Microbial Activity and Nutrient Status in Oak and Pine Oriented Forest
Soil of Mid Altitude Central Himalaya

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Abstract:
Forest tree growth in mid altitude Central Himalayan ecosystem is most commonly limited by shortage of mineral nutrients. Consequently, in these circumstances growth depends on nutrient cycling in the soil in rhizosphere region specifically due to presence of microbes that transfer soil organic matter and release mineral nutrients into forms available to plants. Rapid replacement of oak oriented forest with pine oriented forest in Garhwal for its commercial product or non-timber forest product cause a huge losses of various ecological attributes that are associated with oak forest soil. In Mid Altitude Central Himalaya it has been found that in many aspects oak oriented forest is better than pine oriented forest, whether it is on the basis of nutrient status or microbial activity.
Continuous deforestation of oak oriented forest causes reduction in microbial activity in the soil. Prediction of microbial activity can be done by estimation of Dehydrogenase activity in the soil. The mean Dehydrogenase activity (DHA) obtained in oak forest soil were 106.28 nmol /g dry weight soil/ 2 hr and for pine forest it was 66.37 nmol /g dry weight soil/ 2 hr respectively, indicate a higher microbial activity in oak oriented forest than pine oriented forest.

**Keywords:** DHA, Deforestation, Soil Nutrients

**Introduction**

The objective of forestry in harsh and fragile ecosystem of the Central Himalaya is to increase productivity with sustainable land use, exploiting the beneficial effects of trees on the soil. Forest trees help in improving soil fertility through biological nitrogen fixation, Phosphorus solubilization and decomposition of organic matter in their rhizosphere zone (RZ) and non rhizosphere zone (NRZ). These RZ and NRZ processes play an important role in plant nutrition and maintaining soil fertility (Prasad and Mertia, 2005). Microbiological activity has been defined as "a general term that includes all the metabolic reactions and interactions conducted by the microflora and microfauna in soil" (Nannipieri et al. 1990). Soil DHA reflects the total range of oxidative activity of soil microflora and, consequently it may be a good indicator of microbiological activity in the soil (Skujins, 1976, Brzezinska et al., 2001, Xiao et al., 2008). High microbial activity in the soil indicates high oxidative metabolism in soils (Skujins 1973), because, being exclusively intracellular, it is linked to viable cells. Intracellular DHA in soil is a common method of estimating potential soil microbiological activity (Casida et al. 1964; Thalmann 1966). Availability of sufficient soil moisture and favorable micro-site factors
developed by water absorption had further increased the dehydrogenase activity (Badejo et al. 1998). Various intracellular dehydrogenases in different soil microorganisms contribute to the total DHA (Burns 1978). Soil enzyme activity plays an important role in the maintenance of soil fertility (Dkhar and Mishra, 1983). Dehydrogenases are enzymes of respiration pathways of aerobic as well as of anaerobic microorganisms (Stevenson, 1959, Burns 1978). Microbial biomass and enzyme activities were sensitive to soil compaction and Forest floor removal and that such disturbances had negative consequences for forest soil N and P cycling and fertility (Xiao et al., 2008). Soil enzyme activities in a forest floor are influenced by several variables such as nutrient availability, and input of leaf litter (Kang et al., 2009). Although the studies by (Quilchano and Maranon, 2002) have indicated presence of dehydrogenase activity in soils providing correlative information on biological activity and microbial population but information on forestry trees in relation to Soil nutrient status & DHA, particularly of mid altitude Central Himalaya, is lacking.

Statistical analysis involves Correlation and Residuals error analysis; it is difference between an observed value of the response variable and the value predicted by the model (Moore and McCabe, 1993). In short,

\[
\text{Residual} = \text{Observed } Y - \text{Predicted } Y
\]

The mean of the residuals is always zero. As a result plotting of residuals enables the data to be viewed from a standard orientation point. Residual plots show the deviation from the expected value for each x value in the model. If we knew the random errors, e, we could just plot them against a + bX. A random scatter would indicate that the errors do not depend on a + bX; i.e., the errors are free of a + bX. Thus the model is good.
However, we don't know the errors; we only know Y and X. But using Y and X we estimate a and b. This leads to an estimate of $a + bX$, the predicted value of Y, which we label as $\hat{Y}$. Our estimate of the error ($\hat{e}$) is $(Y - \hat{Y})$, this is called the residual, literally, what's left. We will denote the residual by $\hat{e}$, that is

$$\hat{e} = Y - (a + bX)$$

Then we can check our model assumption by plotting $\hat{e}$ versus $\hat{Y}$. This is called the residual plot. A random scatter indicates a good model. If it is not a random scatter then we need to rethink the model. Thus, the main objectives of this study were (1) Assessing Dehydrogenase Activity (DHA) in soils of both the forest type (2) To study the relations between DHA and various other soil parameters using statistical analysis.

**Materials and methods**

**Study Area**

This study was conducted near Kotma village of Garhwal Himalaya in Uttaranchal state. The landscape covers an elevation range of 1900-2000 meters and receives annual rainfall of 1200mm. The study area lies between 79°03' to 79°09'E longitudes and 30°35' to 30°35'N latitudes, in the mid altitude Central Himalaya. The region can be seen from the confluence with the Mandakini River up to Lenkh in the Madhmaheswar valley, and up to Khunnu (Kotma) in Kaliganga valley. Parent material of this region comprises of quartz, schist, quartz muscovite, feldspar and can be classified as Dystric-cambisol (USDA Soil Classification System). The forest chosen for sampling was east facing.

Forest fires are seen to be frequent in pine forest.

**Soil Sampling and Analysis**
Composite soil samples and fine-root samples were collected from 20 cm depth (once the litter had been removed) for assessing DHA and nutrients. The samples collected were collected from rhizosphere (RZ) and non-rhizosphere (NRZ) zone of oak (*Quercus leucotricophora*) and pine (*Pinus wallichiana*) trees. Total 36 samples were collected, 18 being from each forest type. Some trees of other species present in the study area were not considered for analysis. Once in the laboratory, soil samples were sieved (<2 mm) and then stored at 4°C until the enzyme analysis was performed. To assess the active microbial population in rhizosphere of different tree species, the DHA was assayed with the TTC reduction, modified method of (Casida et al 1964). Three determinations per sample were made for DHA.

**Procedure for DHA estimation:** Mix thoroughly 20g of air dried soil and 0.2g of calcium carbonate. Place 6g of this mixture in each test tube. In each tube add 1ml of 3% TTC (triphenyl tetrazolium chloride) and 2.5ml distilled water. After mixing stopper the tube and incubate it at 37°C. After 2 hrs remove stoppers and add 10ml of methanol shake it for 1 min. Unstopper the tube, filter suspension through a glass funnel plugged with absorbent cotton into a 100ml volumetric flask. Wash the tube by additional methanol until the reddish color has disappeared from cotton. Dilute the filtrate to 100ml volume by methanol. Measure the intensity of reddish color by using spectrophotometer at a wavelength of 485nm using 1-cm cuvette with methanol as a blank. Amount of TPF (triphenyl formazan) can be calculated by reference to a calibration graph prepared from TPF standards.

The other soil parameters that were analyzed are – Soil pH (pH meter 1:2.5), Soil Texture, Soil Moisture, Organic Carbon (Walkey and Black method), Organic Matter, Available Nitrogen (Kjedahl’s Method) and Available Phosphorus (Olsen’s Method). The parameters were determined using the manual of Tropical Soil Biology and Fertility: A Handbook of Methods (1993), edited by Anderson and Ingram.
Results and discussion:

The soils were found to be loamy sand in texture having pH(1:2): 5.25 (oak), 5.35 (pine); Organic carbon: 5.57% (oak), 3.62% (pine); Organic matter: 9.61% (oak), 6.59% (pine) Available P: 6.3 (oak) and 11.2 (pine) mg/kg soil; Available Nitrate: 23.2 (oak) and 15.7 (pine) mg/kg soil; Soil Moisture: 30.49% (oak) and 25.61% (pine), C/N ratio: 0.24 (Oak) and 0.23 (Pine), DHA: 106.28 (Oak) and 66.37 (Pine) expressed as nmol reduced triphenyl formazan (g dry weight soil)⁻¹ 2 h⁻¹. The values are shown in the Fig.1

![Fig.1 Graph indicating soil nutrient status and DHA of Oak and pine soil](image)

![Chart showing DHA in OAK and PINE forest](image)

![Standards of TPF](image)
All the sampling points show significantly higher DHA in rhizosphere and non-rhizosphere soil in oak forest as compared to pine forest (Fig.1 and Fig.2). The higher microbial activities in tree rhizosphere in oak oriented forest as compared to pine oriented forest, soil might be due to increased supply of carbon and nutrients from dead root cells and rhizodeposition (Huxley, 1999, Kang et al., 2009). and less forest floor removal (Xiao et al., 2008). Soil DHA was shown to be positively related to the moisture status of the soil. So it may be the reason that almost all oak forest are found by the river side and Pine forest is usually found at higher elevation and not by river side where the moisture level of soil is less. It appears that availability of sufficient soil moisture and favorable micro-site factors developed by water absorption had further increased the dehydrogenase activity in oak forest in comparison to Pine forest. These enzymes are produced by the soil microbial community to enable them to breakdown organic matter. Mid-Central Himalayan soils are normally found to be acidic in nature (Valdiya, 1989) that was well supported by the data. RZ and NRZ soil of oak forest show C: N value higher than pine forest, it may due to the release of C substrates from roots, most commonly resulting in an excess of available C and a shortage of N. The increased availability of C relative to N has led to the conclusion that competition between plants and microbes for N is intense within the rhizosphere. Statistical analysis shows a negative coefficient of correlation(r) existed between DHA and available nitrogen in both oak and pine forest ($r_{oak} =-0.142$, $r_{pine} = -0.604$), whereas in case of available phosphorus Pine have high positive
coefficient of correlation than Oak ($r_{\text{oak}} = .135$, $r_{\text{pine}} = 0.394$), for pH ($r_{\text{oak}} = 0.774$, $r_{\text{pine}} = 0.113$), soil moisture ($r_{\text{oak}} = 0.514$, $r_{\text{pine}} = 0.473$) and Organic carbon ($r_{\text{oak}} = 0.399$, $r_{\text{pine}} = 0.5$) at $P < 0.05$ level of significance. One way Analysis of Variance (ANOVA) of oak forest soil DHA shows that calculated values of F is more than the table value at 5% level of significance [$F_{\text{cal}} = 10.21$, $P(F<=F_{\text{cal}} = 0.0030)$, $F(0.05) = 4.13$] and thus there exist a significant difference in mean between dehydrogenase activity of soil of both the forest types.

![Fig.3 Residual error analysis of Oak oriented forest soil.](image)

**Fig.3 Residual error analysis of Oak oriented forest soil.**

Residual plot analysis of Oak forest soil(Fig.3) indicate that random scattered points are only obtained for O.C., and Moisture percentage so both are good for predicting the DHA activity since these two parameters show less error in their estimation but rest two, i.e. Phosphorus and pH there is necessity of rethinking the model. Similarly in case of Pine
(Fig.4), O.C., pH and Moisture percentage, shows a good random scattered points and thus it indicates a good model, so these three can be use for predicting DHA in pine forest soil. Overall finding suggest that there exist strong relationship between DHA and Soil nutrients in Oak forest soil than Pine forest soil so deforestation of oak should be avoided because the above gain from the Oak forest can never be accomplished from the plantation of Pine forest.

**Conclusion:**
In mid altitude central Himalaya reveals that in many aspects oak forestry is better than pine forestry, whether it is on the basis of nutrient status or microbial activity. Continuous deforestation of oak forest for commercial need decreases microbial activity in the soil leading to decrease in soil nutrient status and other parameters, associated with this forest. On the other hand pine forest is better adapted in low moisture condition, so its plantation is suitable for the area where there is moisture scarcity.

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**References:**


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