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RESEARCH ARTICLE

ASSESSMENT OF MYCOTOXIN AND HEAVY METAL CONTAMINATION (CADMIUM, LEAD AND COPPER) IN SORGHUM GRAINS GROWN IN CENTRAL AND NORTHERN BENIN

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Abstract

To assess the sanitary quality of cereals produced in Benin, the contamination of sorghum grains grown 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), as well as two resilient varieties, Soumalemba and Tiandougou Coura, grown in the North-Central regions (Dassa-Glazoué and Savalou-Ouaké). To quantify mycotoxins, in particular aflatoxin B1 (AFB1) and ochratoxin A (OTA), within a complex matrix such as 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). Concentrations of heavy metals (Cd, Pb, Cu) were determined using atomic absorption spectrometry (AAS). The 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, showing the highest cumulative levels for AFB1 and OTA (1.56 µg/kg and 0.06 µg/kg, respectively). In addition, trace element bioaccumulation profiling demonstrated that the local variety N'Dali was the most vulnerable to the accumulation of toxic contaminants, recording cadmium and lead levels of 0.02 mg/kg and 0.08 mg/kg, respectively.

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Conversely, the local variety Djougou showed the highest concentration of copper (1.98 mg/kg), indicating a higher accumulation capacity for this essential trace element. In light of the international standards established by the Codex Alimentarius (CXS 193-1955), the grains of the four sorghum varieties demonstrated satisfactory sanitary quality for both mycotoxins (AFB1 and OTA) and heavy metals (Cd, Pb, Cu).

Introduction:-

Cereals are 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 key role in formulating flours for infant weaning (Anihouvi et al., 2016; Cruz et al., 2005). In Benin, sorghum is the third most produced cereal, with an estimated production of 150,465 tonnes (DSA, 2024). Although this crop exhibits remarkable agronomic resilience to climate variability (Alohoutade et al., 2025; Kané and M'baye, 2023; Mohammed and Misganaw, 2022), its value chain faces significant sanitary constraints related to environmental pollution and inadequate 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 (TMEs) (Senou et al., 2023). Among these contaminants, lead (Pb), cadmium (Cd), and copper (Cu) diffuse into the rhizosphere, penetrate the root system, and translocate through active bioaccumulation mechanisms to the aerial parts, including the grains. After harvest, exposure of panicles to residual moisture and inadequate post-harvest operations (threshing, winnowing, insufficient drying, and poor storage) create a microclimate conducive to the proliferation of toxigenic 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 ochratoxin A (OTA) (Nguyen et al., 2007).

Heavy metals and mycotoxins therefore represent the main threats to cereal product safety and international 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 and nephrological pathologies (Bereda, 2025; Soltani and Shaheli, 2017).

In the face of these threats, the biological accumulation capacity of chemical pollutants and susceptibility to molds depend on the plant species, growth substrate, and cultivated variety (Arora et al., 2008; Li et al., 2019). Currently, the Beninese agricultural sector's response to climate challenges is reflected in the adoption of new resilient sorghum varieties with widespread dissemination. These resilient varieties have been adopted for their agronomic performance against biotic (e.g., Striga spp.) and climatic stresses, as well as for their suitability for traditional food processing, often replacing local varieties (Agbokou et al., 2014; Kayodé, 2018).

While agronomic selection successfully addresses climate and biotic pressures, it remains unclear whether these newly promoted resilient varieties inadvertently present a higher vulnerability to chemical and biological pollutants compared to traditional local varieties. Specifically, to what extent do genetic and environmental variations between local and resilient sorghum cultivars influence their differential bioaccumulation of toxic heavy metals and their susceptibility to mycotoxigenic mold proliferation?

Consequently, answering this question through a comparative assessment of local versus resilient varieties regarding their simultaneous contamination by heavy metals and mycotoxins has become a critical public health priority. To address this issue, the present study aims to evaluate the contamination of sorghum grains grown in central and northern Benin by mycotoxins (aflatoxin B1 and ochratoxin A) and heavy metals (cadmium, lead, and copper).

Materials and Methods:-

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

Study area:-
Local varieties were collected in N'Dali and Djougou under the designations N'Dali local and Djougou local, while resistant 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 in central Benin, while the municipalities of N'Dali, Djougou, Ouake and Natitingou are in northern Benin. The municipality of Natitingou belongs to Pole 3 of the Territorial Agencies for Agricultural Development (TAAD), while the other municipalities belong to Pole 4 of the TAAD.

The municipalities of Dassa-Zoume and Natitingou have served as introduction areas for resilient sorghum varieties as measures to adapt to the impacts of climate change under PROMISO-2 (Strengthening the Capacity of West African Farmers and Researchers to Jointly Adapt New Millet and Pearl Sorghum Varieties and Innovations in Agricultural Production), funded by the European Union through IFAD (International Fund for Agricultural Development) (Kayodé, 2011).

The municipalities of Savalou and Ouake have been areas of introduction and extension for resilient varieties under the PANA-1 (Integrated Adaptation Programme to Combat the Negative Effects of Climate Change on Agricultural Production and Food Security) programme, funded by the UNDP (United Nations Development Programme) (Kayodé, 2014). In contrast, the municipalities of N'Dali and Djougou were selected as areas where resistant varieties had not been introduced, thus preserving local sorghum varieties.

Sampling and data collection:-
After the field surveys, 5 kg of each of the four varieties selected for the analysis phase were collected from recognized producers. These samples 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 in cardboard bags and kept at room temperature until they arrived at the laboratory.

Plant matter:-
Local and resistant varieties of sorghum (Sorghum bicolor (L.) Moench) available in the study area were used. These included local varieties (local N'Daliandlocal Djougou), as well as resistant varieties (Soumalemba and Tiandougou Coura).

Analytical methods:-
Mycotoxins (aflatoxin B1, ochratoxin A) and heavy metals (cadmium, lead and copper) were assessed. For example, mycotoxin levels were determined by ELISA. The AAS method was used to determine the heavy metal content of the sorghum samples.

ELISA method:-
To quantify toxins (AFB1 and OTA) in a complex matrix such as sorghum, a hybrid approach combining an immunoassay (such as an ELISA assay or an immunoaffinity column based on the same antibody principle) and high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) was used. It includes 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 minutes, then centrifuge or strain to obtain a clear extract.

Immunoaffinity cleaning (immunoaffinity column ELISA - IAC):-
Instead of using a conventional ELISA plate, which is prone to matrix interference, the extract was transferred to an immunoaffinity column (IAD). This column contains specific monoclonal antibodies (anti-AFB1 or anti-OTA) immobilized on a gel. 10 mL aliquots of the crude extract were sampled and diluted in phosphate buffer serum (PBS) at a pH of 7.4. Run the diluted solution slowly through the column (1-2 drops per second). Rinse the column with 10 mL of distilled water or PBS buffer to remove any remaining residue from the matrix. Pass 1.5-2 mL of pure HPLC-grade methanol (or acetonitrile) through the column. The solvent denatures the antibodies, releasing all purified AFB1 and OTAs. Collect this eluate carefully.

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

Quantitation by fluorescence detector:-
The separated molecules pass through the FLD detector adjusted to the specific excitation and emission wavelengths for each toxin. The acquisition software integrates the surface of the chromatographic peaks obtained at the exact moment of retention of each toxin. By comparing this area to a calibration curve constructed according to 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 according to the AAS method follows a strict protocol. Thus, the starch and organic matter of the flour were completely destroyed to release the metals in solution before the AAS analysis. The quantification was carried out in two essential steps:

Mineralization:-
The goal is to transform solid flour into a liquid without organic matter. Microwave-assisted acid digestion in a closed system was used to prevent the loss of cadmium and lead through volatilization. It included the following steps: drying the sorghum flour in the oven (105°C) for 72 hours. Place 0.5 g of flour in a Teflon (PTFE) mineralization container. Add 5 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide. Let the mixture sit in the open air for 15 minutes. Seal the containers tightly. Start the microwave program (turn up to 200°C and hold for 20 minutes under high pressure). After complete cooling, transfer the colourless, clear liquid to a 50 mL volume flange. Filter through an ashless filter paper. Increase the volume with ultrapure water. A white has also been prepared.

Quantification:-
As trace metals have different sensitivities, different atomization techniques were used: for copper, flame mode (air/acetylene) with a standard range of 0.5, 1, 2 and 4 mg/L; for cadmium, graphite furnace mode with a standard range of 0.5, 1, 2 and 5 µg/L; and for lead, graphite furnace mode with a standard range of 5, 10, 20 and 50 µg/L. Depending on the instrument configuration (Cu: 324.8 nm; Pb: 283.3 nm; and CD: 228.8 nm), analytical measurements were performed as follows: aspirate the white reagent for zero the instrument; analyze standard solutions in ascending order of concentration; and analyze digested sorghum meal samples. The instrument displays a reading concentration (Cread) in mg/L for the flame mode or in µg/L for the graphite oven mode. The actual metal content of solid flour in mg/kg (or ppm) is determined by the following formula: C_m

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

C_{read} :Concentration of the solution read (mg/L) ;
V_{Flask} : Final volume of the mineralization flask (L) ; : Exact mass of the sorghum flour weighed (kg)m_{flour}

Data analysis:-
The data were processed using Microsoft Excel version 2010 to generate tables. 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 resistant cereals of sorghum varieties to toxigenic mould 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

Settings	Sorghum varieties			
	Local N'Dali	Local Djougou	Soumalemba	Tiandougou Coura
Aflatoxine B1 (µg/kg)	1.08± 0.02 ^{to}	0.95±0.02 ^{b}	1.4±0.023 ^{c}	1.56±0.02 ^{D}
Ochratoxine A (µg/kg)	0.03± 0.001 ^{to}	0.00 ^{b}	0.00 ^{b}	0.06±0.001 ^{c}

On each line, the means followed by the same alphabetic letters do not show significant differences at the 5% threshold according to the Student Newman-Keuls test. From the analysis of this table, we can say that the resilient variety Tiandougou showed the highest concentration of aflatoxin B1 (1.56 µg/kg), while the localvariety Djougou showed the lowest (0.95 µg/kg). These distinct values of aflatoxin B1 concentration showed a significant difference at the 5% threshold, indicating that each variety possessed a specific resistance to the infestation of toxigenic molds.

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 Soumalembashowed the lowest (0.00 µg/kg). These concentration values showed a significant difference at the 5% threshold. The significant variation in mycotoxin contents reflects a difference in the genetic sensitivity or resistance of the varieties to the development of toxigenic moulds (in particular species of the genera Aspergillus or Penicillium producing AFB1 and OTA). The Local Djougou variety appears to be the most resistant or the least favourable to the development of these toxins (lowest AFB1 content and absence of OTA). The Tiandougou Coura variety is the most sensitive, recording the highest concentrations for both Aflatoxin B1 (1.56 µg/kg) and Ochratoxin A (0.06 µg/kg). From a health point of view, the levels recorded for all varieties remain very low and well below the maximum international regulatory limits (for example, the European or Codex Alimentarius standard often sets the AFB1 limit at 2µg/kg or 5µg/kg depending on the use of the cereals). To assess the degree of bioaccumulation of heavy metals, the concentrations of heavy metals (Cd, Pb and Cu) of the different sorghum varieties were determined and presented in Table 2.

Table 2 : Heavy metal content of sorghum varieties

Settings		Cadmium (mg/kg)	Lead (mg/kg)	Copper (mg/kg)
Sorghum varieties	Local N'Dali	0.02±0.0001 ^{to}	0.08±0.0017 ^{to}	1.90±0.006 ^{to}
	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, the averages followed by the same letter do not differ significantly 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), while 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 differences were also found at the 5% threshold. The local variety Local N'Dali again showed the highest contamination (0.08 mg/kg), while the resilient varieties recorded the lowest values. The significant differences observed between the varieties suggest a genetic effect specific to each variety on the capacity to accumulate heavy metals, or variations related to the nature of the soil and the cultivation practices specific to the areas of origin (such as N'Dali or Djougou).

With regard to the bioaccumulation of copper (Cu), an essential element for plant metabolism, the Student-Newman Keuls test showed strict statistical disparities between all varieties. In contrast to the previous toxic metals, the local variety Djougou had the highest concentration (1.98 mg/kg), and the resilient variety Soumalemba the lowest (1.80 mg/kg). The Local N'Dali variety is distinguished by a higher capacity for accumulation of potentially toxic elements (Cadmium and Lead) than the other varieties. Conversely, Soumalembarecorded the lowest concentrations for all three elements overall. For example,copper, which is a trace element essential for plant growth, is present in much higher concentrations (1.80 to 1.98 mg/kg) compared to lead and cadmium, which are non-essential and toxic contaminants. In short,although the actual values are low for all samples, these results make it possible to identify the varieties that accumulate the fewest contaminants, which is decisive for the selection of more health-safe cultivars.

Discussion:-

The evaluation of 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 between these concentrations of aflatoxin B1 can be explained by intrinsic genetic factors specific to each sorghum variety. Indeed, the structure of the grains, the hardness of the endosperms or the polyphenol/tannin content (known for their antifungal properties) may have influenced the susceptibility of the grains to infestation by molds of the genus Aspergillus (in particular Aspergillus flavus) and the subsequent synthesis of AFB1. Resilient varieties seemed to be more vulnerable to this biosynthesis than local varieties. This differential accumulation dynamic, linked to genotype and regional agroclimatic 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, mainly due to the metabolism of fungi belonging to the genera Aspergillus and Penicillium, may result from differences in the texture of the pericarp and the moisture content of the grains. 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 probably made them vulnerable. Indeed, grains reaching maturity during periods of high humidity could not escape the proliferation of OTA-producing moulds. As highlighted in recent studies on the prevalence of toxins, OTA expression is closely related to the porosity of the outer shell 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 Tiandougouwas more susceptible to fungal contamination, accumulating a higher AFB1 and OTA content. In contrast, the local variety Local Djougou showed greater resistance to toxigenic mould infestation. From a food safety point of view, 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 the CXS 193-1995standard amended in 2025, which applies to sorghum grains intended for further processing). The ochratoxin values assessed were also below the maximum limits set by the Codex Alimentarius (<5 µg/kg according to the CODEX STAN 193-1995 standard, corresponding to the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC/RCP 51-2003)). These values are also in line with European Union standards: Commission Regulation (EU) 2022/1370 amending the criteria for ochratoxin A in certain foods, setting a specific maximum limit of 3.0 µg/kg, and Commission Regulation (EU) 2023/915 concerning maximum levels for certain contaminants in food (replacing the former Regulation 1881/2006), set at 2.0 µg/kg. As a result, all four sorghum varieties demonstrated satisfactory sanitary quality for these two mycotoxins.

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

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

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 has thus demonstrated a better ability to accumulate this nutritional trace element in its grains, which may represent an advantage from the point of view of nutritional value for flour.

Overall, the assessment of trace metal elements showed that the local variety N'Dali was the most vulnerable to the accumulation of toxic contaminants (Cd and Pb), while the Soumalemba variety had the lowest bioaccumulation profile for all metals tested. From a food safety point of view, these results are in line with the international Codex Alimentarius standards (CXS 193-1995), which set maximum limits of 0.1 mg/kg for cadmium and 0.2 mg/kg for lead in cereals. Similarly, the copper content remained perfectly within the plant's usual physiological ranges. As a result, the flours made from these four varieties of sorghum have demonstrated an irreproachable sanitary quality regarding the risks associated with heavy metals.

Conclusion:-

The joint assessment of biological and chemical contaminants highlights a major influence of the varietal factor on the sanitary quality of sorghum. The TiandougouCoura variety is the most sensitive to mycotoxin contamination (AFB1 and OTA), while N'Dali Local accumulates the highest levels of xenobiotic heavy metals (Cd, Pb). On the other hand, the Local Djougou variety has the most balanced and safest profile, with the lowest AFB1 content, an absence of OTA and low levels of heavy metals. The Soumalemba variety is distinguished by the most basal concentrations of cadmium and lead. From a food safety point of view, all values meet the critical toxicity thresholds. The maximum concentrations of AFB1 (1.56 µg/kg) and OTA (0.06 µg/kg) are well below the international standards of the Codex Alimentarius (CXS 193-1955) and the European Union (EU Regulation 2023/915), set at 10 µg/kg (total aflatoxins) and 3 to 5 µg/kg (OTA) respectively. Similarly, the maximum levels of cadmium (0.02 mg/kg) and lead (0.08 mg/kg) remain well below the Codex regulatory limits, set at 0.1 mg/kg for Cd and 0.2 mg/kg for Pb in cereals. As a result, the flours made from these four varieties of sorghum demonstrate a fully satisfactory sanitary and commercial quality for human consumption.

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