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Influence of Depth and Temperature on Rhizospheric and Non-Rhizospheric Bacterial Population of Apple Orchard Soils of Kashmir Valley

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ABSTRACT

The present investigation on apple orchards of Kashmir Valley was undertaken during March 2004 to Oct 2004 and deals with soil bacteriological study. The main aim of the study was to find out the influence of depth and temperature on both rhizospheric and non-rhizospheric bacterial populations. The results of the study revealed a decrease in the bacterial population with soil depth. Moreover, the bacterial population was found to be more during spring months while there was a sharp decline during the summer.

Key words: Bacteriological study, rhizosphere, non-rhizosphere, bacterial population, soil-depth, temperature.

INTRODUCTION

Soil is a universal natural culture medium supporting an abundant and extremely diverse population of micro-organisms (Bakerspigel,1953). Soil is constantly changing with the change of environment and consequently its microbial population is controlled by various factors, which include edaphic (soil-type, soil-depth, soil moisture and soil reaction), seasonal changes (temperature, rainfall and light) and type of ground vegetation.

Soil bacteria play a major role in organic matter decomposition and are therefore central to soil ecosystem processes maintaining plant primary productivity (Griffiths *et al*, 2003). In the light of recent concerns regarding the impact of agriculture and climate change on biodiversity and ecosystem functioning (Wardle *et al*, 1998; Tilman *et al*, 2001), it is imperative to gain a detailed understanding of the bacterial community ecology in our native orchards. As a pre-requisite for studies examining the effects of agriculture practice and other perturbations on soil bacteria, an appreciation of the natural temporal and spatial variation at different depths of both rhizospheric and non-rhizospheric soil profiles of our orchards is desirable.

The physico-chemical and structural characteristics of soil provide many microenvironments in

which complex bacterial populations can evolve (Ranjard and Richaume, 2001). Besides the physical properties of the soil, bacteria are also influenced by nutrient availability resulting from rhizodeposition and decomposition (Lynch and Whipps,1990). The diversity, abundance and activity of bacterial communities are therefore structured in relation to depth, since the primary source of input in orchards is above ground. Several authors have attempted to examine the vertical distribution of bacteria in soils (Fritze *et al*, 2000; Ekelund *et al*, 2001). Bardgett *et al* (1997) in their study of grassland sites in Snowdonia reported that microbial biomass and activity (CO_2 evolution) was highest in the top 5 cm of soil and decreased down to a depth of 15 cm. Similarly Fritze *et al* (2000) reported a decrease in microbial biomass together with changes in diversity down forest soil profiles using phospholipid fatty acid (PLFA) analyses, and Ekelund *et al* (2001) showed a decrease in culturable bacterial abundance with depth. Environmental factors such as temperature and moisture regulate bacterial survival, giving rise to temporally variable, but, spatially defined niches within the soil profile. There is a scarcity of material describing temporal variation, particularly in grasslands (Bardgett *et al*.,1997).

Till date no substantive work on the bacterial flora of orchard soils of Kashmir Valley has been carried out. Keeping in view the importance and usefulness of these orchards, it was thought necessary to conduct studies on both rhizospheric and non-rhizospheric bacterial flora of apple orchards. The study is first of its kind in the Valley.

MATERIAL AND METHODS

The present study was conducted on soils from two apple orchards in the northeast suburban area of Srinagar city - one at Malbagh (orchard-I) and the other at Zakura (orchard-II). Composite rhizospheric and non-rhizospheric soil samples were collected from surface (1-6") and subsurface (7-12", 13-18") layers on monthly basis usually during first week of the month from March to October, 2004 (season-wise Spring, Summer and Autumn). Soil corer was pressed / drilled vertically into the soil up to the desired depth to obtain samples, and at least 5-10 sample cores of each layer were taken at random from different spots of the orchard under study.

The pH was tested on the spot by sensitive pH-indicator paper (E. Merck-Universal Indikator Papier) and further confirmation of the accurate pH was done in the laboratory using a potentiometer.

Soil samples were collected in sterile polythene bags. In most cases, samples were immediately analysed in the laboratory, failing which, they were stored at room temperature, until analysed. Several isolation techniques were employed, but the dilution plate method (Waksman,1922b; Waksman & Fred,1922) was followed for quantitative determination of the bacteria. Following Emerson's technique (1925), five dilutions were made (i.e., 1:10, 1:100, 1:1000, 1:10000 and 1:100000) and two replicates were prepared for each dilution. The samples were cultured using nutrient agar medium (Koch, 1882) through spread plate technique (Taylor *et al*, 1983; Kaper *et al*, 1978). The plates

were incubated at a constant temperature of 28±2°C and the colonies were counted after 3rd and 5th day. Some of the bacteria so isolated were also grown in tube slants on suitable media.

A complete record of all the bacterial colonies appearing in each plate was kept, and the bacterial content per gram of soil was calculated (Waksman, 1922b, 1927, 1952).

RESULTS

The data was scored from both the orchard sites on monthly basis from March to October, 2004. Vertical distribution of bacteria was investigated in all rhizospheric and non-rhizospheric soil samples. In both sample types, the bacteria showed a marked decrease in number from upper to lower horizons. The detailed investigation of bacteria for all three seasons spring, summer and autumn represented by April, July and October respectively have indicated that their count goes on decreasing with soil depth (Table 1).

For each sample depth (layer) under investigation, it was found that rhizospheric soil layers had greater number of bacteria than their adjacent non-rhizospheric soil layers. This was observed during almost all monthly counts (Table 2).

Soil samples from both the orchards were studied regularly each month. A monthly record of the bacterial flora content was kept for the year 2004 beginning with the month of March. It is evident from these records that the bacterial population was richest during spring (March, April and May), while a sharp decline was observed during summer, followed by a gradual increase in bacterial content during autumn (Table 2). Season-wise comparative assessment of bacterial population in soils from the two orchards also depicts the same trend (Fig.1).

Table 1: Vertical Distribution of Soil Bacteria in Spring, Summer & Autumn, 2004

Soil orchard	pH	Depth (inches)	No.of bacteria per gram of soil (cfu/gm) x 10^5					
			Spring (April)		*Summer* (July)		*Autumn* (October)	
			R	NR	R	NR	R	NR
I.	7.1	1-6	185	105	12	7	45	29
		7-12	125	72	7	3	21	11
		13-18	95	39	0	0	15	0
II.	7.3	1-6	194	106	13	4	49	26
		7-12	140	35	4	0	30	12
		13-18	78	12	0	0	13	2

R = rhizospheric. NR = non-rhizospheric. cfu = colony forming unit. pH = taken as average.

Table 2: Average Soil Bacterial Content during Different Months of the Year 2004

Months	No. of bacteria per gram of soil (cfu/gm) x 10^5			
	Orchard-I		Orchard-II	
	R	NR	R	NR
March	103	78	95	66
April	135	72	137	51
May	92	45	42	26
June	24	13	28	10
July	6	3	6	1
August	2	2	4	0
September	18	5	15	7
October	27	13	31	13

R = rhizospheric, NR = non-rhizospheric.

DISCUSSION

The findings clearly indicated that the number of bacteria decreased from upper to lower horizons of soil. Upper 6 inches of soil contained highest number of bacteria and the number gradually decreased at the lower depths. Similar trends in the soil bacterial population with depth were earlier reported by many other workers like Tresner *et al* (1954), Saksena (1955), Cooke (1959) and Mukerji (1965). In temperate zones, all bacteria are almost in the top meter, largely in the upper few centimeters. In contrast with field soils, in which the greatest number of bacteria is typically found several centimeters below the upper crust, the highest number in shaded land forest, orchard or meadow is frequently in the top 1 to 2 cm (Alexander, 1985). The variation observed in soil depths also correlates with a decrease in moisture content down the soil cores (Griffiths *et al*, 2003).

Bacterial counts were more during Spring and decreased to nearly half or less during Summer months of the year. In temperate regions, a burst of activity occurs in the Spring months as the soil becomes warm and the organic matter from the previous fall and winter become accessible for decay. Same effect of seasonal variation on soil bacterial population was also reported by Alexander (1985). The numbers commonly diminish in Winter as the cells remain in a state of biochemical inactivity; the bacteria are not eliminated during prolonged periods of freezing so that the microflora is ready for reactivation in the Spring (Alexander,1985).

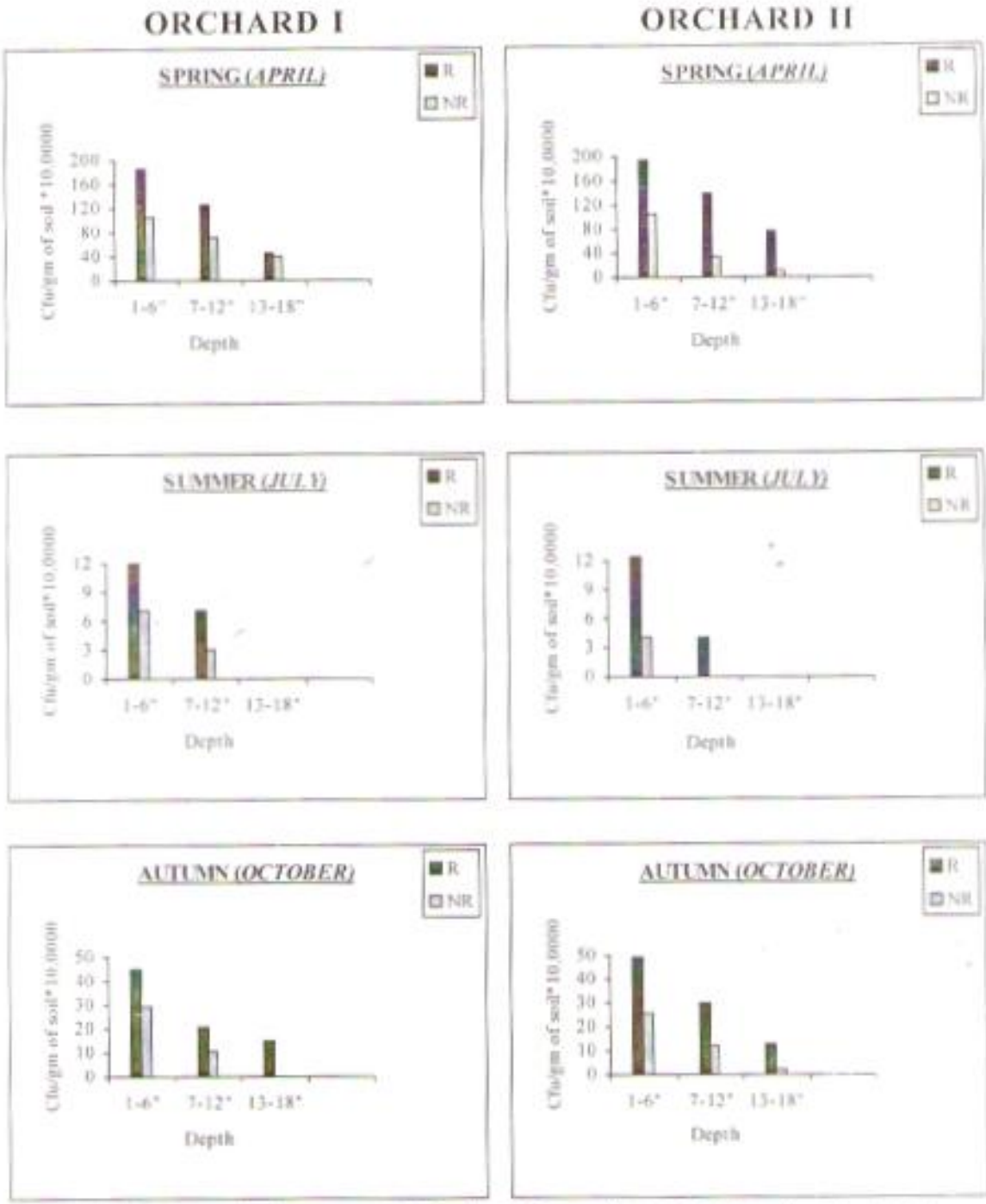

Fig1: Comparative Assessment of Bacterial Population in Soils from two Orchards

The greater number of bacteria in the rhizosphere is in confirmity with the findings of Wardle (1992); which suggest more carbon availability in the rhizosphere as one of the primary determinants of soil bacterial growth. The differential utilization of distinct carboxylic and amino acids in the rhizosphere and at different root zones has also been reported in agricultural crops (Baudoin *et al*, 2001). The increased ability of bacteria to utilize certain organic acids may therefore be due to rhizosphere effects in the form of increased exudation or root length, at the height of plant growth.

In conclusion, the data presented here enhances current knowledge of the influence of depth on soil bacteria by providing evidence for differences in their numbers/counts down the soil profile. The changes with depth were more between the surface (organic) and subsurface (mineral) soils, while temperature influenced changes were more prominent in the surface (organic) layers. In future studies, this would lead to a greater understanding of the effects of natural perturbations on soil bacterial diversity and functioning, a knowledge of which would aid in prediction of the effects of global change and agricultural practice on soil bacteria.

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