Effects of Air Pollution on the Foliar Characters of Asteraceaean Members in Kodungallur

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Abstract

Nowadays, air pollution is a major problem in several countries including India and the number and kinds of air pollutants added to the environment have increased dramatically in the recent decades. Both plants and animals suffer from the hazardous effects of these air pollutants. The prompt detection of plant damage resulting from air pollution is extremely important since plants will often display symptoms of injury before effects are discernible in man. In the present project work, four plants namely *Chromolaena odorata* (L.) R.M. king & H. Rob, *Sphagneticola trilobata* (L.) Pruski, *Vernonia cinerea* (L.) Less. and *Tridax procumbens* L. were selected and the affects of air pollution on the foliar characters were analysed by comparing the plants growing under air polluted and non-polluted habitats. The results of the study reveal that air pollution significantly alters the morphological, anatomical and physiological attributes of all the four selected plant species.

Key words: Pollution, foliar characters, epidermis, stomata, trichome, photosynthetic pigments

Introduction

Among the different types of environmental problems, air pollution is one of the most adverse affects of industrialization. Global advancement in industrialization, urbanization as well as economic growth is invariably associated with increased demand for energy particularly from fossil fuels, which results in the enhanced emission of toxic gases and other substances to the environment (Kalandadze 2003; Uaboi- Egbenni *et al.* 2009). Plants have been observed to be far more sensitive to pollution than animals and man and thus the plants therefore can be used as indicators of air pollution (Rani *et al*. 2006). Pollution due to toxic gaseous emissions engenders deleterious effects on plants, especially those growing in many urban areas (Qadir & Iqbal 1991; Giri *et al.* 2013).

The degree of impact of air pollution in plants depends on pollutants concentration, location of entry into plant and species under consideration. Different species may present varying sensitivity/tolerance levels to different air contaminating agents. Several research works indicated the importance of cuticle and epidermal features in the determination of tolerance/sensitivity of each species to environmental pollutants. Taking into account these characteristics, some authors consider foliar epidemis as a bioindicator of environmental quality (Alves *et al*. 2008; Balasooriya *et al*. 2009).

In the present research work the various effects of air pollution on the foliar characters of selected plants of Asteraceae growing under polluted and non-polluted habitats of Kodungallur were investigated.

Materials and methods

Materials

Plant leaves, polythene bags, distilled water, watch glasses, brush, needle, glass slides, cover slips, compound microscope, stereo microscope attached with camera

Plant material

The research was carried out with the leaves of four plants namely, *Chromolaena odorata* (L.) R.M. king & H. Rob., *Sphagneticolatri lobata* (L.) Pruski, *Vernonia cinerea* (L.) Less. and *Tridax procumbens* L. of Asteraceae family. Plant materials were collected from the polluted and unpolluted areas of Kodungallur during the period June 2017 to May 2018.

Methods

Collection and preparation of plant samples

The four selected plants were collected from different localities in the Kodungallur. They were common along road side. Healthy and mature leaves of each plant were randomly collected from polluted and non-polluted areas in early hours of morning and brought in polythene bags. Plant samples were brought to the laboratory. Leaves of fourth node from the tip were selected for the study. These leaves were washed with water and wiped with clean cotton cloth.

Macroscopic and microscopic foliar characters

Epidermal characters were noted and the size of the leaves was determined with the help of graph paper. For the anatomical study, thin hand sections were prepared. Leaf epidermal peelings from the abaxial side were also prepared. These were stained with saffranin and mounted with glycerine. The mounted section was placed on the stage of compound microscope and the details were noted. Microphotographs were taken with the help of digital camera (Biolinkz) attached to the microscope (Labomed CX_{R3}).

The macroscopic characters include leaf size, stains on leaves, apex, nervation, chlorosis and necrosis. The major microscopic characters studied include length, breadth of trichome, number of trichome, number of stomata, number of epidermal cell, length and breadth of stomata etc. and are measured and noted with the software. Stomatal index was calculated by using the formula:

Stomatal index = S/(S+E) X 100; Where, S = no. of stomata in microscope field area, E = no. of epidermal cells in microscopic field area.

Photosynthetic pigment estimation

Estimation of chlorophyll and carotenoids were done according to the method of Arnon (1949) by using the following formula:

$$\text{Chlorophyll a } \mu g/g \text{ fresh weight} = \frac{12.69\,(A663 - A750) - 2.69\,(A646 - A750)}{\text{Fresh weight of the sample}} \times \text{volume}$$

$$\text{Chlorophyll b } \mu g/g \text{ fresh weight} = \frac{22.9\,(A\,646 - A\,750) - 4.68\,(A663 - A750)}{\text{Fresh weight of the sample}} \times \text{volume.}$$

$$\text{Chlorophyll a+b } \mu g/g \text{ fresh weight} = \frac{20.12\ (A646 - A750) + 8.02\ (A663 - A\ 750)}{\text{Fresh weight of the sample}} \times \text{volume}$$

$$\text{Carotenoid } \mu g/g \text{ fresh weight} = \frac{1000\ (A\ 470) + 3.27\ (\text{chl a - chl b})}{\text{Fresh weight of the sample x 229}} \times \text{volume.}$$

Statistical analysis

Data from observations were recorded and analyzed in the Microsoft office excel sheet. Standard deviation and standard error were determined in the MS Excel programme.

Results

Macroscopic characters

There are many differences in the foliar characters of Asteraceae family members growing in polluted and unpolluted areas. The result demonstrated that the plant species respond in different ways to air pollution depending on the level of pollution in the habitat. The pollution causes changes in the morphological, anatomical and physiological features of plant species.

Plants growing in the industrial areas and near the roads absorb the pollutants through their leaves. The tolerance degree of each plant species towards air pollution is indirectly correlated with the intensity of injuries which occur in plant structures. The size and shape of leaves are the easily observable macroscopic foliar characters. The shape of leaves in the polluted and non polluted plants showed no variation in the present project work. But the size of leaves showed significant variations. Among the four plant species studied, all of them showed reduction in leaf size *Tridax procumbens* showed more reduction in the length and breadth of leaves under polluted habitats when compared to that of non- polluted habitats. Where as in *Chromolaena odorata* such a significant reduction in the leaf size was not observed in the plants under polluted habitats when compared to that of un-polluted ones (Table 1).

PLANTS	Leaf length (cm)	Leaf breadth (cm)
***Chromolaena odorata* NP**	8.4±0.16	4.2±0.05
***Chromolaena odorata* P**	8.3±0.09	4.05±0.03
***Tridax procumbens* NP**	4.3±0.83	2.05±0.07
***Tridax procumbens* P**	2.6±0.02	0.95±0.06
***Vernonia cinerea* NP**	3.7±0.06	1.22±0.01
***Vernonia cinerea* P**	3.5±0.02	0.98±0.07
***Sphagneticola trilobata* P**	5.8±0.03	3.91±0.02
***Sphagneticola trilobata* NP**	5.6±0.01	3.83±0.04

Table 1: Leaf length and breadth of Chromolaena odorata, Tridax procumbens, Vernonia cinerea and Sphagneticola trilobata **under polluted and non-polluted habitats. P-polluted,**

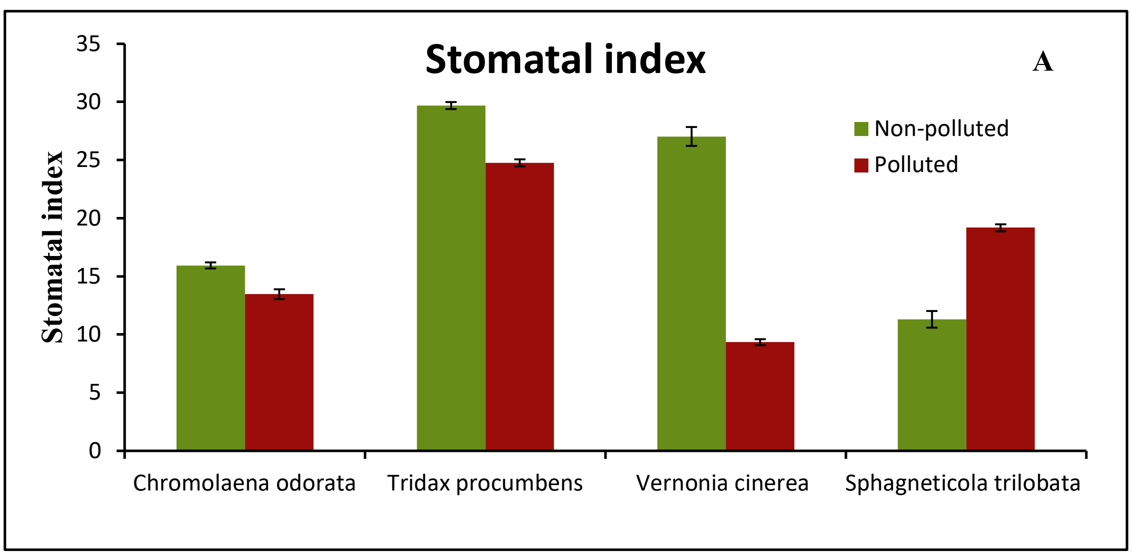
NP- non-polluted. ***The data is an average of recordings from three independent experiments each with three replicates (i.e. n=9). The data represent mean±standard error.***

Microscopic characters

The major microscopic characters which were analysed in the present project work include stomatal number, stomatal length, stomata index, length and breadth of subsidiary cells, length and breadth of guard cells, number of spongy and palisade cells and number of trichomes.

Diacytic type of stomata were found in the leaves of *Chromolaena odorata* while, anisocytic stomata were found in *Tridax procumbens, Vernonia cinerea* and *Sphagneticola trilobata*. Number of stomata found to be decreased in the plants which were exposed to pollution. Significant decline in the stomatal index of plants growing under polluted habitats were observed in the present research work. From the results it was clear that the reduction in stomatal index under polluted condition was higher in the case of *Vernonia cinerea* when compared to other plants studied.

The stomatal index was 27.03 in *Vernonia cinerea* under non polluted habitats and it decreased to 9.34 under vehicular polluted area of Kodungallur. Among the four plants studied, *Chromolaena odorata* showed least decrease in stomata index under polluted habitats. Stomatal index of this plant was 13.47 and 15.94 under under non-polluted and polluted habitats respectively (Figure 1A). Multicellular trichomes were present in all the plant species irrespective of their habitats. All the trichomes showed branching, except that of *Sphagneticola trilobata.* There was no variation in the number and distribution trichomes in *Vernonina cinerea, Tridax procumbens* and *Sphagneticola trilobata*. But, it was noticed that *Chromolaena odorata* showed significant increase in the number of trichomes in plants which were grown under polluted habitats when compared to those of non-polluted habitats (Figure 1B).



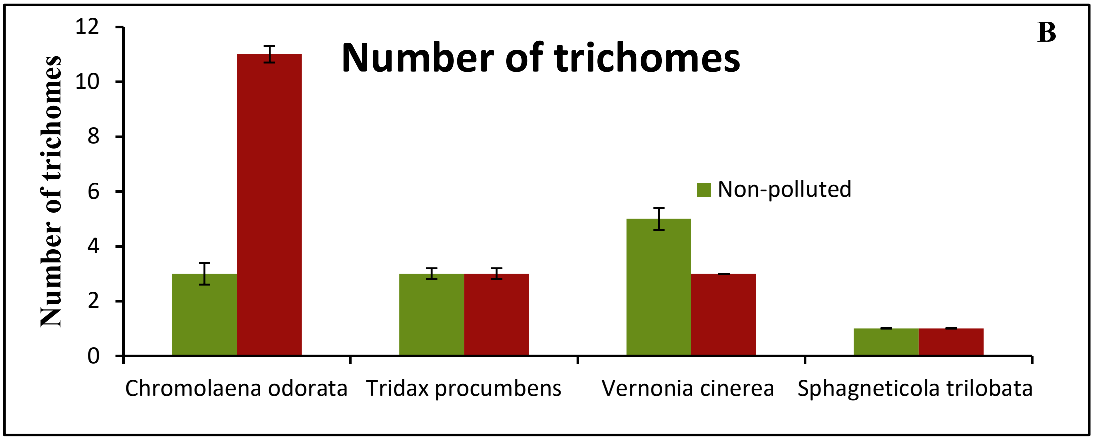
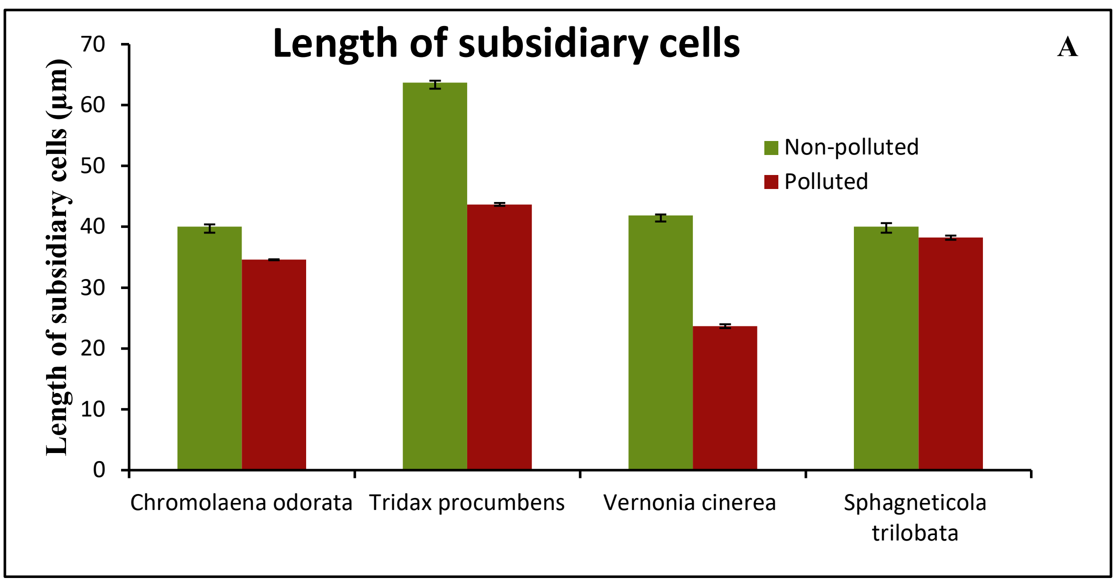


Fig.1: Stomatal index (**A**) and number of trichomes (**B**) of *Chromolaena odorata*, *Tridax procumbens, Vernonia cinerea* and *Sphagneticola trilobata* under polluted and non-polluted habitats. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

As far as the length and breadth of subsidiary cells were concerned, air pollution caused a significant reduction in the length and breadth of subsidiary cells. Length and breadth of subsidiary cells were measured in µm and the value ranged from 23.66 to 63.70 µm. The study revealed that the length of subsidiary cells was significantly high in unpolluted plants, with maximum length noticed in *Tridax* (unpolluted) and minimum in *Vernonia* (polluted) (Figure 2A).

In the case of breadth of subsidiary cells, the value ranged from 20.02 to 58.24 µm and the breadth of subsidiary cell also showed reduction in polluted plants. The maximum breadth of subsidiary cell noticed in *Vernonina cinerea* and least in *Chromolaena odorata.* From the results, it was clear that among the four plants studied, *Vernonia cinerea* showed high reduction in the size of subsidiary cells (Figure 2B).



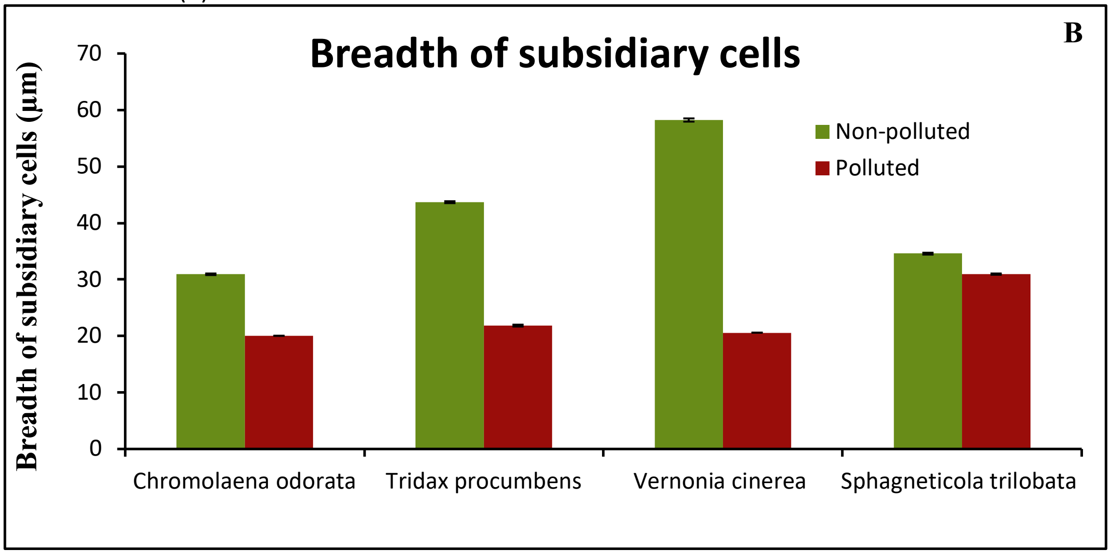
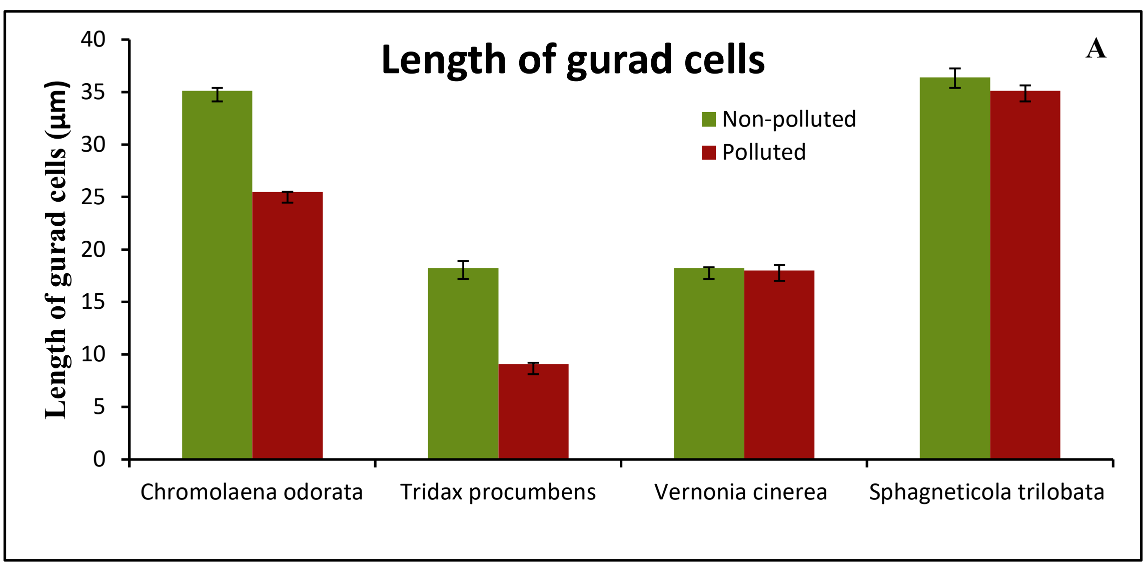


Fig.2: Length (**A**) and breadth (**B**) of subsidiary cells in *Chromolaena odorata*, *Tridax procumbens, Vernonia cinerea* and *Sphagneticola trilobata* under polluted and non-polluted habitats. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

In the case of length of guard cells, the value ranged from 9.1 to 36.4µm. The study revealed that the length of guard cell was significantly high in unpolluted plants with maximum length noticed in *Sphagneticola trilobata* and in *Vernonina cinerea* only a slight difference was noticed between polluted and unpolluted plants (Figure 3A).

In the case of breadth of guard cell, the value ranged from 10.74 to 25.48µm. From the study it was clear that the breadth of guard cell was similar in *Chromolaena odorata, Tridax procumbens* and *Sphagneticola trilobata* growing under non-polluted habitats (18.2µm). But in polluted habitats all the plants showed reduction in the breadth of guard cells (Figure 3B).



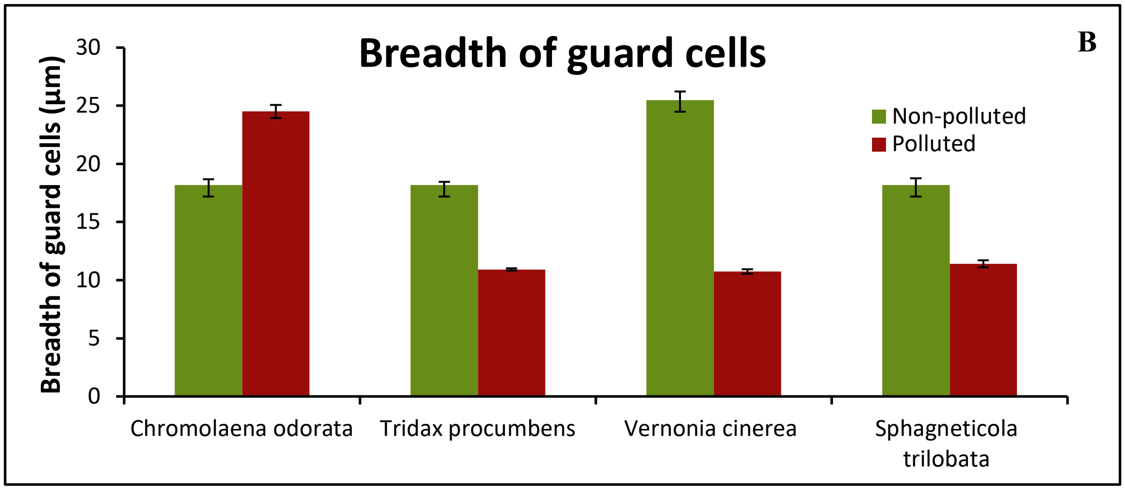
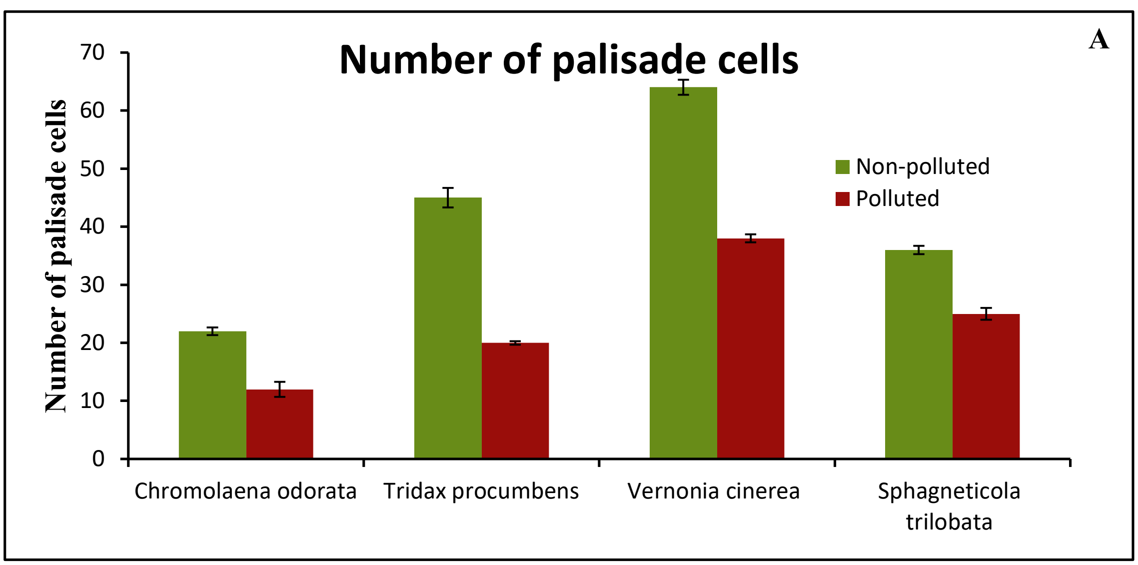


Fig.3: Length (**A**) and breadth (**B**) of guard cells in *Chromolaena odorata*, *Tridax procumbens, Vernonia cinerea* and *Sphagneticola trilobata* under polluted and non-polluted habitats. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

In the present study, mesophyll cells of all the four plants also showed variations under polluted habitats. *Vernonina cinerea* showed high reduction in the number of palisade cells and the number of palisade cells was 64 in the plants which were under non-polluted habitats while in the plants which were under polluted habitats it get reduced to 38.

Among the four plants studied, *Chromolaena odorata* showed least reduction in the case of number of palisade cells (Figure 4A). As far as the number of spongy cells were concerned, high reduction in number was observed in *Tridax procumbens* and it was low in the case of *Chromolaena odorata* (Figure 4B).



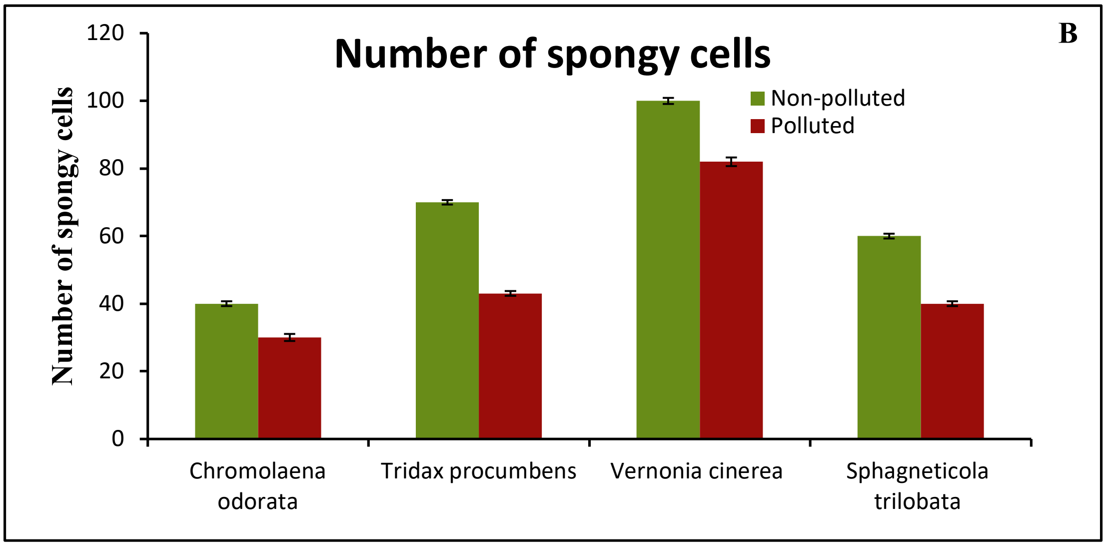


Fig.4: Number of palisade (**A**) and spongy (**B**) cells in *Chromolaena odorata*, *Tridax procumbens, Vernonia cinerea* and *Sphagneticola trilobata* under polluted and non-polluted habitats. The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates

Physiological parameters

The physiological attributes analysed in the present project work include photosynthetic pigment content. From the results it was clear that the air pollution significant caused reduction in Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content of the leaves in all the four plants selected. The maximum reduction in the photosynthetic pigment content was observed in *Tridax procumbens* when compared to other plants under study (Table 2).

Plants	***Chlorophyll a (mg/g fw)***		***Chlorophyll b (mg/g fw)***	
	Non-polluted	**Polluted**	**Non-polluted**	**Polluted**
Chromolaena odorata	469±5.2	255±2.8	471±4.1	190±1.2
Tridax procumbens	542±3.3	328±2.5	704±7.2	149±3.2
Vernonia cinerea	477±4.3	433±3.3	617±5.2	308±2.6
Sphagneticola trilobata	398±1.7	303±1.5	274±4.4	186±2.1
	Total chlorophyll (mg/f dw)		***Carotenoids (mg/g fw)***	
	Non-polluted	**Polluted**	**Non-polluted**	**Polluted**

Chromolaena odorata	939±3.1	444±3.4	129±5.5	79±0.6
Tridax procumbens	1243±9.1	476±1.2	146±3.6	95±1.6
Vernonia cinerea	1091±8.8	739±6.4	134±2.1	120±2.3
Sphagneticola trilobata	670±4.2	489±3.2	108±4.4	86±1.2

Table 2: Photosynthetic pigment content of leaves selected plants under polluted and non-polluted habitats. The data is an average of recordings from three independent experiments each with three replicates (i.e., n=9).

.Discussion

Leaves are the important organs of plants concerned with photosynthesis. These leaves are the immediately exposed to different types of environmental pollutions. In the present project work, it was found that the size of leaves exposed to air pollution get reduced when compared to non-polluted plants. This implies the adaptive modification of these plants under stressed conditions. Stomata play an important role in plant life. Stomatal study is emphasized to know the nature and behaviour of plants in response to air pollution. Low stomatal frequency has been observed in response to polluted air. Similar results were obtained in the present project work also. From the results it was evident that among the four plants studied, *Chromolaena odorata* showed least reduction and *Vernonina cinerea* showed highest reduction in the stomatal index under pollution. This implies the tolerance nature of *Chromolaena odorata* and the sensitive nature of *Vernonina cinerea* as far as stomatal index data was concerned. This result was in accordance with Pawar and Dubey (1982), where, the stomatal index reduced considerably on both the lower and upper surface of leaves under air pollution. As far as the trichomes were concerned, it was found that was no variations in the number of trichomes except in *chromolaena odorata* where about four fold increase in the number of trichomes were observed in the plants under polluted habitats. This again indicates the stress tolerance nature of this species which is trying to avoid the air pollution by producing more number of thrichomes. The shape of the trichomes were different in all the four general selected.

In the present investigation, the size of subsidiary cells and guard cells were found to be decreased in plants which were grown under polluted habitats. This may be an adaptive mechanism of the plants toward their stress tolerance or it may be due to the reduced metabolic efficiency of the plants under air pollution. From the results it was clear that *Vernonina cinerea* from polluted habitats showed maximum variation in the size of guard cells and subsidiary cells when compared to those of plants grown under non-polluted habitats. This again implies the less tolerance nature of *Vernonina cinerea.* Similarly, the number of spongy and palisade cells are found to be low in the plants which were collected from polluted area. These results are in conformity with the study of Iqbal (1985) as well as Jahan & Iqbal (1992), who showed significant reduction in thickness of the cuticle, epidermis, hypodermis, palisade and spongy parenchyma cells in polluted leaves as compared to leaves collected from non-polluted areas. Recently, Stevovic *et al*. (2010) reported that Tansy plants grown in polluted areas showed reduction in the thicknesses of mesophyll parenchyma of upper and lower epidermal tissues.

Photosynthesis is an important physiological process which occurs in leaves. Air pollution significantly alters this important process in plants. Chlorophyll *a* and carotenoids were more severely affected than chlorophyll *b*. This could be due to the induced chlorophyllase activity or inhibition of chlorophyll *b* synthesis.

Summary and Conclusion

In the present research work, foliar characters of *Chromolaena odorata, Sphagneticola trilobata, Vernonia cinerea* and *Tridax procumbens* under polluted and non-polluted habitats were compared. The plants were collected from air polluted and non-polluted habitats. Since the plants are the major receptors of air pollutants and, therefore, they carry a major portion of pollution load along the highways, they show various adaptations when compared to those plants which were grow under normal environmental conditions.

The various parameters examined in the research work include size of stomata, stomatal index, number of trichomes, number of palisade and spongy cells, concentration of different photosynthetic pigments of plants growing in polluted and non-polluted habitats. From the results of the research work it was confirmed that the air pollution caused significant changes in the foliar characters of all of the plants. Air pollution caused reduction in the size of leaves and also reduced the stomatal index of leaves.

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Received: 12th February 2018

Revised and Accepted: 15th April 2018

Published: 30th June 2018