Microbial Population Changes in Pine Forest Soil of Gulmarg as Influenced by Vehicular Pollution

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The present study was conducted during autumn 2010 and spring 2011 at three different locations and each location comprising of disturbed and undisturbed sites in Baramulla District of Jammu and Kashmir, India. From each site five composite soil samples from the rhizosphere of *Pinus wallichiana* were taken in and analysed for soil organic carbon, soil nitrogen and microbial population (Bacteria and Fungi). After analysing the soil samples, it was observed that organic carbon and available nitrogen of the soil was found significantly higher at the undisturbed sites at all the locations in both the seasons. Organic carbon does not show much variation while as available nitrogen in spring season was found significantly higher than that in autumn season. The population of both bacteria and fungi was found significantly higher at undisturbed sites than the disturbed ones in both the seasons. Spring season showed a decline in the microbial population (bacteria and fungi) as compared to autumn season.

Key words: Pollution, Microbial population, rhizosphere, *Pinus wallichiana,* disturbed, Organic carbon.

The transport demand in India has been growing rapidly, which is the second largest consumer of energy, next only to industry. The installing capacity of Indian automobile industry is also increasing over the years, based on the domestic and international demand. Following the trends of urbanization and population growth in Indian cities, people buy more and more vehicles for personal use and thus have perpetuated an increase in vehicles that contribute to vehicular emissions containing pollutants such as sulphur dioxide, nitrogen oxides, carbon monoxide, lead, ozone, benzene, and hydrocarbons (Goyal *et al*., 2005). As a result of urbanization in India, pressure on urban transport is likely to increase substantially in this new millennium. Total vehicle population of India is more than 85 million (about 1 % share of the world) (Economic Survey of India, 2008). The increase in vehicles, as well as the presence of other motorized forms of transportation (taxis, autos, trains, buses, *etc*.), will contribute to the already existent large amount of vehicular emissions. The worst thing about vehicular pollution is that it cannot be avoided as the vehicular emissions are emitted at near-ground level.

Vehicular exhaust severely affect the growth, morphology and metabolism of microorganisms in bulk soils through functional disturbances, protein denaturation or destruction

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of the integrity of cell membranes (Leita *et al*., 1995). Soil microbial population mainly includes bacteria, fungi, actinomycetes and algae which mediate many of the processes that influence soil fertility. The soil with higher humus content harbored the higher fungal density (Thorton, 1956; Brown, 1958). Micro organisms are dynamically involved in many basic ecological processes such as biogeochemical cycling of elements and the mineralization of carbon, nitrogen, sulphur and phosphorus (i.e., organic matter decomposition) (Paul and Clarck, 1996). The increased number of microbes in undisturbed non road side forest soils than disturbed ones was due to the favorable physico-chemical conditions and possible increase in root exudation in the earlier soils than the later ones (Hall and Davies,1971). Studies conducted around point sources of metal pollution (Strojan, 1975; Ebregt and Boldewijn, 1977) have shown suppression of soil respiration activity at the sites closest to the source and having the high concentration of metals. Elevated levels of toxic metals in the soil have the potential to interfere with normal ecological cycles occurring at the important decomposer level of the food chain (Tyler, 1972; Strojan, 1975; Sterritt and Lester, 1980). Post and Beeby (1996) stated that metal contamination of road side plant litter was associated with significant reduction in decomposition rates. Aceves (2005) stated that the amount of microbial biomass in metal contaminated soils was about half of that found in soils from uncontaminated organic manure. Joshi *et al.* (2010) stated that enzyme activities were considerably higher in litter at the less polluted site than at the more polluted site. Significantly higher number of fungi and bacteria were recorded in non road side soil than the effected road side soil (Joshi *et al*., 2010). Trace metal contamination may result in increased uptake by plants, and affect the functions and composition of the soil microbial communities. Total metal concentration in soil is inaccurate for predicting any of these biological facts, since soils vary considerably in their capacity to bind metals in biologically unavailable forms.

The objective of the study is to investigate the effect of vehicular pollution on microbial population, soil organic carbon and soil nitrogen in Pine forest soils of Gulmarg.

MATERIALS AND METHODS

Soil sampling and analytical procedures

The study was carried out on the highway from Tangmarg to Gulmarg, at three different locations *viz*., Tangmarg at an elevation between 2218m to 2240m above sea level, Baba Reshi between 2272m to 2288m above sea level and Gulmarg between 2655m to 2695m above sea levelwith each location comprising of disturbed (up to 5-8m from road) and undisturbed (about 150 m away from road) sites in Baramulla District of Jammu and Kashmir, India during autumn 2010 and spring 2011.

The state of Jammu and Kashmir is the northern most state of India situated between 32.15 degree and 37.05 degree north latitude and 72.35 degree and 83.20 degree east longitude. Gulmarg- the meadow of flowers is a world famous tourist spot in Kashmir located at 34.03 degree north and 74.23 degree east having an average elevation of 8800 feet above sea level surrounded by dense forests of tall conifers. The route to Gulmarg also passes through the dense forest having the dominant pine species of *Pinus wallichaina, Abies pindrow* and *Cedrus deodara*.

In the Kashmir valley increasing trend in the purchase/registration of vehicles has been seen, which can be illustrated from the Table 1 showing the registration of vehicles in the year 2010-11 as compared to previous year (2009-10) vehicular registration which indicates that there is more than 20% increase in the vehicular registration in the year 2010-11 as compared to previous year whereas the average vehicular traffic load on the study site of Gulmarg highway (Table 2).

Soil samples were taken from three different locations viz., Tangmarg, Baba Reshi and Gulmarg with each location comprising of disturbed (up to 5-8m from road) and undisturbed (about 150 m away from road) sites. The sampling was carried out in two seasons, first in autumn season (September 2010) and second in spring season (April 2011). Five composite surface soil samples from upper 0-5 cm soil layer were taken from the rhizosphere of *Pinus wallichaina*, with each composite sample consisting of three sub-samples from three different rhizospheres with each of them taken from five random places within the rhizosphere of the pine tree both at disturbed and undisturbed sites from all the three locations after removing the fallen leaves, debris *etc*., at the sampling site. After collection, these samples were dried in shade, powdered and then sieved through 0.2 mm mesh and then analyzed to assess the following parameters. Samples for determining the moisture content was taken separately in sealed polythene bags.

Soil organic carbon was determined by Walkley and Black's rapid titration method (Walkley and Black, 1947).

Soil available nitrogen was determined by potassium permagnate method (Subbiah and Asija, 1956).

Microbial population (Bacteria and Fungi) was determined by serial dilution agar plating method by Aneja, 1996.

Statistical analysis

The data collected during the experimentation was subjected to appropriate statistical procedures.

Table 1. Vehicular registration in Kashmir valley during 2009-10 & 2010-11

District	Total vehicular registrations 2009-10	2010-11
Srinagar	10972	12935
Budgam	1900	2580
Kupwara	755	964
Baramullah	3147	4444
Anantnag	4087	5248
Pulwama	3747	4052
Total	24608	30223

Source; RTO, Srinagar

RESULTS AND DISCUSSION

Soil organic carbon

The results revealed that highest average soil organic carbon(with 5.27 %) was found to be at undisturbed site of Gulmarg while the lowest organic carbon (1.99 %) was found at disturbed site of Baba Reshi in autumn season of 2010 (Table 3). During spring season of 2011, highest average soil organic carbon(5.15 %) was recorded at undisturbed site of Gulmarg whereas the lowest organic carbon(2.38%.) was found at disturbed site of Baba Reshi (Table 4).The drop in the organic carbon in roadside soils and increase in non roadside soils may be the result of differential accumulation and decomposition of litter. (Joshi *et al.*, 1993; Laskowski *et al.*,1994 and Joshi *et al.*,2010). It may also be due to the presence of dense vegetation at the undisturbed sites than the disturbed ones which may lead to the more and more accumulation and decomposition of litter fall.

Soil available nitrogen

The highest average soil available nitrogen (399.6 kg/ha) was found to be at undisturbed site of Gulmarg while the lowest available nitrogen (112.4 kg/ha) was also found at disturbed site of Gulmarg during autumn season (Table 3). During spring season, the highest average soil available nitrogen (883.5 kg/ha) was found to be at undisturbed site of Gulmarg whereas the lowest available nitrogen (388.0 kg/ha) was found at disturbed site of Baba Reshi (Table 4). Spring season showed higher content of available nitrogen as compared to autumn season. The favorable environmental conditions to the microbial populations at the undisturbed sites which greatly contribute to the nitrogen

Table 2. Average vehicular flow along the study site

Vehicle category	High traffic months(5)*	Medium traffic months(4)**	Low traffic months(3)***
LMV	41,126	19311	5403
HMV	4,512	1218	592
Monthly Total	45,638	20529	5995
Monthly average : (45638x5 + 20529x4 + 5995x3) ÷ 12 =27,357vehicles/month			

Source : Toll post- Tangmarg
* = May, June, July, August and September
** = March, April, October and November
*** = December, January and February

mineralization may be the reason for the higher concentrations at undisturbed sites and also due to the higher populations of microbes there may be more involvement in the nitrogen cycling (Das and Dkhar, 2011).

Microbial population

In the autumn season (2010), the average highest bacterial population was found at undisturbed sites of Tangmarg with 12.32 x 10^7 CFU/g of dry soil and average lowest bacterial population was found at the disturbed site of Gulmarg with an average bacterial population of 6.62 x 10^7 CFU/g of dry soil (Table 3)whereas in spring season (2011), the average highest bacterial population(10.66 x 10^7 CFU/g of dry soil) was found at undisturbed sites of Tangmarg and the lowest average bacterial population was found at disturbed site of Gulmarg with an average population of 5.28 x 10^7 CFU/g of dry soil(Table 4). Fig. 1-2 shows the bacteria identified from the temporary slides made from pure cultures obtained in the slants.

In autumn season of 2010, average highest population of fungi from was found at undisturbed site of Tangmarg with 8.94 x 10^4 CFU/g of dry soil whereas the average lowest fungal population was found at Gulmarg with an average population of 5.66 x 10^4 CFU/g of dry soil (Tale 3).In spring season the average highest population of fungi was recorded from undisturbed site of Tangmarg with 8.36 x 10^4 CFU/g of dry soil and the average lowest at disturbed site of Gulmarg with an average population of 3.34 x 10^4 CFU/g of dry

Table 4. Effect of vehicular disturbance on organic carbon, available nitrogen and microbial population in the pine forest soils along Gulmarg highway in Spring 2011

Location	Site	Organic Carbon (%)	Available Nitrogen (kg/h)	Bacteria (x10^7) CFU/g	Fungi(x10^4) CFU/g
Tangmarg	Undisturbed	4.220±0.465	678.0±138	10.66±1.52	8.36±0.57
	Disturbed	3.560±0.433	586.0±87.4	6.28±0.88	4.94±0.49
	? t ?	2.32*	1.26	5.56**	10.12***
Baba Reshi	Undisturbed	3.724±0.697	599.0±40.9	9.94±1.70	7.40±0.86
	Disturbed	2.388±0.110	388.0±36.7	5.08±0.99	5.02±0.70
	? t ?	4.23*	8.58***	5.52**	4.76**
Gulmarg	Undisturbed	5.152±0.086	883.5±71.6	8.50±1.10	6.84±0.94
	Disturbed	3.760±0.320	596.6±51.5	5.28±0.79	3.34±0.50
	? t ?	9.40**	7.28***	5.32**	7.34***

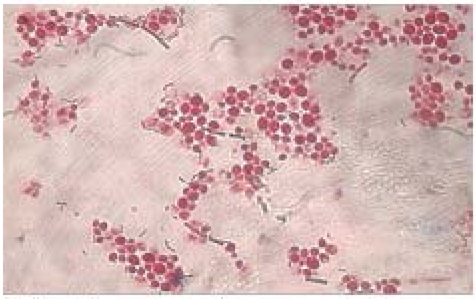
Significance level; *p<0.05 - significant, ** p<0.01-highly significant, ***p<0.001-very highly significant.
Note: The values shown in all tables are Mean ± SD

Table 3. Effect of vehicular disturbance on organic carbon, available nitrogen and microbial population in the pine forest soils along Gulmarg highway in Autumn 2010

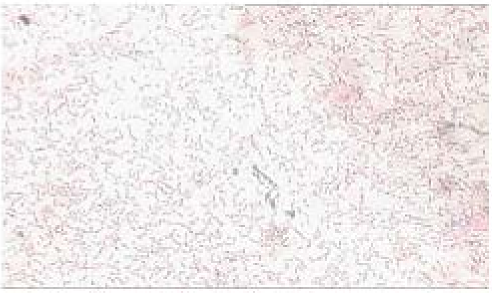
Location	Site	Organic Carbon (%)	Available Nitrogen (kg/h)	Bacteria (x10^7) CFU/g	Fungi(x10^4) CFU/g
Tangmarg	Undisturbed	4.190±0.291	296.0±28.9	12.32±0.716	8.94±0.573
	Disturbed	3.226±0.459	137.1±43.0	8.64±0.850	6.58±0.319
	? t ?	3.97**	6.88***	7.40***	8.05***
Baba Reshi	Undisturbed	4.584±0.410	314.0±27.7	11.82±1.01	8.14±0.93
	Disturbed	1.992±0.590	132.2±24.9	7.26±0.41	6.10±0.40
	? t ?	8.06***	10.91***	9.35***	4.48**
Gulmarg	Undisturbed	5.272±0.323	399.6±94.8	12.10±0.89	8.20±0.86
	Disturbed	4.292±0.234	112.4±12.3	6.62±1.12	5.66±0.32
	? t ?	5.42**	6.71**	8.54***	6.19**

Significance level; *p<0.05 - significant, ** p<0.01-highly significant, ***p<0.001-very highly significant.

- **Bacterial isolates**



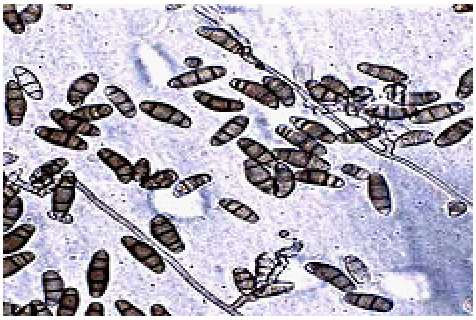
1. Coccal type bacteria



2. Bacillar type bacteria

Fig. 1 and 2

B.Fungal isolates



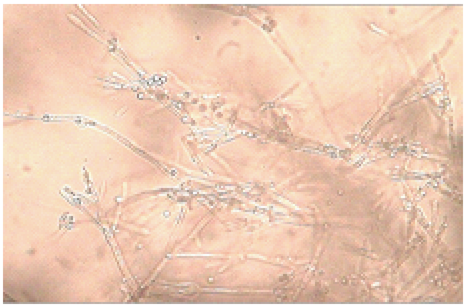
3. *Curvularia* sp.

4. *Fusarium* sp.

5. *Rhizopus* sp.

Fig. 3- 7.

6. *Alternaria* sp.



7. *Pencillium* sp.

soil(Table 4) Fig. 3-7 shows the fungi identified from the temporary slides made from pure cultures obtained in the slants.

The increased number of microbes in undisturbed non roadside soils than disturbed roadside ones may be due to the favorable physico chemical conditions of soil. Higher organic carbon, available nitrogen and also the higher moisture percentage at the undisturbed sites may have greatly contributed to the higher populations of both bacteria and fungi at these sites (Joshi *et al*. 2010). Thorton (1956) and Brown (1958) stated that soils with higher moisture and humus content harbored the higher fungal density. Joshi *et al*. (2010) studied that the 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 metals being produced due to pollution caused by heavy traffic density at the site. The deposition of numerous gaseous and trace metals from road traffic might have resulted 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).

CONCLUSION

It was concluded from the study that vehicular flow along the highway has significantly affected the soil organic carbon, soil nitrogen and populations of both bacteria and fungi .Soil organic carbon was found significantly higher at undisturbed sites than the disturbed ones but on comparing the seasons there was no significant variation. Available nitrogen was also found significantly higher at undisturbed sites as compared to the disturbed ones. In spring season available nitrogen content in the soil was found significantly higher as compared to autumn season. There was observed a decline in the microbial population (bacteria and fungi) at disturbed sites in comparison to undisturbed sites at all the locations in both the seasons. Spring season also recorded a decline in the population of both bacteria and fungi as compared to the autumn season.

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