

**Microbial Community on Leaf Surfaces of Broad-Leaved Alder
(*Alnus nepalensis* D. Don) and Needle-Leaved Khasi Pine
(*Pinus kesiya* Royle Ex Gordon) as Influenced by Atmospheric
Dry Deposition of Roadside Pollution in Eastern Himalayas**

Santa Ram Joshi, Ranjan Kumar Bhagobaty and Rakshak Kumar
Microbiology Laboratory, Department of Biotechnology and Bioinformatics,
North-Eastern Hill University, Permanent Campus, Umshing,
Shillong-793 022, Meghalaya, India

Abstract: Impact of roadside pollution on the population of leaf surface microorganisms of *Alnus nepalensis* D. Don and *Pinus kesiya* Royle Ex Gordon was studied at a hilly terrain in Eastern Himalayas. Changes in the population of microbes especially fungi and bacteria due to roadside pollution was noted. Leaves were collected at sites 8 m and 1 km away from the National Highway No. 44 and were analysed for microbial population, heavy metals and sulphur accumulation. Leaves of both tree species closer to the road contained higher amounts of heavy metals than those at 1 km site. The population of bacteria and most fungi species was higher at 1 km site than the site closer to the highway. Diversity of micro fungal community in phylloplane at the two sites differed significantly. Counts of fungal units (cfu) and bacteria propagules showed significant negative correlation with the concentrations of metals and sulphur. Some fungal forms including *Fusarium oxysporum*, *Mortierella* sp. and *Aureobasidium pollulans* were abundant in the polluted roadside compared to other species of fungi.

Key words: Alder, pine, phylloplane, roadside pollution, leaf surface microorganisms

INTRODUCTION

North Eastern region of India is located in the Eastern Himalayas and roads form the main system of communication and transport owing to the hilly topography of the region. Motor vehicles are the main mode of transport with the nearest rail link located at a distance of 108 km from the site. Automobiles, as the only mode in the transportation system, discharge a number of gaseous and trace metal contaminants due to incomplete combustion of petroleum fuels. Human activities like stone grinding, road construction, sand milling also increase the atmospheric dry deposition and heavy metal contaminant level. These dust and contaminants ultimately get settled on the leaf surfaces of roadside trees and soils. Such interventions may affect the composition and activity of microorganisms in the roadside environment. Leaf surface harbours a definite microbial community by virtue of the presence of leachates. Phylloplane microbes come into direct contact with the gaseous and particulate air pollutants which get deposited on the leaf surfaces. Understanding the importance of relationship between the dry deposition of air pollutants and microorganisms and the impact on the composition of microbial community on leaf surfaces, the study was carried out to compare the nature and composition of microbes on leaf surfaces of alder (*Alnus nepalensis* D. Don) and khasi pine (*Pinus kesiya* Royle Ex Gordon) at the roadside and non-roadside environment. Two sites dominated

Corresponding Author: S.R. Joshi, Microbiology Laboratory, Department of Biotechnology and Bioinformatics, North Eastern Hill University, Permanent Campus, Umshing, Shillong-793022, Meghalaya, India Tel: +91-364-2722406, +91 9436102171

by alder and pine trees were selected, of which one at a busy road intersection near the National Highway No. 44 in Shillong with high traffic density (10,000-12,000 vehicles day⁻¹), taken as the polluted site and the other one in a forested area, approximately 1 km away from the roadside considered as the unpolluted site. Analysis of phylloplane microbes, lead, zinc, copper, cadmium and sulphur was carried out from leaves at the two sites.

The microbial community of the phylloplane may be altered by the gaseous and particulate atmospheric pollutants (Saunders, 1971; Mowll and Gadd, 1985). The relationship between air pollution and microorganisms is an important and incompletely appreciated topic (Saunders, 1973; Manning, 1975). Organisms that exist in the phylloplane are particularly vulnerable to the influence of air contaminants (Smith, 1976a). While there has been some studies on the effects of industrially derived heavy metal pollution on leaf surface microorganisms (Gingell *et al.*, 1976; Bewley, 1980; Bewley and Campbell, 1980), little work has been done on the effects of heavy metal and trace contaminants on leaf surface microbes in the roadside and urban environment in general (Smith, 1971, 1976a, b, 1977) and on the effects of vehicular pollution and roadside deposition on phylloplane microbes in particular (Mowll and Gadd, 1985; Lambais *et al.*, 2006).

While there has been some studies on the effects of industrially derived heavy metal pollution on leaf surface microorganisms (Gingell *et al.*, 1976; Bewley, 1980; Bewley and Campbell, 1980; DeKempeneer *et al.*, 2004; Lambais *et al.*, 2006; Sandhu *et al.*, 2007), little work has been done on the effects of heavy metal and trace contaminants on leaf surface microbes in the roadside urban environment in general (Smith, 1971, 1976a, b, 1977; Swailesh *et al.*, 2004) and on the effects of vehicular pollution on phylloplane microbes in particular (Mowll and Gadd, 1985). Therefore, a study to understand the impact of roadside pollution on the phylloplane microbes of roadside trees was carried out to understand its impact on the population dynamics of microbes in these environment.

MATERIALS AND METHODS

Description of the Study Sites

Two sites dominated by alder trees (*Alnus nepalensis*) and pines (*Pinus kesiya*) of Meghalaya in North-East India were selected for study. One site, located at a busy roadside of the National Highway No. 44 (8 km from the capital town of Meghalaya) Shillong (altitude 1730 m MSL, latitude 25°34', longitude 91°46' E) with traffic density (10,000-12,000 vehicles day⁻¹) was taken as the polluted site while the other one was approximately 1 km away from the National Highway roadside and considered as the unpolluted site. While selecting care was taken to ensure similarity of both sites in topography and micro-environmental conditions. The study area has typical monsoon climate with prevailing South-west monsoon and North-east winter winds. The climate of the study area is characterized by four distinct seasons: (i) the monsoon season with heavy rainfall (May through September) due to south west monsoon. (ii) a transitional period of low rainfall, autumn season (October through November) due to retreating monsoon. (iii) a winter season (December through February) with scattered low rainfall and (iv) a windy dry summer (March through April). The average monthly rainfall ranged from 2.1 to 421.2 mm during the study period (2005-2006). The average minimum and maximum temperature varied from 6.1 to 18.0°C and 15.1 to 24.3°C, respectively. The average relative humidity ranged between 68.5 to 89%.

Sampling Procedure (Including Samples for Microbes and Those for Heavy Metals Analysis)

Ten samples of matured leaves were collected at a bimonthly interval for one year from ten different individuals of each tree species at each site, then stored in sterilized container and brought to the laboratory.

Microbes Isolation and Their Community Traits

The isolation of microfungi and bacteria from the leaf surface was based on dilution plate method (Dickinson, 1971). The microbial assay was carried out within 24 h of sampling. The leaf samples were cut into small pieces with sterilized scissor in a laminar flow chamber to avoid contamination and mixed thoroughly. One gram on wet weight basis sample was taken in a 250 mL sterilized conical flask containing 100 mL of sterilized distilled water. Flasks were shaken vigorously for 30 min to detach the surface propagules. A suspension of 1:100 in volume was then obtained. Ten milliliter of this suspension was transferred aseptically to a 250 mL conical flask containing 90 mL of sterilized distilled water to get a dilution of 1:1000. The process was repeated to get a suspension of 1:10,000 dilution. For the isolation of fungi and bacteria, a 1: 1000 and 1: 10,000 dilutions was used, respectively.

Five milliliter suspension culture were transferred aseptically into sterilized petri plates containing 15 mL of sterilized solidified Rose Bengal agar (Martin, 1950) and nutrient agar medium (Difco-Manual, 1953) for fungi and bacteria, respectively. Three replicates were maintained in each case. The Petri plates for microfungi and bacteria were incubated at 25±1°C and 30±1°C for 5 days and for 24 h, respectively. Number of fungi and bacteria was calculated as colony forming units (cfu) per gram dry leaf, taking into account by the dilution factor and leaf moisture content.

The relative abundance of fungi was calculated by using the following formula:

$$\text{Relative abundance (\%)} = \frac{\text{Total No. of cfu of the individual}}{\text{Total No. of cfu of all the species}} \times 100$$

The index of fungal diversity was calculated by Shannon's index of diversity (Shannon, 1948) as:

$$\text{Shannon's index } (\bar{H}) = \frac{-(ni)}{N} \log \frac{(ni)}{N}$$

Where:

- ni = Relative abundance of one species
- N = Total relative abundance

Similarity index was calculated by Sorenson's similarity index (Sorenson, 1948) as:

$$\text{Similarity index} = \frac{2C}{A + B} \times 100$$

Where:

- A = No. of fungal species in unpolluted site
- B = No. of fungal species in polluted site
- C = Species common in A + B

Species richness index was calculated as:

$$\text{Species richness} = \frac{S-1}{\log N}$$

Where:

- S = No. of fungal species
- N = Total number of fungi (cfu)

Chemical Analysis of Heavy Metals and Trace Elements in Leaves

0.2 g of dried powders of leaves in triplicate was digested (by wet or dry digestion) in 20 mL conc. HNO₃. Extracts were filtered and made up to 50 mL with double distilled water. The extent of metal binding by filters was checked using metal stock solution and was found negligible (Proctor *et al.*, 1980). Samples were analysed on Perkin Elmer 2380 Atomic absorption spectrophotometer after appropriate dilution with distilled water.

The concentration of metal was calculated as:

$$\text{Total metal } (\mu\text{g g}^{-1}) = \frac{c(\text{ppm}) \times \text{solution volume (mL)}}{\text{Sample weight (g)}}$$

Where:

c = ppm metals obtained from the standard curve

The samples for sulphur determination were oven dried at 80°C for 24 h, powdered, ashed at 450°C for 12 h in a muffle furnace and the ash was dissolved in conc. HNO₃. Total sulphur was determined by the turbidimetric method (Allen, 1974).

Total sulphur in leaves was calculated as:

$$\text{Total sulphur } (\mu\text{g g}^{-1}) = \frac{c(\text{ppm}) \times \text{solution volume (mL)}}{\text{Sample weight (g)}}$$

Where:

c = ppm sulphur obtained from the standard curve

RESULTS

Bacterial Community

It showed a significant negative correlation with the concentration of lead, zinc, copper, cadmium and sulphur in leaf at the polluted site. A significant positive correlation between the bacterial population and ambient temperature was also observed.

Microfungal Community

Fungal composition at the two sites varied significantly. A total of 29 microfungal taxa were isolated from the phylloplane of alder trees. Of which, 28 species were recorded from the unpolluted site and 16 species from the polluted one. Species including *Cladosporium cladosporioides*, *Aspergillus fumigatus*, *Aspergillus niger*, *Paecilomyces variotii*, *Sporobolomyces roseus*, *Absidia spinosa*, *Epicoccum purpurascens*, *Geotrichum candidum*, *Cunninghamella echinulata*, *Curvularia lunata*, *Acremonium* sp., *Oidiodendron* sp. and *Chaetomium* sp. were specific to the unpolluted site whereas, *Mortierella* sp. was isolated only from the polluted site. CFU of *Aureobasidium pollulans* and *Fusarium oxysporum* were higher and dominated in the polluted site. Maximum fungal diversity was observed in May and minimum in January in both the sites (Table 1).

In comparison, a total of 24 microfungal taxa were isolated from the phylloplane of pines. Of which, 23 species were recorded from unpolluted site and 11 species from polluted ones. *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Mucor hiemalis*, *Paecilomyces variotii*, *Absidia spinosa*, *Epicoccum purpurascens*, *Botrytis cinerea*,

Table 1: Relative abundance (%) of fungal species on leaf surface of *Alnus nepalensis* at polluted and unpolluted sites

Fungal species	Polluted site (8 m from roadside)						Unpolluted site (1 km from roadside)					
	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep
<i>Absidia spinosa</i>	-	-	-	-	-	-	-	-	-	-	4.3	4.0
<i>Cunninghamella echinulata</i>	-	-	-	-	-	-	-	14.3	-	-	-	-
<i>Mortierella</i> sp.	-	18.5	-	-	-	5.9	-	-	-	-	-	-
<i>Mucor hiemalis</i>	-	-	11.1	6.2	8.3	5.9	-	-	-	1.4	-	8.0
<i>Pythium</i> sp.	-	-	-	8.3	11.1	-	-	-	-	5.8	-	4.0
<i>Rhizopus stolonifer</i>	-	-	7.4	4.2	5.5	7.8	-	-	5.3	4.3	-	-
<i>Sporobolomyces roseus</i>	-	-	-	-	-	-	-	-	-	5.8	-	-
<i>Aureobasidium pull. ulans</i>	-	37.0	7.4	8.3	5.5	9.8	-	-	5.3	4.3	-	-
<i>Alternaria alternata</i>	-	-	14.8	-	-	-	-	-	10.5	8.7	8.6	8.0
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	-	-	-	4.3	4.3	-
<i>A. flavus</i>	-	-	-	10.4	11.1	3.9	-	-	5.3	2.9	2.1	4.0
<i>A. niger</i>	-	-	-	-	-	-	-	-	10.5	4.3	3.2	2.7
<i>Acremonium</i> sp.	-	-	-	-	-	-	14.8	14.3	-	-	-	5.3
<i>Botrytis cinerea</i>	13.3	22.2	-	-	-	-	11.1	16.6	-	-	-	5.3
<i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	2.9	4.3	-
<i>C. herbarum</i>	-	-	11.1	16.7	13.8	13.7	-	-	10.5	7.2	7.5	5.3
<i>Chaetomium</i> sp.	-	-	-	-	-	-	-	-	8.8	-	-	-
<i>Curvularia lunata</i>	-	-	-	-	-	-	-	-	-	-	7.5	6.7
<i>Epicoccum purpurascens</i>	-	-	-	-	-	-	-	-	-	-	-	6.7
<i>Fusarium oxysporum</i>	-	-	18.5	12.5	13.8	11.8	-	-	-	-	-	9.3
<i>Geotrichum candidum</i>	-	-	-	-	-	-	13.0	14.3	10.5	-	6.5	-
<i>Oidiodendron</i> sp.	-	-	-	-	-	-	7.4	11.9	-	-	-	5.3
<i>Paecilomyces variotii</i>	-	-	-	-	-	-	13.0	-	-	7.2	7.5	-
<i>Penicillium chrysogenum</i>	-	-	-	10.4	8.3	11.8	-	-	-	5.8	7.5	4.0
<i>P. citrinum</i>	-	-	-	-	-	-	-	11.9	7.0	2.9	5.4	-
<i>P. rubrum</i>	-	-	7.4	4.2	-	-	11.1	-	8.8	4.3	5.4	-
<i>Trichoderma viride</i>	6.7	7.4	7.4	-	-	13.7	7.4	7.1	-	5.8	5.4	5.3
<i>Verticillium</i> sp.	23.3	-	-	6.2	-	-	9.2	-	7.0	5.8	4.3	5.3

-: Indicates absence of microbes in the sample

Table 2: Relative abundance (%) of fungal species on leaf surface of *Pinus kesiya* at the polluted and unpolluted sites

Fungal species	Polluted site (8 m from roadside)						Unpolluted site (1 km from roadside)					
	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep
<i>Aureobasidium pull. ulans</i>	43.3	45.5	25.9	20.2	2.8	33.3	-	-	15.6	11.1	-	-
<i>Trichoderma viride</i>	13.3	15.0	7.3	-	-	12.5	19.6	16.7	13.7	-	-	8.3
<i>Alternaria alternata</i>	-	-	-	13.6	16.6	-	-	-	7.8	4.8	3.3	5.9
<i>Cladosporium herbarum</i>	-	-	-	-	22.2	14.6	-	-	19.6	11.1	10.0	10.7
<i>C. cladosporioides</i>	-	-	-	-	2.8	-	-	-	-	-	8.3	-
<i>Penicillium chrysogenum</i>	-	-	-	13.3	13.8	14.6	-	-	-	19.0	5.0	-
<i>P. rubrum</i>	-	-	-	6.6	-	-	13.7	9.2	-	7.9	5.5	-
<i>P. citrinum</i>	-	-	-	-	-	-	13.7	11.1	-	1.6	5.3	-
<i>Fusarium oxysporum</i>	-	-	14.8	6.6	2.8	-	-	-	-	-	-	-
<i>F. poae</i>	-	-	-	10.0	13.8	14.6	-	-	-	3.1	6.6	-
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	-	-	-	-	5.0	-
<i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-	-	4.8
<i>A. niger</i>	-	-	-	-	-	-	10.8	-	-	-	5.0	-
<i>Rhizopus stolonifer</i>	-	-	-	-	-	-	-	-	-	1.8	-	-
<i>Mucor hiemalis</i>	-	-	-	-	-	-	-	-	-	1.6	-	3.6
<i>Paecilomyces variotii</i>	-	-	-	-	-	-	9.8	-	-	-	-	7.1
<i>Sporobolomyces roseus</i>	-	-	-	6.6	5.5	-	11.8	29.9	17.6	9.5	6.6	-
<i>Absidia spinosa</i>	-	-	-	-	-	-	-	-	-	-	-	8.3
<i>Epicoccum purpurascens</i>	-	-	-	-	-	-	-	-	-	-	10.0	-
<i>Botrytis cinerea</i>	-	-	-	-	-	-	-	-	-	-	-	3.6
<i>Pythium</i> sp.	-	-	-	-	-	-	-	-	-	4.8	-	-
<i>Acremonium</i> sp.	-	-	-	-	-	-	3.9	-	-	-	-	2.4
<i>Oidiodendron</i> sp.	-	-	-	-	-	-	-	-	-	-	-	5.9
<i>Lophodermium</i> sp.	10.0	18.1	-	-	-	-	5.9	14.8	-	-	-	-

-: Indicates absence of microbes in the sample

Pythium sp., *Acremonium* sp., *Oidiodendron* sp. were specific to the unpolluted site whereas *Fusarium oxysporum* was isolated only from the polluted site. cfu of *Aureobasidium pollulans* and *Fusarium poae* were higher and dominant in polluted site. Maximum fungal diversity was observed in May and minimum in January in both the sites (Table 2).

Fungal diversity and species richness were higher in the unpolluted site than those in the polluted site. (Table 3, 4). This is attributed to less deposition in the leaf surface at the unpolluted site allowing better host and fungal interaction.

A significant negative correlation was observed between the number of cfu and the concentration of Pb, Zn, Cu, Cd and S in leaf at the polluted site. Significant positive correlation between the rainfall and the number of micro-fungi was also observed (Table 4, 5).

Seasonal Change in Pb, Zn, Cu, Cd and S Contents

In case of alder, maximum amount of pollutants was observed in January and minimum in May, exhibiting a negative correlation with the rainfall. While in case of pine, maximum pollutants was observed in November and minimum in March. The variations in levels of Pb, Zn, Cu, Cd and S were not significant between samples collected at different samplings time from unpolluted site as was seen in polluted site (Table 6).

Table 3: Index of diversity, species Richness and similarity of fungi at the two sites in *Abnus nepalensis*

Months	Shannon's index of diversity		Species richness index		Sorensen's similarity index
	Polluted site	Unpolluted site	Polluted site	Unpolluted site	
Nov	0.71	0.97	4.00	5.57	46.15
Jan	0.64	0.88	3.14	5.23	36.36
Mar	0.95	1.05	8.38	7.82	60.00
May	1.03	1.33	8.30	12.48	68.96
Jul	1.01	1.24	7.41	10.05	32.00
Sept	1.01	1.24	7.31	11.44	51.85

Table 4: Index of diversity, species Richness and similarity of fungi at two sites in *Pinus kesiya*

Months	Shannon's index of diversity		Species richness index		Sorensen's similarity index
	Polluted site	Unpolluted site	Polluted site	Unpolluted site	
Nov	0.6	0.9	5.0	7.3	42.8
Jan	0.5	0.7	3.8	4.7	60.0
Mar	0.6	0.7	5.2	4.8	54.5
May	0.9	0.9	9.0	9.1	66.6
Jul	0.8	1.0	9.2	9.9	78.2
Sept	0.7	0.9	4.9	8.2	44.4

Table 5: Correlation coefficients(r) of the concentration of Pb, Zn, Cu, Cd, S, humidity, temperature and rainfall with fungal and bacterial populations on phylloplane of *Abnus nepalensis* at the polluted and unpolluted sites

Sources of variations	df	Polluted site			Unpolluted site		
		Fungal population	Bacterial population	Rainfall	Fungal population	Bacterial population	Rainfall
Pb	4	-0.948***	-0.834*	-0.975***	-0.351	-0.335	-0.551
Zn	4	-0.864*	-0.960**	-0.750	-0.581	-0.662	-0.946**
Cu	4	-0.905*	-0.897*	-0.930**	-0.660	-0.753	-0.984***
Cd	4	-0.905*	-0.897*	-0.930**	-0.660	-0.753	-0.984***
S	4	-0.929**	-0.923**	-0.937**	-0.595	-0.678	-0.972**
Rainfall	4	0.901*	0.771	-	0.577	0.696	-
Temperature	4	0.855*	0.900*	-	0.930**	0.983***	-
Humidity	4	0.539	0.467	-	0.712	0.636	-

-: Indicates non-correlating entities, *: p<0.05, **: p<0.01, ***: p<0.001

Table 6: Correlation coefficients(r) for Pb, Zn, Cu, Cd, S, humidity, temperature, rainfall, fungal and bacterial populations on phylloplane of *Pinus kesiya* at polluted and unpolluted sites

Sources of variations	df	Polluted site			Unpolluted Site		
		Fungal population	Bacterial population	Rainfall	Fungal population	Bacterial population	Rainfall
Pb	4	-0.909*	-0.920**	-0.873*	-0.356	-0.732	-0.706
Zn	4	-0.853*	-0.892*	-0.807	-0.361	0.667	-0.857*
Cu	4	-0.945**	-0.766	-0.908*	-0.663	-0.824*	-0.821*
Cd	4	-0.909*	-0.807	-0.898*	-0.662	-0.824*	-0.827*
S	4	-0.924**	-0.436	-0.887*	-0.556	-0.784	-0.897*
Rainfall	4	0.994***	0.712	-	0.545	0.552	-
Temperature	4	0.752	0.986***	-	0.741	0.842*	-
Humidity	4	0.340	0.667	-	0.650	0.483	-

-: Indicates non-correlating entities, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

DISCUSSION

The number of phylloplane fungi and bacteria was significantly reduced in the polluted site as compared to the unpolluted site and correlation coefficient analysis of data indicated the detrimental effect of the pollutants on the leaf surface microbial community. The findings were supported by industrially derived metal pollution studies (Bewley, 1980; Bewley and Campbell, 1980; Wild *et al.*, 2004) and chemical sprays on the leaf surface (DeJager *et al.*, 2001; Sandhu *et al.*, 2007). Microfungal species composition and their diversity in the phylloplane was directly correlated to the roadside pollution as suggested for bacteria in dusty hemlock leaves and tree canopies (Manning, 1971; Lambais *et al.*, 2006) and airborne phenol in the phyllosphere (Sandhu *et al.*, 2007). Similar change in lichen cover was correlated to air pollution in a tropical forest (Monge-Najera *et al.*, 2002).

The specificity of certain fungi to unpolluted site may be attributed to their sensitivity to the pollution. Whereas, the predominance of *A. pollulans*, *F. oxysporum*, *Mortierella* sp. in the polluted site was due their resistance to pollutants and altered sporulation capacity or spore life span. Stimulatory effect of pollutants on the microbial number but not the species composition supports our findings that the stressed or hostile environmental conditions enhance the production of spores ensuring their survival or movement to suitable environments (Manning, 1971). The greater density of yeast cells and other metal tolerant fungi was attributed to their ability to utilize elements as nutrients within the pollutant dust and the beneficial effects of which might outweigh the effect of pollutant toxicity (Bewley, 1980; Lambais *et al.*, 2006). The complexity of interactions between various phylloplane microbes were affected differentially by the pollutants due to their sporulation capacity (Fritze and Baath, 1993; Lambais *et al.*, 2006).

A noteworthy difference in the diversity of microfungal species between polluted and unpolluted sites was attributed to the inhibitory effect of vehicular exhausts and trace contaminants at the roadside (Mowll and Gadd, 1985). Drastic change in the number of fungi at polluted site was directly correlated to the toxicity level of pollutants. The seasonal fluctuation in cfu was due to change in pollutant's level, temperature, rainfall and relative humidity. Maximum fungal population in the month of May was correlated to the high rainfall reducing the dry deposition level on the leaf surface at the polluted site. Further, the young leaves had less pollutants deposition on their surface during May compared to the matured leaves during winter months. While changes in the number of phylloplane microbes in unpolluted site could be related to the change in the nutritional status of leaves with age due to increased leaf leachates (Tukey, 1971). The main effect of the pollution by heavy metals retained by leaves on the phyllosphere microflora was the strong inhibition of each group of microorganisms (Brighigna *et al.*, 2000; Sandhu *et al.*, 2007). Volatile organic compounds may also accumulate in the leaf cuticle, as indicated by the visible localization of anthracene in the outermost layer of the cuticle (Wild *et al.*, 2004).

The marked reduction in microbial population on leaf surface due to pollution and leaf deposition on the roadside may consequently affect the microbial colonization of leaf litter thus delaying nutrient release and decomposition. This may lead to gradual accumulation of organic matter, loss of fertility of the soil hampering the nutrient cycling and balance in the ecosystem.

REFERENCES

- Allen, S.E., 1974. *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publication, Oxford.
- Bewley, R.J.F., 1980. Effects of heavy metal pollution on oak leaf microorganisms. *Applied Environ. Microbiol.*, 40 (6): 1053-1059.
- Bewley, R.J.F. and R. Campbell, 1980. Influence of zinc, lead and cadmium pollutants on the microflora of Hawthorn leaves. *Microb. Ecol.*, 6 (2): 227-240.
- Brighigna, L., A. Gori, S. Gonnelli and F. Favilli, 2000. The influence of air pollution on the phyllosphere microflora composition of Tillandsia leaves (Bromeliceae). *Rev. Biol. Trop.*, 48: 10-18.
- DeJager, E.S., F.C. Wehner and L. Korsten, 2001. Microbial ecology of the mango phylloplane. *Microb. Ecol.*, 42 (3): 201-207.
- DeKempeneer, L., B. Sercu, W. Vanbrabant, H. Van Langenhove and W. Verstraete, 2004. Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Applied Microbiol. Biotechnol.*, 64: 284-288.
- Dickinson, C.H., 1971. Cultural Studies on Leaf Saprophytes. In: *Ecology of Leaf Surface Microorganisms*, Preece, T.F. and C.H. Dickinson (Eds.). Academic Press, London, pp: 129-137.
- Difco-Manual, 1953. Detroit Mich. 9th Edn. Difco Laboratories Inc.
- Fritze, H. and E. Baath, 1993. Microfungal species composition and fungal biomass in a coniferous forest soil polluted by alkaline deposition. *Microb. Ecol.*, 25 (1): 83-92.
- Gingell, S.M., R. Campbell and M.H. Martin, 1976. The effects of zinc, lead and cadmium pollution on the leaf surface microflora. *Environ. Pollut.*, 11: 25-37.
- Lambais, M.R., D.E. Crowley, J.C. Cury, R.C. Büll and R.R. Rodrigues, 2006. Bacterial diversity in tree canopies of the Atlantic. *For. Sci.*, 312: 1917.
- Manning, W.J., 1971. Effects of limestone dust on leaf condition, foliar disease incidence and leaf surface microflora of native plants. *Environ. Pollut.*, 2: 69-76.
- Manning, W.J., 1975. Interactions between air pollutants and fungal, bacterial and viral plant pathogens. *Environ. Pollut.*, 9: 87-92.
- Martin, J.P., 1950. Use of acid rose Bengal and streptomycin in the plate method for estimating soil fungi. *Soil. Sci.*, 69: 215-232.
- Monge-Najera, J., M.I. Gonzalez, R. Rivas and V.H. Mendez-Estrada, 2002. Twenty years of lichen cover change in a tropical habitat (Costa Rica) and its relation with air pollution. *Rev. Biol. Trop.*, 50: 309-319.
- Mowll, J.L. and G.M. Gadd, 1985. Effect of vehicular lead pollution on phylloplane mycoflora. *Trans. Br. Mycol. Soc.*, 84 (4): 685-689.
- Proctor, J., J. Burrow and G.C. Craig, 1980. Plant and soil chemical analyses from a range of Zimbabwean serpentine sites. *Kirkia*, 12: 127-139.
- Sandhu, A., J.H. Larry and G.A. Beattie, 2007. Bacterial degradation of airborne phenol in the phyllosphere. *Environ. Microbiol.*, 9 (2): 383-392.
- Saunders, P.J.W., 1971. Modification of the Leaf Surface and its Environment by Pollution. In: *Ecology of Leaf Surface Microorganisms*, Preece, T.F. and C.H. Dickinson (Eds.). Academic Press, London, pp: 81-89.

- Saunders, P.J.W., 1973. Effects of atmospheric pollution on leaf surface microflora. *Pestic. Sci.*, 4: 589-595.
- Shannon, C.E., 1948. Mathematical theory of communication. *Bell. System. Technol. J.*, 27: 379-423.
- Smith, W.H., 1971. Lead contamination of roadside white pine. *For. Sci.*, 17: 195-198.
- Smith, W.H., 1976a. Air Pollution Effects on the Structure and Function of Plant-Surface Microbial Ecosystems. In: *Microbiology of Aerial Plant Surfaces*, Preece, T.F. and C.H. Dickinson (Eds.). Academic Press, London, pp: 75-105.
- Smith, W.H., 1976b. Lead contamination of roadside ecosystem. *J. Air Pollut. Contr. Assoc.*, 26: 753-766.
- Smith, W.H., 1977. Influence of heavy metal leaf contaminants on the *in vitro* growth of urban-tree phylloplane fungi. *Microb. Ecol.*, 3: 231-239.
- Sorenson, T., 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. *Det. Kong. Danske. Vidensk. Selsk. Biol. Skr (Copenhagen)*. 5: 1-34.
- Swalesh, K.M., R.M. Hussein and S. Abu-Elhaj, 2004. Assessment of heavy metal contamination in roadside surface soil and vegetation from the West Bank. *Arch. Environ. Contam. Toxicol.*, 47: 23-30.
- Tukey, Jr., H.B., 1971. Leaching of Substrates from Plants. In: *Ecology of Leaf Surface Microorganisms*, Preece, T.F. and C.H. Dickinson (Eds.). Academic Press, London, pp: 67-80.
- Wild, E., J. Dent, J.L. Barber, G.O. Thomas and K.C. Jones, 2004. A novel analytical approach for visualizing and tracking organic chemicals in plants. *Environ. Sci. Technol.*, 38: 4195-4199.