# Exp.1: Contact Slide Assay

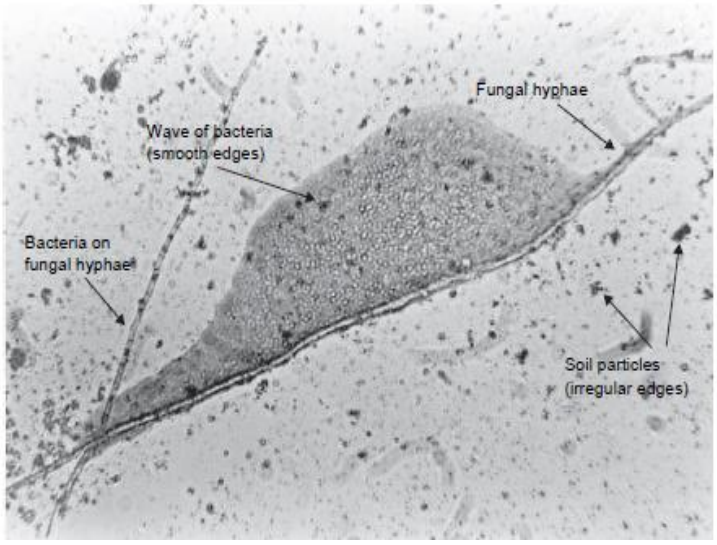
**THEORY:**

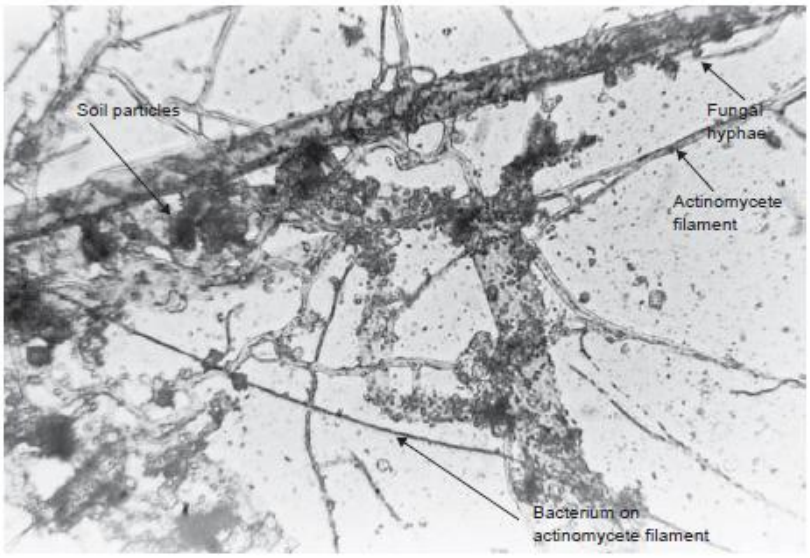
The ability to view soil microbes *in situ* is important since it allows students to view the interrelationships between soil microbes and their interactions with soil particles. However, it is difficult to observe colloidal size microbes that exist within soil. A technique developed back in the 1930s is still a valuable learning tool today. This is the contact slide or buried-slide technique of Rossi et al. (1936), which is a simple technique for qualitatively assessing the spatial relationships between soil microorganisms. It is useful to illustrate the orientation of soil organisms to one another and to soil particles. It also allows students to see bacteria, actinomycetes, fungi and spores; the technique involves burying a glass slide in soil for a defined period of time (Figure 1-1). Nutrient amendments, such as the carbon source glucose and the nitrogen source ammonium nitrate, encourage the rapid proliferation of heterotrophic microorganisms.

After removing the slide from within the soil, the slide is fixed with acetic acid and stained to provide contrast, as the often-colorless organisms would otherwise not be visible under a microscope.

Viewed under a microscope, soil bacteria, actinomycetes, and fungi can be seen growing on soil particles, in pure colonies on the slide, and in juxtaposition to each other, often with bacteria lining the fungal hyphae.

1. Adjust soil moisture to a value close to “field capacity” (value provided by instructor).
2. Insert glass slides into a beaker of moist soil.
3. Incubate for one week.
4. Remove slides, stain with phenolic Rose Bengal.
5. View under microscope




## Materials and Methods:

### Materials First

300 g of each soil 1% glucose

$NH_4NO_3$ 2 polystyrene cups for each soil type, volume 250ml

Label tape and pens Plastic wrap

Four microscope slides for each soil type Rubber bands

Weighing paper Deionized water in a wash bottle

Analytical balance and benchtop balance (±0.01 g) Graduated cylinder

### Method First

**1.** Weigh out 150 g portions of each soil into two cups, recording the mass of the soil you added to each cup. Label one cup as "treatment" and the other as "control." A 100 g sample of soil should be used for soils high in organic matter, as they are less dense than mineral soils.

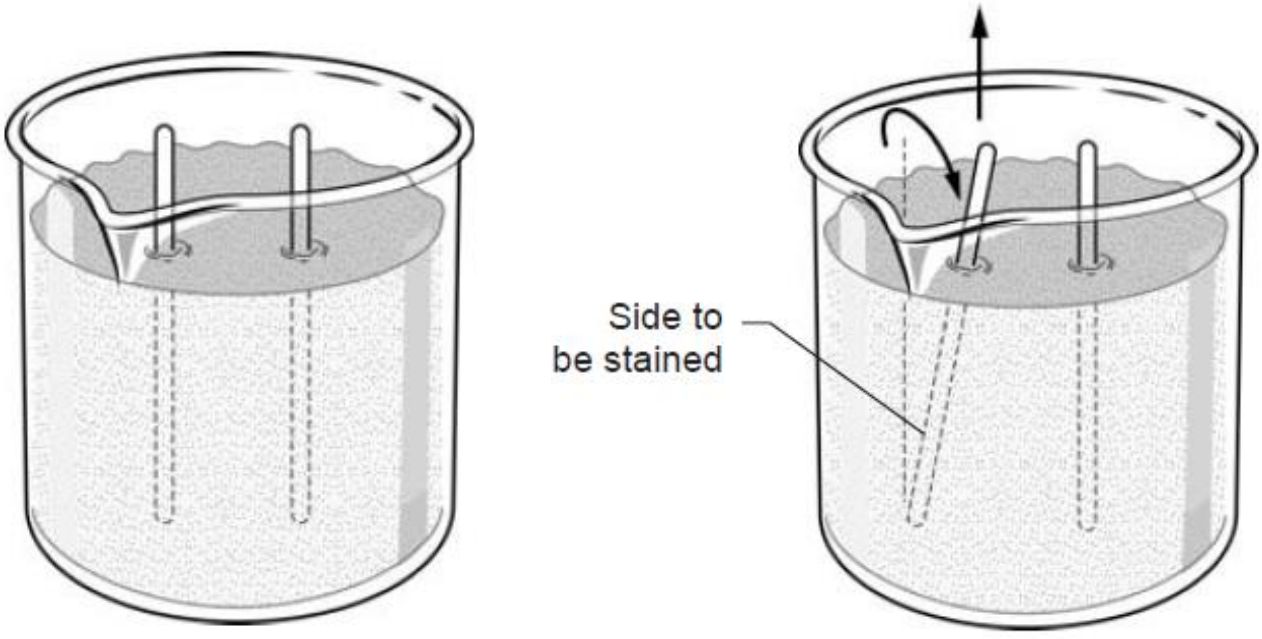
**2.** Calculate the amount of moisture necessary to alter the moisture content of the soil samples to the moisture content specified by your instructor. This soil moisture content is often close to field capacity.

Measure out this much-distilled water with a graduated cylinder and add it to each of two vials. Label one vial "treatment" and the other "control."

**3.** Amend the water in the treatment vial with enough glucose for a final soil glucose concentration of 1% (w/w) on a dry weight basis in the treatment soil above. Also add 200mg of $NH_4NO_3$ to the treatment vial.
Stir to dissolve the amendments. Do not amend the control vial.

**4.** Mix the contents of the treatment and control vials into their respective Cups by adding the liquid to the soil in small aliquots, and mixing with a spatula after each moisture addition. For heavy textured clay, soils avoid mixing, as this will "puddle" the soil.

**5.** For each cup, label two clean microscope slides, designating the soil and treatment for that slide. There will be two slides for each cup. Insert each slide vertically into its respective cup, leaving 2 cm of each slide projecting above the soil surface. Do not force the slides as they will break.



**6.** Cover the cups with plastic wrap, securing with a rubber band. Puncture the wrap or foil several times with a probe to allow air in and yet preclude excessive evaporation of moisture. Weigh each cup. Incubate the soil-filled cups at room temperature in a designated incubator for one week.

**Materials Second**

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|---|---|
| Incubated cups from Period 1 | 40% (v/v) acetic acid |
| Phenolic Rose Bengal stain | |
| Staining racks with a pan to catch excess stain | |
| Protective goggles | microscopes |
| Immersion oil | paper towels |

**Methods Second**

**1.** Re-weigh the cup and calculate the soil moisture at the time of slide removal.

**2.** Remove the two slides from each cup after seven days by pressing each slide to an inclined position and withdrawing in a manner such that the upper face of the slide is not disturbed. Mark and identify the side to be stained

**3.** Gently tap the slide on the bench top to remove large soil particles from the slide surface. Clean the lower face with a damp paper towel and dry the slide at room temperature.

**4.** Wearing protective goggles, immerse the slide in 40% (v/v) acetic acid for 1–3 min under a fume hood, holding the slide with forceps.

**5.** Wash off the excess acid under a gentle stream of water, and cover the surface with phenolic Rose Bengal from a dropper bottle, supporting the slide on a staining rack over a container to catch the excess stain.

Be careful not to wash with such force as to remove microorganisms from the slide surface.

**6.** Stain for 5–10 minutes, *but do not permit the slide to become dry*. Add more stain as needed.

**7.** Gently wash the slide to remove excess stain. Dry and examine the slide microscopically using the oil immersion objective. Compare what you see with.