PW 3: Enumeration of soil microorganisms

The aim of this practical is to isolate and enumerate culturable soil microorganisms (bacteria, and fungi) using serial dilutions and surface plating, and to express their abundance as colony-forming units per gram of soil (CFU/g).

Materials and media

- Soil sample (taken from 0–20 cm depth, placed in a clean bag).
- Sterile peptone water or sterile physiological saline (diluent).
- Dilution tubes (9 ml).
- Sterile pipettes (1 ml) or micropipette with sterile tips.
- Sterile Petri dishes.
- Media: Nutrient agar (total heterotrophic bacteria) and Potato Dextrose Agar (PDA) or Sabouraud agar (filamentous fungi and yeasts).

Experimental procedure

Sample preparation

- Weigh 10 g of soil into an Erlenmeyer flask containing 90 ml of sterile diluent (10^{-1} dilution).
- Shake vigorously (vortex or orbital shaker) for 10–15 min to detach microorganisms from soil particles.

Serial dilutions

- Prepare a series of tubes, each containing 9 ml of sterile diluent.
- Transfer 1 ml from the 10^{-1} suspension into 9 ml to obtain 10^{-2}, then continue stepwise up to 10^{-6} or 10^{-7}, depending on expected soil microbial load.

Plating

For each microbial group, select 2–3 appropriate dilutions and perform at least duplicate platings.

- Total bacteria:
  - Plate 0.1 ml from dilutions 10^{-4} to 10^{-6} onto nutrient agar, spread over the surface.
  - Incubate plates at 30–37 °C for 24–48 h (inverted plates).
- Fungi:
  - Plate 0.1 ml from dilutions 10^{-2} to 10^{-4} onto PDA or Sabouraud agar, spread gently.
  - Incubate at 25–28 °C for 3–7 days; plates may be kept upright to reduce medium dripping where appropriate.

Data processing and calculations

- Only consider plates with 30–300 colonies as countable.
- For each medium and dilution, calculate the mean colony number from replicate plates.
- Use the general formula for enumeration on solid media:

$$CFU/g = N \times FV$$

where N = mean colony count, F = dilution factor, V = plated volume (ml).