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Effects of Re-suspended Roadside Dusts and Its Elemental Constituents on the Phylloplane Microflora

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Abstract: In the present paper the effect of traffic generated dust pollution on phylloplane microflora of *Polyalthia longifolia* was studied. More dust and absence of micro-flora especially fungi was observed in the leaves from roadside site unlike that of non-roadside site. Elemental profiling of dust on leaves has shown the presence of trace elements like Al, Si, Cl, Ti, Cr, Mn, Fe, Ni, Cu and Zn and toxic elements like As and Hg. Presence of such elements in dust may be attributed to retardation of micro-flora on leaf surfaces exposed to traffic generated dust particulates.

Key words: Air pollution, dust, phylloplane microflora, SEM-EDX.

Introduction

Phylloplane or plant leaf surface is a habitat for many microorganisms. The microbial communities of the phylosphere consist of numerous genera of bacteria, fungi, yeasts, algae and in some situations protozoans and nematodes (Morris et al., 2002; Lindow and Brandl 2003). Short-term and long-term changes in the abiotic conditions under which plants grow may affect the growth and productivity of the plants and that of microorganisms living on plant surfaces. Changes in phylosphere microbial populations may, in turn, affect plant growth and the plant's ability to withstand aggressive attacks by pathogens.

It is well known fact that plant leaves absorb re-suspended dust generated by traffic (Samal and Santra, 2002). These re-suspended dust adsorbed on leaves are mainly composed of soot and trace elements, mostly heavy metals (Ordonez et al., 2003; Sawidis et al., 1995). Hence, the micro-flora of the plants are challenged with the thrash condition of dust load. The heavy metals on leaf surfaces of roadside plants come in contact with phylloplane microorganisms and may affect their growth (Joshi, 2008). Varying degrees of growth inhibition by trace metals have been shown in vitro, for different species of pathogenic and saprophytic phylloplane fungi (Smith, 1977; Smith et al., 1978). The present study aims to probe into effect of re-suspended dust on phylloplane microflora of *P. longifolia*.

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Material and Methods

The study was conducted during October-November 2009 in Kolkata, India. Kolkata is one of the oldest, largest, most densely populated and most polluted metropolitan cities of India. The city having approx. 16 million population is passing through the phase of rapid urbanisation. Construction of buildings, bridges, roads as well as increase in transportation have resulted in release of gaseous and particulate contaminants into the atmosphere. Two sites were selected for the present study to make a comparative analysis. One of them is located near a busy road in Salt Lake (22° 35′ N, 88° 25′ E) with high traffic density (5000-6000 vehicles/hour), considered as the roadside site (Site I). The other one is located inside the Raja Bazar campus of Calcutta University, approximately 500 metre away from the roadside (88° 37′ N, 22° 58′ E) and covered by tall buildings, considered as non-roadside site (Site II). *Polyalthia longifolia* was selected for the present study as both the sites are dominated with this plant.

Equally matured leaves (five at each site) of *Polyalthia longifolia* were collected from a height of 10 ft approx. Dust from the leaves were washed into distilled water and vacuum filtered onto a membrane filter of 0.2 μm. Individual leaf area was calculated by tracing out the leaves on graph paper. The amount of dust per unit area was calculated using the equation $W = (Wd - Wb)/A$, where W is dust content (mg cm^{-2}), Wd is weight of filter paper with dust, Wb is initial weight of blank filter paper and A is total area of the leaf (cm^2).

Discs of 1 cm^2 diameter were cut from unwashed leaves with a sharp device, wearing polyethylene gloves and were air-dried in a clean and closed chamber. Small strips were trimmed from areas between the margin and midrib of leaves. Each leaf strip was mounted on an aluminum stub, over double-sided stick tape. The specimens were coated with a thin conductive film of gold (about 200Å), in an ion sputter coater (*Hitachi E-1010*). Coated specimens were examined and photographed under a scanning electron microscope (SEM) (Hitachi S-3400N) at an accelerating voltage of 15 kV, at the magnification range of 200-14000×. The same scanning electron microscope (Hitachi S-3400N) with Energy Dispersive X-ray Spectrometer (EDX) attachment (Thermo SuperDry II) was used to carry out semi-quantitative elemental analysis of the samples. The air quality at the study sites was obtained from the website of West Bengal Pollution Control Board (WBPCB).

WBPCB has an air quality monitoring station at Salt Lake near the study site and the data available on website were considered for the present study. However there is no monitoring station at the study site near Raza Bazar. The average values of four different sites surrounding Raza Bazar namely Beliaghata, Moulali, Shyam Bazar and Ultadanga were considered for the non-roadside site.

Results and Discussion

Table 1 shows the air quality of the study sites. Air quality at both the sites is found to be above the national air-quality standard with respect to SPM and RSPM. Table 2 shows the dust adsorbed per cm^2 on the leaves of *Polyalthia longifolia* collected from both

Table 1: Pollution level at the study sites and standard parameters

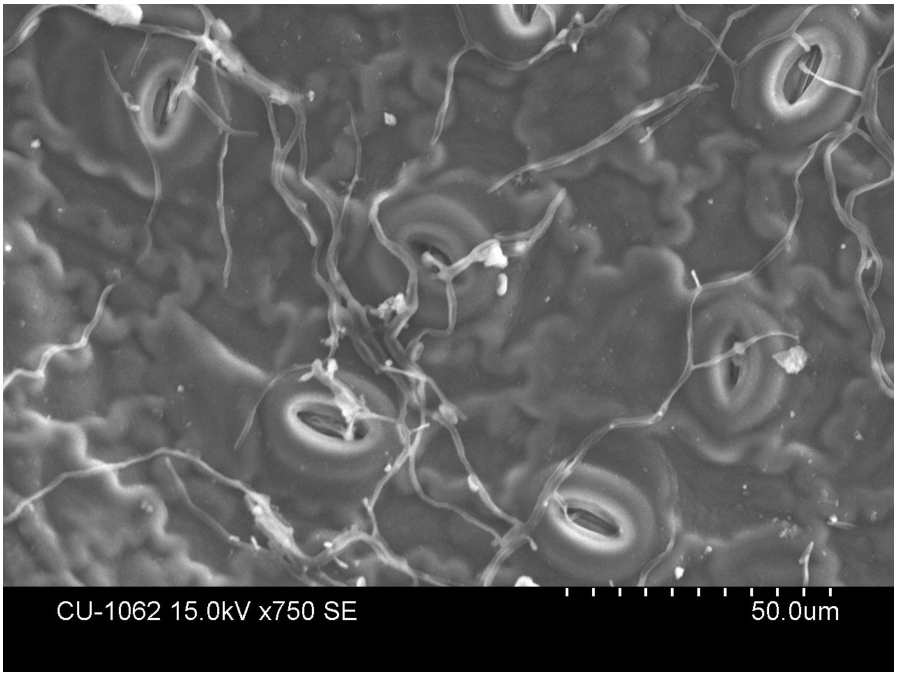
Sites	*SPM*	*RSPM*	*SO_2*	*NO_2*
Salt Lake	185.6097	86.74074	7.034259	67.05023
Raza Bazar	204.8825	94.29575	7.355	67.3465
*Kolkata	190.1251	89.84562	6.995622	61.07292
**Standard	140	60	60	60

All values in μg per m^3.
* Average value of all the monitoring sites in Kolkata.
** Air quality standard values of West Bengal Pollution Control Board.

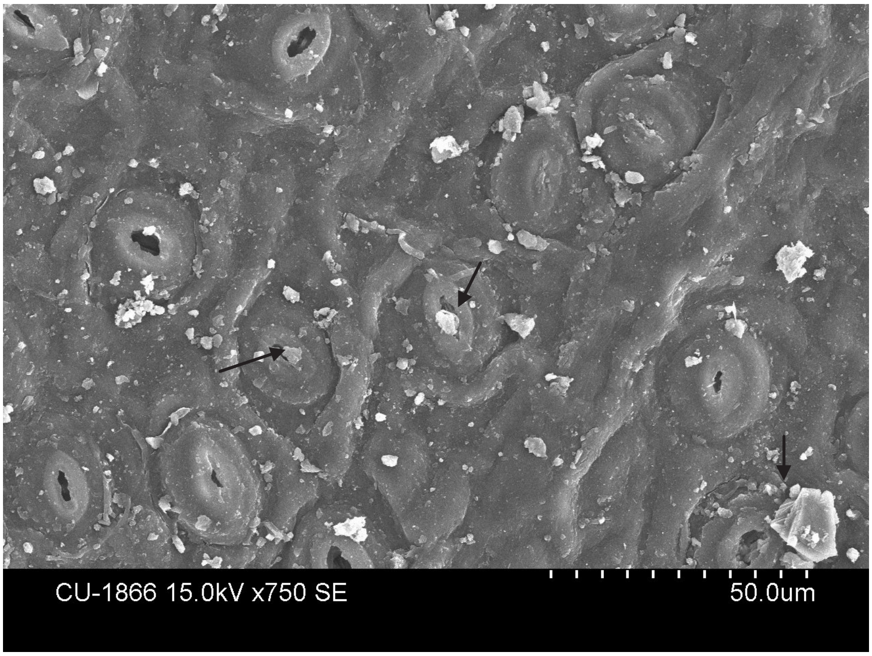
Table 2: Dusts adsorbed on the leaves of P. longifolia at two sites

Sites	*Dust adsorbed mg/cm^2*
Salt Lake	0.314 ± 0.068
Raza Bazar	0.058 ± 0.012

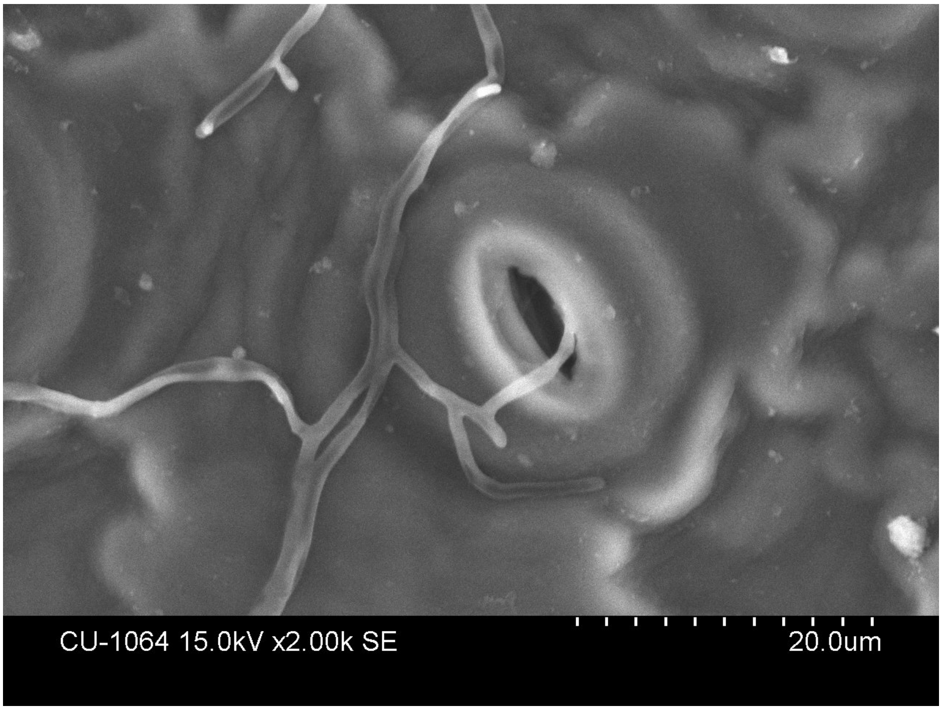
sites. Dust particulates were found to be adsorbed on both abaxial and adaxial surface of leaves. Presence of fungal community has been observed on the lower surface of leaves (Figure 1a), collected from Raza Bazar (non-roadside environment). However, there is absence of any such fungal community on the lower surface of leaves (Figure 1b) collected from Salt Lake (roadside environment). The fungal hyphae were seen to be inserted into the pores of stomata (Figure 1c). However stomata on the leaves from plants roadside environment were seen to be blocked by dust particles (Figure 1b). Analysis of distribution of trace elements in dust adsorbed over leaves revealed the presence of Na, Mg, Al, Si, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu and Zn (Figure 2a-h). Si and Al were found to be most abundant elements in the dust over leaves collected from both



(a) Fungal community on the lower surface of leaves



(b) Dust particles blocking the stomata



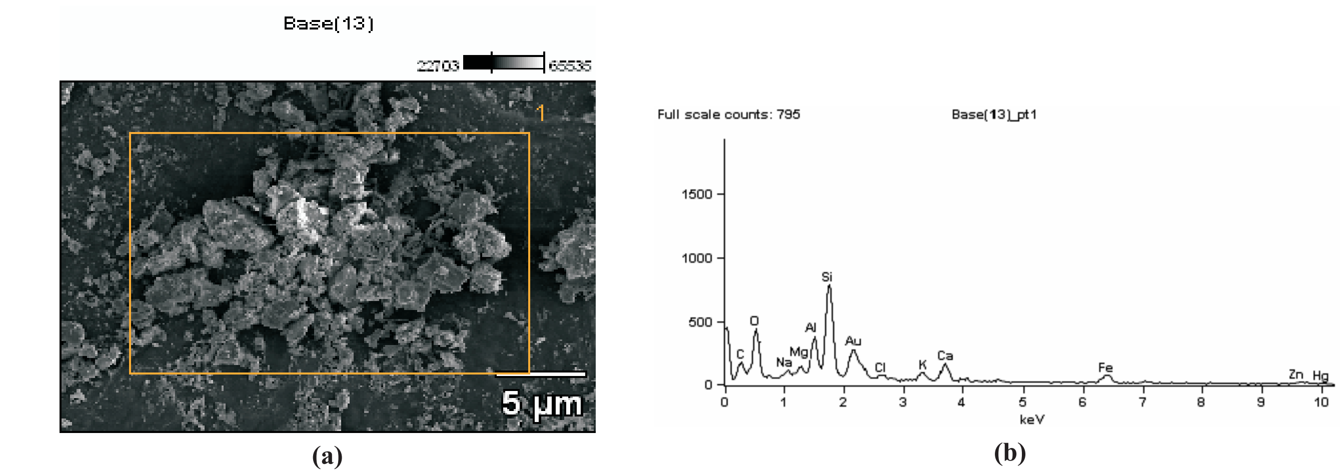
(c) Growth of fungal hyphae into the stomata

Figure 1: Scanning electron micrograph of P.longifolia leaves.

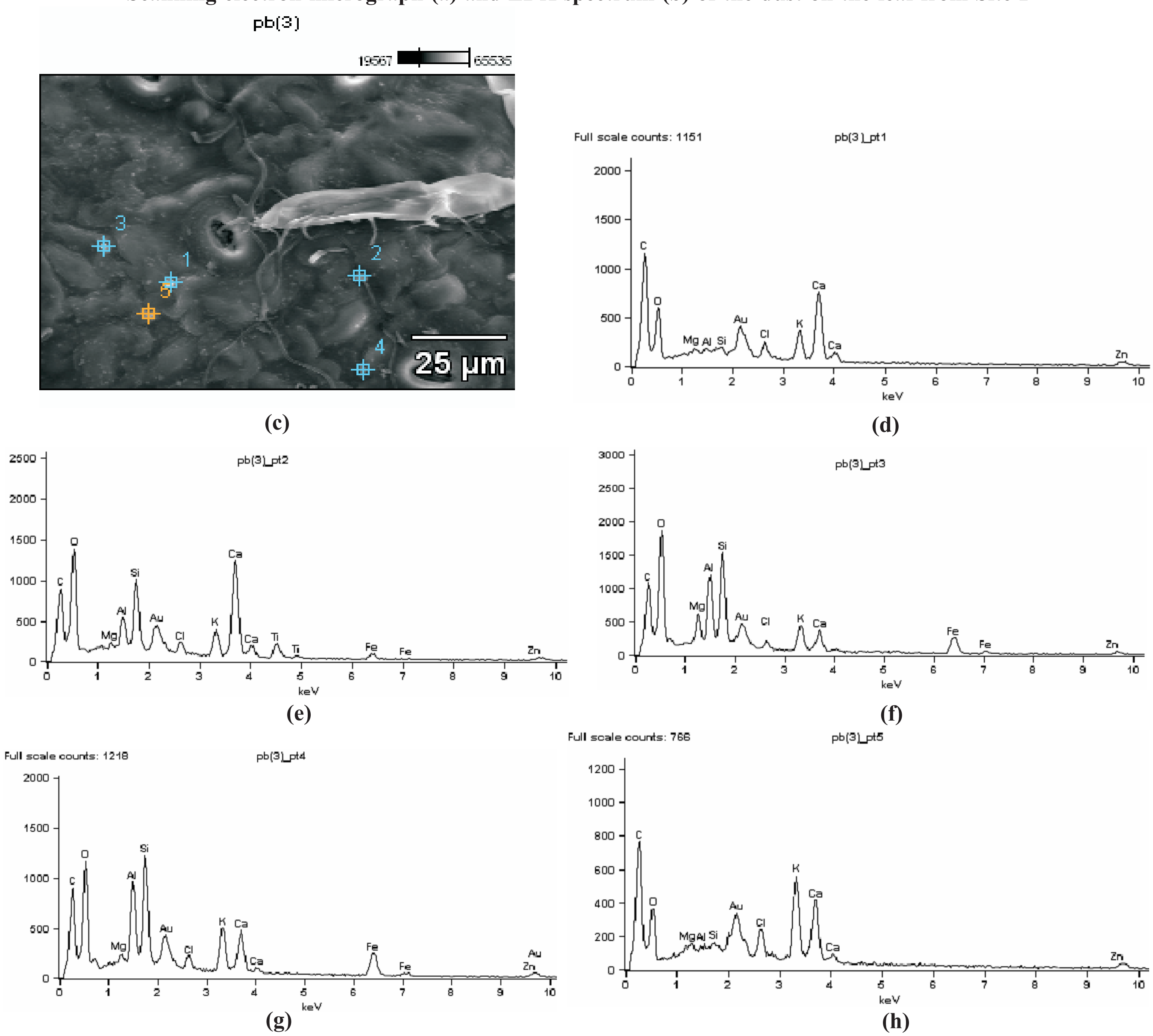
sites. Toxic elements such as Hg and As were detected on the leaves from roadside plants. Abundance of Fe, K, Ca, Cu and Zn were found to be more in the dust over leaves collected from roadside plants in comparison to that of non-roadside plants.

Important observations need to be discussed are: (i) presence of fungal community on the phylloplane of plants on non-roadside environment and its absence in plants near roadside environment; (ii) presence of fungal community on the lower surface of leaves and growth of fungal hyphae into the stomata; and (iii) effect of dust and trace elements/heavy metals on the phylloplane microflora. Microbial communities on the phylloplane are sensitive to both gaseous and particulate pollutants. Findings of our study suggest, presence of phylloplane fungi was almost reduced to nil in roadside plants as compared to the non-roadside plants. Dust deposition analysis has indicated the damaging effect of the dust on the leaf surface microbial community. Similar to our observation, reduction in numbers and species diversity of micro-organisms was reported after chemical sprays on leaves (De Jager et al., 2001). Our findings are also supported by Brighigna et al. (2000), who have reported that the phylloplane microflora of *Tillandsia* plants living in a tropical urban environment changes in terms of number and species composition of yeasts and bacteria. Joshi (2008) has observed a significant reduction in numbers of phylloplane fungi and bacteria in polluted sites.

The pattern of distribution of microflora on the phylloplane is not even, as the fungal communities were observed on the lower surface of leaves. The presence of fungal communities on the lower surface of leaf is supported by the observations of Mafia et al. (2009), who have reported that infection of *Quambalaria eucalypti* in *Eucalyptus* spp took place preferentially on the abaxial leaf surface. Leben (1988) and Surico (1993) have also reported a greater number of bacteria on lower than upper leaf surfaces. This might be attributed to the fact that lower leaf surfaces have a greater density of stomata, trichomes, and/or a thinner cuticular layer (Beattie and Lindow, 1999). Growth of fungal hyphae around or into the stomata was observed and similar observation has been reported elsewhere (Mafia et al., 2009). Researchers have suggested that the most common site of bacterial colonisation is near stomata and at the base of trichomes (Mew and Vera Cruz, 1986; Mansvelt and Hattingh, 1987; Mariano and McCarter, 1993). Not all of the microorganisms that are present over the phylosphere are able to colonise and grow. A low level of water availability and nutrients are key limiting factors for microbial growth on the



Scanning electron micrograph (a) and EDX spectrum (b) of the dust on the leaf from Site I



Scanning electron micrograph (a) and EDX spectrum (d-h) of the dust on the leaf from Site II

Figure 2: EDAX spectra of dust on the leaves from both sites.

phylosphere. However, epiphytes display a variety of mechanisms to overcome these limitations. Blodgett and Swart (2002) have reported differences in colonisation over leaf surfaces due to variation in stomatal frequency. In our study the stomata in the leaves collected from plants at the roadside environment are found to be blocked by the dust particles and hence unavailable for the fungal hyphae. The growth of fungal hyphae around or into the stomata may be one of the mechanisms to draw water and nutrient from the leaf cells.

As far as the air quality at the study sites are considered, the particulate matter load is above the national air-quality standard. However, the level of gaseous pollutant is either below or near to the national standards. Hence gaseous pollutants cannot be reasoned for the absence of fungal microflora on the phylloplane. Now the question arises: is it the dust in general responsible for the microbial retardation or its chemical composition has any role to play? Roadside re-suspended dust contains toxic heavy metals (Aksoy and Ozturk, 1997; Rossini and Valdes, 2004; Rossini and Rautio, 2004), which might affect the phylloplane microflora (Sawidis et al., 1995). Elements detected in the present study, such as Na, Mg, Al, Si, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu and Zn are general components of roadside dusts found over leaf surfaces which has been reported elsewhere (Ram et al., 2010). These elements at certain concentration act as nutrient element; however above certain concentration becomes toxic. Hence leaves with more dust load might have larger concentration of such elements and damage microbial community on the phylloplane. Moreover, dusts deposited on the leaves collected from plants near roadside environment have toxic elements such as As and Hg. Presence of As in roadside dusts of Kolkata metropolis has already been reported by Kar et al. (2006). Accumulation and distribution of Hg on the leaves near chlor-alkali factory in India has also been reported by Shaw et al. (1986). The sources of mercury in the atmosphere are waste disposal, including municipal and hazardous waste, crematoria, and sewage sludge incineration, batteries (Bache et al., 1991), as well as e-wastes. Hence the possible sources of Hg at Salt Lake can be attributed to the waste disposal site as well as e-waste dumping sites near to our study site. Hence it can be summarized that the absence of fungal hyphae on phylloplane of *Polyalthia longifolia* at roadside environment could possibly be due to the dust load in general and presence of toxic elements in dust in particular.

Conclusion

Our preliminary results show the absence of certain fungi at the polluted site and this may be attributed to their sensitivity to air pollutants. Present finding suggests that phylloplane fungi draw nutrient and moisture from plant cell via stomata using hyphae. There is absence of fungal community on the leaf surface where stomata were blocked by dust particles. However, further investigation is needed in order to establish the mechanism by which the habitual members of the phylospheric microflora of roadside plants, is influenced by pollutants. Moreover, the complex relationship between microflora and heavy metal pollution needs to be revealed.

References

Aksoy, A. and M.A. Ozturk (1997). *Nerium oleander* L. as biomonitor of lead and other heavy metal pollution in Mediterranean environment. *Sci Total Environ*, **205:** 145-150.

Bache, C.A., Gutenmann, W.H., Rutzke, M., Chu, G., Elfving, D.C. and D.J. Lisk (1991). Concentrations of metals in grasses in the vicinity of a municipal refuse incinerator. *Arch Environ Con Tox*, **20(**4): 538-542.

Beattie, G.A. and S.E. Lindow (1999). Bacterial colonization of leaves: A spectrum of strategies. *Phytopathology*, **89:** 353-359.

Blodgett, J.T. and W.J. Swart (2002). Infection, colonization, and disease of *Amaranthus hybridus* leaves by the *Alternaria tenuissima* group. *Plant Disease*, **86:** 1199-1205.

Brighigna, L., Gori, A., Gonnelli, S. and F. Favilli (2000). The influence of air pollution on the phyllosphere micro-flora composition of *Tillandsia* leaves (Bromeliaceae). *Rev Biol Trop*, **48(2-3):** 511-517.

De Jager, E.S., Wehner, F.C. and L. Korsten (2001). Microbial ecology of the mango phylloplane. *Microb. Ecol.*, **42:** 201-207.

Joshi, S.R. (2008). Influence of roadside pollution on the phylloplane microbial community of *Alnus nepalensis* (Betulaceae). *Rev Biol Trop*, **56(3):** 1521-1529.

Kar, S., Nath, B., Samal, A.C. and S.C. Santra (2006). Arsenic in urban particulates – A case study in Kolkata metropolis. *Current Science*, **90(2):** 158-160.

Leben, C. (1988). Relative humidity and the survival of epiphytic bacteria with buds and leaves of cucumber plants. *Phytopathology*, **78:** 179-185.

Lindow, S.E. and M.T. Brandl (2003). Microbiology of the phyllosphere. *Appl Environ Microbiol*, **69:** 1875-1883.

Mafia, R.G., Alfenas, A.C., Ferreira, E.M., Andrade, G.C.G., Vanetti, C.A. and D.H.B. Binoti (2009). Effects of leaf position, surface, and entry sites on *Quambalaria eucalypti* infection in eucalypt. *Tropical Plant Pathology*, **34(1):** 3-9.

Mansvelt, E.L. and M.J. Hattingh (1987). Scanning electron microscopy of colonization of pear leaves by *Pseudomonas syringae* pv *syringae*. *Can J Bot*, **65:** 2517-2522.

Mariano, R.L.R. and S.M. McCarter (1993). Epiphytic survival of *Pseudomonas viridiflava* on tomato and selected weed species. *Microbial Ecol*, **26:** 47-58.

Mew, T.W. and C.M. Vera Cruz (1986). Microbiology of the phyllosphere. Fokkema, N.J. and Van den, H.J. (eds), Cambridge University Press, Cambridge.

Morris, C.E. and L.L. Kinkel (2002). Fifty years of phyllosphere microbiology: Significant contributions to research in related fields. *In:* Lindow, S.E., Hecht-Poinar, E.I., Elliott, V.J. (eds), Phyllosphere Microbiology. APS press, St. Paul, USA.

Ordonez, A., Loredo, J., De Miguel, E. and S. Charlesworth (2003). Distribution of heavy metals in street dust and soils of an industrial city in Northern Spain. *Arch Environ Con Tox*, **44:** 160-170.

Ram, S.S., Majumder, S., Chaudhuri, P., Chanda, S., Santra, S., Chakraborty, A. and M. Sudarshan (2010). Characterization of dust particulates deposited on plant leaf surfaces using EDXRF: An approach for pollution monitoring. *Int J Env Sci*, **1(2):** 233-238.

Rossini, O.S. and P. Rautio (2004). Could ornamental plants serve as passive biomonitors in urban areas? *J Atmos Chem*, **49:** 137-148.

Rossini, O.S. and B. Valdes (2004). *Ligustrum lucidum* Ait. F. leaves as a bioindicator of the airquality in a Mediterranean city. *Environ Monit Assess*, **96:** 221-232.

Samal, A.K. and S.C. Santra (2002). Air quality of Kalyani township (Nadia, West Bengal) and its impact on surrounding vegetation**.** *Ind J Environ Health*, **44:** 71-76.

Sawidis, T., Marnasidis, A., Zachariadis, G. and J. Stratis (1995). A study of air pollution with heavy metals in Thessaloniki city (Greece) using trees as biological indicators. *Arch Environ Con Tox*, **28:** 118-124.

Shaw, B.P. and A.K. Panigrahi (1986). Uptake and tissue distribution of mercury in some plant species collected from a contaminated area in India: Its ecological implications. *Arch. Environ Con Tox*, **15:** 439-446.

Smith, W.H. (1977). Influence of heavy metal leaf contaminants on the *in vitro* growth of urban tree phylloplane fungi. *Microbial Ecology*, **3:** 231-239.

Smith, W.H., Staskawicbz, J. and S. Harkovr (1978). Trace metal pollutants and urban-tree leaf pathogens. *Transactions of the British Mycological Society*, **70:** 29-33.

Surico, G. (1993). Scanning electron microscopy of olive and oleander leaves colonized by *Pseudomonas syringae* subsp *savastanoi*. *J Phytopathol*, **138:** 31-40.

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