



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	Asian Journal of Environment & Ecology
Manuscript Number:	Ms_AJEE_43545
Title of the Manuscript:	Enumeration of total heterotrophic bacteria counts and petroleum degrading bacteria counts in water and sediments from Diobu Creek, Port Harcourt, Nigeria
Type of Article:	Original Research Article

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
The authors did not address most of my concerns in their response and in the revised manuscript. Major shortcomings of the study include the use of nutrient agar for bacterial enumeration (rather than the much more appropriate plate count agar or R2A agar) and the complete lack of any molecular data, such as a straightforward phylogenetic analysis. This study has several significant flaws. First, I question the control sampling station, simply because so little information is given concerning it. The authors state that it was upstream with "relatively little human activities and pollution." What does this mean exactly? How far upstream was the sample site? How do the authors know it contains relatively little pollution? Was the water tested for pollutants and compared to other sample sites? Nothing is said of this. Second, the authors state that sample containers were sterile, but they provide no details as to what type of vessel was used or how sterilization was achieved, nor do they provide details as to how aseptic conditions were maintained in the field during sampling. This information must be included. A huge deficiency in the manuscript concerns culturing techniques. The authors state that nutrient agar was used to determine total heterotrophic bacteria. Why was NA chosen for this purpose? Standard media for such experiments include plate count agar and, even more appropriate, R2A agar. The use of NA for this purpose will almost certainly cause an underestimation of THB counts. Further underestimation would occur by incubating at 37 degrees C for just 18-24 hours. A lower incubation temperature, such as 20-30 degrees C and longer incubation time would likely have yielded a higher number of CFUs. To enrich petroleum-degrading bacteria (PDB), the authors should include details of the medium used, including a full list of ingredients and method of preparation. Another major issue is that the authors include no detail whatsoever in the methods used to identify isolates, except to say that Gram stains and "chemical and biochemical tests" were carried out. What chemical and biochemical tests were done? These must be included. Also, a major deficiency in this section is the lack of phylogenetic data. It is standard to include 16S rRNA gene sequence analyses (if not full genome analysis) to determine microbial taxa. The lack of any sequence analysis at all to verify taxonomic conclusions makes these determinations very weak and unsupported. With no sequence analysis, the authors should, at the very least, include details of what biochemical tests were performed to support their conclusions.	1. Nutrient agar is a general purpose media and it may not be rich as R2A agar but no media can satisfy the growth requirement of bacteria from an environment. 2. What authors meant was that human activities and direct discharges to station one (1) were very minimal. 3. The distance from station 2 to 1 is included. 4. The process of sterilization of sample containers is spelled out. 5. Aseptic procedures for water collection were stated. 6. Incubation was at 35°C not 37°C. 7. Details of ingredients used for petroleum degrading bacteria agar were listed. 8. Identification table for isolated bacteria were included. 9. There were more counts in wet season but not significant as stated. 10. Line 154- cfu is corrected. 11. Y-axis scale on figure 1 - corrected. 12. Y-axis scale on figure 3 – corrected.



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Finally, the results do not seem to support the conclusions in a substantive way. The authors state that the highest bacterial counts corresponded to the wet (monsoon) season. However, the data provided are very erratic and don't seem to show a clear seasonal pattern. I expect it is true that higher counts occur after each rainfall due to erosion into waterways, but if a clear seasonal link can be tied to these data, it escapes me.

The manuscript is riddled with typos, grammatical errors, and oddities, such as the unconventional formatting in many places where scientific notation is used (e.g., "17.6 x 10⁶ CFU/g" instead of "1.76 x 10⁷ CFU/g" in line 154). Also, there are no callouts for figures 1 and 2 in the text, and the figure legends are very brief, with little detail. The Y-axis scale in figure 1 should be 0, 2, 4, 6, 8, with the units being 10⁴ instead of 10³. In figure 3, the Y-axis units are 10³, but the accompanying text says 10⁵. Which is correct? It seems like there are just too many problems to fix to support publication of this manuscript, beginning with the experimental design and lack of phylogenetic data.