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# DETERMINATION OF SIZE-NUMBER RELATIONSHIP AMONG LIVING MICROORGANISMS IN SOIL BY SELECTIVE MEDIA

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**Abstract**

When selective media were used to determine microbial population, the average numbers of bacteria and actinomycetes were found to be about 346 and 108 times, respectively, greater than those of fungi in 15 soil samples tested. There was an inverse, linear relationship on the logarithmic basis between propagule volume and population density among the 45 groups of microorganisms isolated from these soil samples. The total biovolume of culturable microorganisms ranged from $9.9 \times 10^6$ to $142 \times 10^6$ $\mu m^3/g$, that was about 0.001% to 0.145% of the total weight of soil.

## Introduction

A community is always composed of many small organisms and few large ones (Clarke, 1954; Kendeigh, 1961; Odum, 1959). Such general phenomenon among animals and higher plants is usually expressed as "pyramid of numbers". Very little is known about the size distribution of microorganisms in nature. The number of bacteria and actinomycetes in soil is always greater than that of fungi in spite of the differences in climate and cultural practices among soil studied (Alexander, 1977; Burges, 1958; Waksman, 1952). The only exceptions are found in extreme environments such as soil with low pH values which is detrimental to bacteria and actinomycetes (Burges, 1958). This seems to indicate the existence of a similar size-number relationship among microorganisms in soil because fungi are much larger than bacteria and actinomycetes. Using the method of direct microscopic observation, Jenkinson *et al.* (1976) showed that there was an inverse relationship between size and number of microorganisms in soil. However, the direct microscopic observation can not distinguish living from dead cells, nor can it distinguish bacteria from actinomycetes (Gray and Williams, 1972). In this paper we report the investigation on size-frequency relationships of microorganisms in soil using selective media to determine the numbers and organisms developing on those media to determine the sizes. A brief account of this study has been published (Chuang and Ko, 1979).

## Materials and Methods

PDA (Potato dextrose agar)-surfactant (Steiner and Watson, 1965), PCNB (Pentachloronitrobenzene)-soil extract agar (Farley and Lockwood, 1968), and water agar (Lingappa and Lockwood, 1962) were used for determining numbers of fungi, bacteria, and actinomycetes, respectively, in soil. Fifteen soil samples from the depth of 0 to 15 cm beneath vegetation such as corn, bean, sweetpotato, tomato, ginger, grass and ohia trees were collected. Soil suspension was prepared by grinding 12.5 g of soil with 100 ml sterile distilled water in an Omni-Mixer (Sorvall Instruments, Newton, Connecticut 06470, U. S. A.) at 4,500 rpm for 30 sec. Before planting on agar media, the suspension was diluted to $2\times10^{-4}$ for fungi and $10^{-6}$ for both bacteria and actinomycetes. Plates were incubated at room temperature (about 25–28°C) for 7 days for fungi and bacteria and 14 days for actinomycetes. Six plates were used for each treatment. For fungi 5 to 10 distinct types based on colony characteristics were recognized per soil sample. These were species belonging to different or the same species. Since most fungi isolated by the dilution plate method exist in soil as spores (Gray and Williams, 1972), 50 spores were measured for each type. To measure the size of bacteria and actinomycetes, 10 colonies were selected randomly for each soil sample, and 20 bacterial cells or actinomycetes spores were measured per colony. For each soil samples, the average propagule size was based on 5–10 colonies of fungi or 10 colonies of bacteria or actinomycetes. The volume of microorganisms was calculated using the formula of $V=4/3\,\pi r^3$ for spherical propagules and $V=0.5236ab^2$ ($a$=length of propagule; $b$=width of propagule) for ellipsoid propagules (McCallean, 1958). Data were used for computation of regression equations and correlation coefficients after logarithmic transformation.

## Results

For all the 15 soil samples tested, the numbers of bacteria and actinomycetes were far greater than those of fungi (Table 1). The average numbers of bacteria and actinomycetes were about 346 and 108 times greater than those of fungi, respectively. The differences in population density among these microorganisms were correlated with their individual size, since the

**Table 1.** *Total numbers and biovolumes of microorganisms in one gram of dry soil*

| Location | Bacterium | | | Actinomycete | | | Fungus | | | Gross Volume ($X10^6 \mu m^3$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number ($X10^6$) | Volume ($\mu m^3$) | Total Volume ($X10^6 \mu m^3$) | Number ($X10^6$) | Volume ($\mu m^3$) | Total Volume ($X10^6 \mu m^3$) | Number ($X10^6$) | Volume ($\mu m^3$) | Total Volume ($X10^6 \mu m^3$) | |
| # 1 (Puna, Hawaii) | 263 | 0.24 | 63.15 | 13 | 1.75 | 22.75 | 0.10 | 20 | 2.00 | 87.87 |
| # 2 (Puna, Hawaii) | 99 | 0.22 | 21.78 | 13 | 1.36 | 17.68 | 0.66 | 22 | 14.52 | 53.98 |
| # 3 (Puna, Hawaii) | 167 | 0.21 | 35.07 | 26 | 0.58 | 15.08 | 0.53 | 13 | 6.89 | 57.04 |
| # 4 (Hilo, Hawaii) | 167 | 0.36 | 60.12 | 5 | 0.40 | 2.00 | 0.19 | 43 | 8.17 | 70.29 |
| # 5 (Hilo, Hawaii) | 63 | 0.50 | 31.50 | 5 | 3.35 | 16.75 | 0.15 | 183 | 27.45 | 75.70 |
| # 6 (Volcano, Hawaii) | 19 | 0.16 | 3.04 | 5 | 0.52 | 2.60 | 0.04 | 299 | 11.96 | 17.60 |
| # 7 (Volcano, Hawaii) | 100 | 1.27 | 127.00 | 13 | 0.22 | 2.86 | 0.18 | 67 | 12.06 | 141.92 |
| # 8 (Kohala, Hawaii) | 45 | 0.28 | 12.60 | 24 | 1.06 | 25.44 | 0.19 | 48 | 9.12 | 47.16 |
| # 9 (Kawaihae, Hawaii) | 3 | 4.00 | 12.00 | 5 | 0.25 | 1.25 | 0.01 | 11 | 0.11 | 13.36 |
| #10 (Pepeekeo, Hawaii) | 2 | 0.24 | 0.48 | 3 | 3.08 | 9.42 | 0.01 | 13 | 0.13 | 9.85 |
| #11 (Waimanalo, Oahu) | 56 | 0.21 | 11.76 | 22 | 0.47 | 10.34 | 0.14 | 16 | 2.24 | 24.34 |
| #12 (Waimanalo, Oahu) | 58 | 0.26 | 15.08 | 26 | 0.43 | 11.18 | 0.31 | 17 | 5.27 | 31.53 |
| #13 (Waimanalo, Oahu) | 80 | 0.20 | 16.00 | 75 | 0.42 | 31.50 | 0.55 | 17 | 9.35 | 56.85 |
| #14 (Waimanalo, Oahu) | 108 | 0.23 | 24.84 | 143 | 0.51 | 72.93 | 0.33 | 12 | 3.96 | 101.73 |
| #15 (Waimanalo, Oahu) | 12 | 0.31 | 3.72 | 8 | 0.54 | 4.32 | 0.23 | 14 | 3.22 | 11.26 |
| Average | 83 | 0.58 | 29.21 | 26 | 1.00 | 16.40 | 0.24 | 53 | 7.76 | 53.37 |

average populations of bacteria, actinomycetes, and fungi were $83\times10^6$, $26\times10^6$, and $24\times10^4$ per g of dry soil, respectively, while their respective volumes were 0.58, 1, and 53 $\mu m^3$. Moreover, there was an inverse, linear relationship between the logarithm of propagule volume and the logarithm of population density among the 45 groups of microorganisms isolated from these soil samples (Fig. 1). The calculated regression equation, $Y=9.189-1.118\,X$ (in which Y is the log numbers of propagules/g dry soil and X is log volume/100 propagules), had a correlation coefficient of $-0.855$ which is significant at $P=0.01$. The total volumes of these culturable microorganisms ranged from $9.9\times10^6$ to $142\times10^6$ $\mu m^3$/g of soil, respectively about $9.9\times10^{-6}$ to $142\times10^{-6}$ g or 0.001 to 0.014% assuming that the density of microorganisms was 1. As expected these values are much lower than those estimated by the direct microscopic measurements mainly because not all the microorganisms in soil are culturable (Gray and Williams, 1972).

## Discussion

Our results of size distribution of microorganisms in soil are compatible with those obtained with the direct microscopic observations by Jenkinson

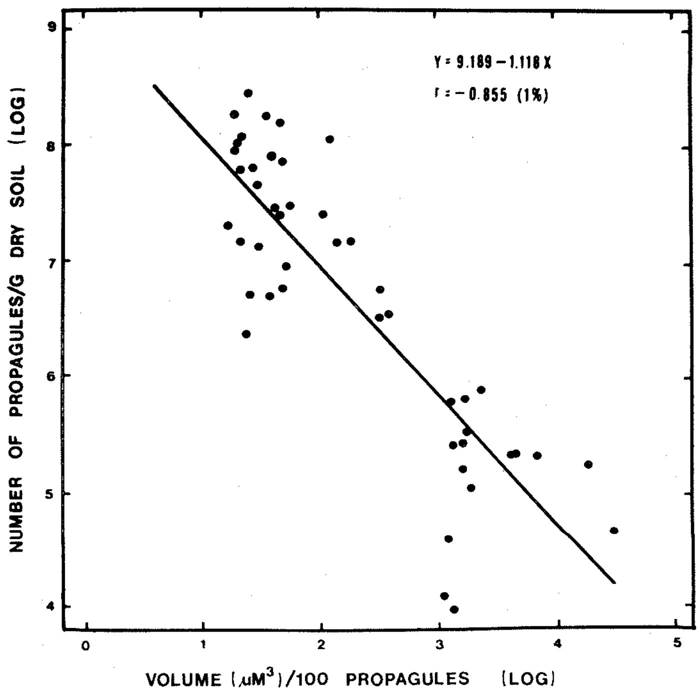


Fig. 1. Relationship between number and size of propagules of microorganisms in 15 soil samples. The correlation coefficient is significant at $P=0.01$.

*et al.* (1976). Their data showed that on the logarithmic basis the relationship between numbers and sizes of spherical organisms in soil is linear and with a slope about −1 over an appreciable range. Zvyagintsev (1964) used fluorescence microscopes to determine the number and to measure the size of microorganisms in soil. When we analyzed Zvyagintsev's data in Table 1 of his paper by converting the cell diameter to cell volvme, we also found an inverse, linear relationship between size and number on the logarithmic scale. The calculated regression equation, $Y=1.98-0.58X$ (in which Y is log numbers and X is log volumes), had a correlation coefficient of −0.995 which is significant at $P=0.01$. By analyzing data obtained from published reports through a literature survey Chuang and Ko (1981) also revealed an inverse linear relationship between the logarithmic of propagule size and the logarithmic of population density among microorganisms in soils from various countries.

The present results show that the size-number relationship among higher animals and plants in communities also exists among living microorganisms in soil. The "pyramid-of-numbers" concept states that the organism mass of volume within a life form group is potentially limited on a given habitat (Odum, 1959). This implies that the organism mass is an expression of the "carrying capacity" of the habitat and that this carrying capacity can be made up of a relatively few large-sized individuals or numerous small-sized ones, or combinations of different sizes. In macroorganisms the phenomenon of food chain is usually considered an important factor contributing to the pattern of many small organisms and few large ones (Odum, 1959). However, in microorganisms the contribution of food-chain phenomenon to the pyramid of numbers is minimal and insignificant.

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# 選擇性培養基所分離土壤微生物菌體大小之分佈

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利用選擇性培養基測定土壤微生物族羣密度，在15個土壤樣品中，細菌與放射菌之平均數目分別爲眞菌的346和108倍。將這些土壤樣品中所分離45羣微生物之菌體體積和族羣密度分別轉換爲對數值，發現兩者之間有直線負相關。在每克土壤樣品中，可培養之微生物總體積範圍爲 $9.9\times10^6$–$142\times10^6$ $\mu m^3$，此約佔土壤總重量之 0.001–0.14%。