

REVIEWER'S REPORT

Manuscript No.: IJAR- 58880

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

Recommendation:

Accept as it is

Accept after Major revision.....

Accept after minor to Moderate revision

Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality		✓		
Clarity			✓	
Significance			✓	

Reviewer Name: **ANAPANA GOPAL**

Reviewer's Comment for Publication.

General Comments

The manuscript addresses an important food-safety issue: the occurrence of mycotoxins and heavy metals in sorghum produced in Central and Northern Benin. The topic is relevant to food security, public health, sorghum breeding, and agricultural adaptation to climate change. The manuscript also has the merit of assessing both biological contaminants (AFB1 and OTA) and chemical contaminants (Cd, Pb and Cu) in the same crop and comparing local and resilient varieties.

However, substantial methodological, statistical, and interpretative issues need to be addressed before the manuscript can be considered for publication. The most important concern is that variety and geographical location are confounded: the varieties appear to have been collected from different municipalities, making it difficult to determine whether observed differences are attributable to genotype, environment/soil, storage conditions, or their interaction. In addition, several statements in the Results and Conclusion contradict the numerical results in Table 1 and Table 2.

Content and Originality

Strengths:

- The study focuses on a relevant and under-investigated food-safety issue in Benin.
- Simultaneous evaluation of AFB1, OTA, Cd, Pb, and Cu provides a broader contamination profile.
- Comparing local and resilient sorghum varieties is potentially valuable in the context of climate adaptation.
- The reported concentrations are presented quantitatively and compared among varieties.

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Moderate concerns:

1. **The originality is not sufficiently established.** The Introduction should more clearly identify what is unknown about mycotoxin/heavy-metal contamination of Beninese sorghum and what specifically distinguishes this study from previous studies.
2. The manuscript repeatedly interprets differences as **varietal effects**, but the sampling design does not adequately support this conclusion. Local N'Dali, Local Djougou, Soumalemba, and Tiandougou Coura were collected from different locations. Therefore, genotype, soil characteristics, climate, agricultural practices, and storage environment may all contribute to the differences.
3. The study does not report important environmental variables such as **soil Cd/Pb/Cu concentrations, soil pH, organic matter, moisture, pesticide/fertilizer history, or storage duration**, despite using these factors to explain contamination.
4. The manuscript's conclusion that a particular variety is "safest" is too strong given the limited sampling design.

Technical Quality

This is the section requiring the most substantial revision.

1. Sampling design and sample size

The manuscript states that **5 kg of each of the four varieties** were collected, but it does not clearly state:

- the number of independent farms sampled per variety;
- the number of sampling locations per variety;
- how farms were selected;
- whether the 5 kg represented one composite sample or several independent samples;
- the number of analytical replicates;
- whether samples were collected from multiple farmers.

These details are essential for assessing statistical validity.

2. Variety versus location confounding

This is the **principal scientific weakness**.

Because varieties were associated with particular municipalities, a significant difference between varieties cannot necessarily be interpreted as a genetic difference. For example, differences in soil contamination, rainfall, storage practices, farming inputs, or microclimate could produce the observed differences.

The Discussion nevertheless attributes differences to genotype, root transporters, vacuolar sequestration, grain structure, tannins, etc. These mechanisms are plausible hypotheses, but they were **not measured in this study**. They should therefore be presented as possible explanations rather than conclusions.

3. Analytical methodology needs validation information

The manuscript provides a detailed procedural description of extraction and AAS digestion, which is useful. However, essential analytical-quality parameters are missing, including:

- limits of detection (LOD);
- limits of quantification (LOQ);
- recovery percentages;
- precision/repeatability;

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- certified reference materials or quality-control samples;
- calibration characteristics;
- number of analytical replicates;
- measurement uncertainty;
- criteria for accepting/rejecting analytical runs.

This is particularly important because some OTA values are reported as **0.00 µg/kg**. A zero result should normally be reported in relation to the analytical detection/quantification limit (e.g., <LOQ), unless genuine zero concentration can be demonstrated.

4. ELISA/HPLC-FLD description is confusing

The manuscript initially states that ELISA was used, but the described procedure is actually based on **immunoaffinity-column cleanup followed by HPLC-FLD**.

The authors should clearly identify:

- the exact analytical method used for AFB1;
- the exact analytical method used for OTA;
- whether ELISA was actually performed;
- manufacturer and model of the IAC;
- HPLC instrument and column specifications;
- detector wavelengths;
- calibration range;
- derivatization procedure.

The statement that AFB1 is converted into AFB2 by TFA treatment should also be technically clarified and supported by an appropriate analytical reference.

5. Statistical analysis

Only ANOVA and the Student-Newman-Keuls test are described. The manuscript should explicitly report:

- experimental/biological replication;
- degrees of freedom;
- F statistics;
- p-values;
- assumptions of ANOVA and whether they were tested;
- whether data transformation was necessary;
- whether the reported $\pm$ values represent SD, SE, or something else.

The very small error values reported in the tables raise questions about whether the analysis is based on **technical analytical replicates rather than independent biological samples**. If so, using these replicates as independent observations would constitute pseudoreplication.

6. Moderate internal inconsistency in the results and conclusion

There is a clear contradiction.

Table 1 shows that **Tiandougou Coura has the highest AFB1 (1.56 µg/kg) and OTA (0.06 µg/kg)**, while Local N'Dali has 1.08 µg/kg AFB1 and 0.03 µg/kg OTA.

Yet the Conclusion states:

"Local N'Dali appears to be the most susceptible to both chemical and biological pollution"

and claims it accumulated the highest contents for both mycotoxins and heavy metals.

This is **factually inconsistent with Table 1**. The conclusion needs to be corrected.

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Similarly, the abstract correctly states that Tiandougou Coura had the highest AFB1 and OTA, while identifying N'Dali as having the highest Cd and Pb. The abstract and conclusion therefore do not convey the same interpretation.

7. Interpretation of heavy-metal results

The heavy-metal results themselves are internally consistent: N'Dali has the highest Cd and Pb, while Djougou has the highest Cu.

However, calling Cu accumulation a nutritional advantage is premature. Higher Cu concentration does not automatically demonstrate improved nutritional value; dietary bioavailability and nutritional requirements would need to be considered.

Language and Presentation

The manuscript requires **substantial English-language editing**.

Examples include:

- "Center and Northern Benin" → preferably "Central and Northern Benin";
- "chemical pollutants" → "chemical pollutants";
- "ochratoxine A" → "ochratoxin A";
- inconsistent spacing and formatting such as "LocalN'Daliand", "Centerof Benin", "Ouakewere", etc.;
- inconsistent use of "Local N'Dali", "local N'Dali", and "LocalN'Dali";
- "Student Newman Keuls" should be consistently written as **Student-Newman-Keuls**;
- "Territorials Agencies" should be corrected to the appropriate institutional name.

The manuscript also contains several long sentences with excessive technical detail in the Methods and repetitive statements in the Discussion and Conclusion.

The formatting of the tables should be improved. Table 2, in particular, would benefit from clearer column/row organization and consistent decimal precision. The superscript statistical letters should also be explained directly in the table footnote.

Structure and Organization

The overall IMRaD structure is appropriate: Introduction → Materials and Methods → Results → Discussion → Conclusion.

Nevertheless, the manuscript would benefit from restructuring.

Recommended changes:

1. Introduction

- Establish the knowledge gap more explicitly.
- Separate the food-safety rationale from the climate-resilience background.
- State precise hypotheses or research questions.

2. Materials and Methods

- Add a subsection on experimental design and sample size.
- Clearly distinguish biological replicates from analytical replicates.
- Provide detailed sampling procedures.
- Add analytical validation/QC information.
- Clarify the statistical model.

3. Results

- Present statistical results more rigorously, including p-values where appropriate.
- Avoid interpretation in the Results section.

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- Correct the statements concerning which variety has the highest contamination.
- 4. **Discussion**
 - Explicitly acknowledge the confounding between variety and location.
 - Avoid presenting unmeasured physiological mechanisms as demonstrated mechanisms.
 - Compare findings systematically with previous studies.
- 5. **Conclusion**
 - Correct the contradiction concerning N'Dali and Tiandougou Coura.
 - Avoid the phrase "**completely satisfactory**" or "**irreproachable sanitary quality**", which is stronger than warranted by the sampling design and analytical information.

References and Citations

The reference list is reasonably extensive, but there are significant issues requiring careful checking.

1. **Citation–claim matching needs improvement.** Some references appear to be used for claims that are not obviously supported by their titles or stated subject matter. For example, the manuscript cites Djouina et al. (2023) in connection with DON-induced colorectal cancer and enteritis, whereas the reference listed concerns plant extracts against *Sitophilus oryzae* during sorghum storage. This citation should be checked and likely replaced.
2. Soltani & Shaheli (2017) is cited for heavy-metal-associated neurodegenerative/nephrological effects, but the listed reference concerns modification of starch digestibility through protein–polyphenol interactions. This appears to be another citation mismatch.
3. The manuscript should use **primary analytical and toxicological literature** when discussing mechanisms and health effects.
4. The two Codex references should be checked for duplication and consistency.
5. Reference formatting is inconsistent, particularly punctuation, DOI presentation, spacing, journal formatting, and capitalization.
6. Regulatory claims should be checked against the **exact applicable Codex/EU commodity and contaminant provisions** rather than relying on generalized limits.

Overall Recommendation

The manuscript addresses a worthwhile and potentially publishable research question, and the analytical results could provide useful baseline information on contaminant levels in Beninese sorghum. However, the current version has **Moderate methodological and interpretive weaknesses**.

The most important revisions are:

- clarify the sampling design and biological replication;
- address the variety/location confounding;
- provide analytical validation and quality-control data;
- clarify the ELISA/IAC/HPLC methodology;
- strengthen the statistical analysis;
- correct the contradiction between Table 1 and the Conclusion;
- moderate unsupported mechanistic claims;

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- verify regulatory limits;
- carefully audit all references and citations;
- undertake comprehensive English-language editing.

Final Decision:

Moderate Revision

I would recommend reconsideration after Moderate revision. The central dataset may be valuable, but the authors need to demonstrate that the analytical and statistical conclusions are adequately supported by the sampling design and correct the substantial inconsistencies in interpretation.