NOTES

PROBLEMS IN THE USE OF AGAR FOR THE ENUMERATION OF SOIL MICROORGANISMS

During an investigation of the biological decomposition of herbicides, a study was initiated to ascertain the numbers of microorganisms in soil capable of utilizing these pesticidal chemicals as the sole carbon source. Since the apparent population size observed was far in excess of that expected, many of the colonies were tested in liquid media in order to verify the apparent utilization and decomposition of the herbicidal compounds. These cultures failed to grow in solution culture, however, suggesting that they were using the agar or impurities contained therein as sources of carbon. A number of similar observations have been reported in other studies of herbicide decomposition. As a result of these preliminary observations, an investigation was made of the relative abundance in soil of microorganisms capable of growth in a medium with no source of carbon other than agar.

For counting, ten-fold serial dilutions of the soils were plated upon an inorganic salts-agar medium of the following composition: \(\text{NH}_4\text{NO}_3, 0.5\) g.; \(\text{K}_2\text{HPO}_4, 0.8\) g.; \(\text{KH}_2\text{PO}_4, 0.2\) g.; \(\text{MgSO}_4\cdot7\text{H}_2\text{O}, 0.2\) g.; \(\text{FeSO}_4\cdot7\text{H}_2\text{O}, 0.05\) g.; \(\text{CaCl}_2\cdot2\text{H}_2\text{O}, 0.1\) g.; agar, 18 g.; demineralized, distilled water, 1,000 ml. The solidifying preparations used included commercial grade Bacto agar (Difco) which has a smaller percentage of contaminating materials and a highly purified preparation available by Dr. W. Yaphe of the Atlantic Laboratory, Halifax, N. S.

Exceptionally high counts were obtained when soil dilutions were plated upon media containing any one of the three types of solidifying preparations. The population density varied from \(10^5\) to \(3.1 \times 10^8\) per g. of soil from New York, Honduras and Panama. Consistently higher values were obtained on Bacto agar than with Noble agar which in turn gave higher counts than the highly purified preparation. This is not unexpected when the degree of purity is considered, the highly purified material containing 98% or more of the gelling component whereas the commercial product contained only about 85% agarose (W. Yaphe, personal communication). The differences between the numbers determined using the different agar preparations may therefore be a result of the presence of additional available sources of carbon.

In order to determine the effect of substrate purification, 23 colonies from the Bacto plates were subcultured to Noble agar. Of these, 43% grew equally well on both media while 35% showed reduced development and 22% virtually no growth; apparently some readily available carbonaceous material is removed in the washing and purification. Of 46 soil isolates developing on Bacto and Noble agar, 74% were represented by \textit{Streptomyces} and some of the more developed nocardias, 13% were true bacteria and 13% fungi.

\(^{1}\text{Contribution from the Department of Agronomy, New York State College of Agriculture, Cornell University, Ithaca, New York, as Agronomy Paper No. 461. Research supported in part by grants from the United Fruit Company and regional project NE-42. Received Aug. 17, 1959. Approved Aug. 28, 1959.}\)