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Original article

Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration

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ABSTRACT

In recent Anthropocene, biodiversity of urban roadside plants is now increasingly being realized as an eco-sustainable tool for monitoring and mitigation of air pollution. The present study aimed to investigate the impact of particulate matter (PM) pollutants on leaf morphology (stomata), biochemical (heavy metals, protein, and sugars) parameters and enzyme activity (peroxidase and catalase) of 12 common roadside plant species, growing at two different sites of Aizawl City, i.e. the Ramrikawn (RKN-Med; polluted peri-urban) site and the Mizoram University (MZU-Low; less polluted rural) site. The highest dust deposition was noted for the RKN-Med site on *Ficus benghalensis* and the lowest in *Bauhinia variegate*. The plant species growing at the RKN-Med site showed significant decreases in stomatal size and stomatal index ($p < 0.05$). Further, increased concentration of heavy metals (Fe, Cu, and Zn) was recorded at the RKN-Med site. Moreover, tolerant roadside plants find their suitability for plantation in ecologically sensitive regions, having implications for urban ecosystem restoration.

Introduction

Biodiversity of urban roadside plants acts as an eco-sustainable filter for air pollution. Air pollution originating from rapid industrialization, urbanization, population growth, and economic development has perturbed the pristine environment of urban ecosystems. Unfortunately, urban ecosystems of ecologically sensitive and biodiversity rich regions like the Indo-Burma hotspot are under severe air pollution stress (Panda and Rai 2015; Rai 2012a; Rai and Chutia 2014; Rai and Panda 2014, 2015a,b; Rai et al 2014b).

Air pollutants comprised of both particulate matter (PM) and gaseous pollutants may cause adverse health effects in humans, affect plant life, and impact the global environment by changing the atmosphere of the earth (Raabe 1999; Rai 2013, 2015b; Rai and Panda 2014; Rai et al 2013, 2014b). Air pollution emanating from PM is particularly deleterious as it leads to various cardiopulmonary diseases through oxidative stress (Rai 2013, 2015b).

In quest of an alternative eco-friendly technology pertaining to urban ecosystem restoration, impacts of air pollutants on morphological, physiological, and biochemical parameters of plants of an urban forest are now being investigated as an integral part of air pollution science. Instead of the existing plethora of policies as well as instrumentation technologies with high cost issues and other limitations (Rai 2013), urban roadside plants are inextricably linked with eco-sustainability (Panda and Rai 2015, Rai 2013; Rai et al 2014a).

Foliar surface of urban roadside plants acts as a sink for PM deposition and through their deposition they show specific morphological, physiological, and biochemical responses. Deposition of PM pollutants on a leaf surface induces structural and functional changes (Panda and Rai 2015).

Although plants are very important to maintain urban ecosystem health, they may, however, be severely affected by PM pollution (Agbaire 2009; Panda and Rai 2015; Rai 2013; Rai and Panda 2014a, 2015a,b; Rai et al 2013, 2014a,b; Randhi and Reddy 2012; Shweta 2012; Steubing et al 1989).

Further, effects of PM pollutants occur at various scales in the plant system, beginning at the biochemical level and progressing up to the landscape level (Panda and Rai 2015). Also, urban roadside plants demonstrate a wide array of responses when

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exposed to pollutants in the form of photosynthesis, respiration, enzymatic reactions, stomatal behavior, membrane disruption, senescence, and ultimately death (Panda and Rai 2015; Rai and Panda 2014b, 2015a,b; Rai et al 2014a; Shweta 2012; Thakar and Mishra 2010).

The foliar injury (foliar effect) along with the significant changes in plant anatomy, physiology, and biochemistry indicates polluted urban environment (Panda and Rai 2015; Rai and Panda 2015a). Therefore, urban roadside plants should be considered as an integral part of any comprehensive plan aimed at improving overall urban air quality and concomitantly they assist in following an eco-sustainable approach (Abida et al 2009; Panda and Rai 2015; Rai 2013; Rai et al 2014b).

The impacts of PM on the morphological, biochemical, and physiological features of urban roadside plants (species) have been recorded by several researchers (Hirano and Aiga 1995; Joshi and Bora 2011; Joshi and Swami 2009; Kulshreshtha et al 2009; Malhotra and Khan 1984; Panda and Rai 2015; Pandey and Agrawal 1992; Rai 2013; Rai and Panda 2014a, 2015a,b; Rai et al 2013, 2014a; Shweta 2012; Thambavani and Sabitha 2011). Concomitantly, the aforesaid researches assist in screening of tolerant plants. By analyzing morphological, physiological, and biochemical parameters, an early diagnosis of urban air quality may be evaluated and eco-sustainable mitigation approaches or options may be investigated (Panda and Rai 2015).

Several studies have been performed on the impacts of air pollution with selected plants only in urban polluted regions (Hirano and Aiga 1995; Joshi and Bora 2011; Joshi and Swami 2009; Malhotra and Khan 1984; Pandey and Agrawal 1992; Shweta 2012; Rai 2013; Rai and Panda 2015b, Thambavani and Sabitha 2011), however, no systematic study has been done in an urban portion of ecologically sensitive hilly regions like Aizawl, Mizoram, North-East India, which is also an integral part of the extremely diverse Indo-Burma hotspot region of Myers (Rai 2009a, 2012b; Rai and Chutia 2014). Further, a plant's response may alter under varying pollution stress; however, until now, no study has been done in ecologically sensitive hilly regions of the Indo-Burma hotspot region to study the impacts of PM pollution on urban roadside plants with their possible phyto-technological innovation.

In the light of abovementioned discussion, the present study deals with the quantification of air pollutants in the ambient air, and assesses the impacts of air pollutants with special reference to PM on morphological, physiological, biochemical changes, and enzymatic activities of some common urban roadside plant species in an Indo-Burma hotspot region.

Material and methods

The study area

The present study was conducted in Aizawl, the capital of Mizoram state (North East India), (21°58'–21°85' North and 90°30'–90°60' East), at 1132 m above sea level (Figure 1). The altitude in Aizawl district varies from 800 m to 1200 m. The climate of the area is monsoonal. The annual average rainfall amounts to ~2350 mm. The area experiences distinct seasons. The ambient air temperature normally ranges from 20°C to 35°C in summer and from 9°C to 21°C in winter. It is well known that meteorological data may also affect the air pollutants including dust or PM, therefore the monthly average minimum and maximum temperatures of the study area are recorded during the study period, i.e. from November 2011 to February 2012. The average minimum temperature during the study period was 13.82°C while the maximum was recorded as 25.52°C (Rai 2015a).

Sampling site

Two sampling sites were selected within Aizawl to assess the impact of air pollution on morphological and biochemical plant parameters. These sampling sites include Mizoram University (MZU) campus (an institutional/rural area) and Ramrikawn (residential and commercial/peri-urban area). Ramrikawn is a very densely constructed commercial area with markets, bus and taxi stands, and the Food Corporation of India (FCI). The FCI provides space for food storage for the whole of Mizoram state. Due to existence of the FCI in the Ramrikawn area, there are frequently heavy duty vehicles coming from all parts of India through the National highway of Pushpak (NH-54). As there is a public bus and taxi stand, vehicular movement is usually high in the Ramrikawn area. Stone quarrying activity is also found in this area, which leads to emission of dust or PM particles (Panda and Rai 2015). Biomass burning through shifting cultivation is very common in this region (Panda and Rai 2015; Rai 2009a, 2012; Rai and Chutia 2014) and may also be a source of suspended PM pollution. In view of these pollution sources, we selected Ramrikawn as the polluted area for investigation. Twelve selected urban roadside plants were sampled from the roadside of the Ramrikawn site over a 7 km stretch of road.

MZU campus is an institutional area. University buses, taxis, trucks, or trollies coming with construction materials are the main sources of pollution in MZU campus. However, the load of vehicles is comparatively low. Therefore, we selected MZU as reference or control site in order to compare with the results recorded from the Ramrikawn site. Twelve selected plants were sampled from the roadside of the MZU site over the stretch of 5 km. We confined our quest on impact of air pollution to two sites, as both covered the range of 12 km. Thus, the present study was carried out for comparative assessment of two differentially polluted sites (in peri-urban and rural locations) in relation to morphological and biochemical parameters of 12 common roadside plant species during the winter season (November 2011–February 2012).

Dust or PM tends to concentrate during the winter season through atmospheric inversion (Panda and Rai 2015; Verma and Singh 2006) particularly during the morning hours. Further, in our recent research (Rai and Panda 2014b) we recorded maximum dust deposition during winter seasons for the 12 plants used for the present study. Moreover, since the leaves were formed well before the air quality sampling, the morphology is more likely related to air qualities prior to the beginning of the air sampling period, therefore, there might be an argument that the prior growth season has an impact on the extent of dust deposition as well. The aforesaid fact cannot be totally overlooked. However, to address this issue, we started the air and plant sampling immediately after rainy season cessation, as rain tends to wash off the dust particles from the leaf surface and therefore we assume that air pollutant impact on leaf morphology and physiology is negligible. Moreover, we point out that average rainfall recorded during the winter season in our study period is zero; therefore, there is no net removal of dust particles through rain during the winter season.

Air quality analysis

Ambient air quality in terms of common air pollutants such as SO_2, NO_2, and suspended PM was analyzed for the selected sites (Table 1). Sampling was conducted over 24 hours, and twice in a week during the winter season of the study period. A high volume air sampler (Envirotech model, APM-460NL; Envirotech Instruments Pvt. Ltd., India) with a gaseous attachment (Envirotech model, APM-411TE; Envirotech Instruments Pvt. Ltd., New Delhi)

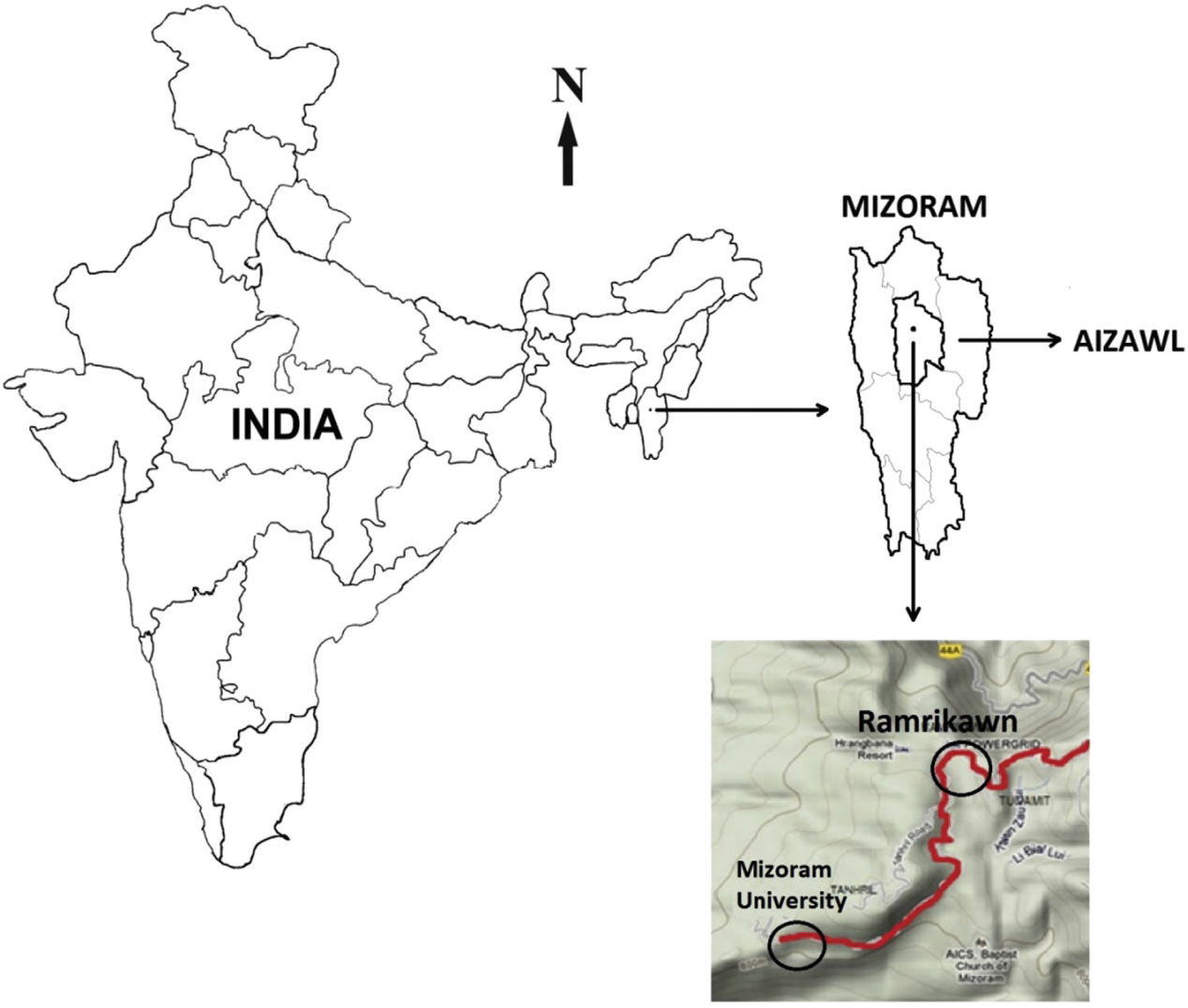

Figure 1. Location of two study sites in Aizawl, Mizoram, North East India-Ramrikawn (RKN-Med polluted peri-urban site) and Mizoram University (MZU-Low polluted rural site).

was used to monitor the air quality. For the collection of samples for suspended particulate matter from ambient air, GF/A filter paper was used in the high volume sampler at a flow rate of 1.0–1.5 m^3/min. Suspended PM was computed as per the standard method. Filter paper was weighed before and after sampling. The West and Gaeke (1956) method and modified Jacob and Hochheiser (1958) method were used for analysis of SO_2 and NO_2, respectively.

Table 1. Ambient air quality (concentration of suspended particulate matter, NO_2, and SO_2 during November 2011 to February 2012) and air pollution index (API) for two sites at Aizawl, Mizoram, North East India.*

Serial No.	Site	SPM ($\mu g/m^3$)	NO_2 ($\mu g/m^3$)	SO_2 ($\mu g/m^3$)	API†
1	Ramrikawn	250.07 ± 0.6	22.14 ± 0.8	2.09 ± 0.4	72.96 (MAP)
2	Mizoram University	130.12 ± 0.2	12.02 ± 0.6	1.03 ± 0.1	38.20 (LAP)

Data are presented as mean of three replicates ± standard error.
CA = clean air; HAP = heavy air pollution; LAP = light air pollution, MAP = moderate air pollution; SAP = severe air pollution (rating scale for indices).
* Ambient air quality standards used for calculation of API, i.e. 140 $\mu g/m^3$ for SPM, 60 $\mu g/m^3$ for $SO_2$and 60 $\mu g/m^3$ for NO_2.
† API range: 0–25 = CA, 26–50 = LAP, 51–75 = MAP, 76–100 = HAP, > 100 = SAP.

Air pollution index

The averages of the sum of the ratios of three major pollutant concentrations to their respective air quality standards (mentioned under Table 1 as table footnotes) were obtained. The average was then multiplied by 100 to get the index (Rao and Rao, 1989):

$$\text{API} = 1/3(\text{SPM}/S_{\text{SPM}}) + (SO_2/S_{SO2}) + (NO_X/S_{NOx}) \times 100 \quad (1)$$

where API = air pollution index, and S_{SPM}, S_{SO2}, and S_{NOx} are the ambient air quality standards for SPM, SO_2, and NO_2, respectively.

The API of both sites were developed on the basis of ambient air quality analyzed at specified study sites through instrumental monitoring of SPM, SO_2, and NO_2 and correlated with the variation in biochemical indicators.

Plant sampling and biochemical analysis

Thirty-six total plant species per site, namely, *Ficus benghalensis*, *Ficus religiosa*, *Mangifera indica*, *Bougainvillea spectabilis*, *Psidium guajava*, *Hibiscus rosasinensis*, *Lantana camara*, *Delonix regia*, *Artocarpus heterophyllus*, *Cassia auriculata*, *Bauhinia variegate*, and

Lagerstroemia speciosa were selected for this study, as they are common roadside trees in the region. Further, it is worth mentioning that *F. benghalensis* and *F. religiosa* are of ecological relevance as keystone species and their removal leads to extinction cascade, thus perturbing the urban ecosystem (Pandey et al 2005; Rai 2013; Rai and Chutia 2015). Also, these two species along with *M. indica*, *P. guajava*, *A. heterophyllus*, and *L. speciosa* have already been investigated for their suitability in efficient dust capture (Rai and Panda 2014a; Rai et al 2013). Finally, these species are of socioeconomic importance to the local people of Mizoram (Rai and Panda 2015a), and therefore are selected for the present study.

Twenty leaves over three plants per species were collected from the branches facing towards the roadside in the early hours of morning (8:30 AM to 12:00 AM) through random selection and were put in polythene bags. Leaf samples were collected thrice during a month, i.e. at 10 day intervals (10th day, 20th day, and 30th day of the month) during the months of November 2011 to February 2012. Leaf samples were kept in an ice box, brought to the laboratory, and enzymatic activities were studied immediately. The leaf samples were preserved at −20°C in freeze until they were analyzed for various morphological and biochemical parameters within 24 hours of their harvesting.

Determination of stomatal size and index

Ten mature leaves of the 20 leaves were prepared as specimens for anatomical study. A leaf segment of an area of 1 cm^2 from each specimen was cut and immersed in concentrated solution of nitric acid for 5–10 minutes. The upper (adaxial) and the lower (abaxial) surfaces were separated with dissecting needle and forceps and rinsed with clean water. Each specimen was stained with 1% aqueous safranin for 3–10 minutes. Excess stains were rinsed off with clean water. The stained cuticle was mounted in glycerine jelly for microscopic observation using transmission electron microscope (TEM) with EDAX TECHNAI G^2 (5350 NE Dawson Creek Drive, Hillsboro, Oregon, 97124, USA). The number and size of stomata were measured with the help of an ocular and stage micrometer, and stomatal index was calculated as per the Salisbury (1927) equation, i.e.:

$$\text{S.I.} = \text{S} \times 100/\text{S} + \text{E} \quad (2)$$

where S.I. = stomatal index, S = number of stomata/unit area of leaf, and E = number of epidermal cells/unit area of leaf.

Dust deposition

The leaf dust deposition was calculated by taking the initial and final weight of the beaker in which the leaf samples were washed. It was calculated by using the following formula:

$$\text{W} = \frac{\text{W}_2 - \text{W}_1}{\text{A}} \quad (3)$$

where W = dust content (mg/cm^2), W_1 = weight of beaker without dust, W_2 = weight of beaker with dust, and A = total area of leaf in cm^2.

Heavy metal analysis

Trace elements (Fe, Cu, and Zn) were extracted by digesting leaf samples with 1 mL of 50% perchloric acid, 5 mL concentrated nitric acid, and 1 mL concentrated sulfuric acid at moderate heat (Moore and Chapman 1986). The concentrations of Fe, Cu, and Zn in the extract were determined by using an atomic absorption spectrophotometer (model 370A, Perkin Elmer, 940 Winter St.,Waltham, Massachusetts 02451, USA).

Estimation of total soluble sugar content

Total soluble sugar content was analyzed using the anthrone method described by Irigoyen et al (1992). Absorbance of the resulting solution was read at 625 nm and a calibration curve with D-glucose (concentration range: 200–1000 μL) was performed as a standard.

Estimation of total protein content

Protein content was determined by the method of Lowry et al (1951). Leaves of the plant sample (0.2 g) were homogenized in 10 mL of 20% trichloroacetic acid. The homogenate was centrifuged for 20 minutes at 10,000 rpm with the temperature at 4°C (Eppendorf centrifuge with a maximum limit of 10,000 rpm: model 5810 R; Eppendorf AG, Barkhausenweg 1, 22339 Hamburg, Germany.). The supernatant was discharged and a pellet was re-extracted with 5 mL of 0.1 N NaOH. A 1 mL amount of the extract was taken in a test tube and 5000 μL of reagent "C" (protein reagent) was added. This solution was mixed well and kept in the dark for 10 minutes. Later, 0.5 mL of folin phenol reagent was added and the mixture was kept in the dark for 30 minutes. The sample was read at 660 nm. The total protein content was calculated from a standard curve of bovine serum albumin.

Estimation of enzyme activity (catalase and peroxidase)

For the determination of some antioxidant enzymatic activities, the enzyme extraction procedure was prepared according to Nayyar and Gupta (2006) with some modification in relation to quantity of reaction mixture (1000 μL), enzyme extract (50 μL), and concentration of H_2O_2 (10mM). The aforesaid components may vary in quantitative perspective in varying protocols. Catalase activity was determined according to Aebi (1984) by monitoring the decomposition of H_2O_2. In 1 mL of reaction mixture containing potassium phosphate buffer (pH 7.0), 50 μL of enzyme extract and 10mM H_2O_2 were added to initiate the reaction. The reaction was measured at 240 nm for 5 minutes and H_2O_2 consumption was calculated using extinction coefficient, 43.6 $M^{-1}cm^{-1}$.

Peroxidase activity was determined using the guaiacol oxidation method by Chance and Machly (1955). A 3 mL reaction mixture contains 10mM potassium phosphate buffer (pH 7.0), 8mM guaiacol, and 50 μL enzyme extract. The reaction was initiated by adding 10mM H_2O_2. The increase in absorbance was recorded within 5 minutes at 470 nm due to the formation of tetraguaiacol. A unit of peroxidase activity was expressed as the change in absorbance per minute and specific activity as enzymes units per mg soluble protein (extinction coefficient 6.39$mM^{-1}cm^{-1}$).

Statistical analysis

Dust deposition was correlated to the different tree responses across species within a site. For the aforesaid objective, data was subjected to Pearson's correlation to find out the coefficient of correlation and all statistical calculations were performed using SPSS version 11 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Air quality index for the selected sites in Aizawl City is shown in Table 1. On the basis of API, the Ramrikawn site was categorized as a

moderate air pollution site (RKN-Med) with API 72.96 and the MZU site (MZU-Low) as a light air pollution site (API 38.20; Table 1). The values of all of the three air pollutants were lowest at the MZU-Low site because it is an institutional area with a low density of vehicles, whereas the RKN-Med site is located in a residential area with some commercial complexes.

High dust deposition was recorded for all the plant species at the RKN-Med site and the trend of dust trapping capacity among the plant species was: *F. bengalensis* > *P. guajava* > *B. spectabilis* > *M. indica* > *L. camara* > *H. rosasinensis* > *L. speciosa* > *A. heterophyllus* > *F. religiosa* > *D. regia* > *C. auriculata* > *B. variegate* (Figure 2).

Analysis of the dust or PM collected from the leaf surface indicated the presence of some toxic heavy metals such as Fe, Cu, and Zn. The comparison between the accumulation levels of Fe, Cu, and Zn in the leaves of the RKN-Med site and the MZU-Low site are shown in Figures 3A–C. A significant positive correlation was recorded between dust deposition and heavy metals (Fe, Cu, and Zn) at both the MZU-Low and RKN-Med sites (Table 2). High concentrations of heavy metals in plants of the RKN-Med site may be due to heavy traffic frequency and more commercial and domestic activities when compared to the MZU-Low site. Verma and Singh (2006) also demonstrated high metal concentrations at heavy polluted sites in Lucknow, India compared to the low polluted site when using *F. religiosa* and *Thevetia neriifolia* as a biomonitoring tool.

The accumulated metal concentrations in leaves of plants differ from one species to another in response to dust or PM accumulation (Panda and Rai 2015). Foliage of *F. benghalensis* recorded a maximum accumulation of Fe (26.1 mg/kg) and Cu (19.5 mg/kg) while *C. auriculata* (12.1 mg/kg) showed the lowest accumulation of Fe. The lowest accumulation of Cu was recorded with *B. variegate* (1.88 mg/kg). However, as far as accumulation of Zn was concerned, the maximum (48.2 mg/kg) was recorded in *M. indica* while *B. variegate* showed the lowest accumulation of 11.3 mg/kg Cu at the Ramrikawn site.

Higher plants act as biomonitors of aerial heavy metal contamination because of their bioaccumulative properties (Rai 2008, 2009b, 2012b; Verma and Singh 2006). Higher plants not only intercept metals from atmospheric deposition, but also accumulate them from the soil (Verma and Singh 2006). Airborne heavy metals, when deposited on soil, are taken up by the plants through their root system and translocated to other parts of the plant through an active uptake mechanism (Panda and Rai 2015; Rai 2008, 2009b, 2012b; Shparyk and Parpan 1990; Verma and Singh 2006).

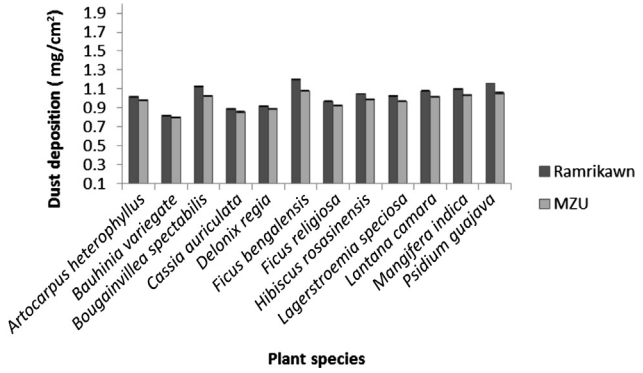

Figure 2. Dust deposition capacity of selected plant species at Mizoram University (MZU)-Low and Ramrikawn (RKN)-Med site during the study period (November 2011–February, 2012). Data are presented as mean ± standard error ($n = 3$).

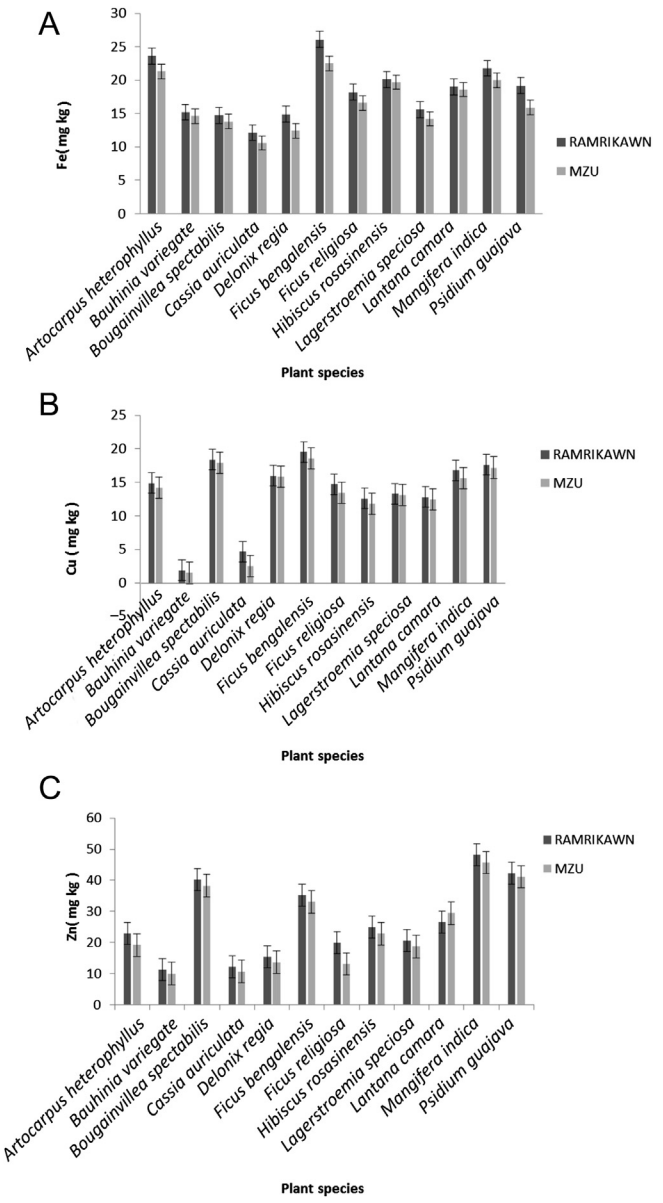

Figure 3. Accumulation of heavy metals by 12 selected plant species at MZU-Low and RKN-Med site during the study period (November 2011–February 2012): A, concentration of Fe in the leaf sample of the study sites during the study period; B, concentration of Cu in the leaf sample of the study sites during the study period; C, concentration of Zn in the leaf sample of the study sites during the study period. MZU = Mizoram University; RKN = Ramrikawn.

However, in certain cases through other mechanisms, metals may adhere as plaques on root surfaces without uptake and translocation to other parts of the plant (Panda and Rai 2015). Alfani et al (1996) observed that metal concentration was significantly higher in leaves from the urban roadside plants. They (Alfani et al 1996) also observed a positive correlation between leaf deposition and leaf metal accumulations. Therefore, plants growing along the roadside may also work as phytoremediators of airborne metals released from vehicles and street dust (Panda and Rai 2015; Rai 2008, 2013; Rai et al 2014a; Verma and Singh 2006). The presence of these heavy metals in the dust or PM may also play an important role in disturbing the various physiological, biochemical, and metabolic processes in plants (Panda and Rai 2015; Rai 2013; Rai et al 2014b; Verma and Singh 2006).

Table 2. Table showing Pearson correlation coefficient of dust deposition with stomata size, sugar content, protein content, heavy metals (Fe, Cu, and Zn), and enzyme (catalase and peroxidase) for two study sites. Dust deposition was correlated to the different tree responses across species within a site.

Sites	Stomata size	Sugar content	Protein content	Fe	Cu	Zn	Catalase	Peroxidase
RKN-Med	−0.504972*	−0.516823†	−0.828953*	0.669664*	0.830325†	0.887953*	0.501343*	0.55105*
MZU-Low	0.473552*	0.379058†	0.283591*	0.624713†	0.806215†	0.865972*	0.532697*	0.42992*

Significant at: *$p < 0.05$, † $p < 0.01$.
Copper = Cu; Iron = Fe; MZU-Low = Mizoram University; RKN-Med = Ramrikawn; Zn = zinc.

The suspended PM as well as gaseous pollutants such as SO_2 and NO_2 enter through stomata and interfere with the metabolism, resulting in reduction of the biochemical parameters (Panda and Rai 2015; Rai and Panda 2015b). The PM enters the leaf through stomata opening and their toxicity may perturb the physiological activity of plants (Farmer 1993; Panda and Rai 2015; Rai and Panda 2015b; Rai et al 2010; Verma and Singh 2006). Further, PM are reported to affect stomatal movement, leaf temperature (Borka 1984; Naidoo and Chirkoot 2004; Panda and Rai 2015; Rai and Panda 2015b), photosynthesis, transpiration (Ernst 1982; Panda and Rai 2015; Rai and Panda 2015b), penetration of toxins, and also degradation of epicuticular wax (Bystrom et al 1968; Eveling 1986; Panda and Rai 2015; Rai and Panda 2015b; Sauter and Pambor 1989; Verma and Singh 2006). The aforesaid facts may be attributed to significant differences in stomata size and frequency as demonstrated in Table 3.

Results demonstrated that air pollution caused significant changes in foliar morphology. Almost all of the species showed variations in their biochemical parameters and leaf morphology at the polluted RKN-Med site as compared to the MZU-Low site. While studying the micro morphological parameters (Table 3), it was observed that there was considerable reduction in stomatal characteristics (stomatal size and stomatal index) for all of the species growing at the RKN-Med site ($p < 0.05$). *Ficus benghalensis* showed more reduction percentage in its stomatal characteristics, i.e. stomatal size (43.87%), stomatal index adaxial (48.09%), and abaxial (30.40%), respectively, whereas the least reduction percentage was recorded in *B. variegate*, stomatal size (3.91%) and stomatal index adaxial (27.28%) and abaxial (6.45%). A significant and negative correlation between dust deposition and stomata size at the RKN-Med site was observed (Table 2). Reduction in stomatal size and index at the RKN-Med site may be attributed to the heavily polluted air, containing mainly suspended PM in conjunction with gaseous pollutants emitted from vehicles and other multifaceted sources.

The concentrations of total sugar content markedly decreased with increasing pollution load at the RKN-Med site (Figure 4) for all of the plant species except *F. benghalensis* (0.55 ± 0.02 mg/g), *B. spectabilis* (0.12 ± 0.02 mg/g), and *P. guajava* (0.38 ± 0.02 mg/g), which showed a considerable increase ($p < 0.01$) in the sugar content when compared with the MZU-Low site. A significant negative correlation was observed between dust deposition and total sugar content for the RKN-Med site (Table 2). Reduction in soluble sugar content at the polluted site can be attributed to increased respiration and decreased carbon dioxide fixation because of chlorophyll deterioration (Davison and Barnes 1986; Panda and Rai 2015; Tripathi and Gautam 2007). Pollutants like SO_2, NO_2, and H_2S under stress conditions may cause more depletion of soluble sugar in the leaves of plants grown in a polluted area (Davison and Barnes 1986). The reaction of sulfite with aldehydes and ketones of carbohydrates can also cause reduction in carbohydrate content (Tripathi and Gautam 2007). The observed increase in the concentration of sugar content in *F. benghalensis*, *B. spectabilis*, and *P. guajava* may be attributed to osmoregulation and tolerance contributing to plant survival (Hare et al 1998).

The present study observed a reduction in total protein content for all of the selected plant species at the RKN-Med site when compared to the MZU-Low site (Figure 4). A significant negative correlation was observed between dust deposition and protein content for the RKN-Med site (Table 2). Maximum reduction was observed in *M. indica* (52.30%) and *H. rosasinensis* (50.70%). Reduction in protein content of plants at the Ramrikawn site may be due to the enhanced rate of protein denaturation and breakdown of existing protein to amino acid, which is also supported by the findings of Constantinidou and Kozlowski (1979), Panda and Rai (2015), Prasad and Inamdar (1990), Saha and Padhy (2011), and Tripathi and Gautam (2007). Declines in total protein content due to SO_2 and NO_2 pollutants have also been reported by several workers (Agarwal and Deepak 2003; Panda and Rai 2015; Rai and Panda 2014a; Rai and Panda 2015a). Agarwal and Deepak (2003) determined that SO_2 enrichment results in diminished leaf protein levels in two cultivars of wheat by 13% and the decrease is attributed to breakdown of existing protein and reduction in protein synthesis.

Figures 5 and 6 demonstrated the catalase and peroxidase enzyme activities in the plant leaf samples. The average activity of catalase and peroxidase enzymes was increased in all of the plant

Table 3. Stomatal size (μm) and stomatal index of the selected plant species pertaining to two study sites of study area, i.e. Aizawl (significant at: $p < 0.05$, $p < 0.01$).

Plant species	Stomatal size (μm)		Stomatal index (adaxial)		Stomatal index (abaxial)	
	RKN-Med site	MZU-Low site	RKN-Med site	MZU-Low site	RKN-Med site	MZU-Low site
Artocarpus heterophyllus	78.76 ± 14.00	108 ± 18.21	21.00 ± 2.61	25.01 ± 5.32	14.68 ± 2.47	16.75 ± 3.47
Bauhinia variegate	66.28 ± 12.60	68.98 ± 14.02	19.72 ± 6.62	27.12 ± 2.76	19.72 ± 6.62	21.08 ± 4.07
Bougainvillea spectabilis	42.16 ± 10.81	90.29 ± 17.03	19.21 ± 2.66	35.72 ± 8.30	21.63 ± 9.80	26.74 ± 4.80
Cassia auriculata	130.1 ± 17.04	138 ± 16.23	20.46 ± 3.92	16.20 ± 5.15	16.20 ± 5.15	19.31 ± 2.11
Delonix regia	104.3 ± 16.77	120.4 ± 15.39	24.11 ± 7.60	27.13 ± 2.76	10.71 ± 4.41	20.62 ± 7.80
Ficus benghalensis	80.61 ± 17.44	143.6 ± 14.66	18.00 ± 2.11	34.68 ± 11.10	19.82 ± 2.10	28.48 ± 3.40
Ficus religiosa	60.57 ± 13.02	83.66 ± 18.30	29.08 ± 3.50	32.53 ± 8.30	24.68 ± 4.87	30.18 ± 6.08
Hibiscus rosasinensis	46.35 ± 15.21	76.36 ± 13.00	22.30 ± 6.14	28.10 ± 5.85	22.61 ± 7.17	32.58 ± 12.10
Lagerstroemia speciosa	86.20 ± 17.30	116.2 ± 16.00	23.26 ± 5.08	28.17 ± 4.20	30.78 ± 6.70	24.68 ± 4.87
Lantana camara	122.1 ± 17.03	153.5 ± 16.31	25.31 ± 6.02	34.10 ± 5.00	23.68 ± 4.87	31.78 ± 6.70
Mangifera indica	82.34 ± 20.00	118.6 ± 15.39	19.70 ± 4.11	28.74 ± 3.93	14.94 ± 3.92	21.63 ± 9.80
Psidium guajava	125.4 ± 15.21	178.9 ± 18.00	18.34 ± 3.55	30.22 ± 6.00	18.65 ± 4.02	20.20 ± 2.10

Data are presented as mean ± standard error ($n = 10$).

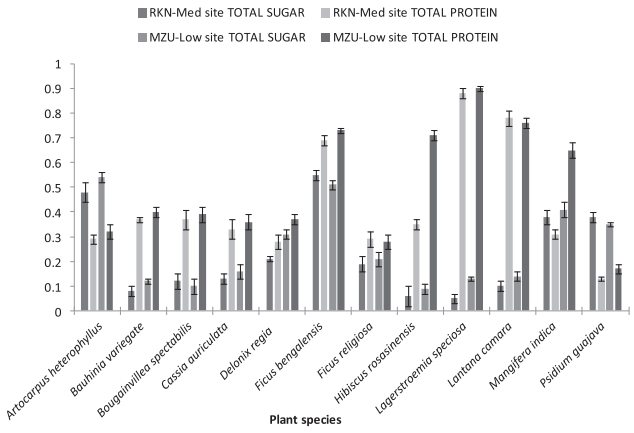

Figure 4. Total soluble sugar content (mg/g) and total protein content (mg/g) of 12 plant species at RKN-Med site (peri-urban) and MZU-Low site (rural) site during the study period (November 2011–February 2012). Data are presented as mean ± standard error ($n = 3$). MZU = Mizoram University; RKN = Ramrikawn.

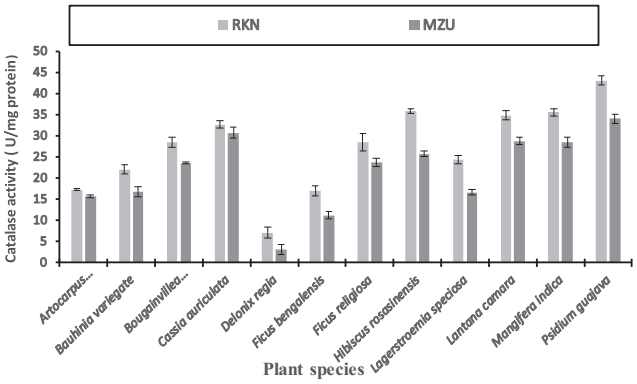

Figure 5. Catalase activity of 12 plant species at RKN-Med (peri-urban) and MZU-Low (rural) site during the study period (November 2011–February 2012). Data are presented as mean ± standard error ($n = 3$). MZU = Mizoram University; RKN = Ramrikawn.

species at the RKN-Med site. A significant and positive correlation of enzyme catalase and peroxidase with dust deposition of plant species at both the MZU-Low site and the RKN-Med site was observed (Table 2). *P. guajava* (36.11 ± 0.06 U/mg protein) showed the highest catalase concentration and *D. regia* (4.10 ± 0.01 U/mg protein) showed the lowest catalase concentration. The highest peroxidase concentration was recorded in *D. regia* (0.18 ± 0.02 U/mg protein) and the lowest in *L. speciosa* (0.04 ± 0.03 U/mg protein).

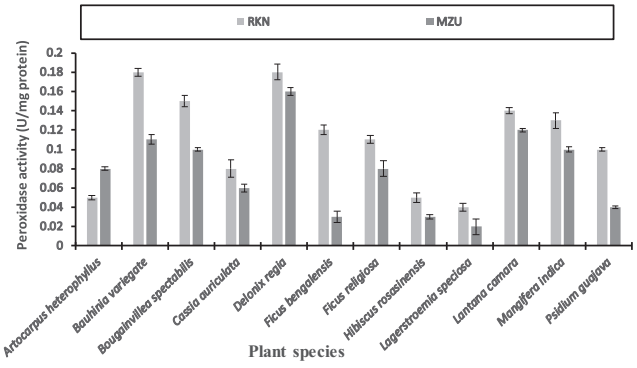

Figure 6. Peroxidase activity of 12 plant species at RKN-Med (peri-urban) and MZU-Low (rural) site during the study period (November 2011–February 2012). Data are presented as mean ± standard error ($n = 3$). MZU = Mizoram University; RKN = Ramrikawn.

In plant cells, electrons may be transferred through chloroplast or the mitochondrial electron transfer system. These electrons can produce reactive oxygen species (ROS) when they come into contact with oxygen molecules (Panda and Rai 2015; Rai and Panda 2015b). ROS are extremely reactive and cytotoxic to all organisms (Pukacka and Pukacki 2000) causing peroxidative destruction of cellular constituents (Lee et al 2007; Tiwari et al 2006). Stress such as air pollution enhances ROS formation in plant cells, resulting in oxidative stress (Dat et al 2000; Miller et al 2010; Mitteler 2002).

The plant cells have several antioxidative enzymes (superoxide dismutase, glutathione reductase, catalase, and peroxidase) and low molecular antioxidants such as ascorbic acid, glutathione, α-tocopherol, flavonoids, and carotenoids to protect plants against these oxidative stressors (Ghorbanli et al 2007; Kangasjarvi et al 1994; Noctor and Foyer 1998; Pell et al 1997; Sandermann et al 1998).

Pollution load increases catalase and peroxidase content in this study and may be a function of ROS production in response to air pollution stress. ROS or free radical production under pollution stress would increase the scavenging properties of enzymatic and nonenzymatic metabolites, particularly catalase and peroxidase, as well as other compounds such as ascorbate, carotenoid, and superoxide dismutase (Bowler et al 1992; Elstner and Osswald 1994) based on dosage and plant physiological status. Varshney and Varshney (1985) reported an increase in peroxides activity in plants under a variety of stresses like mechanical injury and attack by pathogens or an influence of environmental pollution. The increase in peroxidase and catalase activity varies with the plant species and the concentration of pollutants (Panda and Rai 2015; Rai and Panda 2015b).

The present study concluded that common roadside plants growing at the RKN-Med (peri-urban) site of Aizawl City are adversely affected due to higher concentrations of air pollutants as compared to the MZU-Low (rural) site. Higher suspended PM levels have induced several morphological and biochemical changes in the roadside plants. However, despite these changes, plants were thriving well at the peri-urban site as well as the rural site. Therefore, roadside plants of the present study, i.e. *F. benghalensis*, *F. religiosa*, *M. indica*, *B. spectabilis*, *P. guajava*, *H. rosasinensis*, *L. camara*, *D. regia*, *A. heterophyllus*, *C. auriculata*, *B. variegate*, and *L. speciosa* may be used as control of PM pollutants originating from multifaceted sources after further research at heavily polluted urban sites. The present study is a strong first step and warrants further effort, which may pave the way to screen the feasibility of these plants in context of their potentiality to be planted in other urban areas with varying pollution load. In a nutshell, the use of urban roadside plants as bioindicators or biomarkers is an inexpensive and convenient technique and thus offers an eco-sustainable green tool for urban ecosystem restoration.

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