Exp.2: Microbial population counts in soil

Soils contain enormous numbers and kinds of microorganisms. In addition to the multitudes of bacteria, there are protozoans, yeasts, molds, algae, and microscopic worms in unbelievable numbers. Types that predominate will depend on the composition of the soil, moisture, pH, and other related environmental factors. No one technique can be used for counting all organisms since such great variability in types exists.

In this exercise, we will use the plate count procedure that was used in many bacteriological studies require that we be able to determine the number of organisms that are present in a given unit of volume. Several different methods are available to us for such population counts.

To get by with a minimum of equipment, it is possible to do a population count by diluting out the organisms and counting the organisms in a number of microscopic fields on a slide.

In this exercise, we will use **quantitative plating** (Standard Plate Count, or SPC) and **turbidity measurements** to determine the number of bacteria in a culture sample. Although the two methods are some-what parallel in the results they yield, there are distinct differences. For one thing, the SPC reveals information only as related to viable organisms; that is, colonies that are seen on the plates after incubation represent only living organisms, not dead ones, turbidimetry results, on the other hand, reflect the presence of all organisms in a culture, dead and living.

The quantitative plating (Standard Plate Count, or SPC) procedure consists of diluting the organisms with a series of sterile water

Generally, only three bottles are needed, but more could be used if necessary by using the dilution procedure indicated here, a final dilution of 1:1,000,000 occurs in blank C. From blanks B and C, measured amounts of

the diluted organisms are transferred into empty Petri plates. Nutrient agar, cooled to 50° C, is then poured into each plate. After the nutrient agar has solidified, the plates are incubated for 24 to 48 hours and examined.

A plate that has between 30 and 300 colonies is selected for counting. From the count, it is a simple matter to calculate the number of organisms per milliliter of the original culture. It should be pointed out that greater accuracy can be achieved by pouring two plates for each dilution and averaging the counts. Duplicate plating, however, has been avoided for obvious economic reasons.

Procedure

From soil samples, a suspension must be prepared first and diluted. Usually a 10-fold dilution series is applied. Different media can be used for a given sample. A known volume (0.1 mL) of the dilutions is plated onto the surface of a suitable growth medium. After the infected plates had been incubated up to one week, the average number of colonies on plates can be determined.

The number of viable microbes per (g) of the initial sample can be calculated from the average of colony numbers on parallel plates and the known dilution factor (CFU/ g sample).

Calculate the number of bacteria per ml of undiluted culture using the data recorded in section A of the Laboratory Report. Multiply the number of colonies counted by the dilution factor (the reciprocal of the dilution).

Example: If you counted 220 colonies on the plate that received 1.0 ml of the 1:1,000,000 dilution: 220 _ 1,000,000 (or $2.2 * 10^8$) bacteria per ml. If 220 colonies were counted on the plate that received 0.1 ml of the 1:1,000,000 dilution, then the above results would be multiplied by 10 to

convert from number of bacteria per 0.1 ml to number of bacteria per 1.0 ml (2,200,000,000, or 2.2 *10^9).