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SEASONAL VARIATION IN DEHYDROGENASE ACTIVITY AND BIOMASS CARBON IN FOREST ECOSYSTEM

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ABSTRACT

Assessing microbial populations in soil necessitates quantitative information about numbers of organisms, biomass of population, rate of activities, rates of growth and death and cycling and transfer rates of materials within ecosystems. The dynamics of microbial population in terms of biomass carbon and dehydrogenase activity was studied in the forest soil of Raisen district in central part if India. In this investigation ATP was used as an indicator of biomass carbon. Determination of dehydrogenase activity reflected microbial activity in soil samples. Soil samples from 15 forest ecosystem sites containing surface as well as 20 cm depth was collected in three different seasons viz. winter (December, 2011), summer (April, 2012) and rainy (June-July, 2012). Soil microbial biomass and dehydrogenase activity was significantly higher at surface soil than at 20 cm soil depth along with seasonal variation. A positive correlation existed between microbial biomass and dehydrogenase activity. Dehydrogenase activity in winter season was more pronounced with a value of 110.45 µg TPF hr^{-1} g^{-1} soil. Although in summer season, at all sampling stations as well as at soil of 20 cm depth lowering in dehydrogenase activity was gradually decreased with a value of 23.32 µg TPF hr^{-1} g^{-1} soil. The litter, as well as availability of humus in the subsoil also appears to regulate microbial activity thus influencing population of biomass estimation. The upcoming deciduous teak dominated forest flora under favorable environmental conditions in rainy season also shares soil oxygen by fast growing system thereby consequent lowering of oxygen occurs and is the main source of frequency of fluctuating oxygen concentration in soil at various depths. Biomass in winter season in the present study appears to be higher than rainy season with a value of 480 µg C g^{-1} soil. Pronounced activity of microbial system in winter climate is favored by microbial growth supported by availability of litter with very thin layer of humus deposition. Extreme reduction of biomass during summer season with a value of 58.80 µg C g^{-1} soil supports the unavailability of moisture content in the forest vegetation of Raisen district as forest stand is dominated by deciduous flora. The objective of this study was to quantify seasonal changes in soil microbial biomass and the enzyme activity of forest soils in view to understand structural and functional relationships between soil microbial communities.

Key words: Biomass carbon, dehydrogenase activity, ATP content, Luciferin, luciferase.

[I] INTRODUCTION

Soil bacteria and fungi play pivotal roles in various biogeochemical cycles [30; 33] and are responsible for the cycling of organic compounds. Soil microorganisms influence above and belowground ecosystems by contributing to plant nutrition [8; 29], plant

health [26; 7;25] soil structure [36;6] and soil fertility [37;17]. Raisen district with dry tropical deciduous vegetation composed of teak vegetation comprises approximately 13% of forest area of India. Information on density and diversity of soil microorganisms is scarce under dry land tropical conditions especially in forest soils. The microorganisms in forest soil are strongly influenced by various chemical and physical factors, including nutrient availability, organic matter, soil moisture and temperature [2; 27; 34]. Bacteria constitute the principal group of microorganisms and are responsible for biological and biochemical properties of soil. Soil enzyme activities are microbial in origin and can be considered effective indicators of soil quality changes resulting from environmental stress [3]. The activity of dehydrogenase is considered an indicator of oxidative metabolism in soils and thus of microbial activity [24]. However, the relationship between an individual biochemical property and the total microbial activity is not always obvious, especially in case of complex systems like soils, where the microorganisms and processes involved in the degradation of organic compounds are highly diverse [15]. Nevertheless, dehydrogenase activity has been used as an indicator of microbial activity in Mediterranean arid soils (Garcia), and in agricultural soils of more humid regions of northwest Germany [1]. The microbial biomass of soil is defined as the part of the organic matter in the soil that constitutes living organisms smaller than the 5-10 micrometer ^{3} (μm^3). A measure of the total microbial biomass in soil is often required when studying productivity or fertility of soils. Seasonal changes in soil moisture, soil temperature and C input from crop roots, rhizosphere products (i. e. root exudates, mucilage, sloughed cells, etc.), and crop residues can have large effect on soil microbial biomass and its activity [22], which in turn, affect the ability of soil to supply nutrients to plants through soil organic matter turnover. Soil microbial biomass is both a labile nutrient pool and an agent of transformation and cycling of organic matter and plant nutrients in soil; so, it is one of the most important microbiological properties. Several studies indicated that microbial biomass responds more rapidly to seasonal changes and, consequently may be an early and sensitive indicator of soil quality change. ATP is considered to be a useful indicator of microbial biomass in aquatic and terrestrial environments [5; 9;12;4]. ATP content is determined to calculate energy charge level and in turn it reflects active microbial biomass [13]. Microbial concentration of ATP in forest soil was examined using luciferin-luciferase system. As evident from previous studies, the method incorporates individual steps of the firefly catalytic cycle and could be regulated by stimulators to obtain a light emission rather than a peak light emission [11]. The main objectives in this study were: (1) to measure microbial biochemical properties in tropical forests soils of Raisen district, during conditions of three contrasting seasons; (2) to study the effect of changes in soil physico-chemical properties with soil depth and seasons on microbial biochemical properties.

[II] MATERIAL AND METHODS

2.1 Study site and sampling site

Soil samples were collected from fifteen sampling sites in forest area of Raisen district (Division Bhopal, Madhya Pradesh, India), 47 km away from Bhopal, capital city of Madhya Pradesh. The district is situated between latitude $22^0$47' and $23^0$33 north and longitutde $77^0$21' and $78^0$49' east. Eight samples contained surface soil while seven samples contained soil at 20 cm depth. Raisen district is dominated by tropical dry deciduous forest with predominance of teak trees. Temperature ranges between maximum values of 42^0C to minimum of 5^0C with an

average rainfall of 1200mm. For sampling surface litter was scrapped away and soil was collected in presterilized HDPE bags.

2.2 Physico- chemical analysis of soil:

To study the physico-chemical characteristics of forest soil, soil was passed through 2mm sieve to have homogenous particles and stored at 4^0C until needed. Sub samples for the determination of physico-chemical parameters were air dried before analysis.

(i) Mechanical Analysis of soil

Mechanical analysis was carried out by Boucos hydrometer methods and modified by Piper [20].

(ii) Soil pH

The pH of the soil was determined by using pH meter, Elico- 111-E model. Analysis was carried out with 1:2 soil water suspension prepared in triple distilled water.

(iii) Water holding capacity

Gravimetric method was performed for determining percent saturation or water holding capacity of soil [20].

(iv) Percent Organic carbon

The percentage of organic carbon content was determined by Walkley and Black's rapid titration method using $K_2Cr_2O_7$ and H_2SO_4 [20].

(v) Available Nitrogen

The available nitrogen content of soil was determined according to Subbiah [27].

(vi) Available Phosphorus

Phosphorus content of soil was measured colorimetrically using Na_2CO_3 (sodium carbonate) solution [18].

2.3 Dehydrogenase activity

Three samples from each site were collected in random fashion and average value of three readings were used in calculations. Sampling was done in three different seasons namely rainy (August), winter (December) and summer (April) in the year 2011-2012. Succinate dehydrogenase assay was determined using 2,3,5-triphenyltetrazolium chloride (TTC) as a terminal acceptor of protons and electrons from organic compounds (Thalman 1967). Triphenylformazon formed was extracted with methanol and measured (UV-visible Spectrophotometer; Shimadzu 1601) at a wavelength of 480 nm.

2.4 Biomass estimation

Adenosine tri phosphate (ATP) is an important energy compound in the metabolism of all living organisms. Soil ATP content is closely correlated with other indices of biomass, e.g. Carbon, Nitrogen, etc. and can serve as an independent estimate of soil biomass content. ATP was extracted using tricholoro acetic acid [10] and ultrasonication to distrupt microbial cells. Extracted ATP was quantified using luciferin-luciferase, firefly assay and was converted to biomass carbon [11].

[III] RESULTS

3.1 Physico chemical analysis

In general, soil at sampling sites was black cotton in texture and slightly alkaline (Table: 1) and normal with respect to fertility measured in terms of organic carbon, phosphorus and nitrogen content. The percent sand, silt and clay is illustrated in Fig: 1. Water holding capacity ranged between 50.1% to 68.6%. Color of soil was mostly black with slight intensity variation upto grey at 20 cm depth. However, organic carbon ranged from 0.1% to 0.7% (Fig: 2) reflecting predominance of vegetation in relation to retention capacity of water thus making supporting microbial activity. Phosphorus being stimulator of growth to plant as well as microbial cells ranges between 0.18 ppm to 2.98 ppm (Fig. 3), whereas, availability of nitrogen, falls between 0.08 ppm to 1.6 ppm (Fig.4).

[3.2] Dehydrogenase activity

Variation in dehydrogenase activity was observed with season and soil depth during the course of study at all sampling sites starting from SS1 to SS8 (Table 2). The data presented in (Fig: 5) is an average value of triplicate samples taken from each sampling sites. Dehydrogenase activity was more pronounced in winter season

as compared to rainy and summer season. However, it also varies with depth of soil highest being on surface soil. Succinate dehydrogenase activity in winter at SS1 sampling site was with highest value of 110.45 µg TPF hr^{-1} g^{-1} soil. At lower depths designated as DS1 to DS7, the enzyme activity showed remarkable reduction from 86.32 µg TPF hr^{-1} g^{-1} to 60.16 µg TPF hr^{-1} g^{-1} soil. In rainy season maximum enzyme activity was 76.21 µg TPF hr^{-1} g^{-1} soil at SS1 and lowest being 40.42 µg TPF hr^{-1} g^{-1} soil at DS7. Whereas, in summer at all sampling stations as well as at soil of 20 cm depth TTC reduction was found to be gradually decreased from 44.62 µg TPF hr^{-1} g^{-1} soil at SS4 to 23.42 µg TPF hr^{-1} g^{-1} soil at DS5. [3.3] ATP and Biomass estimation Seasonal variation in ATP content and microbial biomass carbon was observed (Table: 3) in tropical dry deciduous forest of Raisen district. ATP content of microbial population at surface level was seen to be slightly higher than in soil at 20 cm depth. The overall observation in rainy season shows variation between 2.74 ATP micro grams / gram soil (µg /g) to 3.06 ATP µg /g soil at surface soil at all sampling sites. ATP content at lower depths at respective sampling stations represents lesser microbial activity as it is clear from the observation that value of ATP was found to slightly reduce from 2.17 ATP µg /g soil to 2.61 ATP µg /g soil. Maximum value of ATP recorded was 3.12 ATP µg /g soil at SS5 sampling point. Winter season observations reflect enhanced ATP as compared to rainy season. ATP content of surface soil at all the 08 sampling sites ranges between 3.21 ATP µg /g soil to 4.00 ATP µg /g soil. In contrast, at depth of 20 cm ATP content at all the subsequent sampling sites ranges between 2.41 ATP µg /g soil to 3.25 ATP µg /g soil. In extreme summer at 37^0C – 44^0C ATP content ranges between 0.73 to 0.92 ATP µg /g soil. ATP is a well-accepted indicator of microbial biomass carbon. The microbial biomass examined by calculating biomass carbon in micro grams / gram soil (C µg /g soil) showed variation in relation to sampling seasons such as winter, rainy and summer (Fig: 6). Microbial biomass in winter season was maximum at SS4 i.e. 480.00 C µg /g soil in contrast to deeper soil where maximum biomass recorded was 390.05 C µg /g soil at DS1. In rainy season at SS4, 333.60 C µg /g soil was estimated which shows reduced microbial viability activity in rainy season in contrast to winter. At depth of 20 cm ranging between sampling sites DS1 to DS7 biomass C was observed to be 260.4 C µg /g soil to maximum of 313.2 C µg /g soil. However, a sharp reduction in biomass content in relation to C measurement was seen in summer season at all the sampling sites. The surface biomass was shown to fall between 90 C µg /g soil to 110.40 C µg /g soil. At lower depth of 20 cm the biomass was reflected as 58.8 C µg /g soil to 79.2 C µg /g soil.

[IV] DISCUSSIONS

4.1 Dehydrogenase activity

A knowledge of the spectrum of enzyme activity of a soil is important since it indicate the potential of soil to permit the basic biochemical processes necessary for maintaining soil fertility. Enzyme assays is related to several characteristics of soil, including microbial growth, activity and geochemical profiles [32]. Through acting as both source and sink of nutrients, and as the mediator of carbon turnover, soil microorganisms and their activity are immediately linked to the carbon status of soil. This has been shown to be of particular importance for seasonal tropical climates [23; 38]. Dehydrogenases are considered to play an essential role in initial stages of oxidation of the soil organic matter by transferring hydrogen and electrons between substrates to acceptors [22]. The parameters selected for study shows diverse observations in relation to increasing depth from surface area. In forest ecosystem variation in

microbial activity is regulated by modifying biotic and abiotic stress. Populations of microclimate in the upper bed of forest ecosystem is regulated directly by climatic change. The formation of humus layer in the forest ecosystem appears to be modulated by vegetation type and environmental characteristics. However, phenomenal change in dehydrogenase activity was observed with depth and season during the course of study at all sampling sites thus confirms the presence of microbial flora.

[4.2] ATP and biomass estimation

Seasonal variation controls the soil microbial biomass and is directly related to availability of moisture in the soil. It has been shown that soil microbial biomass decline upon drying and increases upon rewetting [19; 34; 35], whereas drying and rewetting of soil caused a decrease in microbial biomass. Nevertheless, the positive correlations between soil moisture and microbial biomass are not universal and negative relations between these variables, mostly in conditions of high soil moisture, is being recorded [21; 34]. The lesser ATP content at 20 cm soil depth might be due to less moisture content or less availability of organic matter as source of energy. However, aeration of soil at lower depth in forest ecosystem, sometimes, directly influence microbial biomass. The litter, as well as availability of humus in the subsoil also appears to regulate microbial activity thus influencing population of biomass estimation. The upcoming forest flora under favorable environmental conditions in rainy season also shares soil oxygen by fast growing system thereby consequent lowering of oxygen occurs and is the main source of frequency of fluctuating oxygen concentration in soil at depth of 20 cm. The presence of biomass in winter season in the present study appears to be more than rainy season. Pronounced activity of microbial system in winter climate is favored by microbial growth supported by availability of litter with very thin layer of humus deposition. The established fact for relationship between soil moisture content and microbial biomass is not universal as under of high soil moisture content lowering of biomass is also detected [21; 34]. The present observation supports the earlier concept as the fluctuating moisture contents leads to selection of microbial population effecting nutrient cycling in Raisen forest stand dominated by teak vegetation. The remarkable decline of biomass count during summer season might be directly related to high temperature with least or no moisture content as the forest stand is occupied by deciduous vegetation. The microbial bioprocessing of minerals is also reduced in response to environmental changes. The measurement of ecological parameter indicates the functional interaction between microbes and with the surroundings they are enclosed [31]. The present data supports that microbial biomass as observed during summer i.e. 90 μg C g^{-1} soil fails to participate in active mineralization because of presence of least moisture content. Further, isolation of bacterial flora from 20 cm depth of forest bed with successful examination of genetic diversity to help to elucidate adaptive capability in stress situations. Potentials of mineralization by bacterial flora could be confirmed genetically to establish genetic diversity.

[V] CONCLUSION

The properties of forest bed depends on the relationship between soil ecological parameters and other environmental factors. To study the quality and fertility of soil, samples were collected from forest floor of Raisen district, 47 km. away from Bhopal. Samples contained surface soil (SS), as well as, soil at 20 cm depth (DS). Samples were collected in triplicate from each sampling site in a random fashion. Soil samples thus collected were subjected to physico-chemical analysis. In general, soil at sampling site was black cotton in texture and

slightly alkaline. Water holding capacity ranged between 50.1% to 68.6%, organic carbon varied from 0.1% to 0.7%. Soil was normal with respect to fertility as revealed by phosphorus content, which falls between 0.18 ppm to 2.98 ppm and Nitrogen content, which ranged between 0.08 ppm to 1.6 ppm.

The longevity of biomass in soil was estimated by comparing ATP content, biomass C and dehydrogenase activity in the collected soil samples. The ATP content of microbial population at surface level was seen to be slightly higher than soil at 20 cm depth. Sampling was done in three different seasons namely, rainy, winter and summer. The overall observation in rainy season shows variation between 2.74 ATP μg/g soil to 3.12 ATP μg/g soil. The ATP content at lower depth represents less ATP which varied from 2.17 ATP μg/g soil to 2.61 ATP μg/g soil. Maximum ATP content was recorded at SS5, which was 3.12 ATP μg/g soil. The lesser ATP content at lower depths might be due to decreased moisture content or less availability of organic matter as source of energy. Winter season observations reflects enhanced ATP content as compared to rainy season records. The ATP content of surface soil at all eight sampling stations ranged between 3.21 ATP μg/g soil to 4.00 ATP μg/g soil. In contrast, at the depth of 20 cm ATP content at all the sites ranged between 2.41 ATP μg/g soil to 3.25 ATP μg/g soil.

However, in summer season the forest soil ATP content ranged between 0.73 ATP μg/g soil to 0.92 ATP μg/g soil. Pronounced activity of microbial system in winter climate (18^0C to 22^0C) is favored by microbial growth supported by availability of litter with very thin layer of humus deposition. Extreme reduction of ATP content during summer season support the inavailabilty of moisture to forest vegetation of Raisen district.

The microbial biomass was calculated in terms of carbon μg/g soil which exhibited variation with respect to sampling season namely, rainy, winter and summer. Microbial biomass in winter season was maximum at SS5 i. e. 481.94 C μg/g soil in contrast to deeper soil (DS5) where maximum biomass recorded was 390.05 C μg/g soil at DS1. In rainy season at SS5, 374.4 C μg /g soil estimated reflected reduced microbial viability/ activity in rainy season in contrast to winter. At DS1 level 313.2 C μg /g soil was recorded. At depth of 20 cm ranging between sampling sites DS1 to DS7 biomass C was observed to be 260.4 C μg /g soil to maximum of 313.2 C μg /g soil. However, a sharp reduction in biomass content in relation to C measurement was seen during summer season at all the sampling sites. The surface biomass was shown to fall between 90 C μg /g soil to 110.40 C μg /g soil. At lower depth of 20 cm the biomass reflected as 58.8 C μg /g soil to 79.2 C μg /g soil. The remarkable decline of biomass concentration in summer season might be directly related to availability of soil moisture. The microbial bioprocessing of minerals is also reduced in response to environmental changes. The measurement of biomass carbon indicates functional interaction between microbes and with the surroundings they are enclosed.

The adequate documentation of microbial biomass can also be performed by enzymatic activities, which are directly or indirectly involved in oxidation-reduction reactions. Variation in activity of dehydrogenase enzyme was observed during the course of study at all sampling sites starting from SS1 to SS8. In the present survey total soil collected was subjected to dehydrogenase test. The method confirms the presence of microbial flora including actinomycetes and fungi. The succinate dehydrogenase activity was measured in terms of μg of TPF formed per gram dry weight of soil per hour from the calibration curve of TTC and

absorbance was recorded at 480 nm. In contrast to rainy season succinate dehydrogenase activity in winter was more pronounced at SS1 sampling site i.e. 110.45 TPF µg /g/ hr soil. At lower depths designated as DS1 to DS7, the activity showed remarkable reduction, reflecting population density of microbes present in forest soil. There was sharp decrease in dehydrogenase activity in rainy and summer season. In rainy season maximum activity was at SS1 i. e. 76.21 TPF µg/g soil. In summer season at all sampling stations as well as at soil of 20 cm depth TTC reduction was gradually decreased. Maximum activity was recorded at SS4 i. e. 44.62 TPF µg/g soil. The variation in population density as measured by biomass C supports the level of extracellular enzyme activity.

The bacterial activity measured in terms of biochemical approaches such as, ATP content, biomass C etc. significantly proves that the community structure in the soil of forest stand is directly correlated with availability of oxygen and organic matter. Thus, by measuring the above mentioned parameters there is a better understanding of the relations between microbial diversity and soil functions.

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Table 1: Soil analysis outcome of forest region of Raisen District

S.No	Sample No.	Soil Color	Water holding capacity (%)	Soil pH	Mechanical Analysis
01	SS1	Gray	50.1%	6.7	Sand: 10% Silt: 30% Clay: 36%
02	SS2	Black	53.6%	5.5	Sand: 20% Silt: 23.70% Clay: 55.90%
03.	SS3	Black	52.5%	6.9	Sand: 22.3% Silt: 31.80% Clay: 31.80%
04.	SS4	Tan	53.7%	7.1	Sand: 22% Silt: 15% Clay:55%
05.	SS5	Black	57.9%	6.0	Sand: 26% Silt: 18.60% Clay: 43.90%
06.	SS6	Gray	50.3%	6.4	Sand: 2.90% Silt:22.60% Clay: 57.90%
07.	SS7	Black	54.3%	5.9	Sand: 5.90% Silt: 30% Clay: 56.30%
08.	SS8	Black	53.9%	6.2	Sand: 1.80% Silt: 25.70% Clay: 54.30%
09.	DS1	Gray	60.4%	6.4	Sand: 4.60% Silt: 24%; Clay: 57.60%
10.	DS2	Black, wet	67.4%	5.5	Sand: 5.60% Silt: 25% Clay: 59.40%
11.	DS3	Black	62.9%	7.2	Sand: 17% Silt: 28% Clay: 60%
12.	DS4	Black	68.6%	7.5	Sand: 13% Silt: 21% Clay: 51%
13.	DS5	Gray	60.3%	6.9	Sand: 19% Silt: 35% Clay: 48%
14.	DS6	Gray	64.5%	6.4	Sand: 26% Silt: 15% Clay: 50%
15.	DS7	Gray	66.9%	6.2	Sand: 23% Silt: 18% Clay: 49%

Table: 2 Dehydrogenase activity of different soil samples in different seasons.

	Seasons (2006-2007)		
Sample	*Winter (Dec)	*Summer (April)	*Rainy (Aug)
SS1	110.45	39.21	76.21
SS2	106.52	36.73	73.32
SS3	98.02	42.21	69.21
SS4	96.24	44.62	65.32
SS5	109.54	39.32	63.21
SS6	101.25	31.26	60.24
SS7	99.32	36.31	59.24
SS8	91.73	33.48	55.22
DS1	86.32	30.21	51.21
DS2	80.21	26.31	50.32
DS3	79.32	29.24	51.72
DS4	70.62	24.02	49.02
DS5	69.21	23.42	45.24
DS6	65.21	24.08	40.42
DS7	60.16	26.76	46.78

SS – surface soil; DS – deep soil - value in μg TPF hr^{-1} g^{-1} soil

Table: 3 ATP content and biomass carbon estimation in three different seasons in the forest soil of Raisen district.

Sample no.	Seasons (2006-2007)					
	Winter (Dec.)		Rainy (Aug.)		Summer (April)	
	ATP*	Biomass C #	ATP	Biomass C	ATP	Biomass C
SS 1	3.40	408.00	2.96	355.20	0.75	90.00
SS 2	3.21	385.20	3.06	367.20	0.82	98.40
SS 3	3.63	435.60	2.98	357.60	0.89	106.80
SS 4	4.00	480.00	2.78	333.60	0.76	91.20
SS 5	3.91	469.20	3.12	374.40	0.73	87.60
SS 6	3.86	463.20	3.05	366.00	0.88	105.60
SS 7	3.95	474.00	2.92	350.40	0.92	110.40
SS 8	3.25	390.00	2.74	328.80	0.79	94.80
DS 1	3.25	390.00	2.61	313.20	0.65	78.00
DS 2	2.41	289.20	2.45	294.00	0.62	74.40
DS 3	3.09	370.80	2.32	278.40	0.56	67.20
DS 4	2.80	336.00	2.30	276.00	0.53	63.60
DS 5	2.97	356.40	2.24	268.80	0.49	58.80
DS 6	3.24	388.87	2.29	274.80	0.51	61.20
DS 8	3.03	363.60	2.17	260.40	0.66	79.20

SS – surface soil DS – deep soil * in µg / g soil # in C µg / g soil

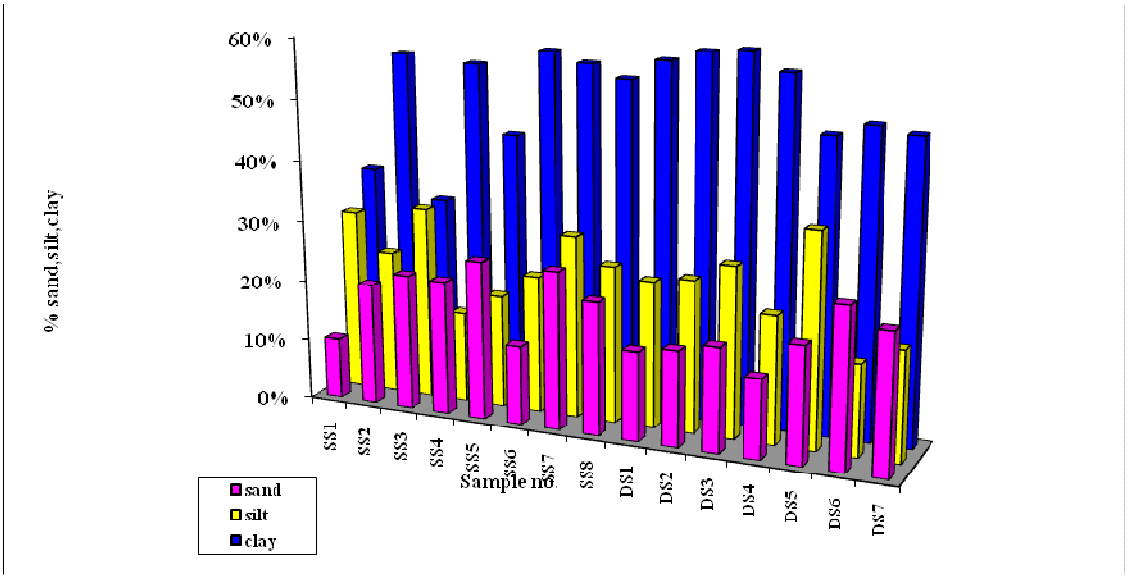

Fig: 1 Sand, silt and clay content of soil samples at Raisen district (Value in %). SS – surface soil DS – deep soil

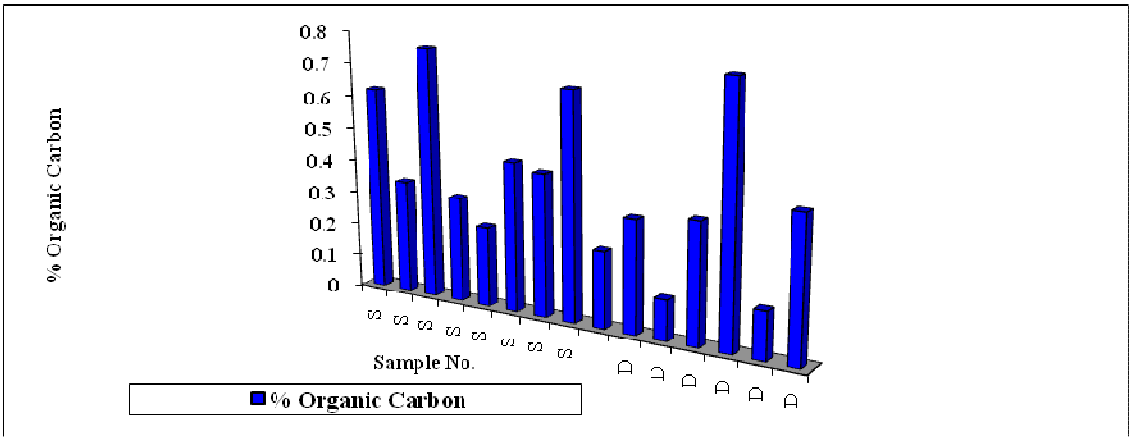

Fig: 2 Percent organic carbon of different sampling sites at Raisen District. (Value in %).
SS – surface soil DS – deep soil

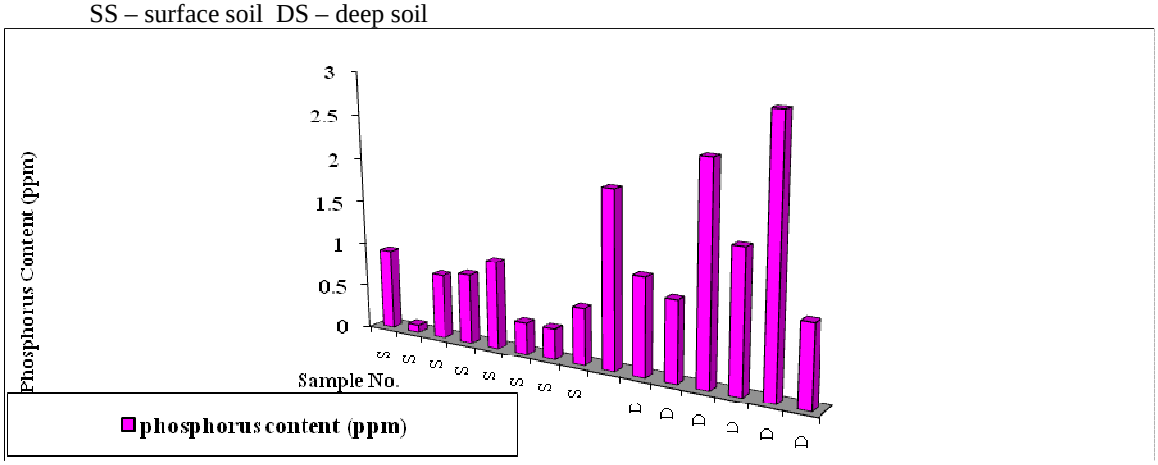

Fig: 3 Phosphorus content of different soil samples at Raisen District. (Value in ppm)
SS – surface soil DS – deep soil

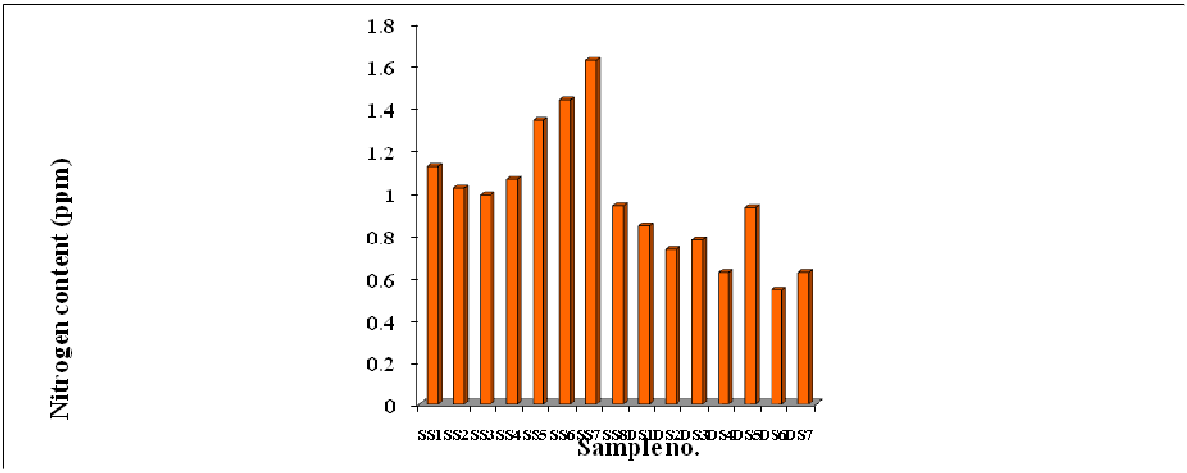

Fig: 4 Nitrogen content of different forest soil samples at Raisen district. (Value in ppm).
SS – surface soil DS – deep soil

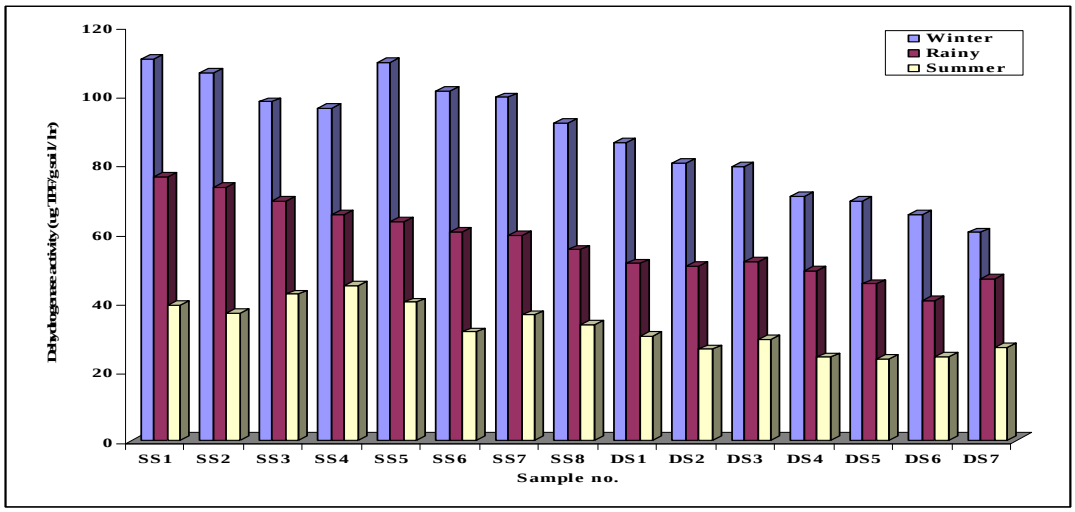

Fig: 5 Variation of dehydrogenase activity of different soil samples in three different seasons viz. winter, rainy and summer. SS – surface soil DS – deep soil

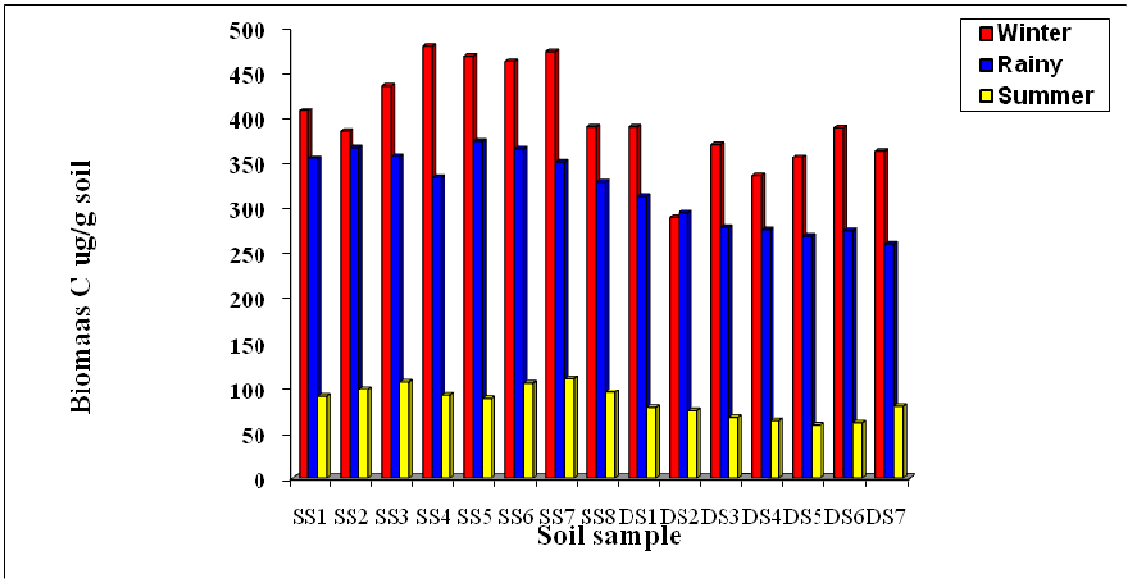

Fig: 6 Variation of microbial biomass C in µg/g soil in soil samples in three different seasons at Raisen District. SS – Surface soil DS – Deep soil