

HISTOLOGICAL CHANGES IN *HIBISCUS ROSA-SINENSIS* ENDORSE ACCLIMATION AND PHYTOREMEDIATION OF INDUSTRIALLY POLLUTED SITES

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ABSTRACT

Addition of undesired effluents to the environment has resulted in massive destruction of the ecosystem due to the persistent nature of the toxicants. The growth pattern of the plants species growing in urban and sub-urban areas are being affected negatively by various pollutants. In order to adapt and survive in hostile environment, different species exhibits a number of transformations and as a result some species restrain better adaptability and growth. The present study was carried out to investigate the histological alterations as an adaptive response as well as soil and air cleaning characteristics of different *Hibiscus* cultivars against industrial effluents. The root and stem fresh samples of *Hibiscus* cultivars were collected from polluted areas of Faisalabad, Punjab, Pakistan. The transverse sections of the root and stem samples were stained and observed by using light microscopy. The presented data clearly depict altered anatomical features depending upon presences of toxicants in the rhizosphere. The modified anatomical attributes like thick stem epidermis, increased epidermal cell area high vascular tissue and enhanced cortical cell area in *H. rosa-sinensis* cv. Cooperi alba and Lemon chiffon respectively confer the better adaptation to polluted conditions. The knowledge is likely to be helpful for devising plant based decontamination strategies for industrially polluted environment.

Key words: Acclimation, *Hibiscus* cultivars, industrial effluents, Tissue modifications, Phytoremediation

INTRODUCTION

The district of Faisalabad is located in the flat plains of northeast Punjab, Pakistan, (31°24'N, 73°04'E). The city is famous for its textile and dyeing industries, where generally weaving, dyeing, printing and finishing of cloth has been extensively carried out. Industrial operations in the city usually produce extremely alkaline liquor containing dissolved materials and suspended particles (Akhtar *et al.*, 2005). Due to absence of ample treatment facilities and efficient drainage system, bulk of the effluents from these industrial units flow into open land and low lying areas (Yasmin *et al.*, 2014) causing severe damage to local flora and fauna. Other contamination sources include direct use of fertilizers in the agriculture sector, herbicides and pesticides in the sub-urban areas.

Designated as primary recipient of solid waste, soil receive million of tons of waste from different sources inclusive of industrial, domestic and agricultural (Nyles and Ray, 1999). Production of such waste is a basic part of industrial activity and may cause heavy metal contamination in soils to the levels which are undesirable and phyto-toxic. However, the inability to

anticipate or predict the type and magnitude of undesired effluents, added to the environment, has resulted in massive destruction of the ecosystem. These effluents are poured into rivers, streams or supplied to open lands without any treatment (Uaboi-Egbenni *et al.*, 2009). Logging of the effluents not only destroy the soil structure and fertility but also result in transfer of toxic elements from soil to plants, and ultimately intake to humans through food chain, thereby causing serious health issues (Muhammad *et al.*, 2011). It is a common observation that plant species growing in urban areas are strongly effected by various pollutants like hydrocarbons, nitrogen as well as sulphur oxides, particulate matters, peroxyacyl nitrates (Stevoic *et al.*, 2010).

Different researchers have studied the influence of effluents on some plant types. In addition to quantitative changes, the plants from polluted sites showed various structural abnormalities (Kapatina, 2002). Differences in morpho-anatomical traits in *Pongamia* sp. have been noticed under cumulative environmental conditions with dominant effects of soil contamination (Shirbhate and Malode, 2012). For example, Cd enriched medium accelerated endodermal development in root (Richter *et al.*, 2009). It was found that the production of a thick suberized endodermis, and

cell wall lignification in root tissues exposed to Cd source could restrict the Cd loading into the xylem by limiting its radial apoplasmic movement (Lux *et al.*, 2011).

Genus *Hibiscus*, a member of Malvaceae, is found mainly in tropical and sub-tropical areas of northern and southern hemispheres (Beers and Howie, 1992) and found to be one of the most appealing ornamental plants. This genus comprised of approximately 250 species and *Hibiscus rosa-sinensis* is a significant one with a wide range of cultivars grown across the globe (Pekamwar *et al.*, 2013, Noman *et al.*, 2014; Khan *et al.*, 2014). *Hibiscus rosa-sinensis* is a potential source of many bioactive natural products which are of significant value in folk medicinal system, especially for curing liver disorders and hypertension (Yasmin, 2010). Furthermore, reports exist that *Hibiscus* species are effective for metal uptake and can be fitted in long term phytoremediation programs for decontamination of toxicants (Bhaduri and Fulekar, 2015).

In order to adapt, survive and clean contaminated environment, different species exhibits a huge number of structural transformations. As a result some species restrain their growth, or the autochthonous vegetation is replaced with the mechanized one-poor in individuals and species numbers that become permanent as an industrial-climax. Specific anatomical and physiological changes in plants facing stressful environments may enable them to thrive well in such environments (Noman *et al.*, 2012; Noman *et al.*, 2014). It is imperative to study the structural changes in various plant parts to understand their survival in the presence of increased environmental risks especially industrial pollution and the role of anatomical alterations in the perspective cleaning of soil and air. A better understanding of modification in structural arrangement provides a clear idea of mechanism enhancing plant resistance to environmental hazards (Vijayakumar and Udayasoorian, 2007). Despite their crystal clear value and role in plant adaptability along with soil and air remediation, many a times, anatomical investigations have been overlooked in comparison with physiological or morphological studies. Therefore, the present study was carried out with the objective to investigate the histological changes in stem and roots as an adaptive response as well as soil and air cleansing role of different *Hibiscus* cultivars to industrial effluents.

MATERIALS AND METHODS

Plant collection: To examine anatomical changes in *Hibiscus rosa-sinensis* and its cultivars growing in industrially polluted environment, different industrial

sites in Faisalabad region were explored for the record and distribution patterns of cultivars (Fig. 1). Six cultivars of *Hibiscus rosa-sinensis* namely *H. rosa-sinensis* cv. Charles September; *H. rosa-sinensis* cv. Cooperi Alba; *H. rosa-sinensis* cv. Mrs. George Davis; *H. rosa-sinensis* cv. Frank Green; *H. rosa-sinensis* cv. Lemon Chiffon and *H. rosa-sinensis* wilder's white were selected. Stem and root of naturally growing plants of approximately same age receiving industrial effluents directly were collected randomly in triplicate. For recording and comparison of histological modifications, parts of mentioned plants were taken from new Botanical Garden in University of Agriculture, Faisalabad, Pakistan. For recording anatomical observations, 2cm² sections of equal thickness from stem and tap root of each *Hibiscus* cultivar were taken. The material was preserved in alcoholic formalin acetic acid (FAA) solution (Formalin 5%, acetic acid 10%, ethyl alcohol 50%, and distilled water 35%) for fixation.

Climatic Conditions: The average climatic conditions of the study area during sampling calculated as means were day/night RH 33.1/75.1% and day and night temperatures 38.28±4°C and 22.82±3.6°C, respectively. The soil of the investigated area was sandy clay consisting of average 65% clay content, 22% sand and 13% silt.

Effluent Analysis: Samples of effluents from different industries i.e printing, dyeing and finishing supplied to plants were collected at the main drain (Paharang drain) which is main collection point where all effluents merge together. Effluents were analyzed for its various physico-chemical and biochemical properties and are presented in Table 1.

Anatomical Studies: The transversally cut sections (T.S) were passed through a graded series of ethanol onward starting with 50% ethanolic solution and finally treated with absolute ethyl alcohol for dehydration. Double stained standard technique was used for preparing the permanent slides of the T.S. of leaves following Ruzin (1999) for light microscopy. Fully prepared individual stem and root sections were placed on the slide containing 1-2 drops of safranin. Subsequently, the extra safranin was removed by addition of few drops of 95% ethanol. Fast green prepared in 95% of absolute alcohol was added respectively for color establishment. Finally, after addition of one drop of xylene, sections were permanently fixed with canada balsam for preservation. Camera photographs were taken by using Carl-Ziess camera equipped microscope. Data were subjected to statistical analysis using ANOVA to find out the significant differences. Standard error was calculated following Steel *et al.*, (1997).

RESULTS

Stem anatomy: A significant intra-specific qualitative anatomical changeability was observed in various cultivars of *H. rosa-sinensis* collected from various areas receiving effluent irrigation from industries. The presented data depict anatomical alterations among plants growing in polluted habitats. The epidermis was found uniseriate in all the examined cultivars. *H. rosa-sinensis* cv. Cooperi alba was remarkable due to its high epidermal cell area and epidermal thickness, collected both from polluted as well as non-polluted site (Fig. 2). Minimum epidermal cell area was recorded in cv. Frank green and Mrs. George Davis receiving untreated industrial effluents. In addition to structural components, black spots in epidermis and cortex were observed in *H. rosa-sinensis*, cv Cooperi alba and Lemon chiffon.

Inter-cultivar variations in relation to stem cortex area were apparent. In all studied cultivars, multilayered cortex was observed irrespective of effluent irrigation source. Significantly high proportion of stem cortical cell area was recorded in cv. Lemon Chiffon (Fig. 2 and 4a) as compared with other *Hibiscus* cultivars, while this attribute was at its lowest value in *H. rosa-sinensis* cv. Wilders white (Fig. 4a). The maximum cortex thickness was recorded in *H. rosa-sinensis* cv. Frank green as compared with other cultivars (Fig. 4a) and the minimum thickness was observed in cv. Cooperi alba growing in polluted and non-polluted site. Data for vascular bundle thickness in Fig. 2 revealed that it is affected significantly in plants growing in polluted area, but this effect was cultivar specific. A significant increase in vascular bundle thickness was recorded in all *Hibiscus* cultivars except in cv. Wilders white in which it remained unaffected. Of all cultivars, maximum increase in vascular bundle thickness was observed in Cooperi alba as compared with the other cultivars from polluted area.

Results regarding metaxylem area presented in Fig. 2 exhibited a substantive increase in cv. Cooperi alba and Lemon chiffon as compared with other cultivars. Among other cultivars, the cv. Wilders white and Charles September exhibited no difference in their anatomy from the plants inhabiting polluted environment. However,

this feature witnessed its minimum value in *H. rosa-sinensis* cv. Charles september. Furthermore, markedly increased xylem region thickness was recorded in cultivar Cooperi alba in comparison with others growing in polluted site. The remaining cvs. exhibited minor differences from cultivars occupying non-polluted sites in this regard, while this character was the lowest in *H. rosa-sinensis*.

Phloem region thickness also varied significantly among cultivars. *H. rosa-sinensis* cv. Wilders white was superior to the rest of cultivars collected from polluted location (Fig. 2). On the other hand, all cultivars show relative difference in this attribute from plants growing in non-polluted conditions. Furthermore, incremented pith cell area was reported in cultivars Cooperi alba as well as Charles september (Fig. 4a) as compared with other cultivars, while cv. Mrs. George Davis showed massive reduction in character during growth in polluted site.

Root Anatomy: Data for various root anatomical feature exhibit presence of significant variation among cultivars collected from polluted and non-polluted situations. Presented in Fig. 3, data clearly indicates the existence of high exodermal cell area and exodermis thickness in *H. rosa-sinensis* cv Lemon chiffon and Frank green respectively. Cultivar Cooperi alba was noticed with least exodermal thickness under polluted conditions (Fig. 4b). Although, root cortex was multiseriate in all cultivars but, cv. Lemon chiffon was significant in having increased cortical region thickness and cortical cell area, followed by cv. Lemon chiffon, under polluted environments. Cooperi alba and Wilders white were approximately at par by having similar thickness irrespective of the site.

With exception to others, Cv. Frank green possessed incremented root metaxylem area under polluted conditions. Overall, remaining cultivars from polluted locations revealed reduction in this attribute. Differences were observed among cultivars in possessing metaxylem number (Fig. 3). On the whole, all plants from non-polluted locations, represented high metaxylem number in comparison with plants irrigated with water containing industrial effluents.

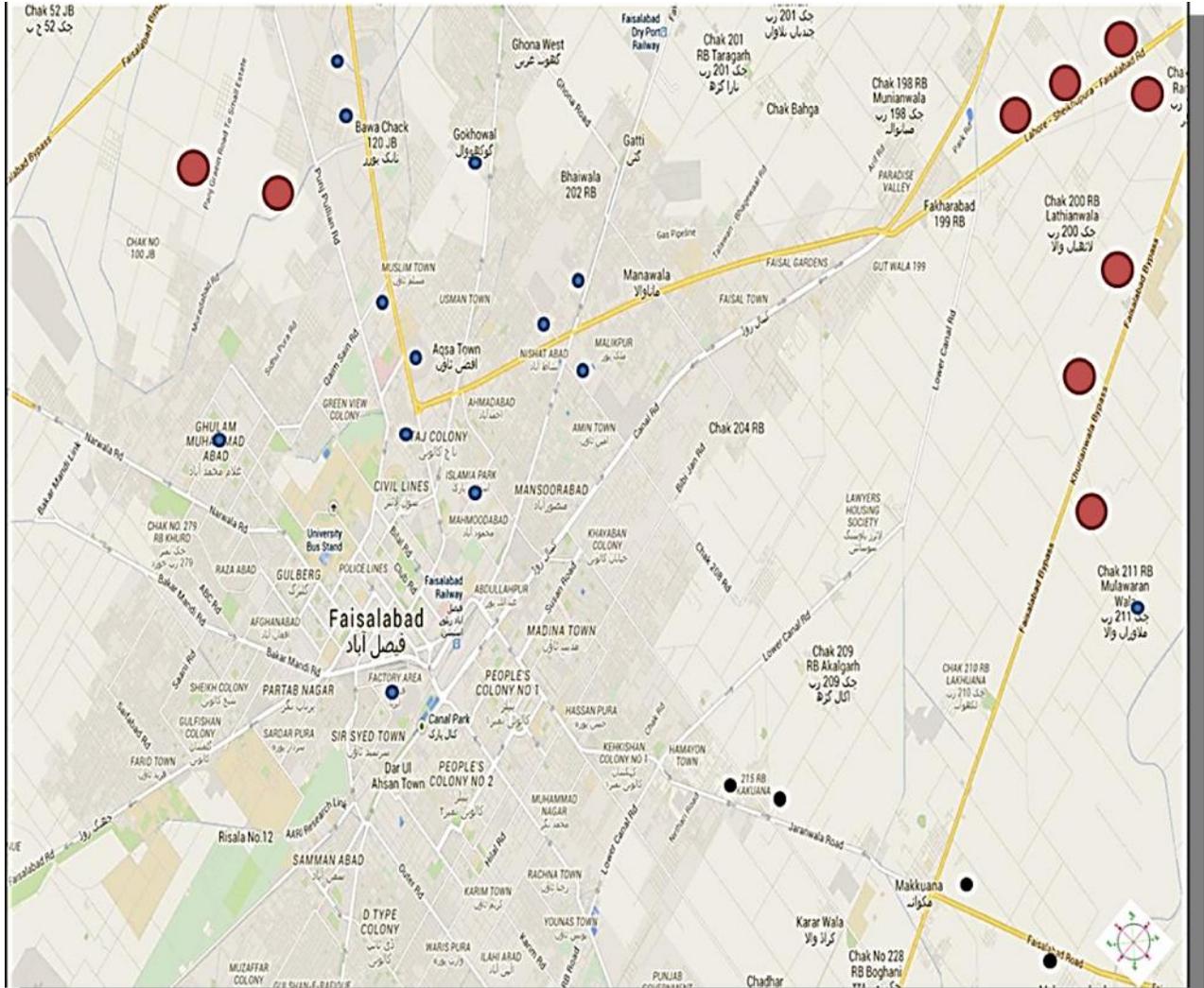
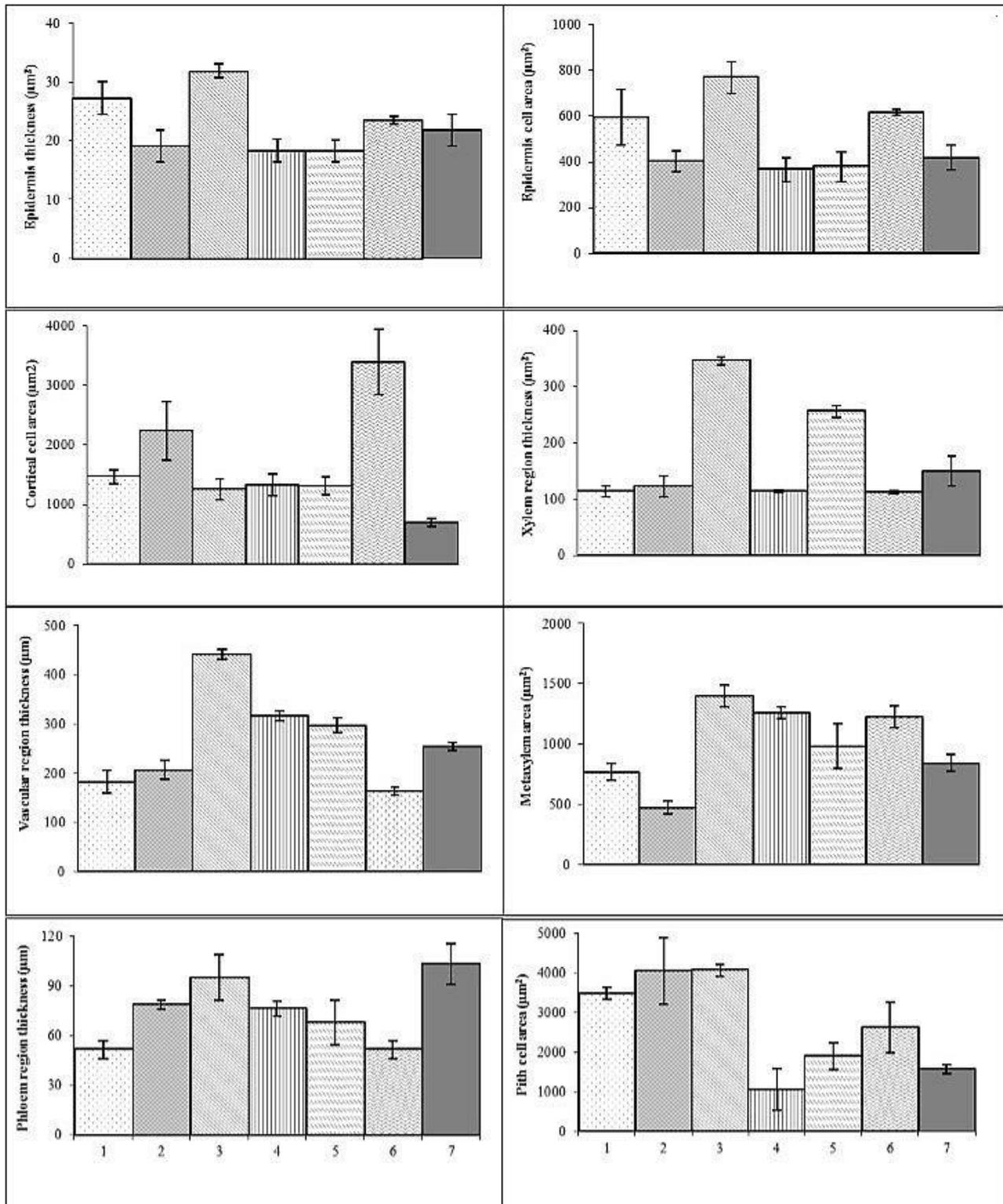


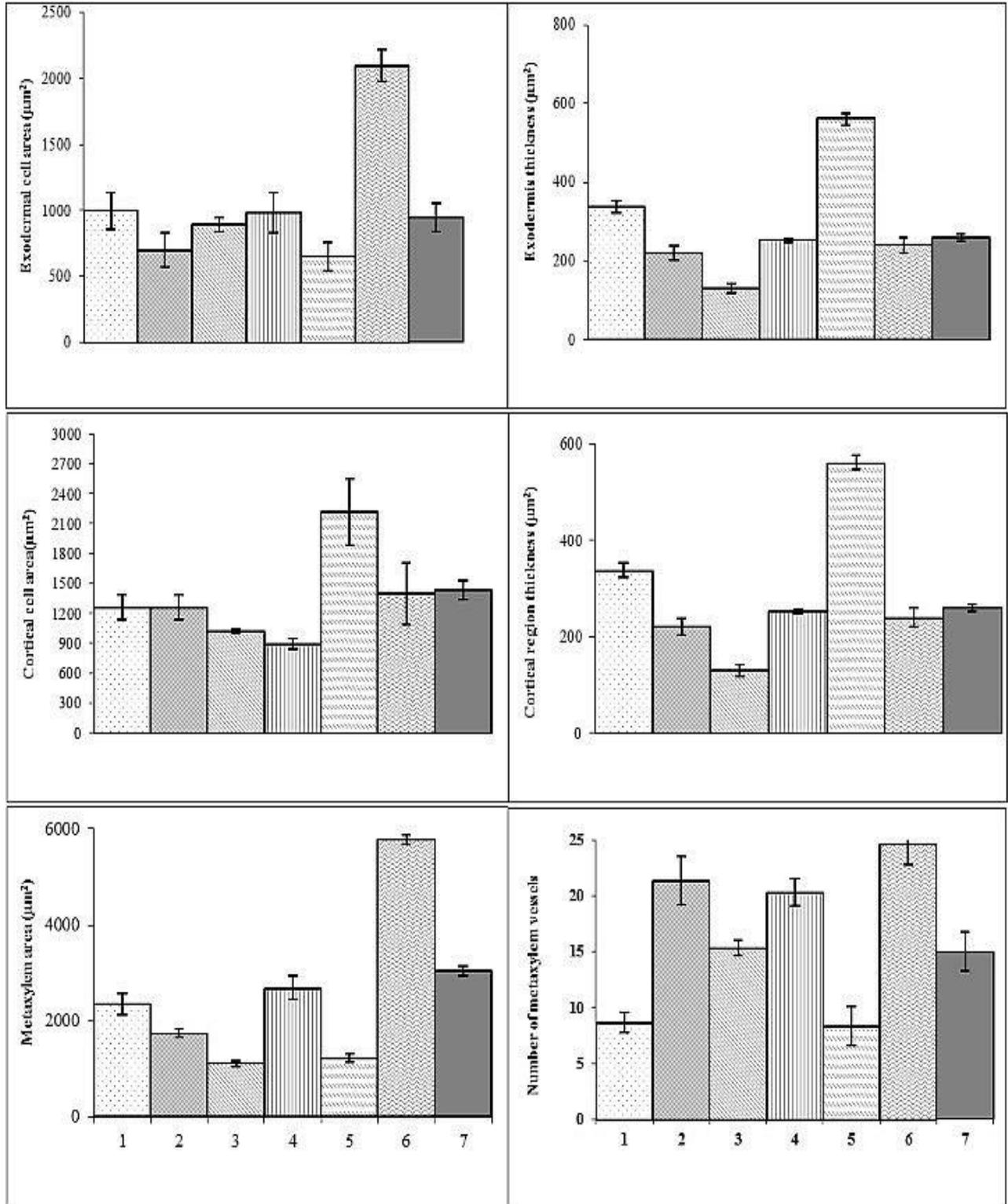
Fig.1. Map showing the *Hibiscus rosa-sinensis* sampling sites from industrial zones of Faisalabad, Punjab, Pakistan.

- Scattered Industries of different types
- Prominent Small and Medium Industries in Populated Area of the city
- Industrial Estates



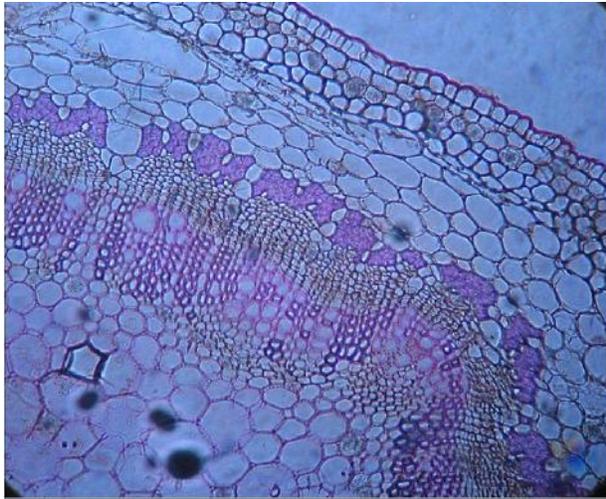
1. *H. rosa-sinensis* L.; 2. *H. rosa-sinensis* cv. Charles September; 3. *H. rosa-sinensis* cv. Cooperi alba; 4. *H. rosa-sinensis* cv. Mrs. George Davis; 5. *H. rosa-sinensis* cv. Frank Green; 6. *H. rosa-sinensis* cv. Lemon Chiffon; 7. *H. rosa-sinensis* wilder's white.

Fig. 2. Statistical analysis of stem anatomical attributes of *Hibiscus rosa-sinensis* cultivars collected from industrial zones of Faisalabad.

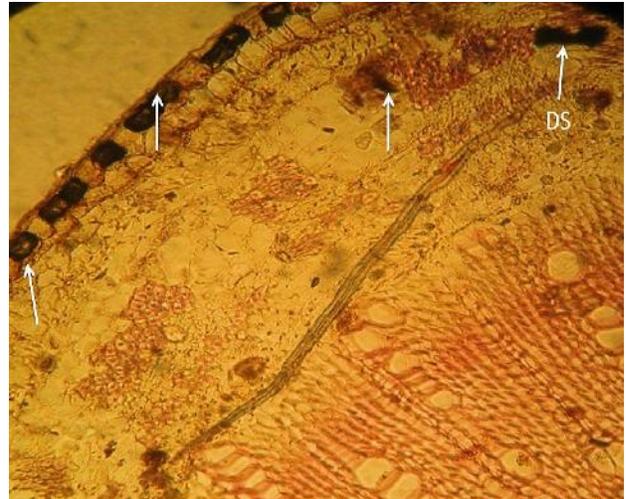


1. *H. rosa-sinensis* L.; 2. *H. rosa-sinensis* cv. Charles September; 3. *H. rosa-sinensis* cv. Cooperi alba; 4. *H. rosa-sinensis* cv. Mrs. George Davis; 5. *H. rosa-sinensis* cv. Frank Green; 6. *H. rosa-sinensis* cv. Lemon Chiffon; 7. *H. rosa-sinensis* wilder's white.

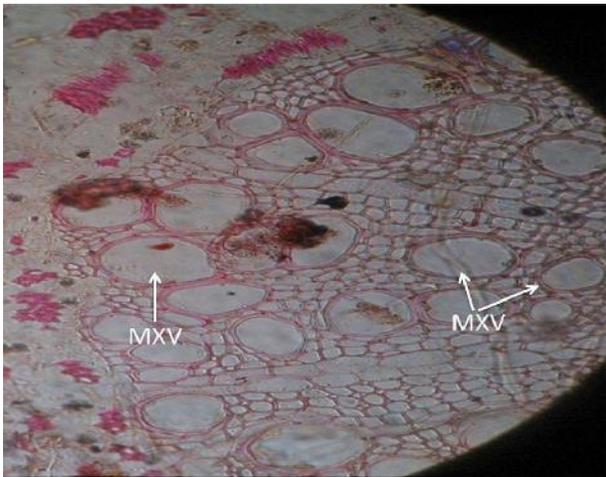
Fig. 3. Statistical analysis of root anatomical attributes of *Hibiscus rosa-sinensis* cultivars collected from industrial zones of Faisalabad.



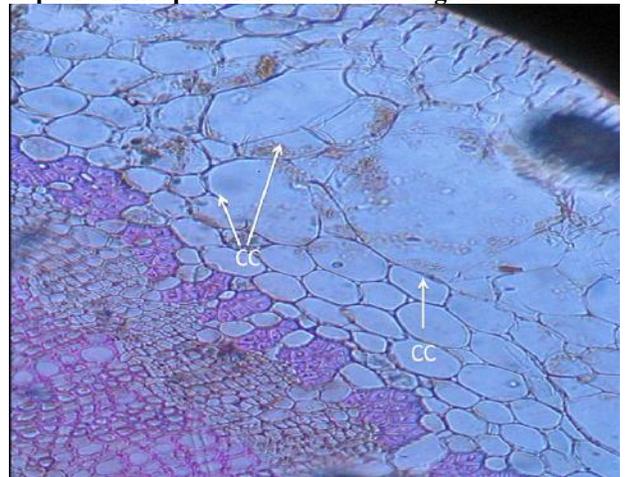
Stem of *H. rosa –sinensis*



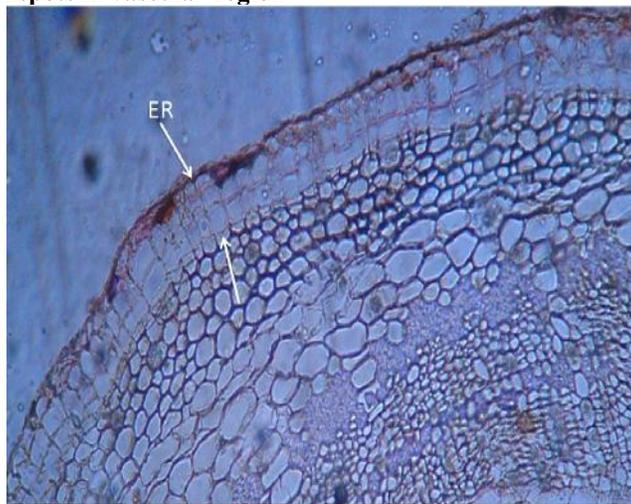
Stem of cv. *Cooperi alba* dark spots showing pollutant deposition in epidermis & vascular region



High metaxylem number and size in stem of *Cooperi alba* along with distinct dark spots in vascular region

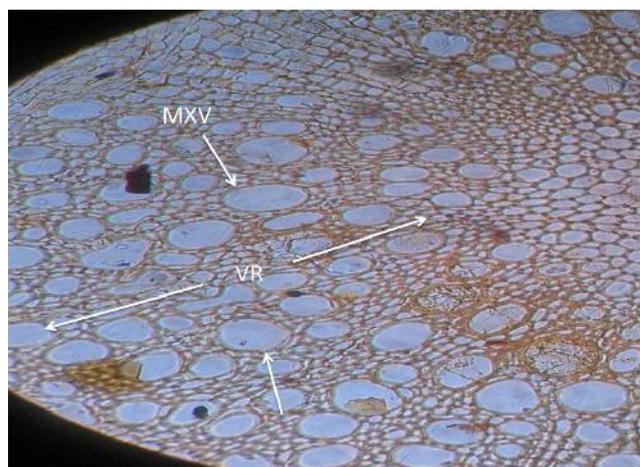


High cortical cell area in cv. *Lemon chiffon*

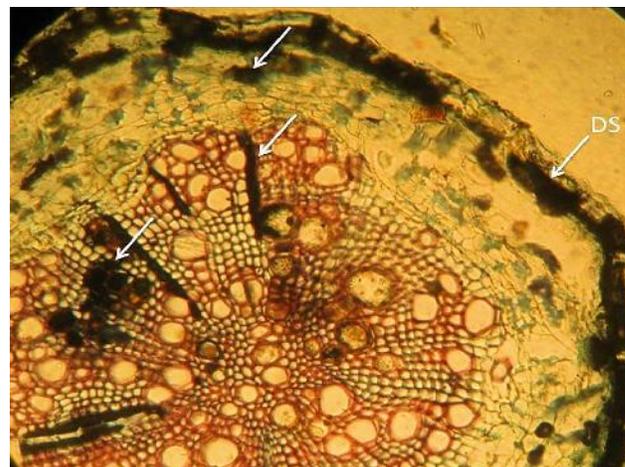


High stem epidermal region thickness in cv. *Cooperi alba*

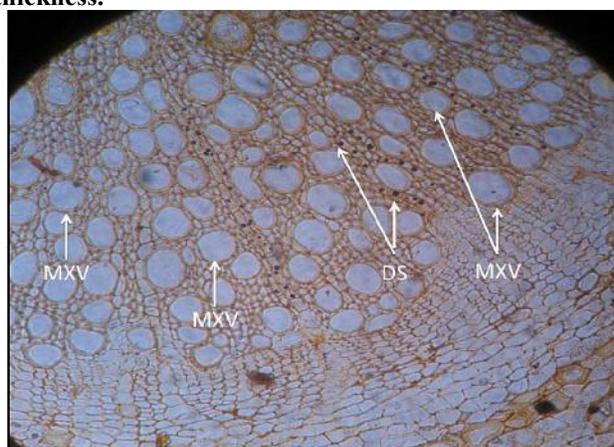
Fig. 4a. Stem anatomical characteristics of some *Hibiscus rosa sinensis* cultivars growing in industrially polluted areas.



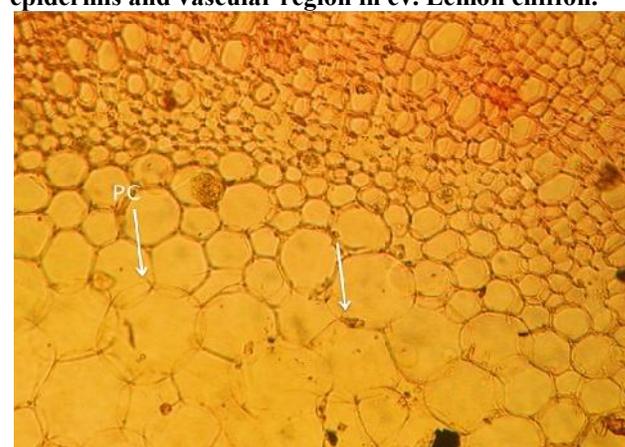
Root high meta-vessel number and vascular region thickness.



Root dark spots showing pollutant deposition in epidermis and vascular region in cv. Lemon chiffon.



Root high metaxylem number with distinct dark black spots in vascular region.



High pith cell area in cv. Cooperi alba.

Fig. 4b. Anatomical characteristics of root some *Hibiscus rosa sinensis* cultivars growing in industrially polluted areas.

Table 1. Physico-chemical properties effluent water collected from industrially polluted areas of Faisalabad, Punjab, Pakistan.

Parameters	Effluent water	NEQS
pH	8.40 ± 0.29	6.00-8.50
EC (dS m ⁻¹)	6.50 ± 0.36	-----
TDS (ppm)	2776.00 ± 81.3	2500
TSS (ppm)	145.00 ± 5.00	400
NO ₃ -N (ppm)	23.50 ± 1.04	-----
PO ₄ (ppm)	10.20 ± 0.76	-----
Cl (ppm)	1010.0 ± 17.32	1000
SO ₄ (ppm)	490.00 ± 17.32	1000
K (ppm)	28.50 ± 1.04	-----
Zn (ppm)	5.00 ± 0.27	5.0
Cr (ppm)	0.78 ± 0.05	1.0
Cu (ppm)	0.77 ± 0.04	1.0
Ni (ppm)	0.64 ± 0.05	1.0

TDS=Total dissolve solids, TSS = Total suspended solids, NEQS = National Environmental Quality Standards

DISCUSSION

Plants, being broadly distributed in array of environmental conditions, experience meticulous physiological, biochemical and morpho-anatomical alterations (Arshad *et al.*, 2015; Anwer *et al.*, 2016; Noman and Aqeel, 2017; Noman *et al.*, 2017). Plants with different anatomical modifications have increased chances of survival under varied environments (Cutler *et al.*, 2007). These customized anatomical characteristics in different plant parts are of supreme significance to cope with adverse environments. The differences in their tolerance level to environmental challenges may not specifically be attributed to physiological adaptations, but also to anatomical alterations in root, stem, and leaf (Wyszkowski and Wyszkowska, 2003; Gostin, 2009; Hameed *et al.*, 2011). Plant's interior can be considerably influenced by environmental variations. The anatomical alterations can be used to assess plant tolerance and survival to environmental stresses (Hameed *et al.*, 2012). In present study, some cultivars of *Hibiscus rosa-sinensis* were investigated anatomically to look at their potential for continued existence under polluted environs.

Results indicated significant inter-cultivar variation with respect to anatomical attributes. These modified anatomical attributes in *H. rosa-sinensis* cv. Cooperi alba including thick stem epidermis, increased epidermal cell area and enhanced cortical cell area are likely to impart higher chances of their survival under polluted conditions. Thick dermal layer accompanied with incremented epidermal cell area was at its acme in this cultivar as compared with the other studied cultivars. The presence of thickened epidermal cell area is consistent with the results of Hameed *et al.*, (2012), who reported that anatomical parameters relating to root and stem in *Asparagus* species/cultivars were not only species specific, but the indicators of habitat ecology. Root length and thickness is one of the reliable traits for successful tolerance level under harsh conditions (Naz *et al.*, 2014). The plant species facing environmental hazards like osmotic stress conditions, showed particular anatomical modifications which are likely to play a vital role in conservation of water, and therefore extremely helpful to cope with environmental hazards (Hameed *et al.*, 2011; Naz *et al.*, 2014). This characteristic is critical under limited moisture availability as thick epidermis is capable of checking water loss through stems (Nawazish *et al.*, 2006). Taleinsik *et al.*, (1999) has described such increase in root exodermis as an aegis as well as a factor controlling radial flow of water along with reducing water loss. Thick walls are in fact active barrier to pollutants that may bore the internal tissue. Reported changes in stem and root epidermal cell structure can be positively coupled with incremented adaptation percentage against pollution and other stress factors (Gostin, 2009). Our recorded observation can be

correlated with the study of Stevovic *et al.*, (2010). They described morphological similarities among plants of one species but major anatomical differences in root, stem and leaf of the plants growing in polluted areas.

One interesting finding was the appearance of dark spots in different tissues of plants like epidermis, vascular region etc. Our results are in line with earlier reports. Resembling dark spots were observed across the different plant parts e.g. in leaf (Gostin, 2009; Noman *et al.*, 2012; Noman *et al.*, 2014), stem and root. Reports elaborate these spots as the deposition of various metals like Pb and Ni (Molas, 1997; Gostin, 2009). Presence of such spots is likely to provide a way of metal accumulation travelled from root zone. This result helped us to suggest a possible soil cleaning effort of studied plant by uptaking and tolerating to soil accumulated metals. Furthermore, it has also been clearly described that under industrial environments, plants accumulate toxic elements like Ni from the rhizosphere. It is estimated that half of such metals retained in root system. The reported accumulation of materials in the form of dark spots can mainly be based upon sequestration in the positive ion exchange sites of the xylem vessel walls parenchyma and immobilization in the root vacuoles (Seregin and Kozhevnikova, 2006). The distribution of metals like Ni and Pb (Gostin, 2009) may describe its good mobility in vascular tissues (Page and Feller, 2005; Riesen and Feller, 2005). However, the presence of dark spots is seemingly the distribution of toxic ions entered in plants. Later on, these ions were translocated to shoot system and distributed preferentially in the dermal cells (Kupper *et al.*, 2001). Such reports make our stance strong about *Hibiscus* cultivars that they can withstand industrially polluted environments and are suitable candidates for phytoremediation/phytostabilization of toxicants.

Moreover, the enhanced cortical cell area and cortical region thickness emerges as adaptability response. These features contribute to successful occupation of harsh habitat and the survival of plant species. Present findings are in line with the studies which reported that large cortical cells in *Eucalyptus microtheca* and *Eucalyptus botryoides* were the indicative of their wide distribution in diverse environmental conditions (Zwieniecki and Newton, 1995; Baloch *et al.*, 1998). Moreover, it is evident that better cortical cell area is thought to be related with improved storage of moisture that is pre-requisite for survival under harsh climatic conditions (Ali *et al.*, 2009). As these parenchyma cells are associated with water storage, hence emerges very crucial under osmotic stress caused by different reasons. Our reports of raised cortical cell size suggest strong capacity of these cultivars to survive in polluted rhizosphere. So, the cultivars from polluted sites with high cortical cell area indicate their genetic potential to resist polluted conditions and thrive well

(Molas, 1997). Enhanced cortical cell area is also the ecologically significant trait to resist adverse environmental conditions (Hameed *et al.*, 2009).

Plants growing at polluted sites usually experience a reduced vascular bundle area. Vascular tissues decrease in size that makes plant vulnerable to injurious effects of different pollutants. This type of variation was noted with reference of xylem area reduction in *Abutilon indicum* samples collected from polluted sites (Sukumaran 2012). Generous increments in vascular bundle area appear as one of the most vital feature supporting plant life cycle under environmental variations. In the present study a substantial increase in vascular bundle area has been recorded in *Hibiscus rosa-sinensis* cv. Cooperi alba from polluted area which represents its adaptation to varying environmental conditions. These findings are in line with the results of Ali *et al.*, (2009) stating direct relation of vascular bundle area to alleviated transport of water and nutrients from the soil, and reported that this anatomical adaptation might be of greater importance under reduced moisture availability. Keeping in parallel, despite decline and definite increase in some parameters, acclimation of plant species mainly relies upon positive changes i.e. increment in size or number to combat pollutant effects. Augmented vascular bundle area is an imperative trait for survival in damaged environments. Awasthi and Pathak (1999) have also reported greater vascular bundle size in saline tolerant genotypes of *Ziziphus*. According to these authors, larger vascular bundles consisting of broad metaxylem vessels along with large phloem may prove vital for conduction of water and nutrients as well as translocation of photosynthates. In addition, Steudle (2000) have also listed presence of such trait crucial for adaptability under deteriorated state of environment.

In present study, significant effects of polluted environment were recorded in *Hibiscus* plants as far as stem metaxylem area was concerned. Contrary to report of Sukumaran (2012) in *Croton sp.*, reporting a high metaxylem vessel length and metaxylem area in some cultivars of *Hibiscus*. Rise in metaxylem area in cultivars Cooperi alba, Lemon chiffon and Frank green collected from polluted environs was observed that appear as an aegis in these plants. Phloem region thickness also varied significantly among all cultivars of *H. rosa-sinensis* collected either from polluted or non-polluted sites. Differentially enlarged phloem area has been recorded in all cultivars where Cooperi alba, Wilder's white and Lemon chiffon exhibited a major increase of phloem area in comparison with the others. The characteristic appears as a comprehensive indicator for the survival of plants under polluted conditions. Similarly, it has been reported that presence of greater phloem area was related with enhanced conduction of assimilates that shows a good reason of ecological accomplishment of this species

under diverse environmental conditions (Hose *et al.*, 2001).

Different tissues in the same plant even respond differentially to one or the other pollutant. This was evident from the studies on *Abutilon indicum*, *Croton sprasiflorus*, *Cassia accidentalis* in which variability was noted for stem diameter and vascular tissue anatomy i.e. xylem and phloem yet cortical cells and pith cells did not vary significantly (Sukumaeran, 2012). Contrary to this, pith area and pith cell size exhibited prominent variation when a comparison was made between samples collected from polluted and non-polluted sites in present study. The unaffectedness of pith area though seems resistant to pollution but it is likely that the rise in pith cell area in certain cultivars represent an acclimation response.

Conclusion: It can be concluded that the studied cultivars of *Hibiscus rosa-sinensis* revealed great diversity in terms of tissue anatomical features. Furthermore, these varied anatomical aspects of the entire *Hibiscus* cultivars are ample indicators of their better adaptability and resistance to varying environmental conditions, therefore, proved its ecological success. Presence of specialized feature in relation to stem and root anatomy in *Hibiscus rosa-sinensis* cv. Lemon chiffon and Cooperi alba appear prudent in determining the fate of these cultivars in multifarious environmental challenges ranging from augmented industrial pollution. Therefore, stem and root anatomical features should be considered while devising long term plant-based pollution remediation plans.

Authors contribution: AN, MA and MTJ planned this study, selected sites for sampling and compiled manuscript. QA, WI, SZ, HK, NK collected samples and performed anatomical analysis. MB, performed photography. MKI and SK performed statistical analysis and made graphs.

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