CIFOR World Agroforestry	**SOP 003**	**Date: 11.09.2023** **Author:** Lukelysia Mwangi **Authorizer:** David Lelei

STANDARD OPERATING PROCEDURE

METHOD FOR ISOLATION AND ENUMERATION OF BACTERIA AND FUNGI RATIOS

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1. Inclusion of workflow
2. Inclusion of quality control document

Contents

ABBREVIATIONS

- CFU - Colony forming unit
- PDA - Potato dextrose agar
- B -Bacteria
- F - Fungi

1. INTRODUCTION

Soil fertility is an essential component of sustainable agriculture and ecosystem health. Understanding the relationship between the bacterial (B) and fungal (F) populations in soil is crucial in determining soil health and fertility. The B:F population ratio is an important metric used to evaluate the status of the soil microbial community and its ability to sustain plant growth. High B:F ratios are generally indicative of healthy soil ecosystems, as fungi are important for nutrient cycling and carbon sequestration, while bacteria are involved in nutrient mineralization and decomposition processes. Therefore, monitoring the B:F ratio can provide valuable insights into the functioning of soil microbial communities, and aid in developing strategies to enhance soil fertility and productivity. In this SOP, we provide guidelines for the quantification of the B:F ratio in soil samples, using a standardized laboratory protocol.

2. SCOPE AND APPLICATION

Soil bacteria and fungi form the soil food web that supports soil health and other living organisms within the soil. This procedure will facilitate the guidelines for isolation and estimation of soil fungi and bacteria populations which after calculation, can aid in knowing the population of these organisms in a landscape. Using the pour plate method, a volume of 1ml of the diluted soil sample is transferred into a sterile Petri dish and then molten agar is poured into the plate and mixed. The inoculated Petri dishes are then incubated for 7 days at 35.5°C and 23°C for bacteria and fungi, respectively. This method yields isolates that form colonies throughout the agar. The colonies are then counted and the colony forming units (CFUs) analysed to give the bacteria: fungi ratios.

3. PRINCIPLE

The soil contains a wide range of micro-organisms whose population changes constantly with diurnal and seasonal fluctuations. These organisms influence the physio-chemical properties of the soil and aid in decaying and decomposition of organic matter hence transformation of the mineral nutrients in the environment. Soil microbes perform many important ecosystem services in the soil including improved soil structure and soil aggregation, recycling of soil nutrients, and water recycling. Soil organisms form microaggregates in the soil by binding soil particles

together with their secretions. These microaggregates are the building blocks for improving soil structure. Improved soil structure increases water infiltration and water holding capacity of the soil. Fungi are the major decomposers in soil and help in nutrient turnover, binding together of soil particles as well as delivering water and nutrients to plant roots. This procedure highlights how to isolate, count colonies and calculate the abundance, diversity and ratios of fungi and bacteria.

4. PROCEDURE

4.1 TOOLS AND EQUIPMENT

a) Laminar flow
b) Autoclave
c) Incubator
d) Colony counter
e) Water still
f) Dispensing bottles
g) Mccartney bottles
h) Soil sample
i) Culture media
j) Water blanks
k) Sterile pipettes
l) Sterile Petri dish
m) Mark pens
n) Spatula
o) Dust coat, nose masks and gloves

4.2 ISOLATING AND ENUMERATING BACTERIA AND FUNGI

a) Serial Dilution

Usually, one gram of soil can have millions of micro-organisms. This makes it hard to count the colonies if they are grown without being diluted because they become too crowded on Petri dishes. Serial dilution is therefore meant to reduce the concentration of microbes in solution for easier counting and estimation of soil fungi and bacteria.

Note: For a detailed description of our methodology please visit or contact us.

b) Isolation of Soil Microbes

The isolation process is done in the laminar flow chamber using the pour plate method for all the microbes.

c) Data Analysis and Reporting

- Determination of the number of bacterial/fungal cells in a soil sample is done as in the equations below:

$$\text{No. of bacterial cells /1gm moist soil} = \frac{\text{number of colonies} \times \text{inverted dilution}}{\text{Weight of dry soil}}$$

$$\text{No. of bacterial cells /1gm dry soil} = \frac{\text{number of colonies} \times \text{inverted dilution}}{\text{Weight of dry soil}}$$

- The unit of measurement here is colony forming units (CFUs)/ g of soil, where the colony may be the yields of the growth and multiplication of a single cell or more.
- The estimation of the ratio of bacteria: fungi (B: F ratio) is done and reported.

5. MAINTENANCE

- All equipment are regularly checked regarding the manufacturer's specifications.
- The results are recorded and in case of repairs, the details about the intervention are recorded.
- Details about maintenance services and repairs are compiled in the Maintenance file, available in the laboratory.

6. OCCUPATIONAL HEALTH AND SAFETY

- All activities performed under this SOP comply with the recommendations of CIFOR-ICRAF Health and Safety policy.
- Laboratory dress code: clean laboratory coats and closed low-heeled closed shoes worn at all times while performing the procedure.

7. QUALITY CONTROL

Quality Control Document for B: F Population Ratio Analysis

1. Sample Collection and Handling
- Samples should be collected and handled according to a standardized protocol to minimize variability and ensure accuracy.
- Samples should be stored properly and transported to the laboratory in a timely manner to prevent degradation.

2. Equipment and Materials
- All equipment and materials used for the analysis should be calibrated and standardized to ensure accuracy and precision.
- Glassware and other laboratory equipment should be properly cleaned and sterilized before use.

3. Data Analysis and Reporting
- All data should be carefully reviewed and analyzed for accuracy and consistency.
- Results should be reported in a clear and concise manner, with appropriate units and statistical measures included.

4. Documentation and Record-Keeping
- All procedures and results should be properly documented and recorded, including sample identification, dates, and personnel involved.
- Any deviations from standard procedures or unexpected results should be thoroughly documented and investigated.

8. REFERENCES

- Ameh, A. A., and Kawo, A. H. (2017). Enumeration, isolation and identification of bacteria and fungi from soil contaminated with petroleum products using layer chicken droppings as an amendment. Bayero Journal of Pure and Applied Sciences, 10(1): 219 - 225.
- Schloss, P. D., & Handelsman, J. (2005). Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. Genome Biology, 6(8), 1-5. https://doi.org/10.1186/gb-2005-6-8-229

8. ANNEX

Workflow