

A ten-fold dilution (Simple dilution):

Principle of the dilution : is used to reduce the concentration of the substance.

A ten-fold dilution, also known as a 1:10 dilution, is a dilution which one part of a stock is mixed with nine parts of diluent. This means the new concentration is one-tenth of the original concentration. It's a common technique in scientific experiments, especially when dealing with very high concentrations.

How to perform a tenfold dilution :

You Take 1 unit of a sample (the "stock").

Add diluent: add 9 units of diluent (e.g., water, saline, buffer) .

Final volume : Transfer one part of the stock into the container with 9 ml of diluent = 10 ml

Example :

- Take 1ml of solution
- Add 9 ml of distilled water $\Rightarrow$ final volume = 10 ml , the concentration becomes 1/10 of original .

$$\frac{1 \text{ ml of sample}}{1 \text{ ml of sample} + 9 \text{ ml of diluent}} = \frac{1 \text{ ml}}{1 \text{ ml} + 9 \text{ ml}} = \frac{1}{10} = 10^{-1}$$

What if the sample is not liquid, for example, a food sample? In this case, we can consider that 1 gram of sample is equal to 1 ml:

$$1:10 \text{ dilution} = \frac{1 \text{ g of sample}}{1 \text{ g of sample} + 9 \text{ ml of diluent}} \Rightarrow 10 \text{ ml of } 1:10 \text{ dilution } (10^{-1})$$

Dilutions are usually made ten-fold, hundred-fold or multiples, it is feasible to use 0.5 ml of sample in 4.5 ml of diluent .

Generally, a dilution of $1/1,000,000$ (10^{-6}) is sufficient to decrease the number of viable cells to the point that an appropriate number of colonies will develop on solid medium.

Serial dilution : multiple repeated dilutions in steps (e.g. $1:10 \rightarrow 1:100 \rightarrow 1:1000$)

$$\text{Previous concentration} \times \frac{\text{Units transferred}}{\text{Total units}} = \text{New Concentration}$$

Isolation and Enumeration of Soil Microorganisms

The soil environment is a microbial community that consists of bacteria, molds ,yeast ,actinomycetes , algae and protozoa. Culture media used the following, according to the type of microorganisms to be isolated.

- 1- **Nutrient agar:** for the enumeration of general bacteria.
- 2- **Jensen's media:** for enumeration of Actinomycetes.

Actinomycetes isolate on Petri dishes are characterized by a dry or dusty or chalky colony. Also, it characterized by a distinctive earthy odor like the smell of rain when falls on dry soil.

- 3 - **Sabouraud agar:** for enumeration of fungi.

Collection of soil samples

- 1- Take into consideration the type of soil, whether fertilized or non-fertilized soil, salty or not.
- 2- Remove the superficial layer of the soil because it's contaminated by so many environmental factors & exposure to UV Light.
- 3- The sample should be taken from **3-5 cm** under the surface of the Soil.
- 4- Clean the samples from stones and remnants of the root of the plants.
- 5- Use clean and sterile tools, ex; clean nylon sac.
- 6- Immediate study and examination of the samples as soon as possible and avoid storage; otherwise, store in a cool place.

Enumeration methods:

1. Direct slide count (Breed Method).
2. Dilution plate count (Viable Plate Count):-

The viable count is widely used for determining the viable number of soil bacteria, and it applies to any Microorganisms that will grow as colonies.

There are two main methods for bacterial counting: spread plate method and pour plate method

- 1- **Spread plate method:** The technique used to quantify the bacteria in a sample.
- Whereas ; 2-**The pour plate method** for counting bacteria from a soil sample is more accurate than the spread plate method.

To find the No. of microbial cells in 1gram of soil sample is calculated using this equation :

$$\text{No of cell / gm soil} = (\text{No. of viable colonies} \times \text{Inverted dilution})$$

That means if the dilution is $1/10 \Rightarrow 10/1$

So ; Inverted dilution" is called reverse dilution

Isolation of Bacteria: -

We are going to isolate bacteria from soil by using Dilution plate count technique (Pouring plate count).

1- First, we have to add 1 gm of the collected soil sample to 9 ml (D.W or N.S) to get 10^{-1} dilution, mix properly. Further Serial dilutions depend on the soil type as follows:

- Domestic soil 6 dilutions
- Vegetable soil 4 dilutions
- Uncultured soil 2 dilutions

2- Then transfer 1 ml from the selected dilution to sterile Petri dishes, after that pour the melted agar into the dishes and thoroughly mix with the sample by a gentle rotation of the dish.

3- Finally incubate plates at $30 - 37^{\circ}\text{C}$ for 24 hr.

Note: count plates which show only about 30-300 colonies.

For isolation spore forming soil bacteria:

heat the sample dilution at 80°C water bath for about 15 min, then transfer 1 ml to Petri dish and continue the same procedure as above.

For isolation spore forming Soil Fungi :

Fungi typically divided into:

1- **Molds:** which are composed of branching filaments termed hyphae, that grow by apical extension to form mycelium.

2- **Yeasts:** which are unicellular and oval or round in shape and reproduce mainly a sexually by budding.