Variation in Some Structural Features and Metal Ion Accumulation at Different Developmental Stages of *Triumfetta rhomboidea* under Environmental Stress

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ABSTRACT

Micro-morphological responses of *Triumfetta rhomboidea* Jacq. to coal-smoke pollution were studied at pre-flowering, first flowering and post-flowering stages of plant development at polluted and non-polluted sites. Size of stomatal apparatus and stomatal aperture on the upper as well as lower epidermis showed a decreasing trend with successive stages of plant development at both study sites. The values were higher for the upper epidermis and relatively low for the lower epidermis at the polluted site than at the reference site. The stomatal density (SD) and stomatal index (SI) in upper as well as lower epidermis decreased with successive stages of development. Under pollution stress, both SD and SI on the upper epidermis, and SD on the lower epidermis, were significantly higher than in normal atmospheric condition at all three stages, whereas SI on the lower epidermis showed irregular variation. Trichome density increased with the successive stages in plant development at both the locations, the values at the polluted site being lower than at the normal site up to flowering stage and higher during the post-flowering phase on the upper epidermis. The reverse was the case for the lower epidermis. Wood fibre length in root and stem decreased, while the length and width of vessel elements increased with advancing plant development at both the polluted and normal sites. However, these cell dimensions were invariably larger at the polluted than at the normal site. Long fibres and wide vessel elements relate to the mechanical strength and the water-conducting capacity of wood, and occurrence of these features may help plant survival under harsh conditions. The accumulation of zinc, copper and iron in roots, stem and leaves showed different trends with reference to plant development and environmental stress. Zinc content generally increased, while the copper and iron contents generally declined, with minor vicissitudes, at successive stages at both the normal and polluted sites.

Key words: Coal-smoke pollution; medicinal plant; metal content; stomatal apparatus; vessel element; wood fibre

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Introduction

Plants are sensitive to environment, and the degree of their sensitivity has a decisive role in their distribution all over the globe. Thermal power plants are a major source of air pollution in India. Most of these are coal-based and emit into the atmosphere a complex mixture of several gases such as CO_2, SO_2, HF, NOx and the fly ash, which result from coal combustion (Nighat et al., 2008). The major gaseous components (SO_2 and NO_2) are injurious to plants, adversely affecting their physio-chemical properties and even structural features. High SO_2 concentrations are phytotoxic and disturb stomatal distribution and behavior, photosynthesis, transpiration and the formation of secondary metabolites, and thus reduce the overall yield (Dhir et al., 2001; Wali et al., 2004, 2007; Ali et al., 2008). In SO_2-exposed plants, sulphur entry occurs mainly in the aerial parts through open stomata on leaves (Iqbal et al., 2005). Once in the

mesophyll, SO_2 readily dissolves in aqueous phases, forming sulphurous acid with dissociation products as sulphite, bisulphite and protons (Rennenberg & Herschbach, 1996). The sulphite and bisulphite anions are phytotoxic. Fly ash, an inorganic by-product of coal combustion in the boilers of thermal power plants, consists of dehydrated and dissociated clay mineral impurities along with small amounts of unburnt coal and forms part of the smoke. Its major constituents are Al_2O_3, SiO_2, FeO_3, CaO, $CaSO_4$, H_2O and trace elements, some of which (like Mo, Hg, Se and Cd) are highly toxic (Singh & Yunus, 2000).

We have studied physio-chemical and morpho-anatomical features of diverse plant species from areas shown to be affected by coal-smoke pollution caused by thermal power plants (Dhir et al., 1999; Husen et al., 1999; Singh et al., 2000; Trag et al., 2001, 2002; Gupta & Iqbal, 2005; Verma et al., 2006; Iqbal et al.,

2010a, 2010b, 2011; Mahmooduzzafar et al., 2010). Physio-chemical responses of the medicinally important *Triumfetta rhomboidea* to pollution stress have been published earlier (Iqbal et al., 2010b). The present article examines the impact of coal-smoke pollution on some anatomical characteristics and the pattern of metal ion accumulation in tissues of different plant parts at various phenological stages of this species.

Material and Methods

Site Description

Material for the present study was collected from Jamia Hamdard campus (non-polluted, reference site) and from around the Kasimpur thermal power plant complex (polluted site) in Aligarh district. Aligarh is located in the north-west of Uttar Pradesh (a northern state of India) in the Ganga-Yamuna doab, between 77°29′ and 78°38′ E longitude. Kasimpur town (the study site) lies about 187 meters above sea level. The power plant at Kasimpur consisted of three power stations having a capacity of 90 MW, 210 MW and 230 MW electricity generations respectively. The complex runs on the bituminous coal that has 2.92% moisture, 22.20% ash, 31.86% volatile matters including 0.49% sulphur, 5.61% hydrogen, 5.24% nitrogen, 20.23% oxygen and 42.45% fixed carbon on an average. About 4194 metric tons coal per day was burnt during the period of study, giving rise to emissions of approx. 0.0169 ppm h^{-1} SO_2, 0.300 ppm h^{-1} NO_x and 6.854 ppm h^{-1} CO_2 (Table 1). The soil of the sites is of a loam and clayey loam type, with high pH (8–8.5) and a poor drainage system. The area experiences dry tropical monsoon climate.

The areas around the Kasimpur thermal power plant complex were surveyed in different seasons for grassland vegetation. The gross community structure at the selected site, with various bushes and some trees planted in patches, remained almost uniform throughout the year, although components of the community used to change with season. Samples were collected from areas about 6 km away from power-plant complex, where coal-smoke emissions from chimneys of the power plant affect the vegetation maximally (Iqbal, 1981; Mahmooduzzafar & Iqbal, 2000).

Plant Description and Sampling

Triumfetta rhomboidea Jacq. (Tiliaceae), an under-shrub with stellate pubescence and spiny fruits, grows wild in areas around the Kasimpur power plant. Young plants of *T. rhomboidea* were collected from the polluted and non-polluted (reference) sites at three phenological stages, *viz.* during the vegetative growth of young plants prior to the first flowering (pre-flowering stage), during the first flowering period (flowering stage), and after the flowering phase was over (post-flowering stage). At each of these phenological stages, three plants were collected from polluted as well as reference sites. Five slides prepared from each of the three plants were examined microscopically to know the effect of pollutants released from chimneys of the power plant on the leaf epidermal tissue and the root and stem woods. At least ten readings were taken from each slide so that n = 3×5×10 = 150. The study was repeated next year and the results showed no significant difference.

Studies of Epidermal Cells

Epidermal peels obtained from five mature leaves of each of the three plants were treated with hot HNO_3 (Ghouse & Yunus, 1972), processed in the customary ethanol series for dehydration and stained with safranin, and then mounted in Canada balsam on glass slides for microscopic study. Stomata, trichomes and normal epidermal cells were counted per microscopic field and measured with the help of an ocular micrometer scale. Density of stomata and trichomes was estimated in relation to epidermal-cell population and expressed in percentage. Stomatal index (SI) was calculated using the Salisbury's formula,

$$SI = \frac{S}{S + E} \times 100,$$

where S and E represent the number of stomata and epidermal cells per mm^2, respectively.

Measurement of Wood Cells

Root and stem of the three collected plants were fixed in FAA [ethyl alcohol: formaldehyde: glacial acetic acid (5:5:90)] for a week, and then preserved either in alco-glycerol mixture (1:1 mixture of 70% ethyl alcohol and glycerol) or in 70% ethyl alcohol only, depending on the hardness of the material. To study the cell size of fibres and vessel elements slices of wood obtained from the third stem internode and from the basal part of root were macerated in hot HNO_3 following the method of Ghouse and Yunus (1972). The macerated fibres and vessel elements were measured with the help of ocular micrometer scale attached to a compound light microscope.

Table 1. Average amounts of major gases released from Kasimpur thermal power plant in different seasons of a year. The gases were measured at a height of 8 m in the stack*.

Season	SO_2		NO_x		CO_2	
	t ha^{-1}	ppm ha^{-1}	t ha^{-1}	ppm ha^{-1}	t ha^{-1}	ppm ha^{-1}
Winter (Nov-March)	18.86	0.019	337.95	0.338	13217.00	13.217
Summer (April-June)	16.69	0.017	299.06	0.299	2304.49	2.307
Monsoon (July-October)	14.25	0.014	255.25	0.255	2311.37	2.312
Annual average	16.78	0.017	300.66	0.300	6853.81	6.854

*Data obtained from office of Kasimpur Power Plant

Estimation of Metal Content

Dried samples of root, stem and leaves of the plants sampled were digested using HNO_3 and $HCIO_4$, following the method of Miller (1998). The digested material was filtered in 100 ml volumetric flask and the volume made up to 100 ml by adding double distilled water. The contents of Zn, Cu and Fe were determined in each sample by using the atomic absorption spectrophotometer and expressed in ppm, as in earlier works (Khudsar et al., 2008; Ansari et al., 2013; Siddiqui et al., 2013).

Student's *t*-test was applied to the data obtained, in order to confirm whether the differences observed between the polluted and the non-polluted plant materials were statistically significant (Khan & Khanum, 2009).

Results

Epidermal Responses

The length and width of stomata in both the upper (adaxial) and lower (abaxial) epidermises showed a decreasing trend with progressive plant development at both the polluted and reference sites, with the maximum values at the vegetative (pre-flowering) stage, slightly less during flowering and the minimum at the post-flowering sage. On the upper epidermis, both the length and width of stomata were larger under pollution stress, than at the reference site, with the difference being statistically significant at the flowering stage only. The stomatal length and width at the pre-flowering stage was 31.9 and 22 μm respectively at the polluted site, and 29.5 and 20.7 μm respectively at the reference site. The same pattern of variation was noted for stomatal aperture, with the maximal values (25.7 μm at the polluted sites; 23 μm at the reference site) recorded at the pre-flowering stage (Fig. 1). On the lower epidermis, in contrast, the length and width of stomatal apparatus as well as the stomatal aperture were smaller at the polluted site than at the reference site.

The largest values for length (28.3 and 29.5 μm) and width (22.2 and 24.3 μm) at the polluted and reference sites, respectively, were recorded at the pre-flowering stage. The maximum difference in stomatal length and stomatal width was observed at the pre-flowering and post-flowering stages respectively. The stomatal aperture on both the upper and lower epidermises displayed a decreasing trend with plant age at the polluted as well as the reference sites. The difference was significant at the flowering stage in the upper epidermis and pre-flowering stage in the lower epidermis. Overall, the aperture was larger on the upper epidermis than on the lower epidermis (Fig. 1).

The stomatal density (SD) and stomatal index (SI) in upper as well as lower epidermis decreased with advancing phenology at both the polluted and non-polluted sites, the difference among the pre-flowering, flowering and post-flowering stages of plant being significant at the reference site but non-significant at the polluted site. On the upper epidermis, the maximum value of SD and SI noted in the pre-flowering stage were 38% and 99.4 respectively at the polluted site, and 27% and 74.2 respectively at the reference site. On the lower epidermis, these values were 45% and 101.4 respectively at the polluted site, and 41% and 91 respectively at the reference site. In general, the values were higher under the pollution stress than at the reference site, except for the SI in lower epidermis, which depicted higher values at the pre-flowering and flowering conditions at the reference site. The difference in stomatal density due to pollution stress was significant at all the three stages of plant development in the upper epidermis, but non-significant in the lower epidermis. As regards the stomatal index, in both the upper and lower epidermises it was non-significantly higher due to pollution stress in the pre-flowering and flowering stages but significantly higher in the post-flowering stage, compared to the reference site (Fig. 2).

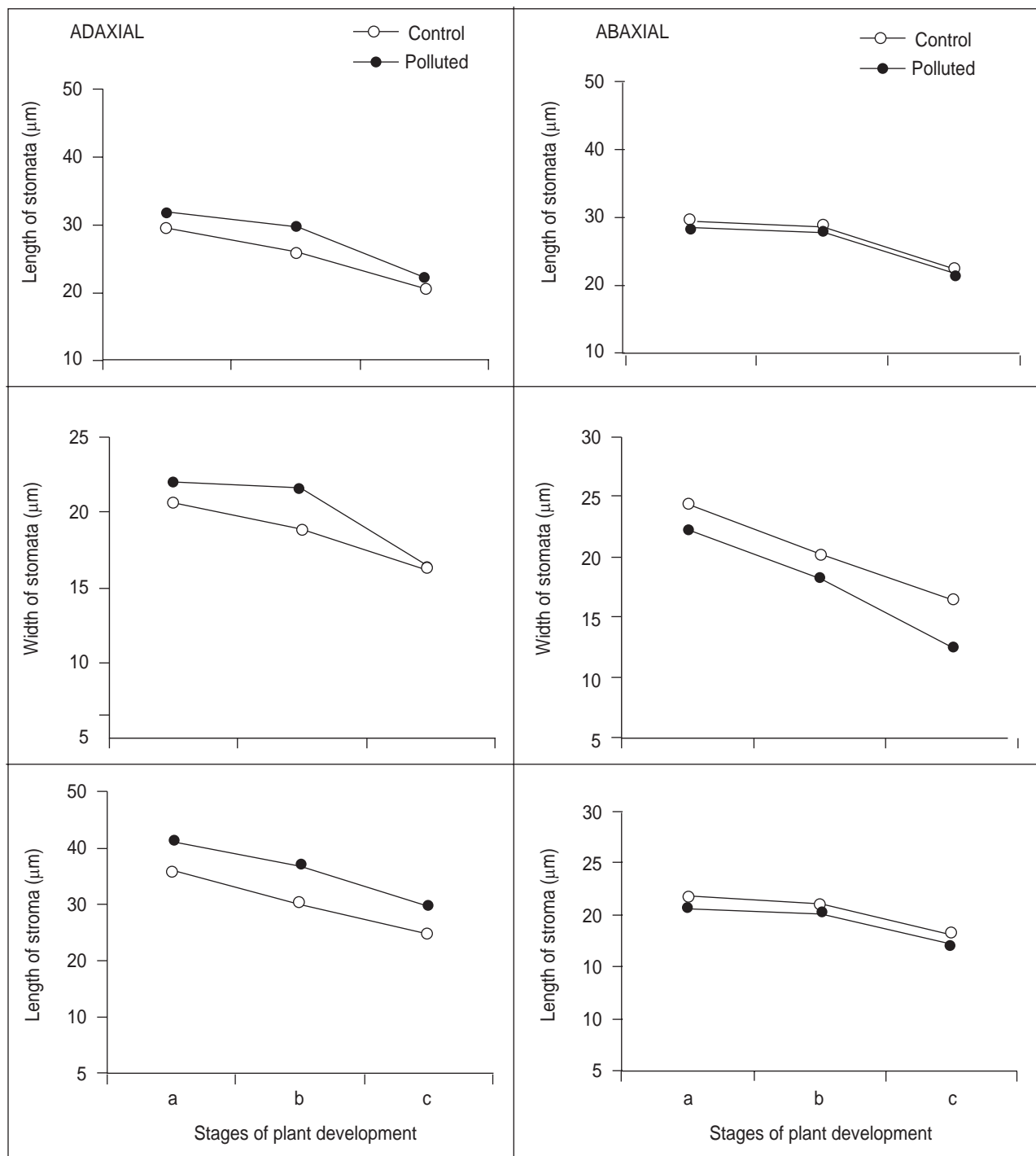Under normal conditions, the density of epidermal

Figure 1. Changes in stomatal dimensions on the adaxial and abaxial epidermises of leaves of *T. rhomboidea* plants under normal and polluted atmospheric conditions, as observed during the (A) pre-flowering; (B) first flowering; and (C) post-flowering phases.

cells (number per microscopic field) of both (upper and lower) epidermal layers increased with advancing plant development. Compared to normal condition, their density in the upper epidermis was non-significantly lower under pollution stress at the pre-flowering and post-flowering stages. However, density was significantly higher at the flowering stage. On the lower surface,

the epidermal cell density was significantly higher at the polluted site in the first two stages only, and was significantly lower at the post-flowering stage. Percent variation was the maximum at flowering stage in the upper epidermis and pre-flowering stage in the lower epidermis (Fig. 3).
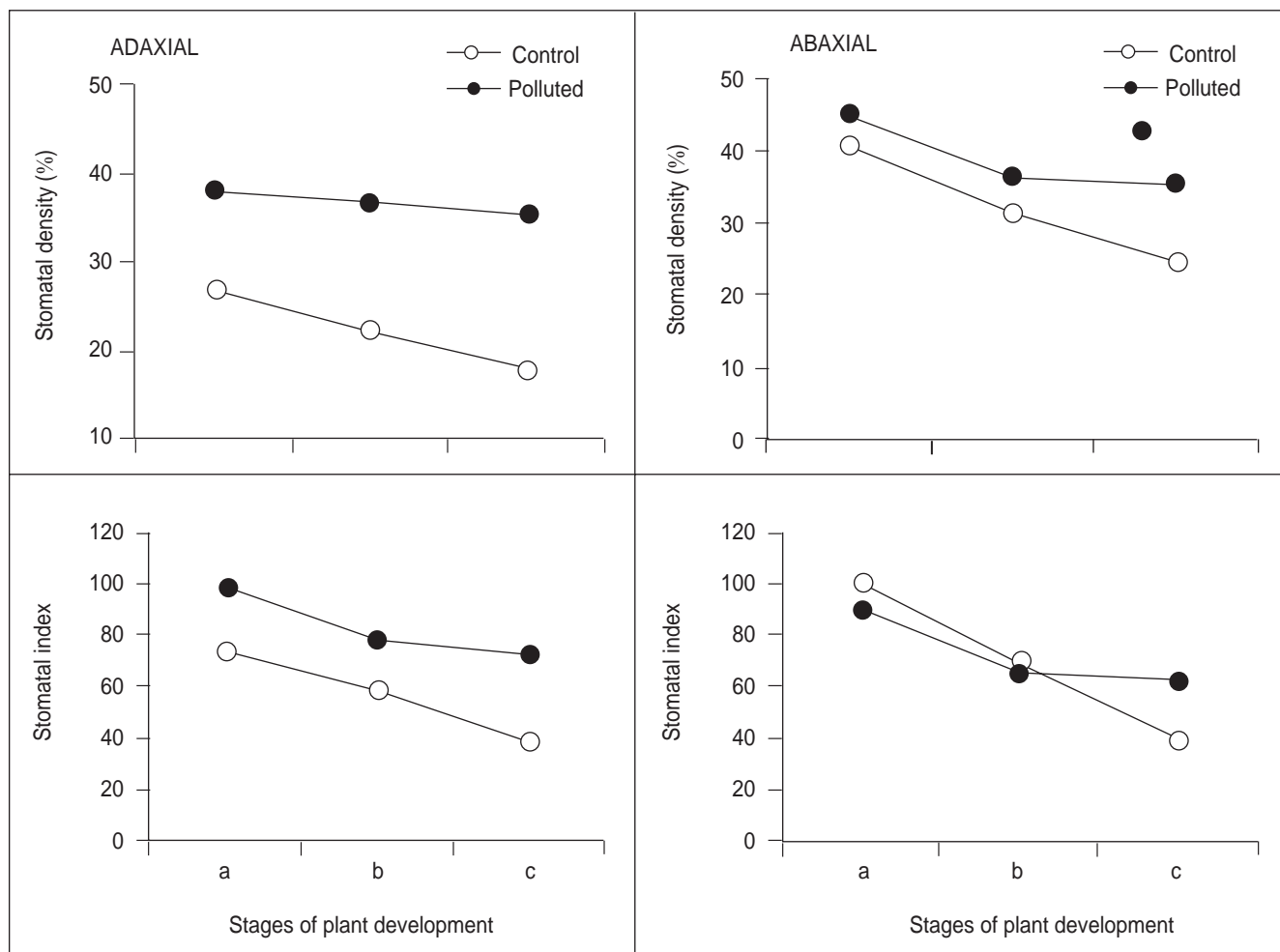
Figure 2. Changes in stomatal density and stomatal index on the adaxial and abaxial epidermises of leaves of *T. rhomboidea* plants under normal normal and polluted atmospheric conditions, as observed during the (A) pre-flowering; (B) first flowering; and (C) post-flowering phases.

Trichome density on both the epidermal layers increased with advancing stages of plant development, at both the polluted and non-polluted sites. At the polluted site, the rise in density was steep (from 20% up to 42%) in the upper epidermis. The extent of variation from the reference site decreased in successive developmental stages of the plant. On the lower epidermis, the pollution stress caused the trichome density to increase slightly at the pre-flowering stage and markedly at the flowering stage, but the density came down marginally in the post-flowering stage, in comparison to the control. The maximum variation from the control was recorded during the flowering stage (Fig. 3).

Vessel Element and Fibre Cell Size

Fibre length in root and stem was found to decrease with advancing plant development at both the collection sites. However, the fibres were significantly longer in all the corresponding collections from the polluted site, compared to the non-polluted site. The maximum variation was recorded at the post-flowering stage (Tables 2, 3). On the contrary, the length of vessel elements increased gradually throughout the course of plant development at both the collection sites. It was always greater at the polluted site than at the reference site, and the difference was significant. Variation from the control was the maximum at pre-flowering stage. Vessel width also showed a similar variation trend. However, the increase in vessel width due to pollution stress was non-significant in the first two samplings of roots and in the first one sampling of the stem. Percent variation showed an increasing trend with advancing plant development (Tables 2, 3).

In roots, the fibre length declined from 931.29 μm (pre-flowering stage) to 655.73 μm (post-flowering stage), whereas the length and width of vessel elements
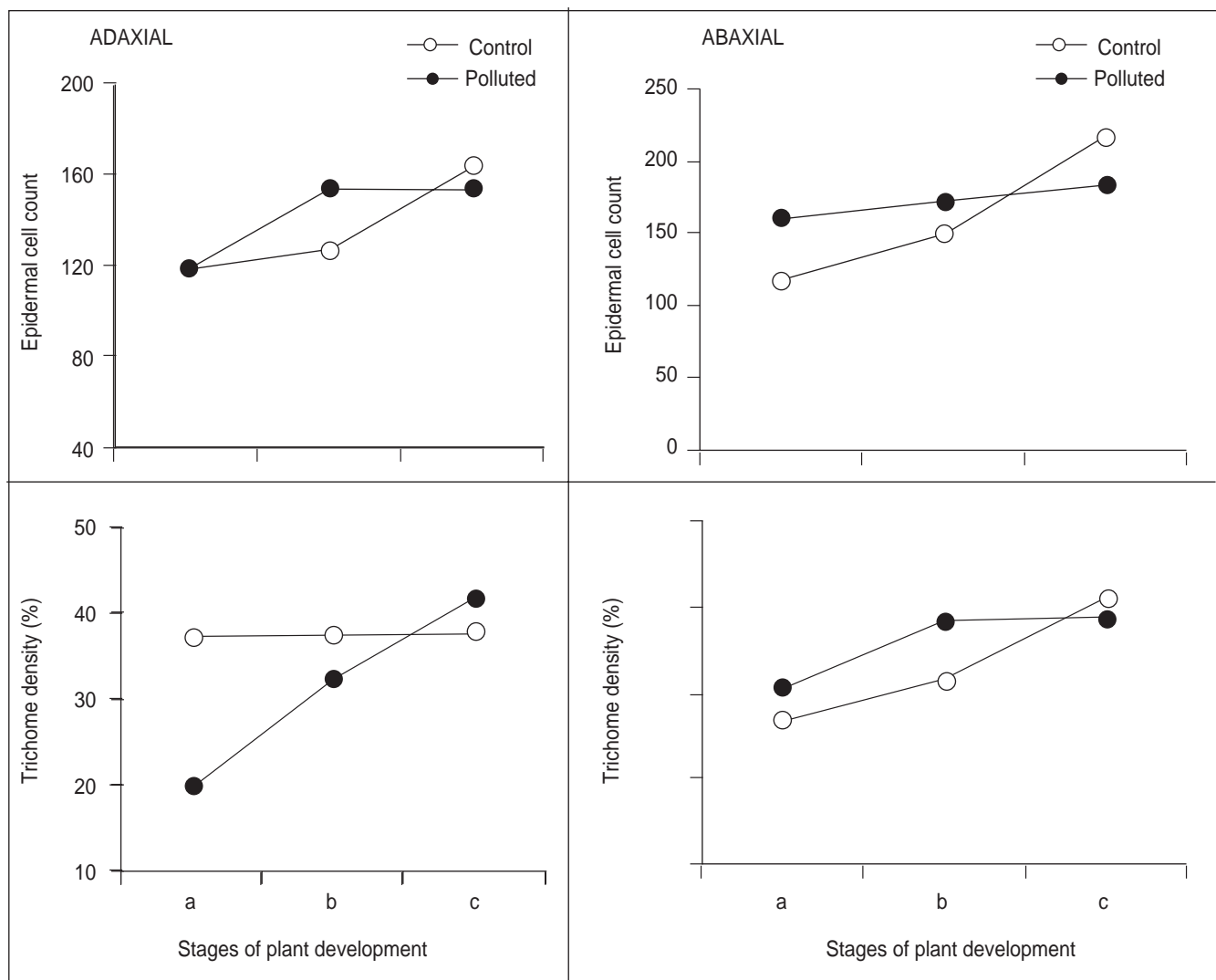
Figure 3. Changes in epidermal cells (per microscopic field) and trichome density on the adaxial and abaxial epidermises of leaves of *T. rhomboidea* plants under normal and polluted atmospheric conditions, as observed during the (A) pre-flowering; (B) first flowering; and (C) post-flowering phases.

increased from 186.42 to 201.58 µm and from 57.62 to 60.97 µm respectively at the reference site. Under polluted conditions, all these dimensions were relatively larger at each developmental stage (Table 2). In the stem also, variation trends in the length of fibres (945 to 758 µm) and in the length (115.21 to 186.59 µm) and width (26.31 to 37.48 µm) of vessel elements, due to plant developmental stage and pollution stress, were similar to those in the root. The difference between values at the polluted and the reference sites was significant throughout, except for the vessel width at pre-flowering stage (Table 3).

Elemental Analysis

At the reference site, concentration of zinc in roots (1.6 to 3.2 ppm), stem (3.2 to 6.4 ppm) as well as

in leaves (0.38 to 5 ppm) normally increased with progress in plant development, thus attaining its maximum at the post-flowering stage. Even under the pollution stress, the pattern of variation was similar except in the stem, where it increased up to flowering but declined in the post-flowering phase. Compared with the control, zinc concentration in roots lowered due to pollution stress at each of the three stages of plant development, the highest loss occurring at pre-flowering and flowering stages. In the stem, however, zinc level increased due to pollution, at the first two stages, but kept relatively low at the post-flowering stage. In leaves, the concentration at the polluted site was slightly higher at pre-flowering stage but lower in the subsequent stages, showing the maximum difference at the flowering stage (Fig. 4).

Table 2. Dimensions of wood cells in the root of *T. rhomboidea* plants growing under normal as well as polluted atmospheric conditions. The values represent a mean of 150 readings.

Parameter	Control	Polluted	% Variation
Fibre length (µm)			
Pre-flowering	931.29 ± 94.15	968.39 ± 98.64	3.98%**
First flowering	797.04 ± 83.14	825.05 ± 93.64	3.51%*
Post-flowering	655.73 ± 79.63	719.87 ± 91.29	9.78%**
Vessel element length (µm)			
Pre-flowering	186.42 ± 39.02	235.86 ± 34.09	26.52%**
First flowering	197.56 ± 51.94	236.79 ± 42.62	19.85%**
Post-flowering	201.58 ± 45.08	248.98 ± 42.55	23.51%**
Vessel width (µm)			
Pre-flowering	57.62 ± 7.12	59.30 ± 7.19	2.91%NS
First flowering	58.80 ± 6.62	60.97 ± 5.49	3.69%NS
Post-flowering	60.97 ± 5.33	65.64 ± 4.40	7.65%**

* Significant at 5% level;   ** Significant at 1% level;   NS=Non-Significant

Table 3. Dimensions of wood cells in the stem of *T. rhomboidea* plants growing under normal or polluted atmospheric conditions. The values represent a mean of 150 readings.

Parameter	Control	Polluted	% Variation
Fibre length (µm)			
Pre-flowering	945.00 ± 84.25	980.62 ± 83.26	3.76%**
First flowering	833.20 ± 93.61	855.30 ± 80.23	2.65%*
Post-flowering	758.03 ± 91.38	790.23 ± 86.24	4.24%**
Vessel element length (µm)			
Pre-flowering	115.21 ± 51.33	150.24 ± 45.15	30.4%**
First flowering	141.92 ± 37.22	165.80 ± 32.76	16.82%**
Post-flowering	186.59 ± 29.70	206.25 ± 42.29	10.53%**
Vessel width (µm)			
Pre-flowering	26.31 ± 5.10	28.30 ± 5.95	7.56%NS
First flowering	28.63 ± 5.75	33.64 ± 5.05	17.49%**
Post-flowering	37.48 ± 6.99	40.60 ± 5.24	8.32%*

* Significant at 5% level;   ** Significant at 1% level;   NS=Non-Significant

Under normal atmospheric conditions, copper content in the three plant parts showed dissimilar variation patterns; in roots, it declines up to flowering stage and then becomes almost constant; in the stem, it increases up to flowering stage and then becomes almost constant, while in leaves it keeps declining up to the post-flowering stage. Under pollution stress, it follows similar variation patterns but at different developmental stages, e.g., a consistent decline in roots, an increase followed by a decline in the stem, and a decline followed by constancy in leaves (Fig. 5). Further, copper content in all the three organs was higher, i.e., 0.3 ppm in roots and leaves and 0.8 ppm in the stem, at the flowering stage under the pollution stress than in normal conditions. The difference between the copper content of the normal and polluted materials

was maximum during the flowering phase in all the three organs studied (Fig. 5).

On the contrary, the iron content in roots (38 to 20 ppm), stem (50 to 20.5 ppm) and leaves (33.2 to 26.5 ppm) showed a declining pattern with advancing plant development at the reference site. At the polluted site, it showed a similar variation pattern in roots and stem, but in the case of leaves it rose up to flowering and then declined in the post-flowering phase. Overall, the iron content was higher at the polluted site than at the reference site except at post-flowering stage in stem and pre-flowering stage in leaves. The maximum difference in iron content between the reference and polluted sites was observed at pre-flowering stage in roots, flowering stage in the stem and post-flowering stage in leaves (Fig. 6).
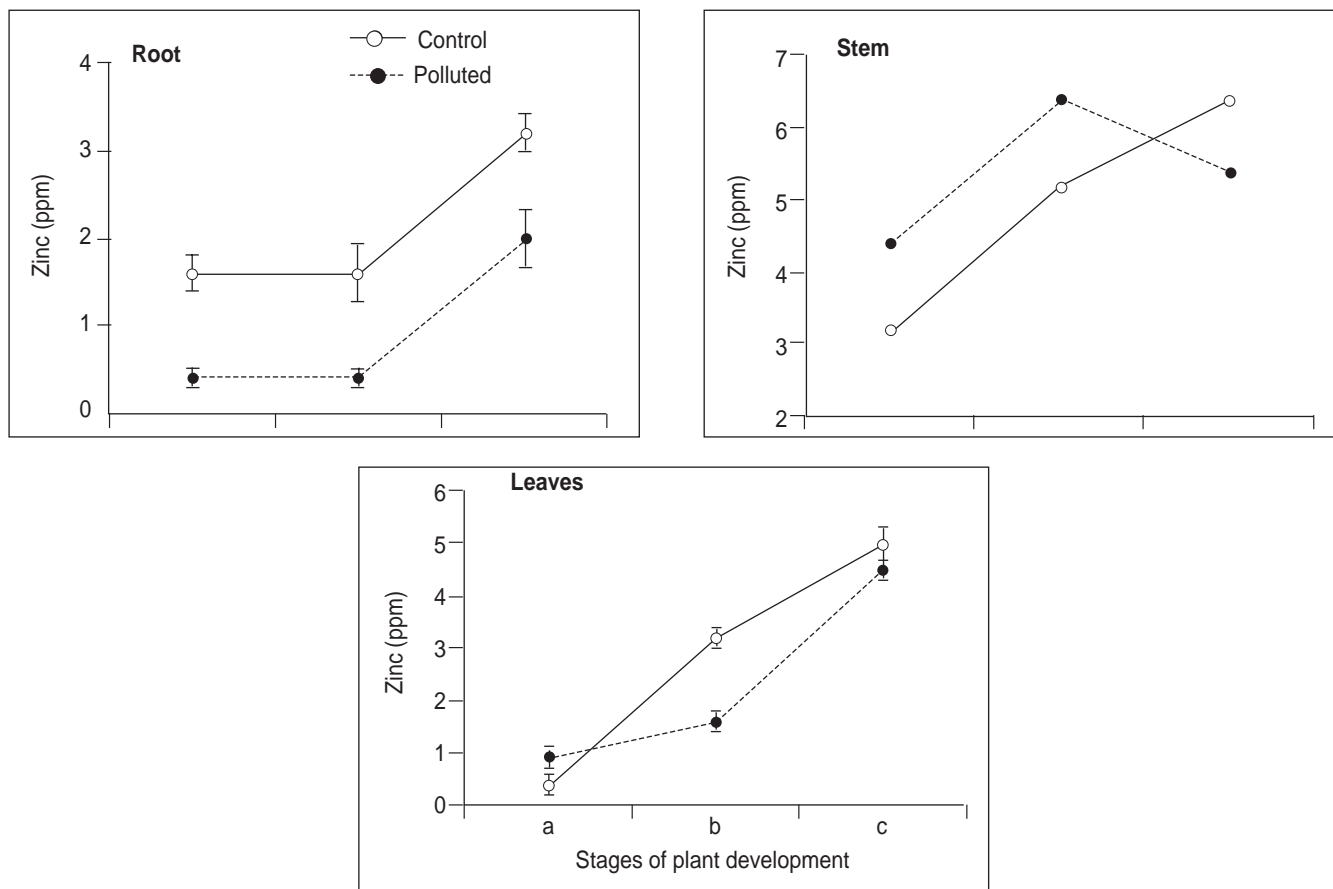
Figure 4. Changes in zinc content (ppm) in roots, stems and leaves of *T. rhomboidea* plants under normal and polluted atmospheric conditions, as recorded during the (A) pre-flowering; (B) first flowering; and (C) post-flowering phases.

Discussion

The stomatal apparatus in *T. rhomboidea* was longer and wider, and the stomatal aperture (stoma) was longer on the upper epidermis under pollution stress than under normal condition, whereas these features on the lower epidermis were not significantly different. No specific reason can perhaps be ascribed convincingly to this curious situation unless the morpho-physiological correlations of the leaf architecture are fully understood with reference to ecological compulsions. Evidence suggests that variations in the pollution-resistance capacity of different plant species are closely linked to stomatal size, which has a bearing on stomatal conductance (Mehindirata et al., 1999; Khudsar et al., 2000). Jensen & Kozlowski (1975) demonstrated that more SO_2 was absorbed by *Fraxinus americana* leaves with large stomata (high stomatal conductance) than by leaves of *Acer saccharum* with small stomata (low stomatal conductance), Similar results on leaf conductance value of ten species (trees and shrubs) in California provided a good index of SO_2 uptake and

resistance (Winner et al., 1982). SO_2 reduces stomatal opening, thus causing a general water stress in plants, which leads to a reduced photosynthesis, increased respiration and overall stunted growth (Iqbal et al., 2010b; Gheorghe & Ion, 2011). SO_2 of the polluted atmosphere enters leaves through stomata, spreads into the intercellular spaces of the mesophyll and combines with water to form sulphurous acid, sulphate and further to sulphite, thus causing accumulation of sulphur content in the affected leaves (Biscoe et al., 1973; Ali et al., 2008). Sulphite plays a vital role in the opening and closing of stomatal aperture. It may reduce the malate content of leaves, which has a close correlation with the size of stoma (Kondo et al., 1984), as it inhibits PEP carboxylase and NADP-malate dehydrogenase (Ziegler, 1974) involved in malate formation.

The present investigation reveals that stomatal density (SD) on *T. rhomboidea,* leaves was significantly higher at the polluted site than at the normal site on both surfaces of leaves at all stages sampled. The effect was more prominent during the post-flowering phase.
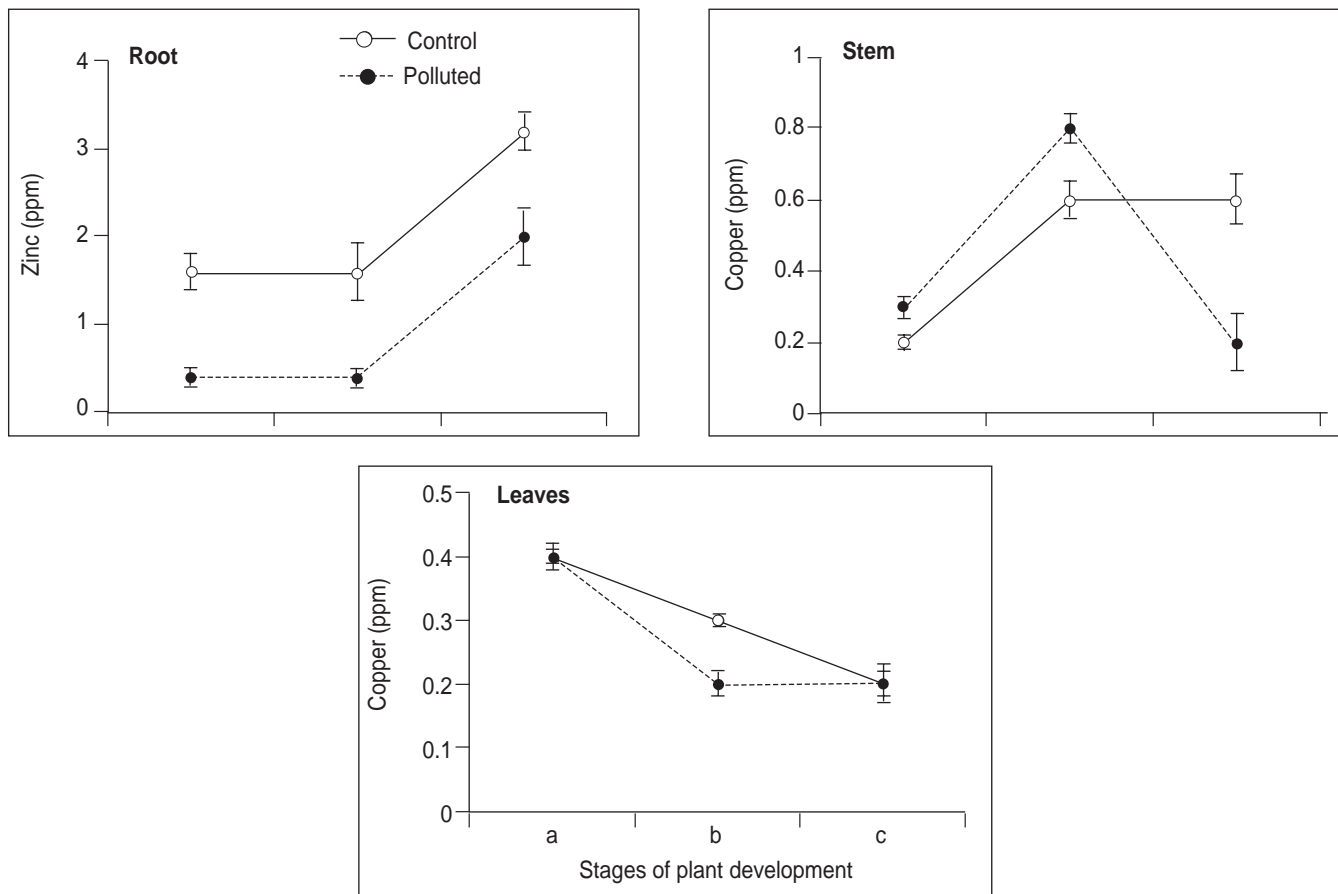
Figure 5. Changes in copper content (ppm) in roots, stems and leaves of *T. rhomboidea* plants under normal and polluted atmospheric conditions, as recorded during the (A) pre-flowering; (B) first flowering; and (C) post-flowering phases.

Formation of more stomata under the environmental stress may be part of the plant strategy to resist the environmental harshness by increasing the stomatal conductance to ensure availability of enough CO_2 for use in photosynthesis. The elevated CO_2 and other environmental variables may cause increases or decreases in SD in different plant species. SD was found to decrease in *Andropogon gerardii* and increase in *Salvia pitcheri* under elevated CO_2 (Knapp et al., 1994). It was reduced in *Peristrophe bicalyculata* under air pollution stress (Nighat et al., 1999). Ceulemans et al. (1995) found a reduced adaxial as well as abaxial SD under elevated CO_2 in expanding leaves of the upper portion of poplar clones, but no such effect appeared on mature leaves of the middle and lower parts of the plant axis.

In the present study, stomatal index (SI) was higher at the polluted site at all stages on both surfaces of leaves, with the difference from the reference site being greater on the upper epidermis than on the lower one. Earlier, a reduced SI was observed under elevated

CO_2 in the expanding leaves of the upper portion of a few clones of *Populus deltoides* and *P. trichocarpa* (Ceulemans et al., 1995). The SI in *Ruellia tuberosa* and *Calendula officinalis* was low under elevated CO_2 and SO_2 during the spring season (Nighat et al. 2000; Wali et al., 2007). It may also vary with the position on the leaf on the plant axis, as in *Lolium perenne* cv. Vigor (Ferris et al., 1996). However, comparative significance of stomatal traits remains ambiguous. Some reports attach importance to stomatal density, as in certain trees of China including *Buxus megistophylla, Populus tomentosa, Robinia pseudoacacia* and *Salix matsudana* (Zhang et al., 2017), while others to stomatal size, as in some conifers (*Cunninghamia lanceolata* and *Pinus massoniana*), and broad-leaf trees including *Broussonetia papyrifera, Ginkgo biloba, Platanus orientalis* and *Magnolia soulangeana* (Liang et al., 2016).

In *T. rhomboidea,* there was a gradual increase in the epidermal cell density with progress in plant development at both the polluted and non-polluted
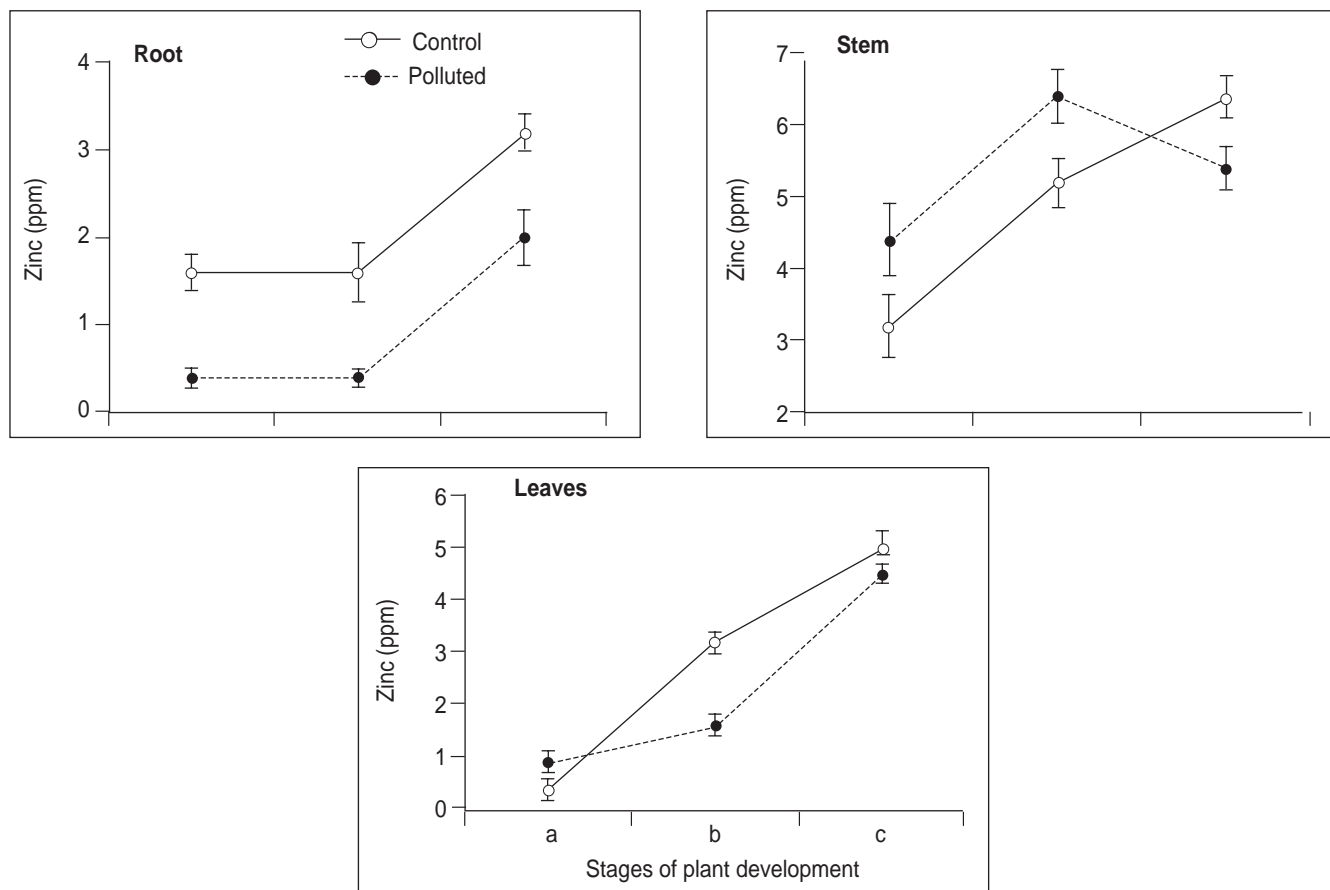
Figure 6. Changes in iron content (ppm) in roots, stems and leaves of T. rhomboidea plants under normal and polluted atmospheric conditions, as recorded during the (A) pre-flowering; (B) first flowering; and (C) post-flowering phases.

sites. Earlier studies have demonstrated both rises and declines in epidermal cell density under pollution stress (Mehindirata et al., 1999; Nighat et al., 1999; Trag et al., 2001, 2002; Wali et al., 2004; Iqbal et al., 2010a). The cell density declined from leaf tip to leaf base under elevated CO_2 conditions, as in *Lolium perenne* and *Ruellia tuberosa* (Ferris et al., 1996; Mahmooduzzafar et al., 2003). Trichome density is regarded as a micro-morphological feature that is most effective for particle capture and deposition (Leonard et al., 2016; Weerakkody et al., 2018). However, in some tree species of China, Liang et al. (2016) found stomatal size to be positively correlated to the amount of $PM_{2.5}$ capture more than the trichome and stomatal densities. Such disagreements among conclusions of similar studies render the current understanding of the situation unclear and should be subject to further comprehensive and intensive research (Barwise & Kumar, 2020). In the present study, trichome density exhibited a more or less consistent increase in relation to progress in plant development at both the study sites.

The cause for higher frequency of trichomes at the post-flowering stage is not known; may be that it has some correlation with the mode of carbon partitioning during different stages of plant development.

Wood structure in roots and stem is significantly influenced by the pollution stress (Pozgaj et al., 1996; Mahmooduzzafar et al., 2010). Wood fibres in the *T. rhomboidea* root and stem were significantly longer under stress throughout the lifecycle of the plant, thus substantiating the earlier findings of Sukumaran (2014) on some tropical plants including *Abutilon indicum, Cassia occidentalis* and *Croton sparsiflorus*. Similar increase in fibre length under the polluted condition was reported for *Calotropis gigantea* (Iqbal et al., 1986b), *Datura innoxia* (Iqbal et al., 1986a), *Achyranthes aspera* (Mahmooduzzafar et al., 1987), *Cajanus cajan* (Ghouse et al., 1989) and *Phyllanthus rhamnoides* (Mahmooduzzafar et al., 1992), among others. The length and width of vessel elements in root and stem of *T. rhomboidea* were also significantly higher in the polluted condition during all developmental stages of

the plant. These observations go in line with those on *Croton bonplandianum* (Ghouse et al., 1986), *Abelmoschus esculentus* (Gupta & Ghouse, 1987), *Lantana camara* (Iqbal et al., 1987b) and *Phyllanthus rhamnoides* (Mahmooduzzafar et al., 1992). The polluted environment caused a widening of vessels in *Sida spinosa* (Mahmooduzzafar et al., 1987), *Cassia tora* (Iqbal et al., 1987a) and *Xanthium strumarium* (Ansari et al., 1993) also. Increase in fibre length and in the vessel-element dimensions is linked to improvement in the mechanical strength and the water-conduction capacity of wood respectively (Pozgaj et al. 1996). Similar tendency in the wood of *T. rhomboidea* under pollution stress enables better survival of individuals that develop these traits under harsh environmental conditions.

The present investigation reveals that, compared with the control, zinc content was lower in roots, higher in the stem (except at post-flowering stage) and again lower in leaves (except at pre-flowering stage) of *T. rhomboidea* plants growing under pollution stress. Zinc accumulation in plant tissues affects the plant physiological processes in different ways. High zinc concentration reduces water uptake, resulting in a water-deficit state of the plant (Paivoke, 1983), and may cause callose deposition on sieve plates in the phloem (Peterson & Rauser, 1979), thus affecting the transport of photosynthates. High doses of zinc proved lethal for *Triticum aestivum*, which did not produce grain and died early (Dudka et al., 1994).

Copper is an essential micronutrient for normal plant growth. It is a component of several enzymes involved in the carbohydrate, nitrogen and cell wall metabolism. In the present study, copper content was significantly higher in roots and stem but significantly lower in leaves at the flowering stage under pollution stress, compared to the control. At the pre- and post-flowering stages, however, it was comparable to the control in roots and leaves, while in the case of stem it went down markedly at the post-flowering stage. Mobility of copper generally decreases with the increase in doses of soil organic matter, and increases with the increase in doses of humic acid (Khan et al., 1997).

Pollution stress greatly increased the iron content in roots, stem (except at post-flowering stage) and leaves (except at pre-flowering stage) of *T. rhomboidea*. Iron is considered to be involved primarily in forming chloroplast protein in leaves (Bashir et al., 2015). It acts as a catalyst for the production of chlorophyll,

and its deficiency affects the iron-porphyin protein complex, which acts as an activator and carrier of oxygen and a transporter of electrons. Lack of iron porphyrin precursor of chlorophyll due to copper deficiency inhibits photosynthesis and respiration, and reduces the chlorophyll content of leaf cells (Balsberg, 1989). In dust-affected atmosphere also, iron content of plants was found to be several times higher than under normal conditions (Mandre & Tuulmets, 1997). Increase in iron content of plant tissues under atmospheric stress also seems to be an adaptive strategy to maintain the chlorophyll production. Although the iron content of roots, stem and leaves of *T. rhomboidea* normally declines with successive stages of plant development, in the case of leaves it goes high during the reproductive phase under the stressed condition. This may be an outcome of mutual interactions and coordination among the phenomena of iron absorption/translocation, chlorophyll formation, net photosynthesis and photosynthate distribution, but the exact situation at the molecular level cannot be speculated upon.

Being an outcome of eco-physiological interactions, ability of plants to absorb metals from the soil and distribute them among different plant parts varies with species/genotype, plant organ, season, stage of plant development and status of plant nutrition (Moinuddin et al., 2004; Umar et al., 2005). Mineral nutrition also modulates the capacity of plants to accumulate and tolerate metals (Siddiqui et al., 2015; Saifullah et al., 2018). While discussing the role of specific proteins in building plant resistance to stress, Cao et al. (2020) focused on actin depolymerising factor (ADF), an actin-binding protein ubiquitous in animal and plant cells. Based on phylogenetic analysis, they distributed the members of *Cajanus cajan ADF* (*CcADF*) family into four subfamilies, which showed specific responses to different stresses. The apparently irregular patterns of metal accumulation in different parts of *T. rhomboidea* at different phenological stages could possibly be a combined effect of metal ions suspended in the polluted air as well as those deposited on the soil and absorbed through root system that may have a diverse affinity for different metals. Future research may likely explain more decisively the differential behaviour of different plant species and plant parts regarding the absorption of different metal ions.

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