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REVIEWER'S REPORT

Manuscript No.: IJAR- 59702

Title: Analyse des contaminants microbiologiques et chimiques de trois types de poissons : Boops boops, Caranx crysos de mer et Bricinus leuciscus dÂ'eau douce consommÃ©s Ã Bamako.

Recommendation:
Accept as it is .....
Accept after Minor revision.....
Accept after Major revision
Do not accept (*Reasons below*)

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

Reviewer Name: Dr. ANAPANA GOPAL

Reviewer's Comment for Publication.

General Comments
The manuscript investigates microbiological and chemical contaminants in three fish species consumed in Bamako, Mali, namely Boops boops, Caranx crysos, and Brycinus leuciscus. The study focuses on the presence of potentially pathogenic microorganisms and heavy metals in different fish tissues, particularly the gills, intestines, and head. It also attempts to evaluate the implications of these contaminants for food safety and human health.
The topic is relevant to food microbiology, environmental health, fisheries, and public health. The investigation of microbial contamination and heavy-metal accumulation in commonly consumed fish is important, particularly in regions where fish handling, transportation, storage, and preparation practices may influence food safety.
The manuscript combines household and fish-vendor surveys, microbiological culture techniques, biochemical identification, and elemental analysis using X-ray fluorescence (XRF) and particle-induced X-ray emission (PIXE). The inclusion of both microbiological and chemical assessments is a positive aspect of the study.
However, the manuscript has several important scientific and methodological limitations that require substantial attention. These include the very limited sample size, insufficient details regarding sampling and laboratory procedures, questionable interpretation of microbial identification results, inconsistencies in the analytical techniques reported, and inadequate justification of the heavy-metal safety comparisons.
The conclusions concerning human health risks and the effectiveness of fish evisceration also need to be supported by stronger scientific evidence. Significant methodological clarification, data verification, and language improvement are necessary before the manuscript can be considered for publication.

Content and Originality

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The manuscript addresses an important food-safety issue by examining microbial and chemical contamination in marine and freshwater fish sold and consumed in Bamako. The simultaneous investigation of microbial indicators, potentially pathogenic microorganisms, and heavy metals provides an interesting multidisciplinary approach. The study also attempts to connect fish-processing practices, particularly the removal of gills and intestines, with the reduction of potential food-safety risks.
However, the scientific contribution and originality of the manuscript should be strengthened.

- The authors should clearly explain the research gap and identify how the study advances existing knowledge of fish contamination and food safety in Mali.
- The rationale for selecting the three fish species should be explained, including their consumption patterns, commercial importance, and potential differences in habitat and contamination exposure.
- The manuscript should distinguish between contamination of internal organs and contamination of edible muscle tissue, as these have different implications for consumer exposure.
- The relationship between fish evisceration and improved food safety should be evaluated more critically. The presence of contaminants in gills and intestines does not automatically establish that evisceration alone makes the remaining fish safe for consumption.
- The authors should discuss possible contamination during harvesting, transportation, storage, handling, and market distribution.
- The discussion of environmental contamination should be strengthened by considering the possible sources of heavy metals in marine and freshwater ecosystems.
- The manuscript should distinguish between microbial contamination detected in fish tissues and actual documented foodborne disease cases among consumers.

The originality of the study would be improved by presenting a more convincing comparison of contamination patterns among the three fish species and their different tissue types.

Technical Quality

The manuscript requires substantial improvement in sampling design, microbiological procedures, chemical analysis, and interpretation of results.

1. Sampling design and sample size: The manuscript mentions random sampling across six communes of Bamako. However, the actual number of fish specimens collected and the number of independent samples analysed are not clearly established. The microbiological section mentions two specimens of each fish type, whereas Tables II-a and II-b present results for three fish species and multiple tissue samples. The authors should clarify the total sample size, number of biological replicates, sampling locations, collection dates, and sample-selection procedures.
2. Sample representativeness: The study covers September 2019 to July 2020, but the number of sampling events and seasonal distribution of samples are not adequately described. The authors should explain whether samples were collected during different seasons and whether the sampling design represents the fish available in Bamako markets.
3. Sample preservation and handling: The manuscript states that biological samples were stored at −20°C. However, the duration of storage, transportation conditions,

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time between collection and laboratory analysis, and procedures used to prevent cross-contamination should be described. The suitability of the preservation conditions for the different microbiological analyses should also be justified.
4. Microbiological identification: The identification of microorganisms appears to rely partly on colony morphology, colour, Gram staining, and biochemical characteristics. These procedures alone are insufficient to establish the identity of several pathogenic microorganisms. The authors should provide detailed confirmation procedures for Salmonella, Shigella, coliforms, and Streptococcus. Appropriate confirmatory biochemical or molecular methods should be considered.
5. Culture media and incubation conditions: The authors should verify the suitability of the selected culture media, incubation temperatures, and incubation periods for each target microorganism. In particular, the interpretation of colonies grown on Salmonella-Shigella agar and deoxycholate lactose agar should be supported by appropriate identification criteria. Colony colour and morphology alone should not be treated as definitive identification.
6. Microbial enumeration: The manuscript reports microbial counts in UFC/ml, but the preparation of the sample suspension, dilution factors, plating procedures, and calculation methods are insufficiently described. The authors should explain how the final microbial concentrations were calculated and whether the reported values represent individual samples or averages of replicates.
7. Identification of Morganella morganii: The authors report identifying Morganella morganii in the gills of Brycinus leuciscus using biochemical identification procedures. However, the description of API 20E and VITEK 2 is inconsistent and requires clarification. The authors should specify the identification system actually used, provide the identification confidence or probability score, and explain the confirmatory criteria.
8. Interpretation of microbial results: The manuscript repeatedly compares microbial counts with a supposed WHO standard of zero colonies/ml. The authors should identify the exact regulatory or technical standard applicable to each microorganism and fish product category. Microbiological acceptance criteria vary according to the organism, sample type, product condition, and analytical method. A universal zero-colony criterion should not be applied without appropriate justification.
9. XRF and PIXE analytical methods: There is a Minor methodological inconsistency in the manuscript. The chemical analysis section initially describes X-ray fluorescence (XRF), including the use of a Siemens instrument and a Niton XRF analyser. However, the results section states that the samples were analysed using PIXE. The authors must clarify which analytical technique was actually used for each sample and provide the corresponding instrumental details.
10. Heavy-metal analysis and quality control: The manuscript does not adequately describe calibration procedures, certified reference materials, analytical blanks, detection limits, quantification limits, recovery percentages, or measurement uncertainty. These details are essential for establishing the reliability of the reported metal concentrations.
11. Elemental analysis and reporting: The methodology lists several elements, including Cr, Mn, Fe, Co, Cu, Zn, and Pb, while the results also report Cd, As, and Ni. The authors should explain which elements were actually measured and why some elements are absent from the tables. Values reported as 1 mg/kg should also be

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clarified, particularly whether they represent measured concentrations, detection limits, or assigned minimum values.
12. Sample coverage in chemical analysis: The chemical analysis tables include Boops boops and Brycinus leuciscus, but the manuscript's stated objective covers three fish species, including Caranx crysos. The authors should explain why chemical results for Caranx crysos are not presented or provide the missing analytical results.
13. Heavy-metal safety assessment: The authors state that cadmium, zinc, and arsenic concentrations exceed WHO standards. However, the reference limits, regulatory source, and corresponding units are not adequately specified. The authors should compare the measured concentrations in fish tissue (mg/kg) with appropriate food-safety limits for fish products, rather than comparing them directly with drinking-water or blood concentration reference values.
14. Human health-risk assessment: The manuscript discusses possible physiological effects associated with copper, zinc, lead, cadmium, arsenic, chromium, and nickel exposure. However, these statements are not supported by a quantitative dietary exposure assessment. The authors should consider estimated daily intake, fish consumption rates, body weight, and relevant toxicological reference values before making conclusions about consumer health risks.
15. Statistical analysis: The manuscript mainly presents descriptive averages. The authors should provide a more detailed statistical analysis, including variation among samples, standard deviations or other suitable measures of dispersion, and comparisons between fish species and tissue types where the sample size permits. The limitations associated with the small sample size should also be acknowledged.
16. Interpretation of fish evisceration: The conclusion recommends eviscerating all fish to reduce microbial and chemical contamination. However, the study does not include a direct experimental comparison of contamination levels in fish before and after evisceration. The authors should avoid presenting this recommendation as a demonstrated intervention unless supported by comparative evidence.
These methodological improvements are essential to establish the validity of the findings and the reliability of the conclusions.

Language and Presentation

The manuscript requires substantial language editing and technical proofreading. Although the subject is scientifically relevant, several grammatical, typographical, and terminology-related problems affect readability.

- Correct grammatical errors, spelling mistakes, punctuation, and inconsistent spacing throughout the manuscript.
- Standardize the scientific names of the fish species. In particular, use Brycinus leuciscus consistently rather than the inconsistent spellings appearing in the manuscript.
- Correct the formatting of scientific names, including italics for genus and species names.
- Standardize microbiological terminology, including colony-forming units (CFU/ml), total coliforms, faecal coliforms, yeasts, moulds, and pathogenic bacteria.
- Correct the definition of UFC/ml and use an internationally accepted equivalent consistently.
- Clarify the abbreviation ppm and avoid defining it incorrectly as "Poids Par Milligramme."

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- Standardize the units used for elemental concentrations, preferably mg/kg for fish tissues.
- Correct inconsistent spellings of X-ray fluorescence (XRF), particle-induced X-ray emission (PIXE), and other analytical terms.
- Improve the readability of Tables II-a, II-b, III-a, and III-b through consistent alignment, headings, units, and numerical formatting.
- Improve the quality and resolution of laboratory photographs and ensure that all images have appropriate captions.
- Correct incomplete sentences and unclear expressions, particularly in the discussion of microbial contamination and human health consequences.
- Remove informal expressions and subjective statements from the Results section.
- Ensure that the abstract accurately reflects the results and does not contain conclusions that are not supported by the experimental data.

The manuscript would benefit from comprehensive scientific English editing. Since the main text is written in French, professional French-language proofreading should also be considered if the journal accepts French manuscripts.

Structure and Organization

The manuscript follows a conventional research-paper structure, with sections covering the introduction, objectives, materials and methods, microbiological results, chemical analysis, discussion, and conclusion. However, several sections require restructuring to improve scientific clarity and logical flow.

- Abstract: Clearly present the study objectives, sample size, sampling locations, analytical methods, principal microbiological findings, and heavy-metal concentrations. Ensure that the abstract includes results for all three fish species or clearly explains any missing data.
- Introduction: Strengthen the scientific background on fish contamination, foodborne pathogens, heavy-metal bioaccumulation, and food safety in Mali. Clearly establish the research gap and justify the selection of the three fish species.
- Objectives: Align the general and specific objectives with the actual laboratory investigations. The objective concerning the impact of fish-viscera disposal should be reconsidered because the study does not appear to include direct environmental measurements.
- Materials and Methods: Provide separate and clearly defined subsections for questionnaire surveys, fish sampling, microbiological analysis, microbial identification, and chemical analysis. Include detailed procedures and quality-control measures.
- Microbiological Results: Present the results separately for gills and intestines, with clear comparisons among the three fish species. Include appropriate statistical summaries and explain the significance of the detected microorganisms.
- Chemical Analysis: Organize the heavy-metal results into a comprehensive table showing sample type, fish species, measured concentrations, analytical detection limits, and applicable food-safety reference limits.
- Discussion: Compare the findings with previous studies on microbial contamination and heavy-metal accumulation in marine and freshwater fish. Explain possible differences among species and tissues.

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- Human Health Implications: Separate potential health hazards associated with microbial contamination from those associated with heavy-metal exposure. Avoid making direct disease-causation claims without supporting evidence.
- Conclusion: Summarize the major findings and provide recommendations that are directly supported by the experimental results. Clearly acknowledge the limitations of the study.
- Figures and Tables: Improve the numbering, captions, resolution, and presentation of all laboratory photographs, analytical tables, and methodological diagrams.

A comparative table showing microbial contamination and heavy-metal concentrations across the three fish species and their respective tissues would significantly improve the scientific presentation.

References and Citations

The manuscript includes references related to food safety, microbiological analysis, fish taxonomy, and chemical contamination. However, the reference list requires substantial improvement.

- Verify the accuracy and completeness of all bibliographic details, including author names, publication titles, years, journal names, volume numbers, page ranges, and DOI information wherever applicable.
- Ensure that every in-text citation corresponds to an appropriate reference-list entry.
- Standardize the reference format according to the journal's guidelines.
- Include more recent scientific literature, particularly from 2021–2026, on microbial contamination of fish, heavy-metal bioaccumulation, and food-safety assessment.
- Include relevant studies conducted in Mali, West Africa, and comparable freshwater and marine environments.
- Support the identification and pathogenic characteristics of Morganella morganii and other reported microorganisms with appropriate microbiological references.
- Provide authoritative references for food-safety limits of cadmium, lead, arsenic, and other metals in fish products.
- Verify the sources used for the claimed WHO microbiological standards.
- Include appropriate technical references for XRF and PIXE analytical procedures.
- Support statements concerning the health effects of heavy metals with reliable toxicological and public-health literature.
- Check the taxonomic references for all three fish species and ensure that their scientific names are correctly presented.

Particular attention should be given to references supporting the quantitative interpretation of heavy-metal concentrations and microbiological safety limits.

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Overall Recommendation
Minor Revision
The manuscript addresses an important public health and food-safety issue by investigating microbiological and chemical contaminants in three fish species consumed in Bamako, Mali. The combination of microbiological culture techniques, biochemical identification, and elemental analysis provides a potentially useful multidisciplinary approach.
The study has practical relevance because fish contamination can affect consumer health, particularly where handling, storage, and processing practices are inadequate.
However, the manuscript has significant methodological limitations that need to be addressed. The limited and inconsistently reported sample size, insufficient validation of microbial identification, lack of clarity regarding XRF and PIXE techniques, incomplete chemical analysis, and inappropriate comparison of heavy-metal concentrations with safety standards raise concerns about the reliability of the findings.
The authors should also strengthen the statistical analysis, provide appropriate food-safety comparisons, and avoid unsupported conclusions regarding human health risks and the effectiveness of fish evisceration.
The manuscript requires substantial scientific revision, data verification, and language editing before it can be considered suitable for publication.

Final Decision
Minor Revision
The manuscript may be reconsidered for publication after the authors address the Minor methodological and scientific concerns, particularly:

1. Clarifying the sampling design, actual sample numbers, and biological replicates.
2. Providing reliable confirmation procedures for the identified microorganisms.
3. Resolving the inconsistencies between XRF and PIXE analytical methods.
4. Verifying the heavy-metal concentrations and comparing them with appropriate food-safety standards.
5. Providing a more rigorous assessment of potential human health risks.
6. Strengthening the statistical analysis and ensuring consistency between tables, results, and conclusions.
7. Improving the scientific discussion, references, and overall presentation.

A further review is recommended after resubmission to determine whether the methodological, analytical, and scientific concerns have been satisfactorily addressed.