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Effects of different environmental pollutants on the anatomical features of roadside plants

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Abstract

Experiments were conducted in order to investigate anatomical changes in leaves and stems of roadsides plants namely Mango (*Mangifera indica*), SilKoroi (*Albizia procera*) and Mahagony (*Sweatenia mahogany*). Plants that grown nearby roadside areas were exposed to various kinds of pollutants. To carry out the experiment, samples (Fresh leaves and Stems) were collected from two polluted sites and one control site. For anatomical study leaves and stems were cut into sections in the laboratory and changes were identified under the light microscopy. Results revealed that leaves of selected roadside plants had reduced cell size with black dot like substance deposited in the epidermis, palisade and spongy parenchyma in the polluted sites. But leaves of control site plants had normal anatomy. Meanwhile stem showed changes in vascular bundle of pollution affected sites but no change was found in control site. These results highlighted the importance of anatomical data for precious diagnosis of injury and to determine the sensitivity of roadside plants to different environmental pollutants.

Key words: Environmental pollutants, anatomical features, Mango, *Sil Koroi*, *Mahagony*

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Introduction

Urbanization and Industrialization, regarded as the most common factors responsible for many negative impacts affecting the well-being of flora and fauna, as well as the environment as a whole. Moreover, the growing number of road vehicles is another major source of regional and global atmospheric pollution, increasing the concentrations of CO_2 in the air and levels of matter in the air and soil (Skrynetska *et al.*, 2018). The roadside vegetation are seriously vulnerable to environmental pollution and the effects of pollutants can easily be detected on plant structure. Among environmental pollutants, air and soil pollution produces inevitable harmful byproduct that is responsible for a variety of deleterious effect on roadside plants. Plants often show symptoms of various injuries, disability and premature ageing due to air pollutants include oxides of nitrogen (NOx), Oxides of sulpher (SOx), (CO_2), Hydrocarbons and Particulate matter (PM) discharged into atmosphere from automobiles, industries and power stations (Sukumaran, 2014). The pollutants that come from various sources such as industrial process, paved and unpaved roadways, construction and demolition sites and so others cause serious injuries internally as well as externally to plants and plant cells. The effects of these pollutants on vegetation especially trees growing at roadsides principally occur through the leaves (Kumar and Nandini, 2013) as leaf is the most sensitive part to be affected by air pollutants instead of all other parts such as stem and roots. The sensitivity rests on the fact that the major portions of the important physiological processes are concerned with leaf. Pollutants came from the auto emission can directly affect the plant by entering in to the leaf, destroying individual cells, and reducing the plant ability to produce food because they are the first receptors (Randhi and Reddy, 2012). Roadside plants exposure to road traffic emissions not only showed changes in leaf anatomy and morphology but also marked alterations in photosynthetic pigments (chlorophyll, carotenoid, and phaeophytin), and relative water content was reduced while antioxidative enzymes like catalase and peroxidase were found to be enhanced (Verma and Chandra, 2014). Besides those roadside plants stems are also vulnerable to various environmental pollutants. Study investigated the effect of coal-smoke pollutants on leaves and stem of plants nearby coal producing areas reported that growth of stem cortex and pith were showed significant decreases due to a greater load of pollutants. Also, area of xylem tissue decreased and found to couple with an increasing number of vessels of reduced sizes (Gupta and Ghouse, 1987).

Several experiments have been carried out in this field to investigate the anatomical, morphological, physiological and biochemical changes of trees (Pourkhabbaz et al. 2010; Maheswari, 2012; Lohe *et al*., 2015; Pandey *et al*., 2015; Deepika and Haritash, 2016). Studies have recorded changes in plants because of a wide range of environmental pollutants and the vast majority of these work allude to physiological modifications (Linster, 1991; Patra and Sharma, 2000). Some studies have also showed that the majority of plants exposed to polluted atmosphere, first have physiological changes before visible damage to the leaves (Abida and Harikrishna, 2010). It has been reported that leaf surface characters, including stomata and epidermal cells, in plant species growing along road sides are considerably modified due to the stress of automobile exhaust emission with high traffic density in urban areas (Rai and Mishra, 2013). Study also reported a conspicuous increase in frequency of stomata, percentage of abnormal stomata, larger stomatal openings and conspicuous circular striations in polluted population of *Ricinus communis* L. (Yunus *et al.*, 1979). Leaves of *Syzygium cumini* L., marked decrease in epidermal cell size, increase in number of epidermal cells and stomata, necrotic lesions and death of epidermal cells (Jafri *et al.*, 1979).The effects of roadside dust on photosynthesis and leaf diffusion resistance over a range of light intensities has also been investigated and it concluded that photosynthesis was reduced tremendously when leaves were dusted with 5 to 10 g of dust m^{-2} of leaf surface (Thompson *et al.*, 1984).Experiments conducted on the extent of circumference of shoot axis, cortex area, pith, xylem area, fibre length of trees nearby clay industry and automobile exhaust and found significant reduction in circumference of shoot axis and xylem area of the plants (Sukumaran, 2014).

Mango (*Mangifera indica*), Sil koroi (*Albizia procera*) and Mahogany (*Sweatenia mahogany*) are three well known plant species found almost everywhere in Bangladesh even in roadside. The present study aims to find out the anatomical changes of this species due to major pollutants of the study area and to compare the changes in anatomical features with these plant species of both polluted and non-polluted area.

Materials and Methods

Study area and Plant materials: The Bangabandhu Sheikh Mujibur Rahman Agricultural University campus in Gazipur was selected as control site, Mauna upazila in Gazipur, and Valukaupazila in Mymensingh as polluted site. The sites for collection of plant samples were located approximately 50m away from these roads. Samples were collected from Mango (*Magnifera indica*), Sil Koroi (*Albizia procera*) and Mahogony (*Swietenia mahagoni*) plant species. The samples were collected during dry seasons.

Sample collection: Fresh leaves at the same leaf stage occurring at the same level of insertion on the stem were collected for each species from the three study sites. Samples of cambial tissue along with inner bark, outer sapwood and heartwood were collected from the main trunk at breast height. A series of small blocks (2 x 2 x 1 cm^3) containing phloem, cambium and some xylem cells were removed with a disposable scalpel and chisel. Samples were removed in a zigzag fashion to eliminate any effects of wounding. Each block was cut into 2-mm-thick samples immediately after removal from the tree. The sampling trees were subjected to sequential observations.

Preparation of samples for light microscopy: Leaf and stem sections were cut in middle, part of the midrib and stems for anatomical study. The leaf samples were cut at the midrib and then washed into fresh water. After that the cutting leaf samples were stained with a solution of 1% safranin water for visualization and then the slides were covered with cover slip. The slides were ready toexamine under a light microscope. The stem samples were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), under a vacuum, for 1 h at room temperature and 90% formaldehyde (FAA) solution. Fixed samples were washed in 0.1 M phosphate buffer and trimmed to 3 mm in length for subsequent preparation of samples. After washing in phosphate buffer, the specimens were dehydrated in a graded ethanol series. The samples were cut into small pieces with anti-cutter and stainless steel blade. The small pieces were washed properly with distilled water for one hour. Transverse sections were cut at a thickness of approximately 10 μm with a stainless steel knife on a cryo-stat microtome. Sections for observations of cambial reactivation (the presence of new cell plates) were stained with a solution of 1% safranin in water for visualization of cambial cell division (Murakami et al. 1999; Nakaba et al. 2006) and examined under a light microscope (Axioscop; Carl Zeiss, Oberkochen, Germany). In each sample, 10 radial files of cambial cells were selected to investigate the presence of new cell plates.

Results

Effects of pollutants on anatomy of leaf and stem of Mango (*Mangifera indica*): Mango leaves of control site (BSMRAU campus) had well-developed xylem, palisade parenchyma, and spongy parenchyma with adequate chlorophyll content having very clear vascular bundle with no deposited dust materials inside the cell. However, leaves from both polluted sites (Mauna and Bhaluka) revealed reduced cell size due to pollutants. Black dot like substances were appeared inside the upper and lower epidermis of mauna mango leaves, whereas palisade and spongy parenchyma of leaves of Bhaluka had black colour appearance due to deposition of pollutant substances (Figure 1).

Besides anatomical features of mango stem showed no change in BSMRAU campus but some pattern of alteration was found in phloem of pollution affected sites (Mauna and Bhaluka). The phloem was highly affected due to deposition of pollutants in phloem parenchyma cells (Figure 2).

Effects of pollutants on anatomy of leaf and stem of Koroi (*Albizia procera*): Koroi leaves of control site (BSMRAU campus) had well-developed xylem, palisade parenchyma, and spongy parenchyma with adequate chlorophyll content, while, vascular bundle was very clear with no deposited dust materials inside the cell. However, leaves from both polluted sites (Mauna and Bhaluka) revealed reduced cell size due to pollutants. Mauna koroi leaf filled with black dot like substances were visualized inside the upper and lower epidermis of muana koroi leaves whereas, palisade and spongy parenchyma of leaves of Bhaluka had black colored appearance due to deposition of pollutant substances (Figure 3).

Anatomical features of koroi stem showed no change in BSMRAU campus but some pattern of alteration was found in phloem of pollution affected sites (Mauna and Bhaluka).The phloem was highly affected due to deposition of pollutants in phloem parenchyma cells (Figure 4).

Effects of pollutants on anatomy of leaf and stem of Mahogany (*Swietenia mahogany*): Mahogany leaves of control site (BSMRAU campus) had well-developed xylem, palisade parenchyma, and spongy parenchyma with adequate chlorophyll content, having very clear vascular bundle with no deposition of dust materials inside the cell, whereas leaves from both polluted sites (Mauna and Bhaluka) revealed reduced cell size due to pollutants. Mahagony leaves from Mauna had an appearance of black dot like substances inside the spongy parenchyma cells, whereas, leaves from Bhaluka had black colour pollutants deposition insidethe xylem of vascular bundle (Figure 5).

Anatomical features of mahagony stem showed no significant change in BSMRAU campus area but some pattern of alteration was found in phloem of pollution affected sites (Mauna and Bhaluka). The phloem was highly affected due to deposition of pollutants in phloem parenchyma cells (Figure 6).

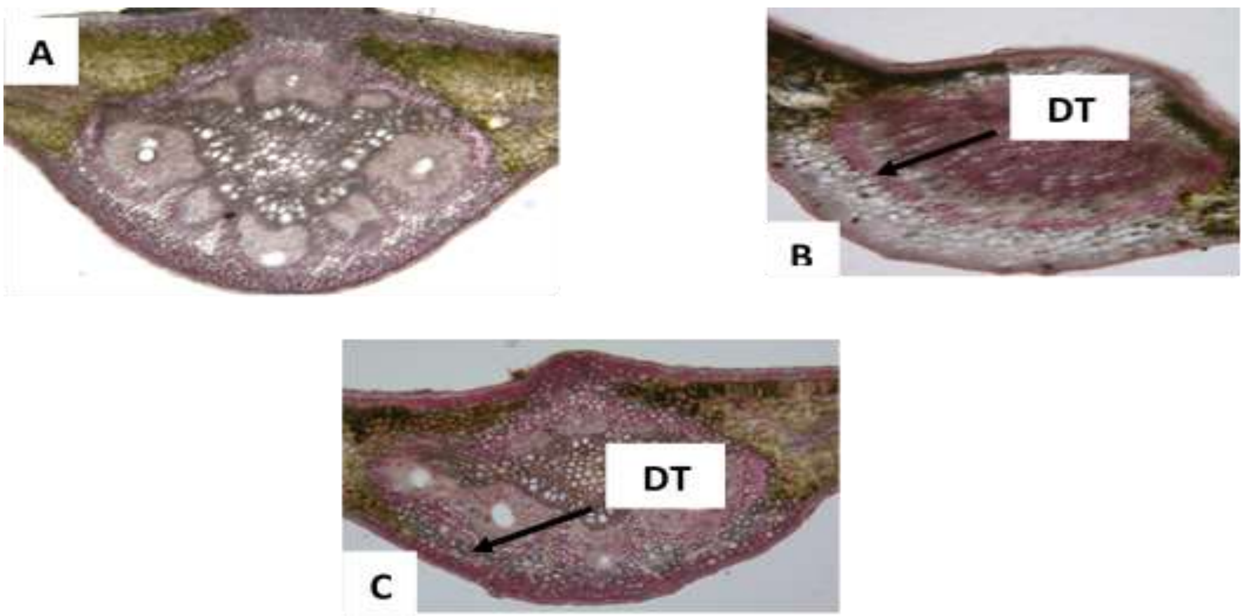

Figure 1. Variations of the anatomy of Mango leaves of study areas. **A** denotes sample from BSMRAU campus. **B** denotes sample from Mauna roadside. **C** denotes sample from Bhaluka roadside**. DT** denotes dust particles.

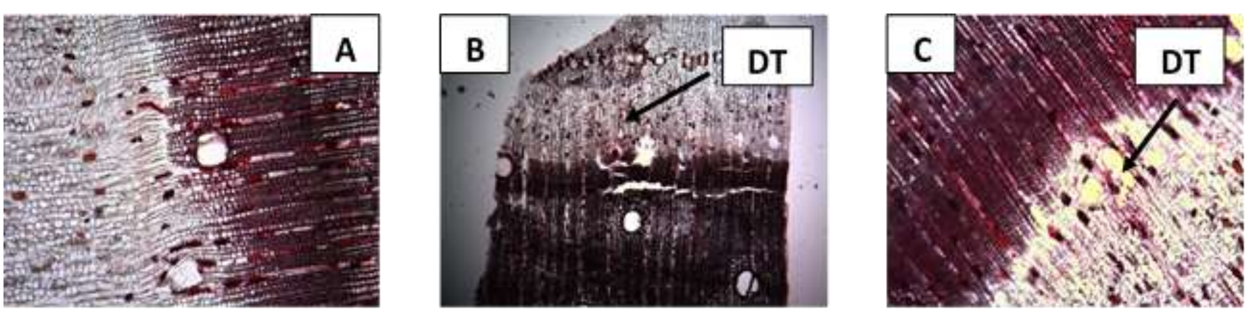

Figure 2. Variations of the anatomy of Mango stems of study areas. **A** denotes sample from BSMRAU campus. **B** denotes sample from Mauna roadside. **C** denotes sample from Bhaluka roadside**. DT** denotes dust particles.

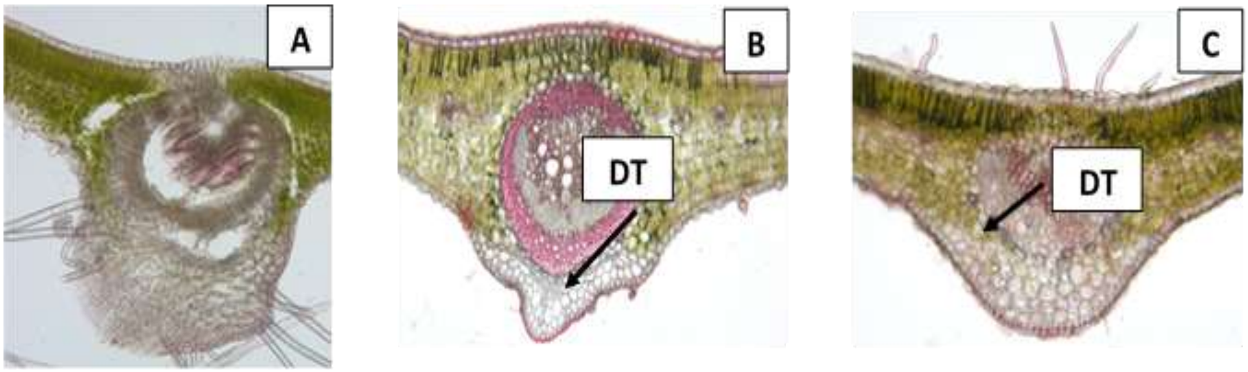

Figure 3. Variations of the anatomy of Koroi leaves of study areas. **A** denotes sample from BSMRAU campus. **B** denotes sample from Mauna roadside. **C** denotes sample from Bhaluka roadside**. DT** denotes dust particles.

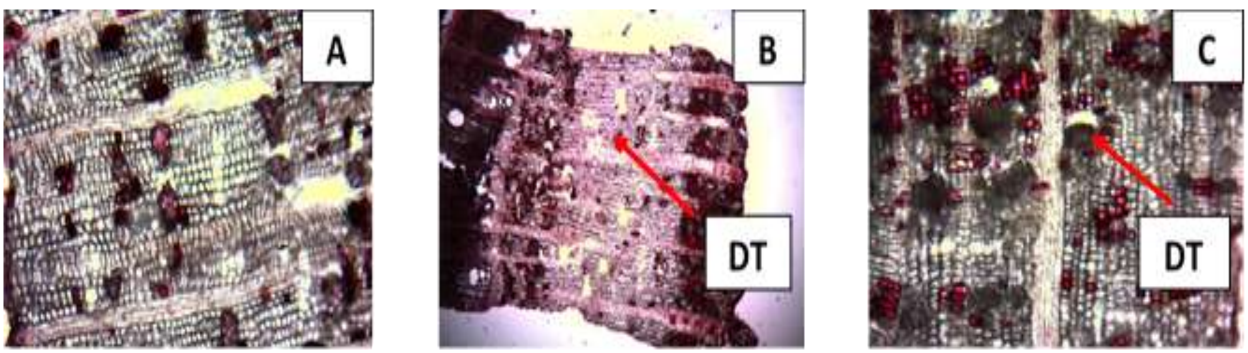

Figure 4. Variations of the anatomy of Koroistems of study areas. **A** denotes sample from BSMRAU campus. **B** denotes sample from Mauna roadside. **C** denotes sample from Bhaluka roadside**. DT** denotes dust particles.

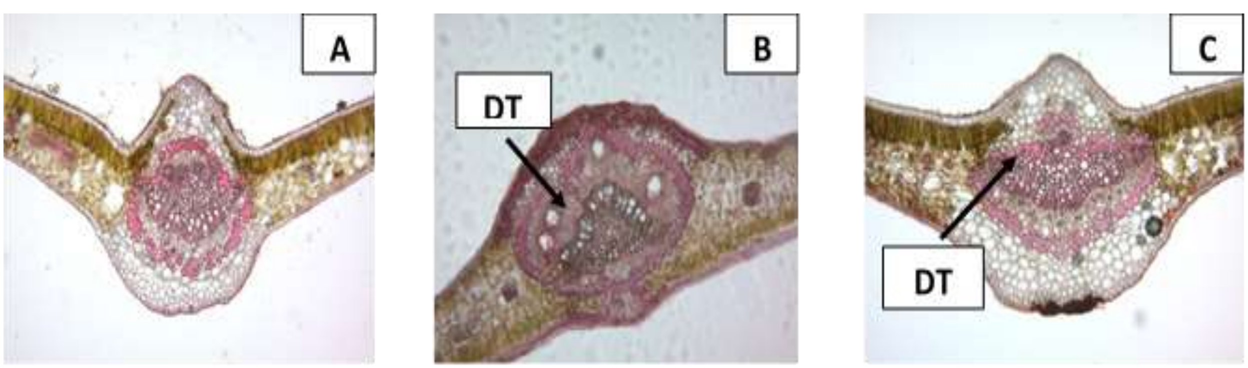

Figure 5. Variations of the anatomy of Mahogany leaves of study areas. **A** denotes sample from BSMRAU campus. **B** denotes sample from Mauna roadside. **C** denotes sample from Bhaluka roadside**. DT** denotes dust particles.

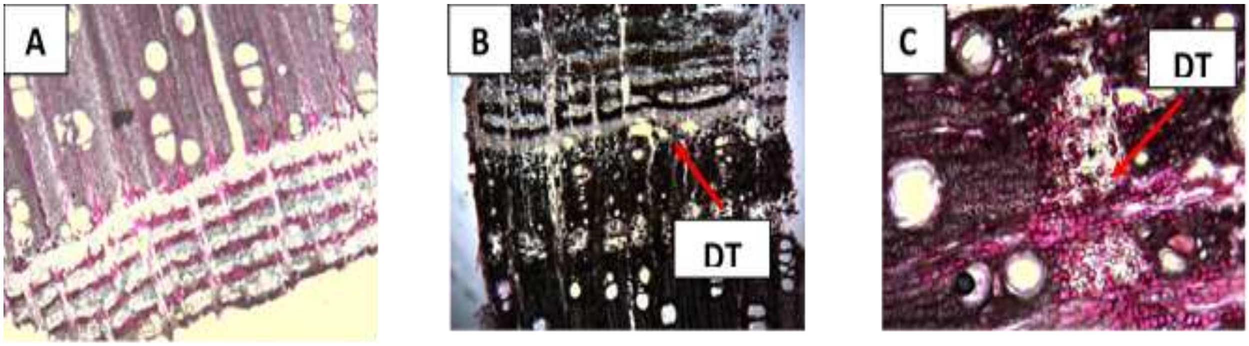

Figure 6. Variations of the anatomy of Mahogany leaves of study areas. **A** denotes sample from BSMRAU campus. **B** denotes sample from Mauna roadside. **C** denotes sample from Bhaluka roadside**. DT** denotes dust particles.

Discussion

Plants growing in extreme environmental conditions specially, besides the roadside have anatomically and morphologically changes in their leaves stems and roots, due to influence of air and soil pollution than plants growing in environmentally friendly conditions (Gielwanowaska *et al.*, 2005). Road traffic pollutants affect the morphological characteristics of plants and the leaf surface characteristics, including stomata and epidermal cells, in plant species growing along road sides are considerably modified due to the stress of automobile exhaust emission with high traffic density in urban areas (Azmat *et al.*, 2009; Rai and Mishra, 2013).

In the current study significant anatomical changes in leaves and stems were observed in spongy parenchyma, lower epidermis, upper epidermis and vascular bundles in Mango, Mahogany and Koroi, respectively due to continuous exposure of pollutants in pollutant sites. Similar results also found for in leaves of white clover of a polluted population whereas significant reduction in palisade and spongy parenchyma was observed (Iqbal, 1985).

A study on Tansy plant demonstrated that leaves of plants growing in polluted site were significantly thinner and with reduced chlorophyll content than those from an unpolluted area (Stevovice *et al.*, 2010). Other studies have demonstrated reductions in leaf area of plants growing in the vicinity of heavy pollutions in many plant species (Bhatti and Iqbal, 1988; Gupta and Ghouse, 1987; Ekpemerechi *et al.*, 2014). Experiments on biodiversity of roadside plants and their response to air pollution in polluted sites of Indo-Burma hotspot region demonstrated that air pollution caused significant changes in foliar morphology along with variations in their biochemical parameters (Rai, 2016).

The absence of anatomical changes in roadside plants in control sites because of low concentration of pollutants in those areas. However, high rate of automobiles, industrial effluents, and other anthropogenic activities going on in the polluted areas results in abnormal changes in leaf and other structures of roadside plants. It would be important to emphasize that a more detailed anatomical as well as physiological assessment should be made to evaluate the potential of these species as bio-indicator of polluted environment since these species represents considerable anatomical and morphological alterations.

Conclusion

The present study was undertaken to investigate effects of different environmental pollutants on the anatomical features of roadside plant. The observations recorded in the present study clearly indicates that pollutants emitted from the industry and automobile exhaust exercised a decisive influence on plant anatomy. From the present investigation it also appeared that all the elements (non-control) from the leaf to shoot to root are affected more in size and proportion compared to other elements (control). In other words, plant cells are more sensitive to pollutants. The result of this study concluded that common road side plants growing near the urban site of Bangladesh are adversely affected due to higher concentrations of automobile pollutants as compared to the lower pollutant (control) sites. Higher suspended pollution levels have induced several morphological and anatomical changes in the roadside plants. This study will helpful to understanding the impact of pollution on foliar and stem anatomy and will also help to bridge the gap on the amount of information available on the impact of pollution on leaf and stem anatomical features, especially in Bangladesh. Further investigation might be needed to carry out to assess the major anatomical and morphological changes and their magnitude of other plants species.

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