Chapter 1
Soil Collection

Soils are discontinuous heterogeneous environments that contain large numbers of diverse microbial populations including bacteria, fungi, algae, protozoa, and viruses. These populations vary with depth and soil type. In general, surface soil horizons have more organisms than subsurface horizons. Thus populations are influenced by many factors such as soil depth, soil type, and natural microsite variations. Natural microsite variations can allow very different microorganisms to coexist side by side in the same region of soil. Because of the great variability in soil microorganisms, it is always necessary to consider more than one sample during a microbial analysis of a site. Otherwise, it is not possible to get the complete picture of a selected soil. Thus, the sampling strategy is influenced by the goal of the analyses, the resources available, the site characteristics, and the history of the soil. The most accurate approach is multiple and individual analysis (MIA), which means taking many samples within a given site and performing a separate analysis of each sample. Another approach is composite analysis. An advantages of this approach is reducing time and effort by combining the multiple samples taken to form a composite sample; this in turn limits the number of analyses that must be performed. Thus composite sampling is better than the MIA approach. Another approach often used is to sample a site sequentially over time from a small defined location to determine effects on microbes. Such effects change over time; thus the effects are temporal.

Soil samples are usually collected using different tools or equipment as needed. For instance, bulk soil samples are easily obtained with a shovel (Fig. 1.1) or, better yet, a soil auger (Fig. 1.2). Soil augers are more precise than simple shovels because they ensure that samples are taken from exactly the same depth on each occasion. This is important, as several soil factors can vary considerably with depth, such as oxygen, moisture, and organic carbon content and soil temperature. Thus, soil augers are useful in characterizing the soils on the basis of depth. A simple hand auger is useful for taking shallow (up to depths of 6 ft) soil samples from areas that are unsaturated.

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Fig. 1.1 Soil shovel

Fig. 1.2 Soil auger

However, when samples are collected for microbial analysis, there is a possibility of sample contamination as the auger is pushed into the soil. Once the auger is inserted into the soil, microbes usually stick to the sides of the auger. When the auger pushes downward, it may contaminate the bottom part of the core. This causes erroneous results in the microbial profile of different soil depths. However, this problem can be overcome by using a sterile spatula to scrape away the outer layer of the core and using the inner part of the core for further analysis. Furthermore, the sample collected in this manner may not be truly representative of the site. This is due to the varied nature of soil and limited diameter of an auger. Thus, it is always better to collect several samples and prepare a composite sample. This greatly reduces the total number of samples and associated costs of the analyses that are performed. Proper procedures should be followed while preparing the composite samples. The foremost requisite for the preparation of a composite sample is the selection of a wide and uniform area. Then equal amounts of soil samples are collected and placed in a sterile bucket or plastic bags. Later, these samples are mixed and become the composite sample. Another advantage of a composite soil sample is that if the sample seems big enough for storage, a portion of the composite sample can be removed, and leftover sample can be analyzed. In order to get the precise data of the soil, samples should be stored on ice until further processed and analyzed. In other incidences, the experimental aim is to test the effect of a soil amendment (such as fertilizer, pesticide, or sewage sludge) on microbial populations over untreated control. In such a case, a sample of each treatment must be analyzed separately and compared with the untreated control. There is also another method of soil collection, called randomized sampling. It involves choosing points randomly within the selected site.

Soil samples can also be collected in different directions of a selected site. For instance, samples are collected in a single direction called transect sampling. This type of sampling is very useful if a sampling site is situated on the bank of a river called a riparian area (Fig. 1.3). Transects could be chosen adjacent to a streambed and at right angles to the streambed. This type of sampling greatly helps in the

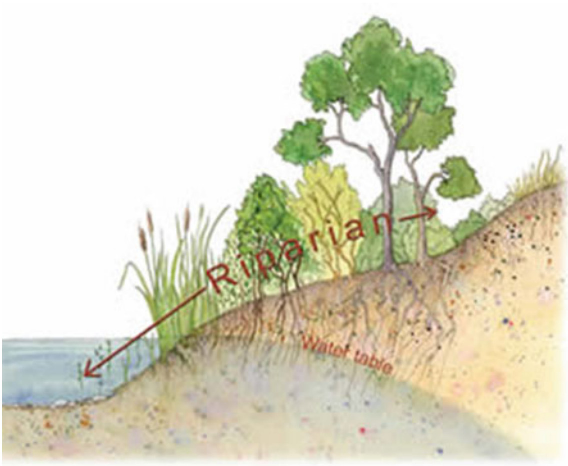

Fig. 1.3 Riparian area

evaluation of influence of a stream on microbial populations. There is another method called two-stage sampling, which is suitable when a site consists of a hillside slope and a level plain. During sampling by this method, the area is broken into regular subunits called primary units. Furthermore, subsamples can be taken randomly or systematically within each primary unit. On the other hand, grid sampling is used when little is known about the variability within the soil of a mapping area. In this type of sampling, samples are taken systematically at regular intervals at a fixed spacing.

In the present investigation, soil samples (test) were collected from different sites, where effluents are being discharged by Sri. Rayalaseema Sugars and Energy (Pvt.) Limited (Nandyal Sugar Factory), Ayyalurimetta village, and Nandyal, Kurnool district of Andhra Pradesh. A soil sample without sugar mill effluents (control) was collected from a site adjacent to the sugar mill. These two soil samples were air dried and mixed thoroughly to increase homogeneity and shifted to <2 mm sieves for determination of soil texture.