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## **Impact of Roadside Pollution on Microbial Activities in Sub-Tropical Forest Soil of North East India**

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**Abstract:** The study was done for a complete year on monthly basis and the differences on the various parameters selected were measured. Significantly higher number of fungi and bacteria were recorded in non-roadside soil than the effected roadside soil. Rate of soil respiration, enzymes activity also followed a similar trend. Other physicochemical characteristics as soil moisture, soil pH, soil temperature and soil carbon revealed similar variations in two conditions. Microbial population and enzyme activities like dehydrogenase, urease and phosphatase were positively correlated ( $p < 0.05$ ) to the edaphic properties of the soil. Microbial populations, soil respiration, enzymes activity and other physicochemical characteristics were determined from the sub-tropical forest soils of roadside (disturbed) and non-roadside (undisturbed) conditions to compare and the adverse effects of anthropogenic activities and roadside vehicular pollution in the busy national highway. The study site is devoid of human settlement and was once a pristine environment prior to the construction of highway through the forest. An average of 10,000 to 12,000 vehicles plies through the road per day.

**Key words:** Sub-tropical forest soil, roadside pollution, microbial pollution, enzyme activity, physicochemical characteristics

### **INTRODUCTION**

North Eastern region of India is undergoing for industrial development at a faster rate during the recent past. Roads form the main system of transportation owing to the hilly topography of the region and the nearest rail link is located at the distance of 108 km from the site. Road construction has been the main activity for development of industrial units. This has led to the loss of forest cover and subsequent loss of soil fertility. Roadside soils often show a high degree of contamination that can be attributed to motor vehicles (Weckwerth, 2001). Various researchers have found that the concentrations of the metals Pb, Cu, Zn, Cd and Ni decrease rapidly within only 10-50 m from the roadside (Pagotto *et al.*, 2001). Vehicular discharge of numerous gaseous and trace metals contaminants due to incomplete combustion of petroleum fuel adversely affects the microbial population and their activities in soil. Contamination of litter and soil with metals of the Class B and borderline elements (e.g., Pb, Zn, Cu, Ni, Cd) can result in reduced rates of litter decomposition, soil respiration, nitrogen mineralization and soil enzyme activity (Tyler *et al.*, 1989). Moderate soil contamination by metals has been shown to reduce the soil microbial biomass and certain indices of its activity (Brookes *et al.*, 1986).

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Microbial population and their activities in soil is a result of complex of interactions existing between the biotic and abiotic factors. This is widely influenced by the vegetation types and forest cover. It is widely accepted that a vegetational community harbours a characteristics micro flora.

The interactions of chemical, physical and biochemical factors are responsible for holding the complex soil system in a dynamic equilibrium. Population dynamics of soil microorganisms is largely regulated by vegetation and soil characteristics. Soil micro flora exerts considerable influence on soil fertility and plant growth. Biochemical processes provide better estimates of the functional attributes of the microorganisms in an ecosystem. These are generally determined by the estimation of the rate of biochemical processes involving microbial enzymes. Enzymes in soil are biologically significant as they are involved in cycling of nutrients and can influence the availability of nutrients to plants thus playing an important role in the initial phase of decomposition of organic matter (Joshi *et al.*, 1993). Enzymes like urease, dehydrogenase and soil respiration provide an index of microbial activity and oxidative-reductive reactions in the soil. A perusal of literature reveals that our knowledge on the ecology of soil microorganisms is largely based on research in agricultural and grassland soils (Grayston *et al.*, 2001; Liao and Boutton, 2008). Tropical and sub-tropical soils have received less attention in relation to microbial dynamics as affected by disturbances (Joshi *et al.*, 1991, 2008; Barbhuiya *et al.*, 2008).

The present study aims to understand in once a pristine forest soil of North-East Himalayan range of India, the influence of roadside pollution caused by vehicular movement on population and activity of microorganisms and physicochemical properties of the soil.

## MATERIALS AND METHODS

### Study Area, Soil, Climate and Vegetation

The study was conducted in forested areas in an around Shillong (Altitude 1680-1700 m MSL, latitude 25E 34°N and longitude 91E 56°E) in East Khasi Hills District of Meghalaya, India during April, 2007 to March, 2008. The hills of Meghalaya are made up of largely of pre-cambrian rocks acutely folded and steeply dipping, with an over turned fringe of mesozoic and tertiary sediments.

The core of the plateau as revealed by the rock distribution is an ancient mass of gneiss much intruded by coarse granite, sandstones and limestones with subordinate clays superimposed over these. The soil of the study site is red sandy loam of laterite (oxisols) and acidic in nature.

The climate of the area is influenced by the South-West monsoon and North-East winter. It can be divided into four marked seasons; windy dry summer (March-April), monsoon season of heavy rainfall (May-September), autumn period of low rainfall (October-November) and winter season (December-February).

The vegetation of the study area was characterized as a wet hill forest type. The forest stand is dominated by early colonizing tree species i.e., *Pinus kesiya*. The other species inhabiting the study area include *Alnus nepalensis*, *Myrica esculenta*, *Elaeagnus latifolia*, *Eurea japonica*, *Rhus japonica* besides the herbaceous weedy *Rubus ellipticus*, *Lantana camara*, *Osbeckia crinata*, *Eupatorium adenophorum* etc.

### Sampling of Soil and Analytical Procedures

Two study sites were considered for the present study i.e., disturbed soil, caused due to heavy traffic motor way (National Highway No. 40) constructed by felling of forest trees taken as the roadside soil (4 m away from the metal of the road) and the other one as undisturbed soil taken from the forest soil about 500 m away from the roadside.

Soil samples were collected from the surface layer (0-5 cm), using a steel corer (6.5 cm inner diameter) from the two different sites at monthly interval from April, 2007 to March, 2008. The data in tables and on figures correspond to mean of three replicate analysis of a mixed sample collected from five random places in each site. The soil samples were brought to the laboratory in the same day to estimate the microbial population, enzymes activity and other physico-chemical characteristics.

Fungal populations were estimated by Warcup's soil plate method using rose bengal agar medium. The inoculated plates were incubated at  $25\pm 1^\circ\text{C}$  and colony forming units were enumerated after 5 days. Dilution plate method was used to estimate bacterial populations developing on nutrient agar medium. The inoculated plates were incubated at  $30\pm 1^\circ\text{C}$  and colony forming units were enumerated after 24 h from the plate of higher dilutions.

Soil organic carbon was determined by the method of Anderson and Ingram (1993). Soil pH was measured in a soil-water suspension (1:5) using an electric digital pH meter (Systronics, India). Moisture content of the soil was assessed by oven dry method at  $105^\circ\text{C}$  (NSW, India). Soil respiration was measured by the estimation of the released  $\text{CO}_2$  during 24 h incubation at room temperature by absorption and titration method using phenolphthalein indicator (Macfadyen, 1970).

Dehydrogenase activity of the soil was determined by 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) reduction technique (Casida, 1977). Urease activity of the soil was assessed by the method of McGarity and Myers (1967). The ammonia released as a result of the urease activity was measured by the indophenol blue method and the optical density of the blue coloured solution was read spectrophotometrically at 630 nm. Phosphatase activity of the soil was assessed by the method of Tabatabai and Bremner (1969). The p-nitrophenol produced due to phosphatase activity using the substrate disodium p-nitrophenyl phosphate gives yellow colour under alkaline conditions for which the intensity is measured spectrophotometrically at 420 nm.

### **Statistical Analysis**

Simple correlation analysis were carried out to find out the relationship between microbial population, enzyme activity and physico-chemical characteristics of soil.

## **RESULTS**

### **Physicochemical Properties of Soil**

Moisture content of soil was higher in undisturbed forest and was maximum during the month of July and minimum during the month of December (Fig. 1). pH of soil ranged from 5.28 to 5.93 in road side (disturbed soil) and from 5.84 to 6.12 in undisturbed forest soil (Fig. 1). No marked spatial variation in pH was observed between the two sites. Soil temperature was higher in the non-roadside undisturbed site ( $23.2^\circ\text{C}$ ) than disturbed ( $21.2^\circ\text{C}$ ) ones (Fig. 1). Organic carbon (C) content was higher in July in undisturbed soil (3.8%) and disturbed soil (2.8%) and earlier site produced more than the later ones (Fig. 2).

### **Microbial Population**

Spatial variation in the number of fungi and bacteria in both forest soils is given in Fig. 2. Number of fungi and bacteria were higher in soil of undisturbed non-roadside condition. The highest number of fungi was observed during the period August and October for undisturbed non- roadside forest soil while in roadside disturbed soil it was observed in the month of July as well as in October. The highest number of bacteria was observed in September in both the undisturbed and disturbed sampling sites.

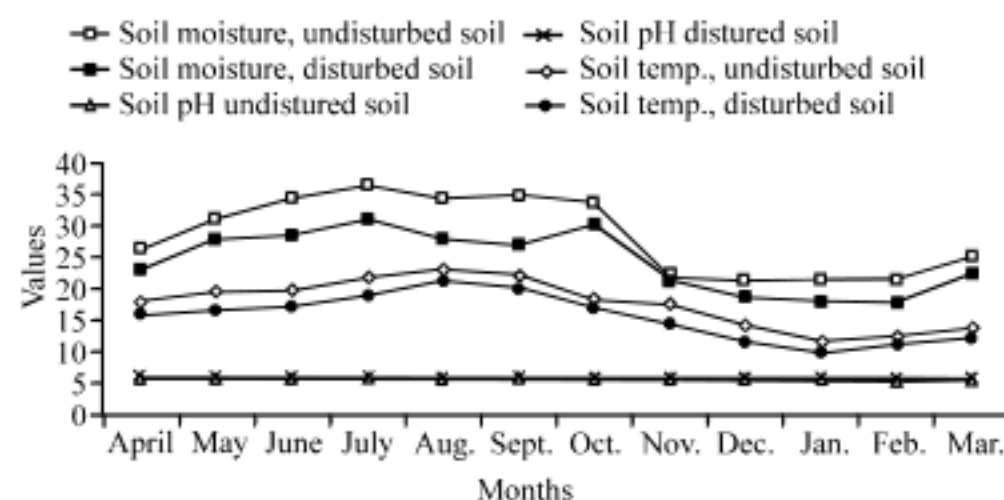


Fig. 1: Monthly variation in soil moisture (%), soil pH and soil temperature of undisturbed and disturbed soil

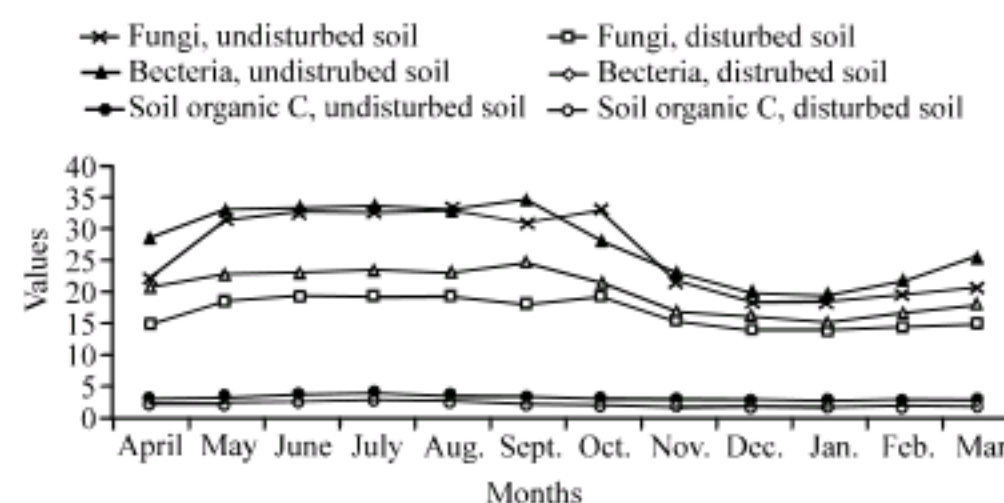


Fig. 2: Monthly variation in the number of Fungi ( $10^3 \text{g}^{-1}$  dry soil), Bacteria ( $10^5 \text{g}^{-1}$  dry soil) and organic carbon (%) of undisturbed and disturbed soil

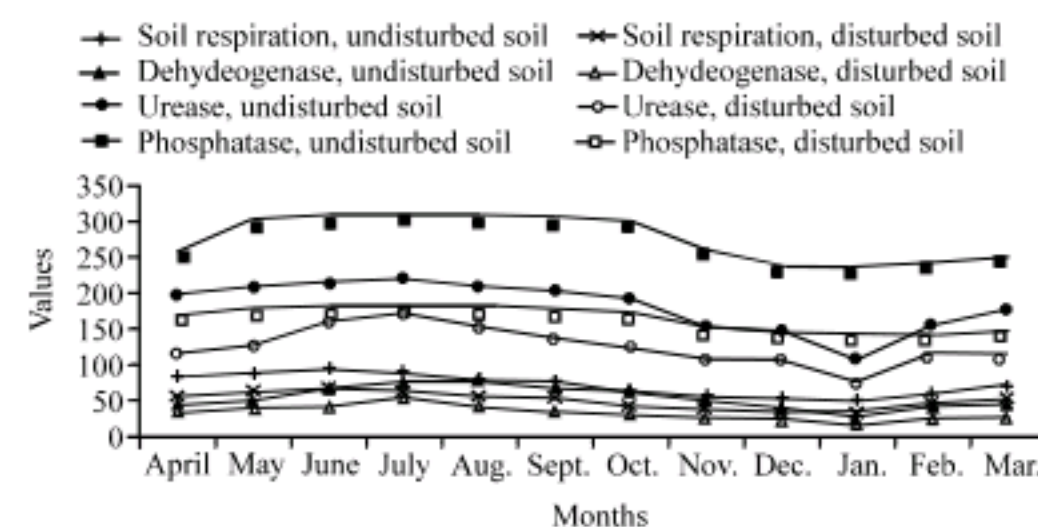


Fig. 3: Monthly variation in soil respiration ( $\text{mg CO}_2 \text{ kg}^{-1}$  dry soil  $24 \text{ h}^{-1}$ ), dehydrogenase ( $\mu\text{g TPF g}^{-1}$  dry soil  $24 \text{ h}^{-1}$ ), urease ( $\mu\text{g NH}_4^+ \text{ g}^{-1}$  dry soil  $3 \text{ h}^{-1}$ ) and Phosphatase ( $\mu\text{g p-nitrophenol g}^{-1}$  dry soil  $\text{h}^{-1}$ ) activity of undisturbed and disturbed soil

### Soil Respiration and Enzyme Activities

Evolution of  $\text{CO}_2$  in undisturbed non-roadside forest soil ranged from 50.8 to  $95 \text{ mg CO}_2 \text{ kg}^{-1}$  dry soil  $24 \text{ h}^{-1}$  and 35.9 to  $68.3 \text{ mg CO}_2 \text{ kg}^{-1}$  dry soil  $24 \text{ h}^{-1}$  in disturbed roadside soil (Fig. 3).

Dehydrogenase activity ranged from 28 to  $78.3 \mu\text{g TPF g}^{-1}$  dry soil  $24 \text{ h}^{-1}$  in undisturbed forest soil and from 18.2 to  $56.75 \mu\text{g TPF g}^{-1}$  dry soil  $24 \text{ h}^{-1}$  in disturbed ones (Fig. 3). Urease activity in non-roadside soil ranged from 108.7 to  $220.6 \mu\text{g NH}_4^+ \text{ g}^{-1}$  dry soil  $3 \text{ h}^{-1}$  and 76.8 to  $174.2 \mu\text{g NH}_4^+ \text{ g}^{-1}$  dry soil  $3 \text{ h}^{-1}$  in roadside soil (Fig. 3).

Table 1: Simple correlation coefficient (r) values between enzymes activity, microbial population and physico-chemical characteristics of undisturbed non-roadside soil

| Variables        | Fungi    | Bacteria | Dehydrogenase | Urease   | Phosphatase | Soil respiration | Soil organic C | Soil moisture | Soil pH  | Soil temp. |
|------------------|----------|----------|---------------|----------|-------------|------------------|----------------|---------------|----------|------------|
| Fungi            |          | 0.904*** | 0.896***      | 0.847*** | 0.991***    | 0.699*           | 0.855***       | 0.969***      | 0.966*** | 0.876***   |
| Bacteria         | 0.904*** |          | 0.851***      | 0.942*** | 0.929***    | 0.888***         | 0.894***       | 0.935***      | 0.916*** | 0.908***   |
| Dehydrogenase    | 0.896*** | 0.852*** |               | 0.849*** | 0.911***    | 0.654*           | 0.915***       | 0.912***      | 0.896*** | 0.915***   |
| Urease           | 0.847*** | 0.942*** | 0.849***      |          | 0.858***    | 0.904***         | 0.878***       | 0.87***       | 0.886*** | 0.850***   |
| Phosphatase      | 0.992*** | 0.929*** | 0.911***      | 0.858*** |             | 0.725**          | 0.886***       | 0.968***      | 0.965*** | 0.914***   |
| Soil respiration | 0.699*   | 0.888*** | 0.654*        | 0.904*** | 0.725**     |                  | 0.845***       | 0.742**       | 0.694*   | 0.716**    |
| Soil organic C   | 0.856*** | 0.894*** | 0.915***      | 0.879*** | 0.886***    | 0.845***         |                | 0.880***      | 0.810**  | 0.880***   |
| Soil moisture    | 0.969*** | 0.935*** | 0.911**       | 0.869*** | 0.968***    | 0.742**          | 0.880***       |               | 0.941*** | 0.878***   |
| Soil pH          | 0.966*** | 0.916*** | 0.896***      | 0.886*** | 0.965***    | 0.694*           | 0.810**        | 0.941***      |          | 0.915***   |
| Soil temp.       | 0.876*** | 0.908*** | 0.915***      | 0.85***  | 0.914***    | 0.716**          | 0.880***       | 0.878***      | 0.915*** |            |

\*, \*\*, \*\*\*Indicates significant levels at&lt;0.05, &lt;0.01 and &lt;0.001 p-values, respectively. N = 12

Table 2: Simple correlation coefficient (r) values between enzymes activity, microbial population and physico-chemical characteristics of disturbed roadside soil

| Variables      | Fungi    | Bacteria | Dehydrogenase | Urease   | Phosphatase | Soil respiration | Soil organic C | Soil moisture | Soil pH  | Soil temp. |
|----------------|----------|----------|---------------|----------|-------------|------------------|----------------|---------------|----------|------------|
| Fungi          |          | 0.887*** | 0.836***      | 0.901*** | 0.636*      | 0.820**          | 0.962***       | 0.702*        | 0.868*** |            |
| Bacteria       | 0.887*** |          | 0.837***      | 0.848*** | 0.969***    | 0.793**          | 0.853***       | 0.911***      | 0.847*** | 0.937***   |
| Dehydrogenase  | 0.787**  | 0.837*** |               | 0.934*** | 0.847***    | 0.838***         | 0.974***       | 0.846***      | 0.688*   | 0.795**    |
| Urease         | 0.836*** | 0.848*** | 0.934***      |          | 0.819**     | 0.819**          | 0.956***       | 0.830***      | 0.647*   | 0.818**    |
| Phosphatase    | 0.901*** | 0.969*** | 0.847***      | 0.819**  |             | 0.758**          | 0.839***       | 0.928***      | 0.807**  | 0.924***   |
| Soil resp.     | 0.636*   | 0.793**  | 0.838***      | 0.819**  | 0.758**     |                  | 0.857***       | 0.683*        | 0.750**  | 0.623*     |
| Soil organic C | 0.82**   | 0.853*** | 0.976***      | 0.956*** | 0.839***    | 0.857***         |                | 0.847***      | 0.665*   | 0.804**    |
| Soil moisture  | 0.962*** | 0.911*** | 0.846***      | 0.830**  | 0.928***    | 0.683*           | 0.847***       |               | 0.814**  | 0.863***   |
| Soil pH        | 0.703**  | 0.847*** | 0.688*        | 0.647*   | 0.807**     | 0.750**          | 0.665*         | 0.814**       |          | 0.739**    |
| Soil temp.     | 0.868*** | 0.937*** | 0.795**       | 0.818**  | 0.924***    | 0.623*           | 0.804**        | 0.863***      | 0.739**  |            |

\*, \*\*, \*\*\* Indicates significant levels at&lt; 0.05, &lt;0.01 and &lt;0.001 p-values, respectively. N=12

Phosphatase activity in undisturbed soil ranged from 238.2 to 312.2  $\mu\text{g p-nitrophenol g}^{-1}$  dry soil  $\text{h}^{-1}$  and 142.8 to 182.6  $\mu\text{g p-nitrophenol g}^{-1}$  dry soil  $\text{h}^{-1}$  in disturbed soil (Fig. 3).

### Statistical Analysis

Table 1 and 2 shows simple correlation coefficient (r) values and significant level (p) of various parameters between the undisturbed and disturbed soil.

## DISCUSSION

### Physicochemical Properties of Soil

Moisture content of soil was higher in undisturbed forest and was maximum during the month of July and minimum during the month of December (Fig. 1). The roadside disturbed forest soil contained less moisture due to higher evapo-transpiration losses of its exposed land resulted by the loss of forest cover. The pH of soil ranged from 5.28 to 5.93 in road side (disturbed soil) and from 5.84 to 6.12 in undisturbed forest soil (Fig. 1). No marked spatial variation in pH was observed between the two sites. Soil temperature was higher in the non-roadside undisturbed site (23.2°C) than disturbed (21.2°C) ones (Fig. 1).

Organic carbon (C) content was higher in July in undisturbed soil (3.8%) and disturbed soil (2.8%) and earlier site produced more than the later ones (Fig. 2). The drop in the organic carbon in the roadside and increase in the non-roadside soil may be as a result of differential accumulation and decomposition of litter (Joshi *et al.*, 1993).

### Microbial Population

Spatial variation in the number of fungi and bacteria in both forest soils is given in Fig. 2. Number of fungi and bacteria were higher in soil of undisturbed non-roadside condition. The highest number of fungi was observed during the period August and October

for undisturbed non- roadside forest soil while in roadside disturbed soil it was observed in the month of July as well as in October. The highest number of bacteria was observed in September in both the undisturbed and disturbed sampling sites.

The decrease in number of soil microbes can be attributed to fewer amounts of minerals. The soils usually with high organic carbon and adequate moisture were acted upon by an array of microorganisms to decompose it into simpler forms; hence the number of microorganisms is higher (Acea and Carballas, 1985). The increased number of microbes in undisturbed non-roadside forest soil than disturbed roadside ones was due to favorable physico-chemical conditions and possible increase in the root exudation in the earlier soil than the later ones (Hall and Davies, 1971). The soil with higher moisture and humus content harboured the higher fungal density (Thorton, 1956; Brown, 1958). The metabolic quotient seems to be an especially good indicator for environmental effects on the soil micro flora (Wardle and Parkinson, 1990). Numerous investigators have suggested that destruction of soil properties may result in a permanent reduction in soil productivity. Generally the effects of soil disturbances are more adverse than beneficial (Anonymous, 1981). The deposition of numerous gaseous and trace metals from road traffic can result in reduced rates of microbially-mediated litter decomposition at roadside, hence proving the point of reduced microbial activity in the roadside soil due to vehicular pollution (Post and Beeby, 1996). Bacterial and Fungal community showed a significant negative correlation with the concentration of lead, zinc, copper, cadmium and sulfur on the phylloplane at the polluted roadside trees, the concentration of trace heavy metal being produced due to pollution caused by heavy traffic density at the site (Joshi *et al.*, 2008).

#### Soil Respiration and Enzyme Activities

Evolution of CO<sub>2</sub> in undisturbed non-roadside forest soil ranged from 50.8 to 95 mg CO<sub>2</sub> kg<sup>-1</sup> dry soil 24 h<sup>-1</sup> and 35.9 to 68.3 mg CO<sub>2</sub> kg<sup>-1</sup> dry soil 24 h<sup>-1</sup> in disturbed roadside soil (Fig. 3). Respiration activity was influenced by microbial number, organic C and physico-chemical properties of the soil (Stroo and Jencks, 1982). This observation was in contrary to that of Ross (1973), who noted that each biochemical activity has its own characteristics distribution in soil.

Dehydrogenase activity ranged from 28 to 78.3 µg TPF g<sup>-1</sup> dry soil 24 h<sup>-1</sup> in undisturbed forest soil and from 18.2 to 56.75 µg TPF g<sup>-1</sup> dry soil 24 h<sup>-1</sup> in disturbed ones (Fig. 3). Dehydrogenase has been used as an indicator of microbial metabolism (Casida, 1977). It followed a similar pattern to organic C and the populations of fungi and bacteria. This observation suggested the possibility that these factors regulate the dehydrogenase activity, individually or in combination. The dehydrogenase activity was positively correlated to organic C an energy driving force to the soil microbes. Zimny and Wieszczyk (1983) also reported reduced dehydrogenase, cellulase and urease enzyme activity in roadside soils which they attributed to the effect of traffic. Urease activity in non-roadside soil ranged from 108.7 to 220.6 µg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> dry soil 3 h<sup>-1</sup> and 76.8 to 174.2 µg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> dry soil 3 h<sup>-1</sup> in roadside soil (Fig. 3). The continued addition of enzymes in the forested soil could be expected from plant roots (Hall and Davies, 1971) and from associated microorganisms (Speir, 1976). It can be inferred that the supplementations from these sources accounted for the increase in urease activity in the non-roadside soil.

Phosphatase activity in undisturbed soil ranged from 238.2 to 312.2 µg p-nitrophenol g<sup>-1</sup> dry soil h<sup>-1</sup> and 142.8 to 182.6 µg p-nitrophenol g<sup>-1</sup> dry soil h<sup>-1</sup> in disturbed soil (Fig. 3). The higher phosphatase activity can be attributed to the higher microbial population and other associated activity like enrichment by litter decomposition.

### **Statistical Analysis**

Simple correlation coefficient (r) values showed positive correlation between microbial population, enzyme activity and physico-chemical characteristics of soil. Table 1 and 2 show simple correlation coefficient (r) values and significant level (p) of various parameters between the undisturbed and disturbed soil.

### **CONCLUSION**

Though there is no human settlement in the study site but the impact of roadside pollution due to heavy traffic has caused the decline in the population as well as the metabolic activities of both the fungi and bacteria in the roadside disturbed soil. The present study showed that single parameter did not determine the biological activity of soil but was influenced considerably by the environmental conditions. Greater number of microbes and their activity in non-roadside undisturbed condition than roadside disturbed ones has suggested that organic C and physico-chemical characteristics of soil are important regulating factors. The present study demonstrated that a higher correlation between abiotic factors and microbes in non-roadside forest soil exists than that of the roadside ones, suggesting that, increased disturbances had an adverse affect on the microbial population and their activity, which may affect the mineralization and immobilization of nutrients in the forest soil.

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