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| Journal Name:  | [**Journal of Advances in Microbiology**](https://journaljamb.com/index.php/JAMB)  |
| Manuscript Number:  | **Ms\_JAMB\_132010**  |
| Title of the Manuscript:  | **Characterization of Microbial Diversity at Watering Points Along the Upper Isiukhu River in Kakamega County, Kenya Using 16S rRNA Sequencing**  |
| Type of the Article  | **Original Research Article**  |

**PART 1: Comments**

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|  | **Reviewer’s comment**

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| **Artificial Intelligence (AI) generated or assisted review comments are strictly prohibited during peer**  |
| **review.** |  |

  | **Author’s Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*  |
| **Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.**  | **This manuscript provides critical insights into the microbial diversity of freshwater ecosystems, specifically in the Upper Isiukhu River, using advanced 16S rRNA sequencing techniques. Understanding microbial communities, including both cultivable and non-cultivable species, is essential for assessing water quality, ecosystem health, and the impact of anthropogenic activities on microbial assemblages. The study’s findings contribute to microbial ecology by identifying key bacterial phyla and their distribution, offering valuable data for conservation efforts and freshwater resource management. Furthermore, this research enhances the scientific community’s ability to monitor and predict ecological changes in response to environmental and human-induced factors, ultimately supporting sustainable water management strategies.**  |   |
| **Is the title of the article suitable?** **(If not please suggest an alternative title)**  | **The current title, "Characterization of Microbial Diversity at Watering Points Along the Upper** **Isiukhu River in Kakamega County, Kenya Using 16S rRNA Sequencing," is informative but could be refined for conciseness and clarity.** **Suggested Alternative Titles:** **"Microbial Diversity in the Upper Isiukhu River, Kenya: A 16S rRNA Sequencing Approach"** **"Assessing Microbial Diversity at Watering Points Along the Isiukhu River, Kenya Using 16S rRNA Sequencing"**  |   |
| **Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.**  | **The study aims to evaluate their influence but does not specify which factors were analyzed (e.g., pollution, agricultural runoff, human activity).** **Including specific variables measured would strengthen the ecological relevance.** **The abstract lists microbial phyla but does not interpret their ecological roles or significance.** **No mention of functional implications, such as potential pathogenic species, bioindicator taxa, or water quality impact.** **The phrase "640,775 sequences passed quality control" lacks context—was this sufficient for robust microbial diversity analysis?** **Percentages of bacterial phyla are provided, but variance across sites is not discussed.** **The conclusion states that the study "reveals significant microbial diversity" but does not specify the key findings that support this claim.** **Needs a stronger link to conservation, public health, or water management applications.**  |   |
| **Is the manuscript scientifically, correct? Please write here.**  | The manuscript presents scientifically valid methods, including 16S rRNA sequencing and bioinformatics analysis, but lacks depth in statistical validation and ecological interpretation. The results are descriptive rather than analytical, failing to rigorously assess the impact of anthropogenic and environmental factors on microbial diversity. Without stronger statistical justification and a clearer discussion of microbial functional roles, the scientific robustness of the study remains questionable.  |   |
| **Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.**  | The references are inconsistently formatted, lacking adherence to any standard citation style, which reflects poor scholarly rigor. Additionally, the inclusion of outdated references, some over a decade old, raises concerns about the relevance and currency of the literature review. The manuscript must incorporate recent, high-impact studies to ensure scientific credibility and alignment with current advancements in microbial ecology.  |   |
| **Is the language/English quality of the article suitable for scholarly communications?**   | Moderate  |   |
| **Optional/General** comments   |  |   |

**PART 2:**

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|   | **Reviewer’s comment**  | **Author’s comment** *(if agreed with the reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*  |
| **Are there ethical issues in this manuscript?**   |   |      |