

REVIEWER'S REPORT

Manuscript No.: IJAR- 59236

Title: Physicochemical and microbiological characterisation of effluent from a faecal-sludge treatment plant in Adjagbo, Benin, West Africa.

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.

1. General Comments

The manuscript addresses an important environmental and public-health issue by evaluating the physicochemical and microbiological quality of effluent from the Adjagbo faecal-sludge treatment plant in Abomey-Calavi, Benin. The study considers treatment stages, seasonal variation, physicochemical parameters, microbiological indicators, ANOVA and principal component analysis. The topic is relevant to wastewater management, environmental protection and agricultural reuse.

However, the manuscript requires **minor revision** before it can be considered for publication. There are important inconsistencies in the sampling design and sample size, methodological details, microbiological procedures, statistical analysis, numerical values, interpretation of standards, PCA presentation, figures and discussion. Some portions of the text also appear to contain unresolved editorial/reviewer comments, which should be removed from the final manuscript.

2. Minor Comments

2.1 Minor inconsistency in sample size and sampling design

The abstract states that **24 samples** were collected seasonally from four locations. However, the Methods section states:

"Three samples were collected at each of four strategically selected points"

and four sampling campaigns were conducted in May, September and December 2023 and February 2024.

If three samples were collected at each of four sites during four campaigns, this appears to imply **48 observations**, not 24.

The authors must clearly explain:

- Total number of samples collected.
- Number of samples per sampling point.

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- Number of samples per season.
- Whether the three samples were technical replicates or independent biological/environmental replicates.
- How the four sampling campaigns were grouped into “rainy” and “dry” seasons.
- Which observations were used for the statistical analysis.

This issue is critical because it directly affects the statistical validity of the study.

2.2 Sampling points and treatment train require clearer description

The study uses B0, B1, B2 and B3, representing different stages of the treatment process.

However, the terminology is not completely consistent. The manuscript should provide a clear schematic of the treatment process:

Raw sludge → buffer basin/pre-treatment → planted drying beds → maturation lagoon → polishing lagoon → final effluent

The exact relationship between B1, B2 and the planted drying-bed/lagoon stages should be clearly described.

A treatment-flow diagram would substantially improve the manuscript.

2.3 Physicochemical analytical methods require additional detail

The manuscript provides useful information about COD, TSS and BOD₅ procedures, but several parameters require more methodological information.

The authors should specify:

- Exact AFNOR standard/reference for each parameter.
- Analytical detection limits.
- Calibration procedures.
- Number of analytical replicates.
- Quality-control procedures.
- Blanks and standards.
- Units used for each parameter.
- Whether measurements were performed on filtered or unfiltered samples.
- Dilution factors, particularly for highly concentrated sludge samples.

The statement that pH was measured “**in diluted sludge and water samples**” also requires clarification because dilution can affect pH measurement and comparability between sampling points.

2.4 Microbiological methodology is inconsistent with the abstract

The abstract states that microbiological parameters were assessed using **membrane-filtration and colony-enumeration methods**.

However, the Methods section describes:

- Surface spreading for faecal coliforms.
- Multiple-tube methods for total coliforms and *E. coli*.

These descriptions need to be reconciled.

The authors should clearly state the exact method used for each organism, including:

- Sample volume.
- Dilution series.
- Culture medium.
- Incubation temperature.
- Incubation duration.

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- Confirmation procedure.
- Enumeration method.
- Detection limit.
- Quality-control procedures.

2.5 Statistical analysis requires substantial revision

The manuscript states that one-way ANOVA was used to evaluate seasonal effects, while PCA and correlation analysis were performed using R.

However, the statistical design is not adequately explained.

The authors need to clarify:

- What constituted the experimental/statistical unit?
- Were the samples independent?
- Were the same sampling points repeatedly sampled across campaigns?
- Was sampling point included as a factor?
- Was season the only factor?
- Were assumptions of normality and homogeneity of variance tested?
- Were data transformed where necessary?
- What post-hoc test was used?
- Why was a **10% significance level** used for some interpretations?
- How were missing values handled?

Because measurements were obtained from fixed locations repeatedly over time, a simple one-way ANOVA may not adequately account for the sampling structure.

The statistical model should reflect the actual experimental design.

2.6 Table 4 requires clearer statistical presentation

Table 4 presents p-values for B0–B3 and marks significance at 10%, 5% and 1%.

However, the table does not clearly identify:

- Sample size used for each comparison.
- The statistical model.
- Degrees of freedom.
- Test statistic.
- Whether assumptions were met.
- Whether multiple comparisons were performed.

The authors should provide sufficient information to allow readers to reproduce the analysis.

2.7 PCA results are incomplete

The manuscript reports that the first two principal components explain **60.51%** of the total variance, with PC1 explaining 41.50% and PC2 explaining 19.01%.

However, the PCA presentation is incomplete.

The authors should provide:

- Eigenvalues.
- Percentage variance explained by all principal components.
- Loading matrix.
- Contribution of each variable.
- Scores or coordinates of observations.
- Description of scaling/standardization used.
- Number of observations included.

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- Criteria for retaining components.

The manuscript itself currently contains a statement that the final version should include a PCA loading table.

This indicates that the PCA section is not yet fully finalized.

2.8 Possible internal reviewer/editorial comments remain in the manuscript

Several sentences appear to be comments or recommendations rather than scientific manuscript text.

For example:

“the manuscript should clearly distinguish between standards for drinking water, irrigation water and wastewater discharge.”

Similarly, the PCA section states that:

“the final manuscript should include the PCA loading table...”

These statements should **not appear in the final research article**. They should be converted into appropriate scientific text and the requested information should actually be incorporated.

A thorough final editorial check is necessary.

3. Important Numerical/Data Consistency Problems

3.1 COD and BOD₅ values appear to be interchanged in the Discussion

The Results section reports maximum COD values of **14,660 mg L⁻¹** and maximum BOD₅ values of **5,521 mg O₂ L⁻¹**.

However, the Discussion states:

“The recorded BOD₅ and COD values reported as 14,660 mg L⁻¹ and 5,521 mg L⁻¹, respectively...”

These values appear to be reversed.

This must be corrected throughout the manuscript.

3.2 COD/BOD₅ ratio should be recalculated

The manuscript reports a COD-to-BOD₅ ratio of **1.74**.

However, the mean rainy-season values presented earlier are approximately:

- COD = 5,195.99 mg L⁻¹
- BOD₅ = 2,805.75 mg L⁻¹

which gives a ratio of approximately **1.85**, not 1.74.

The authors should clearly state which values were used to calculate the ratio and recalculate it accordingly.

3.3 Nitrate interpretation is inconsistent

The Results correctly acknowledge that some nitrate concentrations exceeded 50 mg L⁻¹.

However, the Discussion subsequently states that the nitrate values were below the WHO guideline before later acknowledging that some individual concentrations exceeded 50 mg L⁻¹.

The authors should distinguish between:

- Mean concentration.
- Minimum/maximum concentration.
- Individual observations.
- Applicable regulatory threshold.

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4. Standards and Regulatory Comparisons

The manuscript repeatedly compares results with WHO and Beninese standards. However, the relevant standards must be identified precisely.

The authors should provide:

- Full name of each standard.
- Year/version.
- Parameter-specific limit.
- Intended water use.
- Whether the value applies to wastewater discharge, irrigation or drinking water.

This is particularly important because the manuscript itself recognizes that standards for **drinking water, irrigation water and wastewater discharge are not interchangeable**.

A dedicated table comparing:

Parameter | Measured value | Beninese discharge limit | Irrigation guideline | Applicable interpretation

would be useful.

5. Interpretation of Microbiological Results

The manuscript reports extremely high concentrations of *E. coli* and faecal coliforms, including *E. coli* values reaching 2,710,000 CFU/100 mL and faecal coliforms reaching 4,270,000 CFU/100 mL.

These findings are important, but the authors should be cautious about extrapolating indicator-organism measurements to specific pathogens.

The manuscript appropriately acknowledges that detection of *E. coli* and faecal coliforms does not demonstrate the presence of specific pathogens.

This distinction should be maintained consistently throughout the manuscript.

If pathogen presence was not directly tested, claims concerning *Salmonella*, *Shigella*, viruses, parasites or other pathogens should remain clearly identified as **potential risks**, not demonstrated findings.

6. Treatment Performance Needs Quantitative Assessment

One of the important strengths of the study is that samples were collected at different stages of the treatment process. However, treatment efficiency is not adequately quantified.

The authors should calculate removal efficiency for major parameters:

Removal efficiency (%) = [(Influent – Effluent) / Influent] × 100

for parameters such as:

- TSS
- COD
- BOD₅
- NH₄⁺
- TN
- TP
- *E. coli*
- Faecal coliforms

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This would make the study much more informative because the central question is not only the quality of the final effluent but also **how effectively the treatment plant removes contaminants**.

7. Seasonal Analysis Needs Stronger Interpretation

The manuscript reports seasonal effects for pH, BOD₅, COD, ammonium, total phosphorus and *E. coli* at particular sampling points.

The authors should provide a clearer mechanistic interpretation linking these differences to:

- Rainfall.
- Dilution.
- Hydraulic loading.
- Temperature.
- Treatment residence time.
- Microbial activity.
- Sludge loading.
- Lagoon performance.

If rainfall or hydraulic-loading data were not actually measured, these factors should be described as possible explanations rather than demonstrated causes.

8. Figures Require Minor Improvement

The figures showing seasonal variation of pH, TSS, COD, BOD₅, nitrate, nitrite, ammonium, total nitrogen and total phosphorus require revision.

The figures currently use sampling positions numbered approximately 1–4 on the x-axis rather than clearly identifying **B0, B1, B2 and B3**. This makes interpretation difficult.

The authors should:

- Label x-axes with B0, B1, B2 and B3.
- Clearly identify rainy and dry seasons.
- Include units on every y-axis.
- Improve resolution.
- Use consistent formatting.
- Ensure figure captions correspond to the correct graph.
- Correct the apparent mismatch/ordering of Figures E–I.
- Avoid unnecessary blank spaces.
- Provide statistical significance where appropriate.

The graphs on pages 7–9 should be carefully checked against Table 2.

9. Figure 3/PCA Presentation

The PCA figure on page 14 is potentially useful, but the caption currently reads:

“Figure 3.18. Projection of the physicochemical and microbiological parameters of raw sludge onto the first two factorial axes.”

The numbering **“Figure 3.18”** appears inconsistent with the rest of the manuscript and should be corrected.

The figure should also clearly identify:

- PCA axes.
- Percentage variance explained.
- Variable loadings.

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- Sample scores.
- Scaling method.

10. Discussion Requires Better Scientific Structure

The Discussion contains useful interpretation but is sometimes repetitive and includes unsupported or insufficiently supported explanations.

For example, the manuscript discusses possible effects of suspended solids on water warming and cold-water organisms.

Such statements should be directly relevant to the study system and appropriately supported.

The Discussion should be reorganized around:

1. Treatment efficiency.
2. Organic pollution.
3. Nutrient pollution.
4. Microbiological contamination.
5. Seasonal variation.
6. Environmental implications.
7. Potential irrigation/reuse implications.
8. Limitations of the study.

11. Nitrogen Chemistry and Ecological Interpretation

The discussion of ammonium, nitrite and nitrate requires greater scientific precision.

For example, the manuscript suggests that relatively low nitrate concentrations may partly reflect ammonium volatilisation.

This mechanism should be presented carefully because nitrogen transformations depend on pH, temperature, dissolved oxygen, nitrification, denitrification and other environmental conditions.

Similarly, statements concerning nitrite toxicity and fish mortality should not imply that the present study demonstrated fish mortality caused by the effluent. The manuscript appropriately notes that targeted ecological/toxicological investigations would be needed.

This cautious approach should be maintained throughout.

12. Organic Pollution versus Nutrient Pollution

The manuscript sometimes combines organic and nutrient pollution.

The very high COD and BOD₅ primarily demonstrate a **substantial organic load**, whereas high ammonium, total nitrogen and phosphorus indicate **nutrient enrichment**.

The authors should clearly separate:

- Organic pollution
- Nutrient pollution
- Suspended solids
- Microbiological contamination

This would make the environmental interpretation much clearer. The manuscript itself recognizes this distinction later in the Discussion.

13. Limitations Section Is Needed

A dedicated **Limitations** section should be added.

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Possible limitations evident from the manuscript include:

- Limited number of sampling campaigns.
- Sampling restricted to one treatment plant.
- Lack of direct pathogen analysis.
- Lack of receiving-water measurements.
- Limited information on hydraulic loading.
- Unclear independence of replicates.
- Lack of detailed treatment-performance calculations.
- Possible seasonal classification limitations.

The authors should state these explicitly rather than presenting the findings as universally representative.

14. Language and Technical Editing

The manuscript contains several grammatical and typographical errors.

Examples include:

- “finings above” instead of “findings above.”
- Inconsistent use of “faecal sludge” and “faecal-sludge.”
- Inconsistent capitalization.
- Inconsistent spacing and formatting.
- Some incomplete sentences in the Discussion.
- Missing citations in several statements.
- Inconsistent terminology for treatment units.
- “plan” instead of “plant” in the Figure 1 caption.

A complete professional language edit is strongly recommended.

15. Abstract

The abstract is generally well structured and reports the principal findings, including pH, nitrate, TSS, BOD₅, COD and *E. coli*.

However, the sample-number discrepancy must be resolved before publication.

The abstract should also state the actual number of independent samples and clearly distinguish the rainy and dry-season sampling design.

The statement that the effluent requires additional treatment is supported by the reported high organic and microbiological loads, but the regulatory comparison should be based on clearly identified standards.

16. Conclusion

The conclusion appropriately emphasizes the high concentrations of TSS, COD, BOD₅, ammonium, total phosphorus, *E. coli* and faecal coliforms and the need for additional treatment.

However, it should be shortened and should directly reflect the statistically supported findings.

The authors should avoid introducing information that was not directly evaluated in the study and should distinguish between:

- Demonstrated contamination.
- Potential environmental risk.
- Potential public-health risk.
- Recommendations requiring further investigation.

International Journal of Advanced Research

Publisher's Name: Jana Publication and Research LLP

www.journalijar.com

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17. Overall Assessment

The study has **good practical relevance** because it evaluates the quality of treated faecal-sludge effluent at different stages of a treatment plant and considers both physicochemical and microbiological parameters. The reported data indicate substantial residual contamination at the final outlet and provide potentially useful information for improving treatment and reuse management.

Nevertheless, the manuscript currently has several substantial issues involving **sample-size consistency, experimental design, statistical methodology, analytical methods, data interpretation, regulatory comparisons, PCA reporting, figures and language**. The numerical inconsistency involving COD and BOD₅ and the discrepancy between the stated sample number and sampling design are particularly important and should be resolved before the scientific conclusions are accepted.

Final Recommendation: MINOR REVISION (References are missing)

The manuscript should be reconsidered after the authors have thoroughly revised the methodology, verified all numerical results, corrected the statistical analysis, improved the presentation of PCA and treatment performance, reconciled the regulatory standards, corrected figures/tables, removed embedded editorial comments, and undertaken comprehensive language and technical editing.