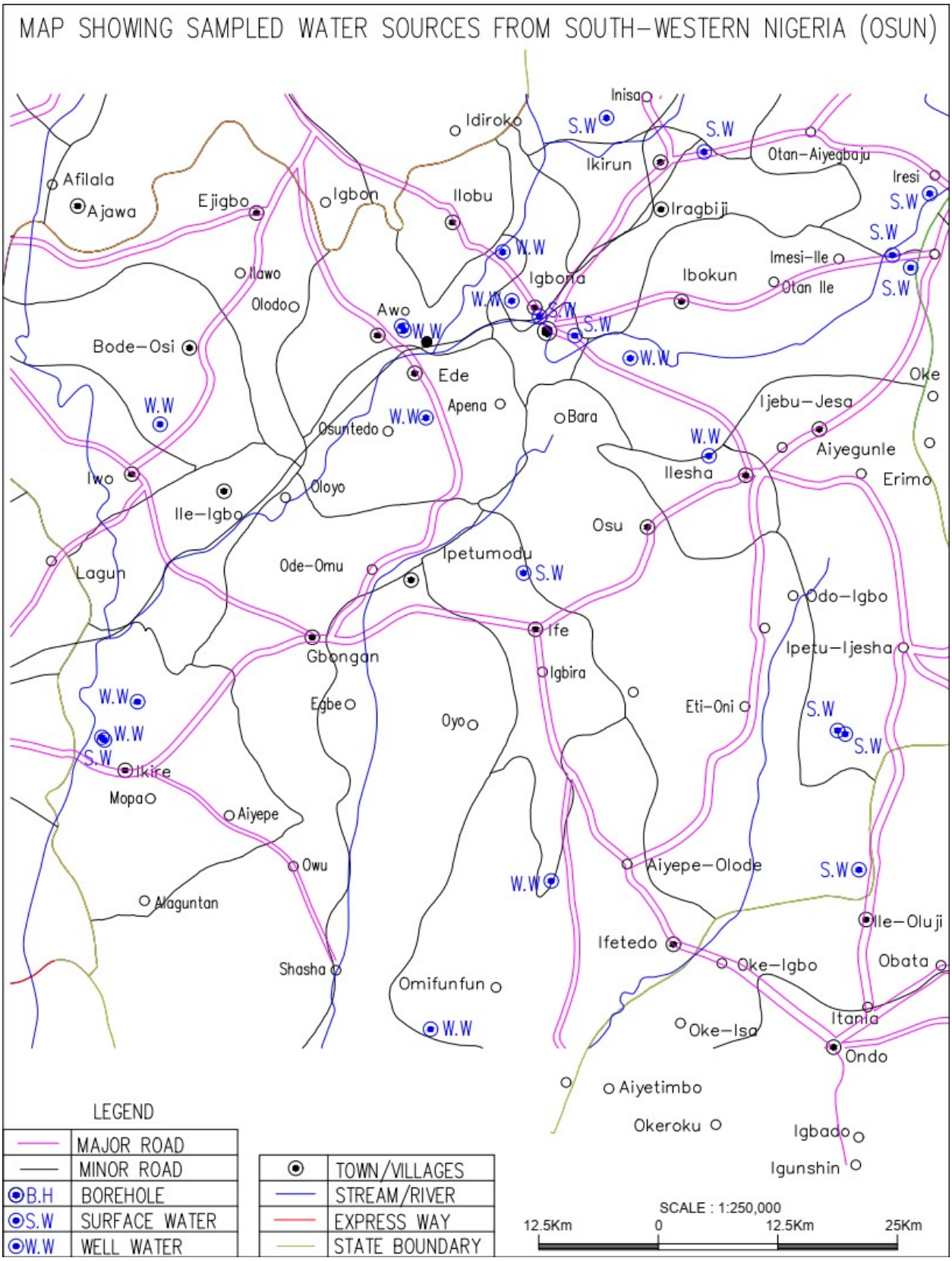


Fig. S1: Map showing sampling points in Osun State Nigeria (Otitoju et al., 2024)

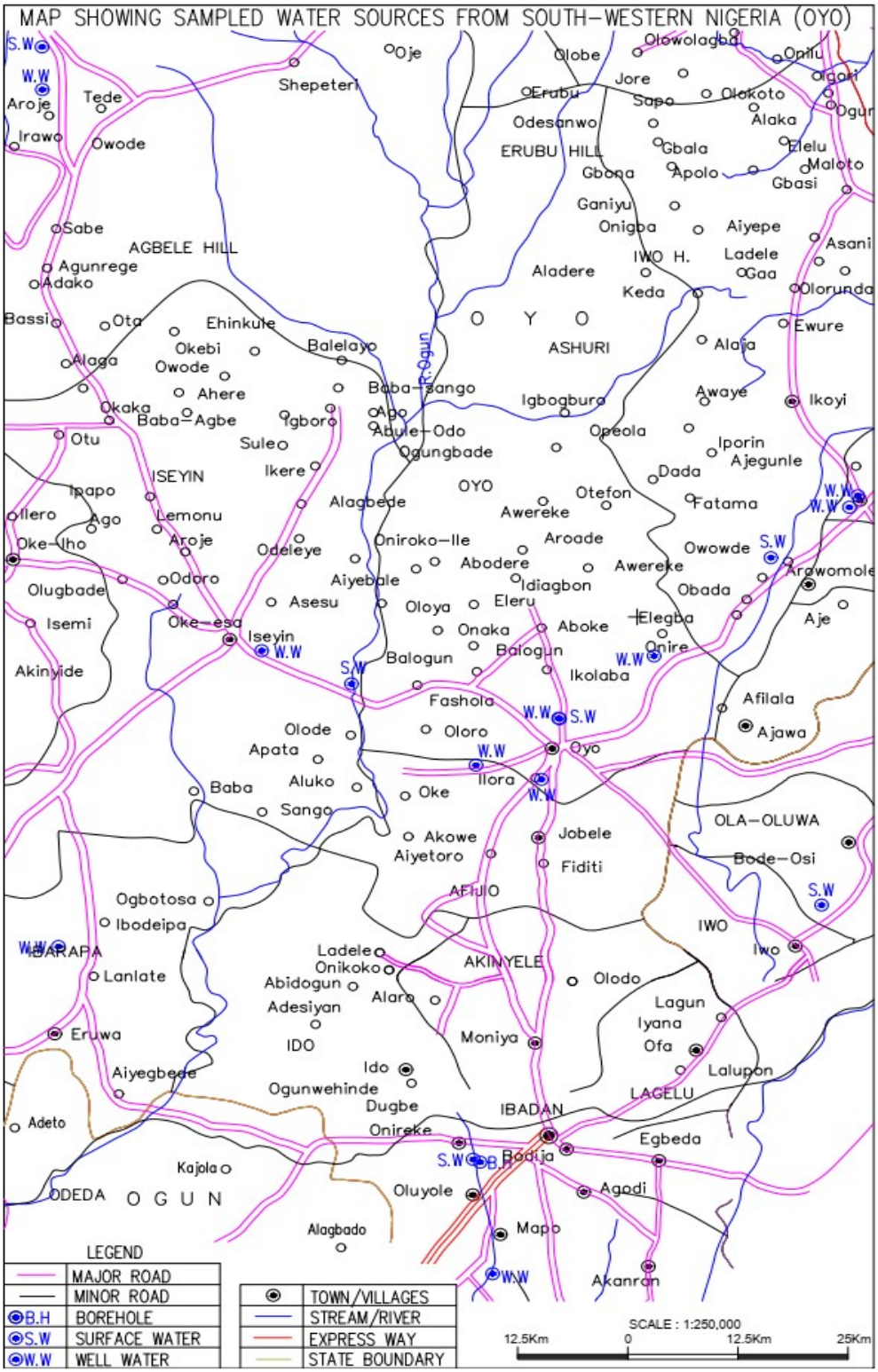


Fig. S2: Map showing sampling points in Oyo State Nigeria (Otitoju et al., 2024)

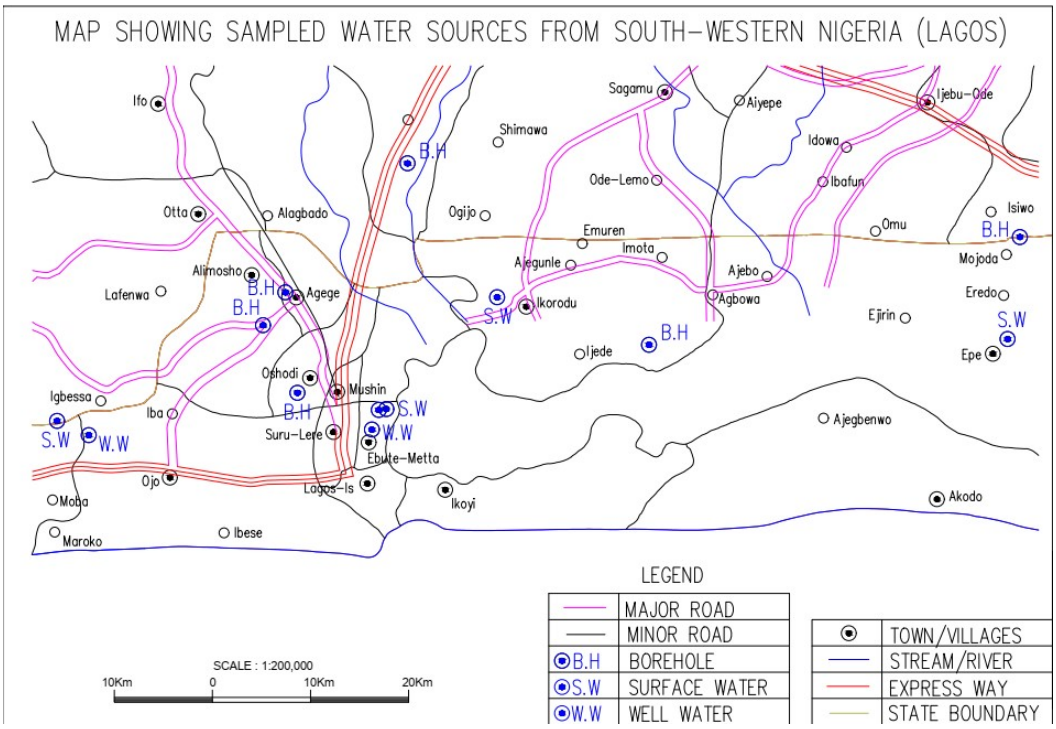


Fig. S3: Map showing sampling points in Lagos State Nigeria (Otitoju et al., 2024)

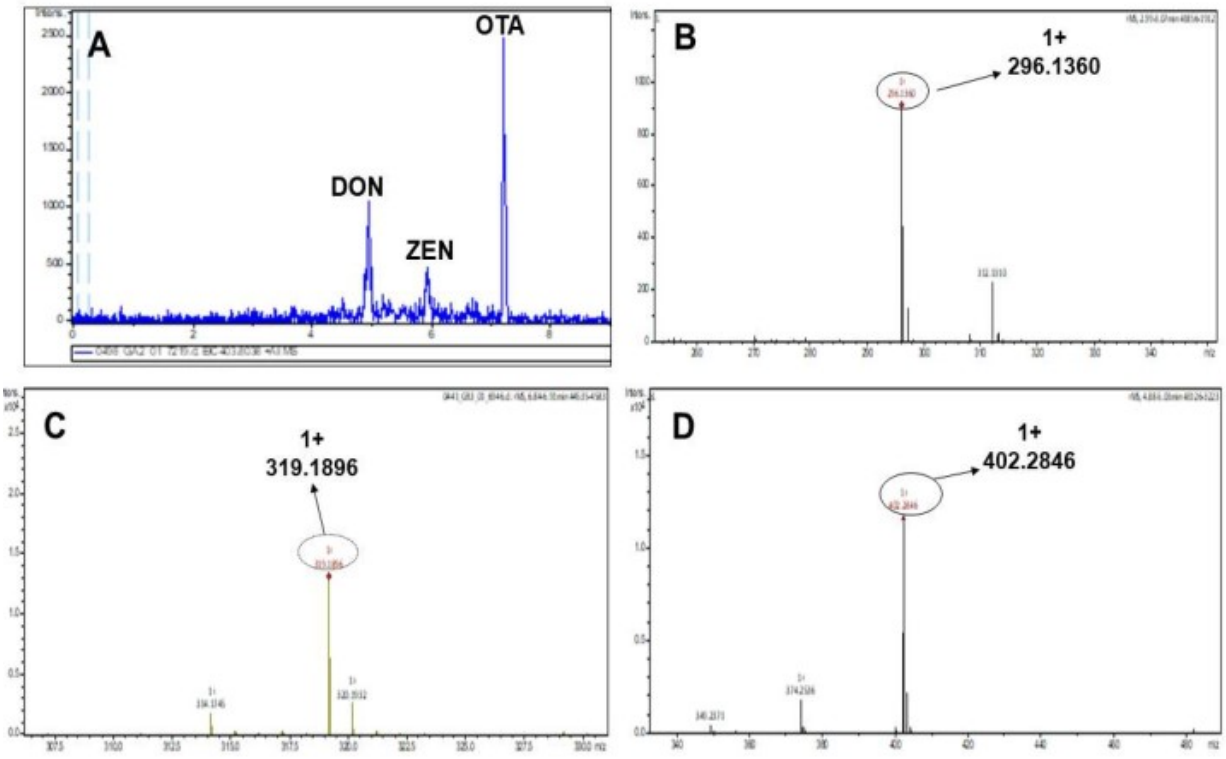


Fig. S4: (A) Chromatogram of Deoxynivalenol (DON), Zearalenone (ZEN) and Ochratoxin A (OTA) standards used in this study and mass spectrum for (B) Deoxynivalenol (C) Zearalenone (D) Ochratoxin A

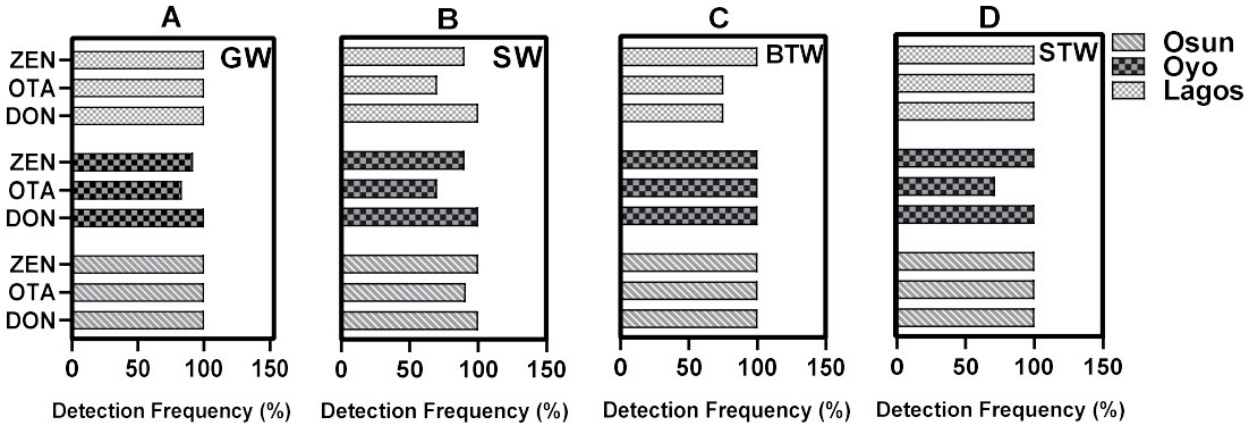


Fig. S5: Frequency of detection of the three mycotoxins Deoxynivalenol (DON), Ochratoxin A (OTA), and Zearalenone (ZEN) in (A) Groundwater-GW (B) Surface water-SW (C) Bottled water-BTW and (D) Sachet water-STW samples across the three States in Southwest, Nigeria.

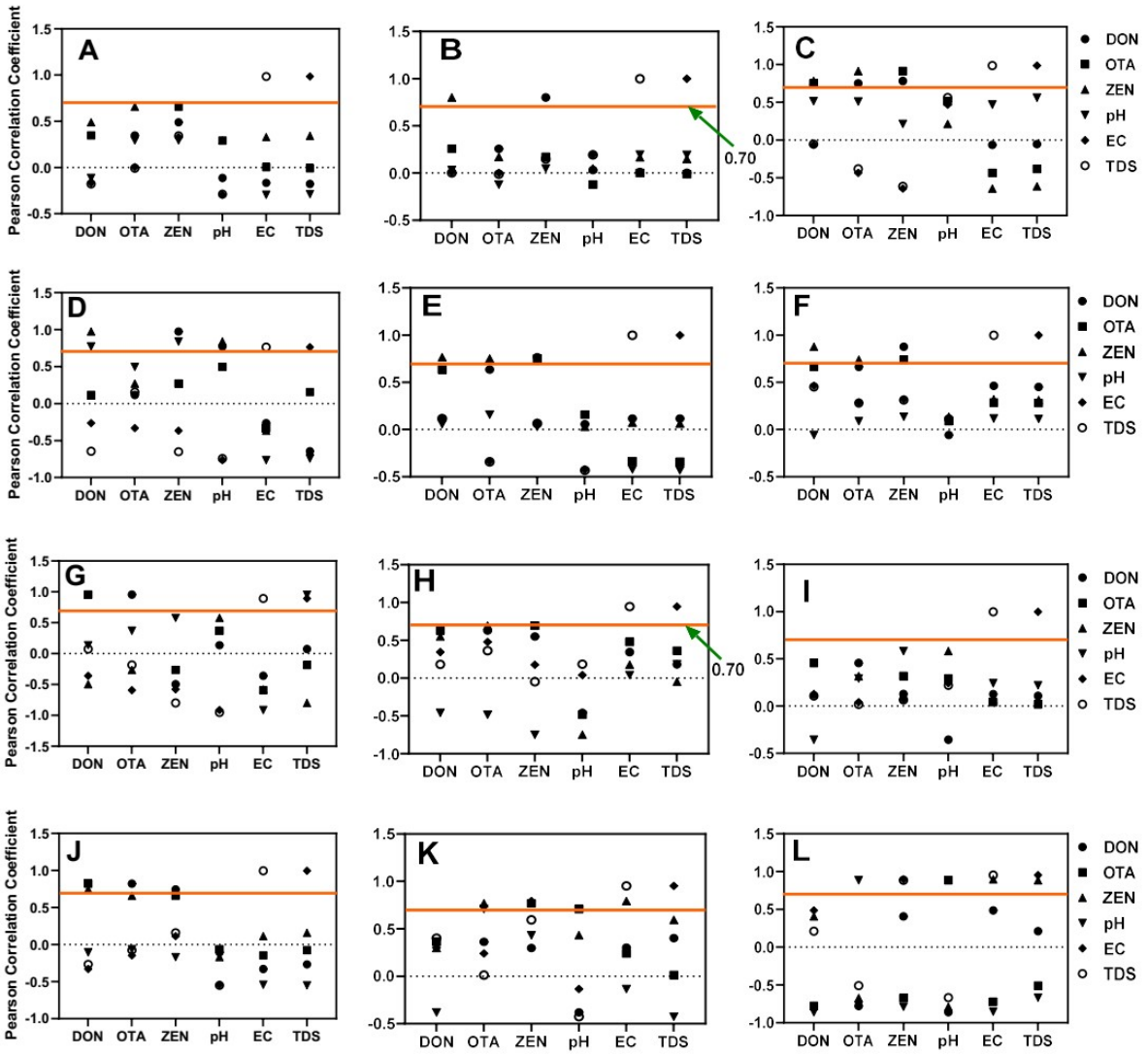


Fig. S6: Pearson Correlation Coefficients for Mycotoxins in (A) Groundwater (B) Surface water (C) Bottled water (D) Sachet water in Osun State (E) Groundwater (F) Surface water (G) Bottled water (H) Sachet water in Oyo State (I) Groundwater (J) Surface water (K) Bottled water (L) Sachet water in Osun State in Lagos State

References

Adesanya, O.O., Ogunlaja, O.O., Agunbiade, F.O., Ogunlaja, A., Unuabonah, E.I., Adebajo, L.O., 2020. Source identification and human health risk assessment of heavy metals in water sources around bitumen field in Ondo State, Nigeria. Environmental Forensics, 1-12.

Awolusi, O., Nasr, M., Kumari, S., Bux, F.J.I.j.o.e.s., technology, 2018. Principal component analysis for interaction of nitrifiers and wastewater environments at a full-scale activated sludge plant. International Journal of Environmental Science 15, 1477-1490.

Bolujoko, N.B., Ogunlaja, O.O., Alfred, M.O., Okewole, D.M., Ogunlaja, A., Olukanni, O.D., Msagati, T.A., Unuabonah, E.I., 2022. Occurrence and human exposure assessment of parabens in water sources in Osun State, Nigeria. Science of The Total Environment 814, 152448.

Eagles, E.J., Benstead, R., MacDonald, S., Handy, R.D., Hutchinson, T.H., 2021. Environmental risks to freshwater organisms from the mycotoxins deoxynivalenol and zearalenone using Species Sensitivity Distributions. Chemosphere 267, 129279.

Faizan, U., Rouster, A.S., 2020. Nutrition and hydration requirements in children and adults. https://www.ncbi.nlm.nih.gov/books/NBK562207/.

Lorusso, L., McEwan, C., Giddings, M., Watt, D., Patel, M., Macey, K., Field, J., Coleman, G., Villeneuve, M., Feeley, M., Jordan, S., Heon, D., Marles, R., Bhuller, Y., Mueller, T., Sharma, R., Gao, S., White, G., 2019. Selection of Body Weights Values for Use in Human Health Risk Assessment (HHRA) Conducted by Health Canada. Task Force on Scientific Risk Assessment's Body Weights Working Group, https://www.canada.ca/en/health-canada.html.

Mata, A., Ferreira, J., Oliveira, B., Batoréu, M., Crespo, M.B., Pereira, V., Bronze, M., 2015. Bottled water: Analysis of mycotoxins by LC–MS/MS. Food Chemistry 176, 455-464.

Muthulakshmi, S., Maharajan, K., Habibi, H.R., Kadirvelu, K., Venkataramana, M., 2018. Zearalenone induced embryo and neurotoxicity in zebrafish model (Danio rerio): Role of oxidative stress revealed by a multi biomarker study. Chemosphere 198, 111-121.

Otitoju, O.B., Alfred, M.O., Ogunlaja, O.O., Olorunnisola, C.G., Olukanni, O.D., Ogunlaja, A., Omorogie, M.O., Unuabonah, E.I., 2023. Pollution and risk assessment of phenolic compounds in drinking water sources from South-Western Nigeria. Environmental Science and Pollution Research 30, 76798–76817

Otitoju, O.B., Alfred, M.O., Olorunnisola, C.G., Aderinola, F.T., Ogunlaja, O.O., Olukanni, O.D., Ogunlaja, A., Omorogie, M.O., Unuabonah, E.I., 2024. Distribution and toxicity of dihydroxybenzenes in drinking water sources in Nigeria. RSC Advances 14, 982-994.

Schenzel, J., Forrer, H.-R., Vogelgsang, S., Hungerbühler, K., Bucheli, T.D., 2012. Mycotoxins in the environment: I. Production and emission from an agricultural test field. Environmental Science and Technology 46, 13067-13075.

Sirot, V., Fremy, J.-M., Leblanc, J.-C., 2013. Dietary exposure to mycotoxins and health risk assessment in the second French total diet study. Food and Chemical Toxicology 52, 1-11.

Tschirren, L., Siebenmann, S., Pietsch, C., 2018. Toxicity of ochratoxin to early life stages of zebrafish (Danio rerio). Toxins 10, 264.