Assessment of mycotoxin and heavy metal (cadmium, lead, and copper) contamination in sorghum grains grown in Center and Northern Benin.

Abstract:

To assess the sanitary quality of cereals produced in Benin, the contamination of sorghum grains cultivated in the Central and Northern regions by mycotoxins and heavy metals (cadmium, lead, and copper) was evaluated. Prior to the analytical phase, a survey of local farmers was conducted to identify the most widely produced varieties. These included two local varieties, *Locale N'Dali* and *Locale Djougou*, grown in the North (N'Dali, Djougou), and two resilient varieties, *Soumalemba* and *Tiandougou Coura*, cultivated in the North-Central regions (Dassa-Natitingou and Savalou-Ouaké). To quantify mycotoxins, specifically aflatoxin B1 (AFB1) and ochratoxin A (OTA), within a complex matrix like sorghum, a hybrid approach was deployed. This method combined an immunoassay (ELISA or immunoaffinity column cleanup, both based on antibody-antigen recognition) with High-Performance Liquid Chromatography coupled with Fluorescence Detection (HPLC-FLD). Heavy metal concentrations (Cd, Pb, Cu) were determined using Atomic Absorption Spectrometry (AAS). Collected data were subjected to statistical analysis, with means compared using the Student-Newman-Keuls test at a 5% significance level. The results revealed that the resilient variety *Tiandougou Coura* was the most susceptible to fungal contamination, exhibiting the highest cumulative levels for both AFB1 and OTA (1.56 µg/kg and 0.06 µg/kg, respectively). Furthermore, the bioaccumulation profiling of trace elements demonstrated that the *locale N'Dali* variety was the most vulnerable to toxic contaminant accumulation, recording cadmium and lead levels of 0.02 mg/kg and 0.08 mg/kg, respectively. Conversely, the *locale Djougou* variety exhibited the highest copper concentration (1.98 mg/kg), indicating a superior accumulation capacity for this essential trace element. In light of the international standards set by the Codex Alimentarius (CXS 193-1995), the grains of all four sorghum varieties demonstrated a satisfactory sanitary quality regarding both mycotoxins (AFB1 and OTA) and heavy metals (Cd, Pb, Cu).

Key words: ELISA methods,Atomic Absorption Spectrometry, cereals, bioaccumulation, chemical polluants.

Introduction

UNDER PEER REVIEW IN IJAR

UNDER PEER REVIEW IN IJAR

Cereals constitute the cornerstone of food security for African populations. In West Africa, they are characterized by their deep socio-cultural roots, their ease of substitution in food processing, and their prominent role in the formulation of infant weaning flours (Anihouvi et *al.*, 2016; Cruz et *al.*, 2005). In Benin, sorghum ranks third in cereal production, with an estimated output of 150,465 tons (DSA, 2024). Although this cereal crop exhibits remarkable agronomic resilience against climate variability (Alohoutade et *al*., 2025; Kané et M'baye, 2023; Mohammed etMisganaw, 2022), its value chain faces major sanitary constraints linked to environmental pollution and poor post-harvest practices.

During the production phase, the intensification of cotton cultivation and the uncontrolled use of inputs (untreated organic waste, chemical fertilizers, pesticides, and herbicides) promote the anthropogenic enrichment of soils with trace metal elements (TME) (Senou et *al*., 2023). Among these contaminants, lead (Pb), cadmium (Cd), and copper (Cu) diffuse into the rhizosphere, penetrate the root system, and translocate to the aerial parts up to the grains through active bioaccumulation mechanisms. Post-harvest, the exposure of panicles to residual moisture and failures during post-harvest operations (threshing, winnowing, insufficient drying, and inadequate storage) create a microclimate conducive to the proliferation of toxinogenic molds (Allogni et *al*., 2010). The genera *Aspergillus*, *Penicillium*, and *Fusarium* preferentially colonize the starchy matrix of cereals in tropical environments, leading to the biosynthesis of highly deleterious secondary metabolites: aflatoxin B1 (AFB1) and ochratoxine A (OTA) (Nguyen et *al*., 2007).

Heavy metals and mycotoxins thus constitute the main threats to the sanitary safety of cereal products and global trade (Bereda, 2025; USDA, 2023). Biologically, heavy metals disrupt photosynthesis and reduce crop yields (Moreno-Jiménez et *al*., 2009). In humans, aflatoxins promote carcinogenesis through the formation of DNA adducts, deoxynivalenol (DON) induces colorectal cancers and enteritis (Djouina et *al*., 2023), while heavy metals cause oxidative stress as well as neurodegenerative or nephrological pathologies (Bereda, 2025; Soltani etShaheli, 2017).

In the face of these threats, the bioaccumulation capacity of chemical pollutants and the susceptibility to molds depend on the plant species, the growth substrate, and the cultivated variety (Li et *al*., 2019; Arora et *al*., 2008). Currently, the response of the Beninese agricultural sector to climate crises is reflected in the introduction of new resilient sorghum varieties with an immediate extension effect. These resilient varieties have been adopted for their agronomic performance against biotic (*Striga* spp.) and climatic stress, and their aptitude for traditional food processing, to the detriment of local varieties (Kayodé, 2018; Agbokou et *al*., 2014). Consequently, the comparative evaluation of the susceptibility of local varieties versus resilient varieties to simultaneous contamination by heavy metals and mycotoxins has become a crucial public health issue. Within this perspective, the present study aims to evaluate the contamination of sorghum grains cultivated in Center and Northern Benin by mycotoxins (aflatoxin B1 and ochratoxin A) and heavy metals (cadmium, lead, and copper).

Material and methods

The methodological approach used in this study include a sorghum sample collection phase and a laboratory analysis phase.

- **Study area**

Local varieties were collected in N'Dali and Djougou under the designations *localN'Dali*and *local Djougou*, whereas resilient varieties were collected in Natitingou, Ouake, Savalou, and Dassa-Zoume. These identified resilient varieties are named *Soumalemba* and *Tiandougou Coura*. The municipalities of Dassa-Zoume and Savalou are located in Centerof Benin, while the municipalities of N'Dali, Djougou, Ouake, and Natitingou are situated in Northern Benin. The municipality of Natitingou belongs to the Territorial Agencies for Agricultural Development (TAAD)Pole 3, whereas the other municipalities belong to the TAAD Pole 4.

The municipalities of Dassa-Zoume and Natitingou served as introduction zones for resilient sorghum varieties as adaptation measures against climate change impacts within the framework of PROMISO-2 (Strengthening West African farmers' and researchers' capacity to jointly adapt new pearl millet and sorghum varieties and crop production innovations), funded by the European Union through IFAD(International Funds for Agricultural Development) (Kayodé, 2011).

The municipalities of Savalou and Ouakewere introduction and extension zones for resilientvarietiesunder the PANA-1 program (Integrated Adaptation Programme to Combat the Adverse Effects of Climate Change on Agricultural Production and Food Security), funded by the UNDP(United Nations Development Program) (Kayodé, 2014).

UNDER PEER REVIEW IN IJAR

In contrast, the municipalities of N'Dali and Djougou were selected as zones where resilient varieties had not been introduced, thereby preserving local sorghum varieties.

- **Sampling and collection of data**

Following the field surveys, 5 kg of each of the four varieties selected for the laboratory analysis phase were collected from recognized producers, with the support of senior crop production technicians (TS) at the municipal cell level, the operational arm of the TAAD (Territorial Agency for Agricultural Development, Pole 3 and Pole 4). These consisted of sorghum samples from the previous harvest (2024 production) and were stored under conventional conditions (granaries) by local producers. These sorghum samples were carefully placed into cardboard bags and kept at room temperature until arrival at the laboratory.

- **Plant material**

The local and resilient varieties of sorghum (*Sorghum bicolor* (L.) Moench) available in the study area were used. These consisted of *local N'Dali* and *local Djougou* (local varieties), and *Soumalemba* and *Tiandougou Coura* (resilient varieties);

- **Analysis methods**

The mycotoxins (aflatoxin B1, ochratoxin A) and heavy metals (cadmium, lead, and copper) were evaluated. The ELISA method was used to assess mycotoxin levels, while the AAS method was used to determine the heavy metal contents of the sorghum samples.

ELISA method

To quantify the toxins (AFB1 and OTA) in a complex matrix such as sorghum, a hybrid approach combining an immunoassay (such as an ELISA test or an immunoaffinity column based on the same antibody principle) and high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) was used. It involves the following steps:

- ✓ **Extraction and filtration**

Weigh 25 g of sorghum flour. Add 100 mL of solvent (methanol/water: 80/20 (v/v) for AFB1 and acetonitrile/water: 60/40 (v/v) for OTA). Shake for 30 min, then centrifuge or filter to obtain a clear extract.

- ✓ **Immunoaffinity cleanup (ELISA principle on Immunoaffinity Column - IAC);**

Instead of using a conventional ELISA plate, which is prone to matrix interferences, the extract was transferred onto an immunoaffinity column (IAC). This column contains specific monoclonal antibodies (anti-AFB1 or anti-OTA) immobilized on a gel.

UNDER PEER REVIEW IN IJAR

Aliquots of 10 mL of the crude extract were sampled and diluted in phosphate-buffered saline (PBS) at pH 7.4. Pass the diluted solution slowly through the column (1 to 2 drops per second). Rinse the column with 10 mL of distilled water or PBS buffer to remove the remaining matrix residues. Pass 1.5 to 2 mL of HPLC-grade pure methanol (or acetonitrile) through the column. The solvent denatures the antibodies, thereby releasing all the purified AFB1 and OTA. Carefully collect this eluate.

✓ **HPLC separation and derivatization (specific to AFB1)**

The purified eluate is injected into the chromatographic system (C18 reverse phase; mobile phase: water/acetonitrile/methanol isocratic mixture). To increase detection sensitivity, a derivatization was performed to convert AFB1 into AFB2 (highly fluorescent). For the pre-column method: add trifluoroacetic acid (TFA) directly into the eluate prior to injection. For the post-column method: use a UV reactor positioned between the column and the detector.

✓ **Quantification by fluorescence detector (FLD)**

The separated molecules pass through the FLD detector adjusted to the specific excitation and emission wavelengths for each toxin. The acquisition software integrates the area of the chromatographic peaks obtained at the exact retention time of each toxin. By comparing this area to a calibration curve constructed with certified standards, the system determines the final concentration of AFB1 or OTA present in the sorghum in µg/kg (ppb).

AAS Method (Atomic Absorption Spectrometry) (NF EN 13805 Standards).

The quantification of cadmium (Cd), lead (Pb), and copper (Cu) in sorghum flour using the AAS method follows a strict protocol. Thus, the starch and organic matter of the flour were fully destroyed to release the metals into solution prior to AAS analysis. The quantification was carried out in two essential steps:

➢ **Mineralization**

The purpose is to transform the solid flour into a liquid free of organic matter. Microwave-assisted acid digestion in a closed system was used to prevent the loss of cadmium and lead through volatilization. It consisted of the following steps: dry the sorghum flour in an oven (105 °C) for 72 hours. Weigh 0.5 g of the flour into a Teflon (PTFE) mineralization vessel. Add 5 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide. Allow the mixture to stand in the open air for 15 min. Hermetically seal the vessels. Run the microwave program (ramp up to 200 °C

UNDER PEER REVIEW IN IJAR

and held for 20 min under high pressure). After complete cooling, transfer the colorless and clear liquid into a 50 mL volumetric flask. Filter through an ashless filter paper. Bring up to volume with ultrapure water. A blank was also prepared.

- **Quantification**

Since trace metals exhibit different sensitivities, distinct atomization techniques were used: for copper, the flame mode (air/acetylene) with a standard range of 0.5, 1, 2, and 4 mg/L; for cadmium, the graphite furnace mode with a standard range of 0.5, 1, 2, and 5 µg/L; and for lead, the graphite furnace mode with a standard range of 5, 10, 20, and 50 µg/L. Following the instrument configuration (Cu: 324.8 nm; Pb: 283.3 nm; and Cd: 228.8 nm), analytical measurements were performed as follows: aspirate the reagent blank to zero the instrument; run the standard solutions in increasing order of concentration; and run the digested sorghum flour samples. The instrument displays a read concentration (C_{read}) in mg/L for the flame mode or in µg/L for the graphite furnace mode. The actual metal content of the solid flour C_m in mg/kg (or ppm) is determined by the following formula:

$$C_m = \frac{C_{read} \text{ x } V_{Flask}}{m_{flour}}$$

C_{read} : Concentration of the read solution (mg/L);

V_{Flask} : Final volume of the mineralization flask (L); m_{flour} : Exact mass of the weighed sorghum flour (kg)

- **Data analysis**

The data were processed using Microsoft Excel version 2010 to generate tables. The R software (v. 4.5.1) was used to perform the analysis of variance (ANOVA).

Results

To assess the degree of resistance of local and resilient sorghum variety grains to toxinogenic mold infestation, the concentrations of secondary metabolites (AFB1 and OTA) were evaluated. These values are presented in Table 1.

Table 1: Mycotoxin content of sorghum varieties

	Sorghumvarieties

UNDER PEER REVIEW IN IJAR

Parameters	*Local N'Dali*	*Local Djougou*	*Soumalemba*	*Tiandougou Coura*
Aflatoxin B1 (µg/kg)	1.08± 0.02 ^{a}	0.95±0.02 ^{b}	1.4±0.023 ^{c}	1.56±0.02 ^{d}
Ochratoxin A (µg/kg)	0.03± 0.001 ^{a}	0.00 ^{b}	0.00 ^{b}	0.06±0.001 ^{c}

On each line, the means followed by the same alphabetical letters do not present significant differences at the 5% threshold according to the Student Newman-Keuls test.

.The resilient variety *Tiandougou* exhibited the highest concentration of aflatoxin B1 (1.56 µg/kg), whereas the local variety *Local Djougou* displayed the lowest (0.95 µg/kg). These distinct aflatoxin B1 concentration values showed a significant difference at the 5% threshold, indicating that each variety possessed a specific resistance to toxinogenic mold infestation.
Based on the ochratoxin A (OTA) concentration values, the resilient variety *Tiandougou Coura* recorded the highest value (0.06 µg/kg), while the local variety *Local Djougou* and the resilient variety *Soumalemba* displayed the lowest (0.00 µg/kg). These concentration values showed a significant difference at the 5% threshold.

To assess the degree of bioaccumulation of heavy metals, the heavy metal concentrations (Cd, Pb, and Cu) of the different sorghum varieties are presented in Table 2.

Table 2: Heavy metal content of sorghum varieties

Parameters		Cadmium (mg/kg)	Lead (mg/kg)	Copper (mg/kg)
Sorghum varieties	*Local N'Dali*	0.02±0.0001 ^{a}	0.08±0.0017 ^{a}	1.90±0.006 ^{a}
	Local Djougou	0.018±0.0002 ^{b}	0.06±0.001 ^{b}	1.98±0.005 ^{b}
	Soumalemba	0.015±0.0002 ^{c}	0.04±0.001 ^{c}	1.80±0.007 ^{c}
	Tiandougou Coura	0.017± 0.0001 ^{d}	0.04± 0.001 ^{c}	1.86±0.004 ^{d}

In each column, means followed by the same letter are not significantly different at the 5% threshold according to the Student-Newman-Keuls test.

The local variety *Local N'Dali* accumulated the highest Cd content (0.02 mg/kg), whereas the resilient variety *Soumalemba* accumulated the lowest (0.015 mg/kg). These cadmium content values showed a significant difference at the 5% threshold. Regarding lead (Pb), significant

UNDER PEER REVIEW IN IJAR

differences were also highlighted at the 5% threshold. The local variety *Local N'Dali* again exhibited the highest contamination (0.08 mg/kg), while the resilient varieties recorded the lowest values. In relation to the bioaccumulation of copper (Cu), an essential element for plant metabolism, the Student-Newman Keuls test showed strict statistical disparities among all varieties. Contrary to the previous toxic metals, the local variety *Local Djougou* displayed the highest concentration (1.98 mg/kg), and the resilient variety *Soumalemba* showed the lowest (1.80 mg/kg).

Discussion

Evaluating the contamination of sorghum varieties by mycotoxins (AFB1 and OTA) and heavy metals aims to assess the sanitary quality of their grains. The variations observed among these aflatoxin B1 concentrations can be explained by intrinsic genetic factors specific to each sorghum variety. Indeed, grain structure, endosperm hardness, or polyphenol/tannin content (recognized for their antifungal properties) may have influenced the susceptibility of the grains to infestation by molds of the genus *Aspergillus* (notably *Aspergillus flavus*) and the subsequent synthesis of AFB1. Resilient varieties appeared more vulnerable to this biosynthesis than local varieties. This differential accumulation dynamic, linked to genotype and regional agro-climatic conditions, has been extensively documented through studies on subsistence cereals in sub-Saharan Africa (Wondafrash et *al*., 2025; Chala et *al*., 2022).

These variations in OTA contamination, arising primarily from the metabolism of fungi belonging to the genera *Aspergillus* and *Penicillium*, may result from differences in pericarp texture and grain moisture content. A low moisture content and a thicker outer film block the development of fungal mycelium and inhibit the production of this toxin. The early maturity of these varieties likely rendered them vulnerable. Indeed, grains that reached maturity during periods of high humidity could not escape the proliferation of OTA-producing molds. As highlighted by recent studies on toxin prevalence, OTA expression is closely linked to the porosity of the outer envelope of the grain, which regulates the penetration rate of fungal mycelia (Gatachew et *al*., 2024; Kortei et *al*., 2023).

This study demonstrates that the resilient variety *Tiandougou* was more susceptible to fungal contamination, accumulating higher contents of both AFB1 and OTA. In contrast, the local variety *Local Djougou* exhibited greater resistance to toxinogenic mold infestation. From a food

UNDER PEER REVIEW IN IJAR

safety perspective, all measured aflatoxin concentration values remained well below the maximum limits established by the Codex Alimentarius (<10 µg/kg for AFB1, AFB2, AFG1, and AFG2, according to standard CXS 193-1995 amended in 2025, which applies to sorghum grains intended for further processing). The evaluated ochratoxin values were also lower than the maximum limits set by the Codex Alimentarius (<5 µg/kg according to standard CODEX STAN 193-1995, corresponding to the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC/RCP 51-2003)). These values also comply with European Union standards: Commission Regulation (EU) 2022/1370 amending the criteria for ochratoxin A in certain foodstuffs, which sets a specific maximum limit of 3.0 µg/kg, and Commission Regulation (EU) 2023/915 concerning maximum levels for certain contaminants in food (which replaces the former Regulation 1881/2006), set at 2.0 µg/kg. Consequently, all four sorghum varieties demonstrated a satisfactory sanitary quality regarding these two mycotoxins.

In relation to the bioaccumulation of heavy metals, the differences observed in cadmium bioaccumulation may be linked to genetic and physiological factors. The root system of the different sorghum varieties may possess varying densities or expressions of membrane transporters (such as proteins from the HMA or NRAMP families involved in metal management and abiotic stress tolerance), thereby altering the uptake efficiency of this toxic element from the soil. Furthermore, the capacity to sequester cadmium within the vacuoles of root or stem cells (the root barrier phenomenon) may have varied from one variety to another, limiting or promoting its final translocation toward the grains. Recent physiological studies on cereals exposed to xenobiotics have confirmed that cadmium translocation to edible parts primarily depends on the vacuolar sequestration capacity at the root level, a trait that is highly variety-dependent (Yıldız et *al*., 2025; Rehman et *al*., 2024).

Regarding the bioaccumulation of lead, which is a highly immobile element within the plant but widespread in the environment, its presence in the aerial parts strongly depends on the root binding capacity. The observed variations suggest that the *Soumalemba* and *Tiandougou Coura* varieties limited the translocation of lead to the xylem conducting vessels, thanks to an increased synthesis of phytochelatins or a stronger binding onto root cell walls. This phenomenon thus limits the transfer of lead along the food chain, as previously observed by Makoae et *al*. (2025) for grasses produced in mining areas. Conversely, local varieties appeared to further facilitate the transfer of this xenobiotic (Pb) toward sorghum grains.

UNDER PEER REVIEW IN IJAR

As for copper, which acts as a major enzymatic cofactor in photosynthesis, respiration, and lignin synthesis, these variations reflect physiological requirements or assimilation efficiencies specific to each genotype. The local variety *Local Djougou* thus demonstrated a better aptitude for accumulating this nutritional trace element in its grains, which may represent an advantage from a nutritional value standpoint for the flour.

Overall, the assessment of trace metal elements highlighted that the local variety *Local N'Dali* was the most vulnerable to the accumulation of toxic contaminants (Cd and Pb), whereas the *Soumalemba* variety showed the lowest bioaccumulation profile for all tested metals. From a food safety perspective, these results comply with international standards of the Codex Alimentarius (standard CXS 193-1995), which establish maximum limits of 0.1 mg/kg for cadmium and 0.2 mg/kg for lead in cereal grains. Similarly, copper contents remained perfectly within the usual physiological ranges of the plant. Consequently, the flours derived from these four sorghum varieties demonstrated an irreproachable sanitary quality regarding heavy metal-related risks.

Conclusion

The joint assessment of biological and chemical contaminants highlights a major influence of the varietal factor on the sanitary quality of sorghum. The local variety *LocalN'Dali* appears to be the most susceptible to both chemical and biological pollution, accumulating the highest contents for both mycotoxins (AFB1, OTA) and xenobiotic heavy metals (Cd, Pb). In contrast, the resilient variety *Soumalemba* exhibits the most advantageous and safest sanitary profile, displaying the lowest concentrations of AFB1, Cd, and Pb, as well as a total absence of ochratoxin A. From a food safety perspective, all values comply with critical toxicity thresholds. The concentrations of AFB1 (maximum 1.56 µg/kg) and OTA (maximum 0.06 µg/kg) comply with international standards of the Codex Alimentarius (CXS 193-1995) and the European Union (Regulation EU 2023/915), which are set at 10 µg/kg (total aflatoxins) and 3 to 5 µg/kg (OTA), respectively. Similarly, for heavy metals, the maximum contents of cadmium (0.02 mg/kg) and lead (0.08 mg/kg) are well below the regulatory maximum limits of the Codex, established at 0.1 mg/kg for Cd and 0.2 mg/kg for Pb in cereals. Consequently, the flours derived from these four sorghum varieties demonstrate a completely satisfactory sanitary and commercial quality for human consumption.

Acknowledgements

UNDER PEER REVIEW IN IJAR

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UNDER PEER REVIEW IN IJAR