ISSN: 2320-5407
ISSN NO. 2320-5407
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

Manuscript No.: IJAR- 59061

Title: ***Impact of Indigenous Mycorrhizal Fungi on Lead Speciation during Phytoremediation of Contaminated Landfill Soils in Bonoua, Côte d'Ivoire.***

Recommendation:
Accept as it is .....
Accept after Major revision.....
Accept after Minor 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 the effect of indigenous arbuscular mycorrhizal fungi (AMF/IMF) on lead (Pb) fractionation during phytoremediation of contaminated landfill soil using *Acacia mangium*. The study uses three Pb levels (0, 250 and 500 mg/kg), with and without fungal inoculation, and evaluates changes in soil Pb fractions using a sequential extraction procedure based on the BCR approach. The experiment consists of six treatments with nine replicates each and five months of cultivation.

The topic is relevant to soil remediation and environmental biotechnology, particularly because the study uses indigenous fungal inoculum obtained from a contaminated landfill environment. The manuscript reports substantial reductions in total soil Pb and generally greater Pb extraction in inoculated treatments. For example, total Pb decreased from 243.46 to 12.92 mg/kg under Pb250 with IMF, compared with 27.56 mg/kg without IMF; under Pb500, it decreased from 494.6 to 18.84 mg/kg with IMF, compared with 52.25 mg/kg without IMF.

The experimental concept is promising, but the manuscript requires **minor revision** before it can be considered for publication. The principal concerns involve the experimental design and statistical description, characterization of the fungal inoculum, interpretation of sequential extraction results, inconsistencies among tables and figures, terminology, and several conclusions that appear stronger than the data presented.

1. Content and Originality

The manuscript addresses an important environmental problem: remediation of Pb-contaminated landfill soils using a plant–mycorrhizal system. The use of indigenous AMF isolated from the same contaminated landfill is a potentially valuable aspect of the study. The Introduction appropriately establishes the importance of Pb contamination and the relevance of phytoremediation and metal speciation.

However, the **specific scientific novelty needs to be stated more clearly**.

The authors should explain:

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1. What is already known about *Acacia mangium*–AMF interactions during Pb phytoremediation.
2. What is specifically unknown about indigenous AMF and Pb speciation.
3. Why sequential extraction provides new information beyond total Pb removal.
4. What distinguishes the present study from the authors' previous work cited as reference 7.
5. Whether the main objective is Pb phytoextraction, Pb immobilization, or alteration of Pb chemical fractions.

At present, these objectives are somewhat mixed.

2. Title and Terminology

The title is relevant, but terminology should be standardized throughout the manuscript.

The manuscript alternates between:

- IMF;
- AMF;
- MIC;
- MFC;
- IFC;
- "fungal consortium";
- "fungal isolates."

For example, the experimental section defines the inoculum as indigenous mycorrhizal fungi, whereas later sections use **MIC**, **MFC**, and **IFC** inconsistently.

This creates substantial confusion.

The authors should select **one primary abbreviation**, preferably **AMF** for arbuscular mycorrhizal fungi, and use it consistently.

If the inoculum is a consortium rather than a single isolate, this should be explicitly stated and maintained throughout the manuscript.

3. Abstract

The Abstract clearly states the overall experimental design and reports the major Pb extraction findings. It describes three Pb concentrations and comparison between inoculated and non-inoculated soils.

However, the Abstract should be revised to include:

- experimental design;
- number of replicates;
- duration of cultivation;
- principal statistical result;
- actual reduction in total Pb;
- principal change in Pb fractions;
- one concise conclusion.

Importantly, the statement that IMF **"enhance lead extraction"** should be supported by the statistical analysis rather than simply by numerical differences.

The Abstract should also distinguish **total Pb removal from Pb speciation changes**.

4. Introduction

The Introduction provides an appropriate background on landfill contamination, Pb toxicity and phytoremediation.

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Nevertheless, the scientific rationale could be considerably strengthened.
The authors should explicitly discuss:

- Pb mobility versus total Pb concentration;
- operationally defined soil Pb fractions;
- why BCR sequential extraction is appropriate;
- how AMF may affect Pb mobility;
- the difference between **phytoextraction** and **phytostabilization**;
- the importance of measuring Pb in plant tissues.

The final paragraph should clearly state a testable hypothesis, for example:
"We hypothesized that inoculation with indigenous AMF would alter Pb fractionation and increase Pb removal from contaminated soil compared with non-inoculated plants."

5. Soil Sampling and Experimental Soil Preparation
The authors collected soil from **35 points at 0–30 cm depth** and prepared a composite sample. The soil was then artificially contaminated with 0, 250 and 500 mg/kg Pb.
This is reasonable for a controlled experiment, but several important details are missing.
The authors should clarify:

1. Whether the 35 samples were composited into **one homogeneous experimental soil**.
2. Whether subsamples were taken randomly from the composite.
3. The initial background Pb concentration.
4. Whether the 0 mg/kg treatment is truly uncontaminated or already contains approximately 16 mg/kg Pb.
5. The exact quantity of lead acetate added to each treatment.
6. Whether Pb concentrations represent **added Pb** or **final measured total Pb**.
7. Whether moisture content was standardized before contamination.
8. Whether soil was mixed periodically during the three-week aging period.

The actual Pb concentration in the nominal Pb0 treatment was approximately **15.96 mg/kg**, demonstrating that the "Pb0" soil was not Pb-free.
Therefore, terminology such as **"non-contaminated soil"** should be used cautiously.

6. Artificial Pb Contamination
The manuscript uses lead acetate to generate 250 and 500 mg/kg treatments and ages the soil for three weeks before experimentation.
The authors should provide a more rigorous description of:

- Pb acetate purity;
- preparation volume;
- final soil moisture;
- mixing procedure;
- aging conditions;
- confirmation of homogeneity;
- measured Pb recovery after contamination.

Artificially spiked Pb may behave differently from historically aged landfill-derived Pb. This distinction is important when extrapolating the findings to actual contaminated landfill soils.

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The Discussion should acknowledge that the experiment evaluates **spiked landfill soil**, rather than naturally contaminated soil alone.

7. Characterization of the Indigenous AMF Inoculum

This is one of the **minor weaknesses of the manuscript**.

The manuscript states that the inoculum consisted of indigenous AMF spores from landfill soil and that the inoculum was multiplied using maize as a host plant.

However, the authors do not provide sufficient information about the inoculum itself.

They should report:

- AMF genera/species identified;
- number of spores per gram of inoculum;
- percentage root colonization;
- method used for AMF identification;
- whether the inoculum contained other microorganisms;
- viability of the inoculum;
- inoculum density standardized across treatments.

Figure 1 illustrates trapping/multiplication, harvesting and conservation of the inoculum, but the figure is largely procedural and does not provide quantitative information about inoculum quality.

A quantitative measure of mycorrhizal colonization in *Acacia mangium* roots at the end of the experiment is also strongly recommended.

8. Experimental Design

The experiment consists of six treatments with **nine replicates per treatment**, which is a positive aspect of the study.

However, the design should be presented more clearly.

The authors should provide a formal factorial description:

- Factor A: Pb concentration — 0, 250, 500 mg/kg.
- Factor B: AMF inoculation — inoculated vs non-inoculated.
- Replication: n = 9.

This is essentially a **3 × 2 factorial experiment**.

The statistical analysis should therefore test:

1. effect of Pb concentration;
2. effect of AMF inoculation;
3. Pb × AMF interaction.

This is preferable to performing multiple independent comparisons without testing interaction effects.

9. Experimental Unit and Independence

The manuscript states that each treatment was replicated nine times, but it is not entirely clear whether each nursery bag represents an independent experimental unit.

The authors should explicitly state:

"Each nursery bag containing one plant was considered one experimental unit."

If more than one seedling was planted per bag, this must be clearly reported.

The number of plants per bag should also be stated.

10. Greenhouse/Shelter Conditions

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The experiment was conducted under a shelter with temperature ranging from **28–36°C** and relative humidity from **37–66%**.
This information is useful, but the authors should also report:

- photoperiod;
- light intensity or approximate natural light conditions;
- watering frequency;
- amount of water applied;
- drainage/leachate management;
- whether bags were randomized or repositioned during the experiment.

This is particularly important because Pb mobility and plant growth can be influenced by moisture conditions.

11. Plant Growth Data Are Missing
The experiment is described as a phytoremediation study using *Acacia mangium*, but the manuscript does not provide adequate plant-growth results.
The authors should report, if measured:

- survival rate;
- plant height;
- biomass;
- root biomass;
- shoot biomass;
- root length;
- biomass response to Pb;
- effect of AMF inoculation on plant growth.

Without plant biomass, the study cannot adequately evaluate **phytoextraction efficiency**.
A decrease in soil Pb concentration alone does not establish how much Pb was actually taken up by plants.

12. Plant Pb Concentration and Pb Mass Balance
This is a **minor scientific issue**.
The manuscript discusses Pb transfer and accumulation in the soil–plant system and states that AMF influences Pb extraction and accumulation.
However, the presented results primarily concern **soil Pb fractions**.
To demonstrate phytoextraction, the authors should provide:

- Pb concentration in roots;
- Pb concentration in shoots;
- root and shoot biomass;
- Pb uptake per plant;
- translocation factor;
- bioconcentration factor, where appropriate.

Most importantly, a **Pb mass balance** should be attempted:
Initial soil Pb = remaining soil Pb + Pb accumulated in plant tissues + Pb potentially lost through other pathways.
Without this information, the reduction in soil Pb cannot automatically be attributed entirely to plant uptake.

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13. Sequential Extraction Method

The use of sequential extraction is appropriate for evaluating operationally defined Pb fractions. The manuscript uses five fractions and measures the extracted Pb using ICP-MS.

However, the method needs greater methodological detail.

The authors should specify:

- exact BCR protocol/version;
- sample mass;
- soil-to-extractant ratio;
- centrifugation time and speed;
- filtration method;
- acidification/storage of extracts;
- ICP-MS calibration;
- detection limit;
- recovery;
- blanks;
- certified reference material;
- analytical precision.

The manuscript calls the procedure the **BCR method**, but the extraction scheme includes a five-step protocol with hydrosoluble and residual fractions. The authors should explain whether this is the original BCR sequential extraction procedure or a modified BCR scheme.

14. Table I Requires Correction and Clarification

Table I contains several presentation and methodological problems.

For example, the table reports:

"16 hours of rotational agitation, 300 minutes, centrifugation at 3000 rpm"

The relationship between the extraction period and the stated 300-minute centrifugation should be clarified.

The authors should verify all:

- extraction durations;
- reagent concentrations;
- reagent volumes;
- centrifugation times;
- temperatures;
- sample masses.

The residual fraction also involves **HF**, which requires particular attention to safety, analytical recovery and appropriate laboratory procedures. The method should be described according to a recognized protocol.

15. Minor Inconsistency in Total Pb Values

There is an important numerical inconsistency that must be corrected.

For **Pb500**, Table V reports a post-phytoremediation total Pb concentration of:

- 52.25 mg/kg without IMF;
- 18.84 mg/kg with IMF.

However, the individual fractions reported in Table VI for Pb500 without IMF are:

- Pb-Hydro = 7.71
- Pb-Exch = 12.00

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- Pb-Oxy = 14.07
- Pb-OM = 6.09
- Pb-Resi = 6.38 mg/kg.

These values sum to approximately **46.25 mg/kg**, not 52.25 mg/kg.
This discrepancy must be investigated and corrected.
Because the manuscript's central conclusions depend on total Pb removal and fractionation, **all tables, calculations and figures must be reconciled**.

16. Extraction-Rate Calculations Need Rechecking
The manuscript reports overall extraction rates of 76%, 88.7% and 89.4% without IMF and 82%, 94.7% and 96.2% with IMF for Pb0, Pb250 and Pb500, respectively.
These values should be recalculated from the corrected initial and final total Pb concentrations.
For example, the authors should clearly define:

$$\text{Pb extraction (\%)} = \frac{\text{Initial Pb} - \text{Final Pb}}{\text{Initial Pb}} \times 100$$

The manuscript should use the same calculation consistently in:

- Table V;
- Figure 3;
- Figure 4;
- Results;
- Discussion;
- Abstract.

17. Figure 4 Interpretation
Figure 4 presents extraction percentages for individual Pb fractions.
However, the text states that the highest extraction under Pb0 occurred in the exchangeable fraction, despite the absolute amount of Pb in that fraction being very small.
This distinction needs to be emphasized.
A high **percentage extraction from a small initial pool** does not necessarily represent the largest contribution to total Pb removal.
The authors should report both:

- percentage extracted;
- absolute amount extracted.

This will prevent misleading interpretation.

18. "Extraction" Versus "Mobilization" Requires Careful Interpretation
The manuscript frequently interprets reduced soil Pb fractions as evidence of fungal-mediated Pb mobilization.
For example, it states that IMF promotes Pb extraction, "most likely through fungal-mediated mobilization or remobilization of lead in the rhizosphere."
This is plausible but **not directly demonstrated** by the present experiment.
The authors did not appear to measure:

- organic acids;
- siderophores;
- rhizosphere pH;
- fungal Pb uptake;

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- Pb concentration in fungal structures;
- root exudates.

Therefore, mechanistic statements should be presented as **possible mechanisms**, not demonstrated mechanisms.

19. Soil pH Interpretation

The manuscript reports pH increases from approximately 6.4 to 6.5–6.7 following cultivation, with somewhat greater increases in inoculated treatments.
The authors attribute this primarily to IMF.
However, there are several possible explanations, including:

- plant nutrient uptake;
- rhizosphere processes;
- changes in organic matter;
- irrigation;
- microbial activity;
- experimental incubation.

Because the pH increase is relatively small, the causal attribution to AMF should be moderated unless supported by appropriate statistical testing.

20. Nitrogen and Phosphorus Interpretation

The manuscript reports increased N and available P after the experiment, particularly in inoculated treatments.
The Discussion then attributes these effects to AMF and, in places, unexpectedly refers to **PGPR**.
This is a minor terminology problem.
If no plant-growth-promoting rhizobacteria were intentionally inoculated or quantified, statements concerning **PGPR effects should be removed**.
The authors should distinguish clearly between:

- AMF effects;
- indigenous bacterial activity;
- plant effects;
- changes caused by cultivation.

21. Lead Speciation Versus Sequentially Extractable Fractions

The title uses the term **"lead speciation."**
Strictly speaking, sequential extraction provides operationally defined fractions rather than definitive molecular/structural speciation.
The manuscript should therefore consider using terminology such as:
"Pb fractionation"
or
"operationally defined Pb fractions"
unless the authors have complementary mineralogical/spectroscopic evidence confirming specific chemical species.
This would make the terminology scientifically more precise.

22. Interpretation of the Residual Fraction

The Discussion states that an increase in the residual fraction suggests stabilization of Pb in a more recalcitrant form.

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This interpretation is potentially reasonable, but sequential extraction alone cannot conclusively establish long-term stabilization.
The authors should use cautious language such as:
"an operationally less extractable fraction"
rather than:
"a stable/recalcitrant form"
unless supported by additional evidence.

23. AMF Mechanism
The mechanistic discussion regarding extraradical mycelium, organic acids, siderophores and glomalin is interesting.
However, these mechanisms are largely inferred from previous literature rather than demonstrated experimentally in this study.
The authors should clearly distinguish:
Observed:
- greater Pb removal in inoculated treatments;
- changes in Pb fractions.

Inferred:
- fungal mobilization;
- organic-acid-mediated dissolution;
- glomalin-mediated sequestration;
- increased root absorption surface.

This distinction would substantially improve the scientific rigor of the Discussion.

24. Statistical Analysis
The statistical section is currently too brief.
The manuscript states that ANOVA was performed and treatment means were compared using the Student–Newman–Keuls test at $P < 0.05$.
The authors should report:
- exact ANOVA model;
- factors included;
- degrees of freedom;
- F values;
- p values;
- interaction effects;
- assumptions of ANOVA;
- whether data were transformed;
- variance homogeneity testing;
- normality testing.

For the factorial design, a **two-way ANOVA** examining Pb concentration, AMF inoculation and their interaction would be more informative.
If comparisons are made among all six treatments, the statistical model should be explicitly justified.

25. Replicate-Level Data and Error Bars
The results mainly present single values for each treatment.
The authors should provide:
- mean ± SD or SE;

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- n;
- error bars in figures;
- statistical grouping letters;
- exact or appropriately reported p-values.

Figure 3 contains significance letters, but the figure should clearly state what the error bars represent and the sample size.

26. Table IV: Soil Chemical Properties

Table IV reports changes in pH, N, available P, OM and CEC.
However, no measures of variability are provided.
For replicated treatments, values should preferably be reported as:
mean ± SD
or
mean ± SE.
If the reported values are single measurements, the manuscript must clearly state this.
Statistical comparisons should also be shown.

27. Table III and Table VI Data Presentation

The tables contain useful information, but their formatting needs substantial improvement.
Problems include:

- inconsistent decimal separators;
- inconsistent abbreviations;
- inconsistent capitalization;
- "Pb-Mo" versus "Pb-OM";
- "oxydable" versus "oxidizable";
- "MIC" versus "IMF";
- unclear superscript letters;
- missing units in some places.

Table VI should also include **total Pb**, allowing readers to verify the sum of the fractions.

28. Figure Quality

The figures are useful but require professional redrawing.
Figure 1
The inoculum-production photograph/flow diagram is informative, but image quality and labeling should be improved.
Figure 2
The stacked fractionation chart is useful, but the statistical lettering is difficult to interpret.
Figure 3
The figure should show mean ± error and clearly distinguish total Pb remaining from extraction percentage.
Figure 4
The four-panel presentation is potentially valuable, but the labels and percentages should be checked against the numerical data.
Most importantly, **all figures should be cross-checked against Tables III–VI**.

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29. Discussion

The Discussion contains relevant literature comparisons and provides plausible explanations for changes in Pb fractionation. The sections on soil properties, Pb fractionation and possible AMF mechanisms provide a logical framework.

However, the Discussion is somewhat repetitive.

Several paragraphs repeat that Pb is predominantly associated with:

- Fe/Mn oxides;
- organic matter;
- residual fractions.

The Discussion could be shortened and made more analytical by focusing on:

1. major finding;
2. comparison with previous studies;
3. mechanism;
4. practical significance;
5. limitations.

30. Environmental Significance

The manuscript concludes that indigenous AMF could be a promising approach for rehabilitation and biorestoration of Pb-contaminated landfill sites.

This is potentially important.

However, the study was conducted in controlled nursery bags for five months.

Therefore, field-scale application requires further investigation regarding:

- persistence of AMF;
- competition with native microorganisms;
- seasonal effects;
- soil heterogeneity;
- plant survival;
- Pb leaching;
- disposal of Pb-rich biomass;
- long-term stability of Pb;
- ecological risk.

The practical recommendation should therefore be phrased as **promising or potentially applicable**, rather than demonstrated field effectiveness.

31. Conclusion

The Conclusion appropriately emphasizes the positive influence of the indigenous fungal consortium on Pb dynamics and soil characteristics.

However, it should be revised to reflect exactly what was measured.

In particular, the statement regarding:

"transfer and accumulation of lead in the soil-plant system"

should be supported by actual plant Pb concentration/biomass data in the Results.

If plant tissue Pb data are not part of this study, that statement should be removed or clearly identified as coming from previous work.

The conclusion should also acknowledge that the experiment demonstrates changes in **soil Pb concentration and operationally defined fractions**, rather than definitive chemical speciation.

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32. References and Citations

The reference list contains relevant literature on:

- Pb toxicity;
- phytoremediation;
- AMF;
- soil Pb fractionation;
- metal mobility;
- fungal mechanisms.

However, reference formatting needs substantial standardization.

For example, some references are complete journal articles, whereas others appear to be:

- theses;
- books;
- technical documents;
- incomplete publication records.

The authors should verify:

- author names;
- article titles;
- journal names;
- volume;
- issue;
- pages;
- DOI;
- publication year;
- consistency with journal reference style.

Some citations also appear questionable in relation to the statements for which they are used and should be checked carefully.

33. Language and Editorial Quality

The manuscript requires **extensive English-language and editorial revision**.

There are numerous problems involving:

- missing spaces;
- incorrect punctuation;
- inconsistent abbreviations;
- grammatical errors;
- inconsistent terminology;
- singular/plural errors;
- French-English translation artifacts;
- inconsistent scientific terminology.

Examples include:

- "manypetri dishes";
- "after contamination and after phytoremediation";
- "oxydable";
- "MIC" used instead of IMF;
- "MFC" and "IFC" used inconsistently;
- "SolPb250" instead of "Soil Pb250";
- "Référencesbibliographiques."

The manuscript should undergo professional language editing before publication.

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Strengths of the Manuscript

The study has several positive features:

1. **Relevant environmental problem** involving Pb-contaminated landfill soil.
2. Use of *Acacia mangium*, a potentially useful phytoremediation species.
3. Use of **indigenous AMF from the contaminated site**, which is potentially novel and environmentally relevant.
4. Controlled Pb concentration gradient of 0, 250 and 500 mg/kg.
5. Six experimental treatments with nine replicates each.
6. Five-month cultivation period.
7. Use of sequential extraction to investigate Pb fractionation.
8. ICP-MS was used for Pb quantification.
9. Clear reduction in total soil Pb following cultivation.
10. Greater overall Pb removal in inoculated treatments.
11. Useful attempt to discuss possible AMF-mediated mechanisms.
12. The study has potential relevance for rehabilitation of contaminated landfill soils.

Overall Recommendation

The manuscript presents a **potentially useful experimental study** on the role of indigenous AMF in Pb removal and fractionation during phytoremediation using *Acacia mangium*. The controlled factorial treatment structure and the observed greater reduction in soil Pb in inoculated treatments are important strengths. The use of sequential extraction also provides potentially valuable information about changes in Pb distribution among operationally defined soil fractions.

Nevertheless, the current version has several substantial issues that prevent a reliable assessment of the strength of the conclusions. The most serious concerns are the **lack of detailed characterization of the AMF inoculum, absence/insufficient reporting of plant Pb uptake, inadequate statistical description, unclear distinction between extraction and mobilization, inconsistent terminology, and numerical discrepancies between the reported tables and figures**. The Pb500 discrepancy between Table V and Table VI is particularly important and must be resolved before publication.

Recommendation: Minor Revision

Final Decision: MINOR REVISION