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Morphological and anatomical study of grasses Triticum aestivum L. and Phalaris minor Retz. a quick tool for assessing the impact of road side pollution

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Abstract

Plants grown close by roadside areas are exposed to variety of pollutants. Air pollution has an adverse effect on plants. Most air pollution comes from Automobiles. Automobiles have increased in number in tandem with population growth, as has pollution from automotive emissions. The first targets of these vehicular pollutants are roadside plants and roadway vegetation. Vehicle emissions contain a variety of contaminants that contribute to the worst types of pollution in the environment. Various contaminants from automobiles changed the properties of the vegetation that are exposed. One of the most obvious roadside plant is grass. This experiment shows how automobile emissions affect grasses anatomy and morphology. Experiments were conducted to investigate morphological as well as anatomical changes in leaves and culms of roadside grasses *Triticum aestivum* L. and *Phalaris minor* Retz.

To carry out the experiment, samples (Fresh leaves and Culms) were collected from polluted sites and non-polluted site acting as a control. For anatomical study leaves and stems were cut into sections and epidermal peeling was performed in the laboratory and changes were identified under the light microscopy. SEM analysis was also done. Results revealed the major anatomical and morphometric variations observed in both *Triticum aestivum*L. and*Phalaris minor* Retz. growing in polluted and non-polluted study sites. Reduction in morphometric parameters for both the specimens growing in polluted study sites indicated a negative effect of pollutants on the growth of these pooideae members. Not only that changes in the anatomy of the polluted site specimens, both in the culm and leaf anatomy was found. SEM images of the abaxial and adaxial surface of the leaves showed micro-morphological changes, with stomatal size and number showing a reduction in case of *Triticum aestivum*L. for the polluted site specimens while the size of stomata showed an increase in the case of *Phalaris minor* Retz. growing in polluted study sites. These results highlighted the importance of anatomical and morphological data to determine the responsiveness of roadside grasses to various environmental pollutants.

Introduction

Grasses (Poaceae; earlier known as Gramineae) are the “tip of the sword” in the evolutionary race, representing a highly evolved and diverse plant group, containing more than 11,000 species spread across 768 genera (Kellog, 2015; Soreng et al., 2017; Hodkinson, 2018). In terms of its size, poaceae is the fifth largest flowering plant family, originating sometime during the early cretaceous while reaching its acme of diversification in the Cenozoic era (Hodkinson, 2018; Zhong et al., 2018).

This diversification coupled with the incorporation of C4 carbon metabolism has led to grasses occupying 1/5th of the earth’s land (Peterson, 2013; Kiran, 2015), thus contributing immensely to the biomass production. Similarly, the economic importance of grasses can be gauged by the fact that the first ten crops that are the mainstay of our dietary needs, all are grasses (Kiran, 2015).Grasses like bamboo are important building material while many grasses provide us with the aesthetically pleasing

lawns and turfs and more recently, they are being used in bio-energy industry such as *Saccharum, Arundo* etc. (Hodkinson, 2018).

From the early 19th century onwards taxonomists like Brown (1814), Bentham (1881), Hitchcock and Chase (1951), Clayton and Renvoize (1986) and many more put forward their classifications based primarily on morphology and anatomy of grasses but with the advancements in molecular studies more robust classifications based on phylogeny have revised these earlier classifications (Peterson, 2013; Smith, 2005; Hodkinson, 2018). Two such grass family classifications based on phylogeny were given by Kellog (2015) and Soreng et al. (2015, 2017) both of which recognized 12 subfamilies of poaceae. Among these 12 subfamilies, the subfamily Pooideae Benth. (1861) occupies the position of being the largest grass subfamily with an estimated 3968 species, not limited to that it also houses some of the most important grasses from an economic point of view(Smith, 2005; Hodkinson, 2018). According to Soreng et al. (2017) both *Triticum aestivum* L. and *Phalaris minor* Retz. belong to the tribe Poeae in the subfamily Pooideae.

Triticum aestivum L. has been intrinsically linked with human civilization, having been cultivated since 8000-10,000 BC (Faltermaier et al., 2014) and today it is one of the three major cereal crops that feed 75% of the world population (Kiran, 2015). While *Phalaris minor* Retz. too has a history with *Triticum aestivum* L., as it is one of the most noxious weed of the Rice-Wheat system (predominantly of wheat), which is a common agriculture system of the Indo-Gangetic plains of India (Singh et al., 1999).

Studying these grasses using morphometrics, wherein data from morphometry is used for statistical analysis to define or de-limit a taxa has been in vogue for quite some time, more since the publication of “Multivariate Morphometrics” by Blackith and Reyment in 1971 (Remagnino et al., 2017). Morphometrics combined with the anatomy provides a powerful tool for studying and describing plants in general. Despite extensive work done in grass anatomy done by Brown et al. (1957, 1959), Metcalfe (1960) and Renvoize (2002), use of morphometrics coupled with anatomy in grasses has been relatively low. Though recent work done by Out et al. (2016) and Chen et al. (2020) has signalled a much-needed change in the use of morphometry and anatomy as a single instrument for taxonomic studies.

Despite being found in cultivated fields, both *Triticum aestivum* L. and *Phalaris minor* Retz. are a common site growing along roadsides (Pourrezaei et al., 2017). This ability of grasses to inhabit areas which are at the opposite end of the spectrum in terms of their environmental conditions only goes to show the adaptability of these grasses. The adaptability in terms of morphometry is observed as a reduction in growth patterns of plants growing in polluted sites due to the abundance of heavy metals and air pollutants which are detrimental to plant growth (Dzhygan et al. 2018). Thus, this present study tries to understand the variation both anatomically and morphometrically in *Triticum aestivum* L. and *Phalaris minor* Retz. inhabiting these two different ecological niches viz. non-polluted/cultivated and polluted/roadside.

Material and Method

Study Area

The study was carried out in Rahya-Suchani located in district Samba of J&K, India (Fig 1A, 1B,). District Samba lies between 75° 11′ N longitude and 32° 56′ E latitude. This area can be divided into two regions with the plains located in the south while northern region is in the foothills of the Shivalik mountains. The actual sampling site in Rahya-Suchani was located between latitude 32°35'N and 32°38' Nand longitude74°57'Eand 75°00' E (Fig. 2). The soil in this area is moderately fertile while the climate here is of Sub-tropical type, though the climate in northern parts of the district gets frigid due to its vicinity to the snow-capped mountains (Bhellum, 2018), while most of the rains are brought by the Monsoons in the months from July to August.

Morphometry

Morphometric studies were done on five specimens each of *Triticum aestivum* L. and *Phalaris minor* Retz. collected from non-polluted/cultivated and polluted/roadside. Forteen quantitative characters (Table 1) were selected for the morphometry based on the study done by Ahmad et al. (2014). Only fresh specimens were used for morphometry so as to prevent erroneous data collection due to drying up of the specimen.

Table 1: Quantitative morphological characters used for morphometry. All scales are in cm.

Character	Character
Total Length	Internode Length
Root Length	Flag Leaf Length
Culm Length	Floret Length
Inflorescence Length	Glume Length
Blade Length	Spikelet Length
Ligule Length	Palea Length
Sheath Length	Lemma Length

Anatomy

For anatomical analysis, the plant samples were first washed using distilled water and then fixed in acetic alcohol solution. Anatomical sections were cut by free hand sectioning of the leaves and culm (stem), while epidermal peeling was also done for the leaves. Sections were stained using safranine and

fast green stains (Ahamd et al., 2014). Micrographs of the stained sections were taken using a hand-held camera.

Statistical Analysis

The morphometric data was subjected to both univariate and multivariate statistical analysis. All the statistical analysis and plots were done using “Past 4.04” (Hammer et al., 2001) and Ms-Excel, 2019.

Results

Morphometry

The morphometric data for *Triticum aestivum* L. and *Phalaris minor* Retz. for the 14 characters was recorded for both polluted and non-polluted sites. Using this data mean values along with the standard error was calculated for both *Triticum aestivum* L. and *Phalaris minor* Retz. (Table 2).

Table 2
Mean and standard error values for morphometric data of *Triticum aestivum* L. and *Phalaris minor* Retz. for all five specimens collected from both the sampling sites.

Character	*Phalaris minor* Retz.		*Triticum aestivum* L.	
	Polluted	Non Polluted	Polluted	Non Polluted
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Total Length	38.86 ± 4.28	54.8 ± 4.09	54.9 ± 6.23	77.02 ± 3.54
Root Length	1.54 ± 0.20	3.02 ± 0.22	3.62 ± 0.19	5.96 ± 1.40
Culm Length	31.58 ± 3.63	47.36 ± 4.22	37.22 ± 6.37	48.64 ± 4.92
Inflorescence Length	3.36 ± 0.49	3.44 ± 0.21	10.4 ± 0.96	19.66 ± 0.98
Blade Length	12.2 ± 1.17	15.8 ± 2.73	16.14 ± 2.62	30.3 ± 1.98
Ligule Length	0.62 ± 0.08	0.7 ± 0.07	0.15 ± 0.03	0.24 ± 0.05
Sheath Length	3.9 ± 0.62	4.54 ± 0.31	9.58 ± 1.03	13.76 ± 2.12
Internode Length	5.16 ± 0.51	8.6 ± 1.16	8.74 ± 1.84	10.96 ± 0.81
Flag Leaf Length	2.3 ± 0.69	3.12 ± 0.8	12.44 ± 0.41	17.44 ± 1.70
Floret Length	0.28 ± 0.01	0.34 ± 0.02	1.08 ± 0.03	1.28 ± 0.03
Glume Length	0.28 ± 0.01	0.40 ± 0.007	1.22 ± 0.08	1.74 ± 0.18
Spikelet Length	0.29 ± 0.01	0.42 ± 0.02	6.06 ± 0.34	10.76 ± 0.44
Palea Length	0.15 ± 0.01	0.23 ± 0.03	0.9 ± 0.04	1.2 ± 0.07
Lemma Length	0.19 ± 0.005	0.33 ± 0.05	0.94 ± 0.05	1.14 ± 0.05

Looking at table 3, a general inference can be drawn that the mean values for both *Triticum aestivum* L. and *Phalaris minor* Retz. collected from non-polluted sites surpasses the mean values of their respective counterparts collected from polluted sites. While the total plant length (mean value) for *Triticum aestivum* L. collected from non-polluted sites was 77.02 cm, the total plant length (mean value) for *Triticum aestivum* L.collected from the polluted site comes out to be only 54.9 cm. Similarly in case of *Phalaris minor* Retz. the values were 54.8 cm and 38.86 cm respectively for samples collected from non-polluted and polluted sites. This increment of values in plant specimens collected from non-polluted sites was not only limited to vegetative parts but also seen in the case of reproductive parts. This variation was much better visualized using box-plots (Fig. 4 and Fig. 5).

The box-plots (Fig. 3 and Fig. 4) clearly show that for each of the 15 characters selected for morphometry, the value for plants collected from non-polluted sites exceed those collected form polluted sites. This can be a clear indication of stunted growth of plants in polluted areas severely

affecting their growth as seen from the box-plots. But in some cases, the values of polluted samples show a slight increase as seen in Inflorescence length and Sheath length in case of *Phalaris minor* Retz.

Anatomy

Transverse section of leaf blade, culm along with epidermal peeling of leaves showed significant variation between the specimens growing in polluted and non-polluted sites for both *Triticum aestivum* L. and *Phalaris minor* Retz.(Fig. 5)

For *Phalaris minor* Retz. transverse section of the leaf blade from both the polluted and non polluted samples showed typical grass anatomy.Though variation was seen with respect to the thickness of sclerenchymatous layer around the vascular bundels.The samples from polluted sites showed a thicker sclerenchymatous layer as compared to that from non polluted site. Also the chlorenchyma cells appeard slightly discloured in the sample collected from the polluted site.Similarly tranverse section of the culm of both the stems showed a hollow pith with vascular bundles arranged in a ring.But in the samples collected from the polluted site the deposition of the sclerenchymatous cells below the epidermis was significantly more than that of non pollued samples.Also number of vascular bundles were considerably more in the samples collected from the polluted sites.While epidermal peeling for the both samples showed certain variations. Leaf epidermal peeling showed a higher number of stoma on the samples collected from the polluted site as compared to the samples collected from the non polluted sites.While both samples showed the presence of phytoliths.

Similarly in case of *Trtiticum aestivum* L. variation was observed in the anatomy for the specimens collected from non-polluted and polluted sites with respect to their culm, leaf and epidermis. (Fig. 5)

In the case of transverse section of culm) both the specimens showed the characteristic grass anatomy having a hollow centre followed by a parenchymatous cortex with vascular bundles scattered. Despite both the culm sections showing sclerenchyma cells inside to the epidermis, the deposition was significant in the samples collected from the polluted sites along with the presence of black debris inside the sclerenchyma cells.While the transverse section of the leaves showed variation with respect to the thickness of sclerenchymatous girders around the vascular bundles, with the specimens collected from the polluted sites showing a significant deposition. Also, discolouration of chlorenchyma was seen in polluted samples.Epidemal peeling revealed the characteristic phytoliths in both the samples while stomata number was slightly more in the sample collected from the non-polluted sites.

SEM Analysis

SEM analysis done for *Phalaris minor* Retz., (Fig. 6) of the dorsal surface of the leaf showed a larger size of stomata for samples collected from polluted sites. The mean size of stomata was 31.12um while for the samples from the non-polluted sites it was 24.70um.Furthermore, SEM analysis or the ventral surface of the leaves collected from both the sites i.e. Non polluted and polluted sites showed variation with respect to the presence of spines. While samples from the non-polluted site clearly showed the

presence of spines on the ventral surface of the leaf whereas such spines were not present in the samples collected from the polluted site.

While SEM images for the dorsal surface (Fig 6) of *Triticum aestivum* L. leaf showed that the size of stomata was bigger in samples collected from the non-polluted sites in contrast to the polluted ones. While the average size of stomata was 40.40 um for non-polluted specimens, it was only 30.49 um for the polluted specimens.Similar to the SEM images of *Phalaris minor* Retz., the ventral surface of *Triticum aestivum* L. showed the presence of spines only in the specimens collected from the non-polluted sites while in the specimens collected from the polluted sites the spines were conspicuously absent.

Discussions

The decrease in morphometric values for *Phalaris minror* Retz. and *Trtiticum astivum* L. collected from polluted/roadside when compared to the samples collected from non-polluted sites, was evident due to high air pollution and occurrence of heavy metals near the road side. Studies conducted by Rocha et al. (2014), Rai (2016), Namrata (2017),Dzhygan et al. (2018), Lugovskaya et al. (2018) and Nykytiyk (2020) all have reported the adverse effect of pollutants on plant morphometry. While Namrata (2017) reported a decrease in size for leaf parameters like leaf area and petiole length for the samples collected from polluted areas (commercial and industrial areas), our results for the leaf blade length for both *Triticum aestivum* L. and*Phalaris minor* Retz. showed similar reduction in samples collected from the polluted sites in contrast to the non-polluted sites. Dzhygan et al. (2018) not only reported a decrease in foliar area for *Rhus typhina* L. growing near roadside but also a significant reduction in annual increment for the specimens growing near the roadside when compared to the control site. This finding corroborates with our own result of total plant length and culm length both of which showed a downward trajectory for *Triticum aestivum* L. and*Phalaris minor* Retz. collected from the polluted sites. Interestingly this decrease in morphometric parameters can be used as a bio-indication for determining the state of pollution as done by Nykytiyk (2020), who observed the decreased morphometric parameters of the fruit of *Robinia pseudoacacia* L. for determining the quality of air. In general, it is safe to say that pollutants have a negative effect on the growth of plants as seen through our morphometric data and corroborated by studies done by Mamieva & Shirnina, (2017), Seyyednejad et al. (2009), Assadi et al. (2011) and many others. Though, it is important to point out that both Efe et al. (1993) and Byres (1992) stated that decrease in morphometric values for leaf parameters like leaf area, leaf breadth and leaf number was a response/adaptation rather a direct consequence of the pollutants. Dineva (2004) in particular stated the morphological changes observed in plants growing in polluted sites help plants minimize stress and maximize the use of internal and external resources.

Anatomical variations in plants growing in polluted sites are often more profound in leaves as compared to the stems (Kumar and Nandini, 2013; Mitu et al. 2019), partially due to fact that the functioning of major physiological processes is the prerogative of leaves, causing significant anatomical changes. Though, stems are not immune to anatomical variations due to pollutants as seen by the work done by Gupta and Ghouse (1987) wherein they observed a significant decrease in stem cortex and pith due to

coal-smoke pollutants. In our study the leaf sections for both *Triticum aestivum* L. and *Phalaris minor* Retz. collected from the polluted sites showed a marked discoloration of the chlorenchyma cells along with the presence of a thick sclerenchymatous deposition below the epidermis. Similar leaf anatomical variations were seen by Mitu et al. (2019) in dicot plants wherein due to pollutants black dot like substances appeared in both upper and lower epidermis of the plants. It is interesting to note that Giełwanowskaet al. (2005) observed that *Deschampsia antarctica*É.Desv. another pooideae member showed deposition of thick sclerenchymatous fibers below the epidermis when grown in climatic conditions simulating high stress response. Thus, presence of thick sclerenchyamtous cells in our study for plants growing in polluted sites can be considered akin to a high stress response as seen by Giełwanowskaet al. (2005). Similar studies showing the role of pollutants in causing anatomical changes in the foliar characteristics of plants was done by Muthu et al. (2021), Uka et al. (2017), Jahan and Iqbal (1992), Bhatti and Iqbal (1988) and Verma and Chandra (2014). In case of sections of stem both *Triticum aestivum* L. and *Phalaris minor* Retz. collected from the polluted sites showed thick sclerenchymatous layer beneath the epidermis but in case of both *Triticum aestivum* L. black dust particles were also seen in the sclerenchymatous layer. Sukumaran (2014) also observed anatomical changes in stem due to roadside pollutants, decrease in circumference of stem with xylem vessels being more profoundly affected by the pollutants. While Stevovice et al.(2010) observed thatthe vascular bundles for Tansy plant growing in polluted areas showed variation in sizes when compared to non-polluted ones. Though such size differences were not quite visible in our study, the number of vascular bundles seen in the case of *Phalaris minor* Retz. growing in polluted sites were significantly more than that of those growing in non-polluted sites which was most likely due to the difference in age for the two specimens collected rather than the consequence of pollutants which will require a more detailed study. Epidermal peeling showed that both the samples growing in the polluted sites showed a marked increase in stomatal frequency when compared to their respective samples collected from non-polluted sites. Our result is in consonance with the findings of Wagoner (1975) and Namrata (2017).Ekpemerechi et al. (2017) went further and stated the possible reason for increase in number of stomata as a strategic decision by plant to allocate more resources for the production of stomata in order to increase the absorption of CO2 from an already polluted environment.

SEM images for the foliar epidermal characteristics for both both *Triticum aestivum* L. and *Phalaris minor* Retz. showed variations. While the stomata size in *Triticum aestivum* L. growing in polluted sites showed a marked reduction in size when compared to the one growing in non-polluted site, the stomata size for *Phalaris minor* Retz. in a complete volte face showed a bigger size for the samples growing in the polluted sites. Studies carried out by Uka et al. (2017), Rai (2016), Kulshrestha (1980), Allahnouri et al. (2018), Raina and Sharma (2006), Raina and Bala(2007) and Akbarian et al. (2011) indicated reduced leaf size due to exposure to pollutants. This decrease in the size of the stomata as seen in the case of *Triticum aestivum* L. growing in polluted sites can be explained as the result of a favourable adaptation by the plant to prevent the entry of harmful pollutants inside the plant itself (Mishra, 1982; Sharma & Tyree, 1973, Sharma & Butler 1975 and Garg & Varshney,1980). Despite the contrasting results seen in the case of *Phalaris minor* Retz., the variation in stomata size can be explained by the simple reasoning

that individual plants react differently to different air pollutants. Further increase in size of stomata for *Phalaris minor* Retz. growing in polluted sites were in conformity with Verma and Chandra (2014) who also noticed an increase size of epidermal cells and stomata in plants growing in polluted sites. Furthermore, epidermal cells in case of *Phalaris minor* Retz. growing in polluted sites were larger than those of non-polluted sites, due to the fact that differentiation of stomata mother cells requires division of epidermal cells, therefore, a decrease in the frequency of stomata as seen in our results, should be normally accompanied by an increase in the size of epidermal cells.Similarly, absence or spines on the ventral surface of both *Triticum aestivum* L. and*Phalaris minor* Retz. collected from the polluted sites in contrast to the specimens collected from non-polluted sites showed a clear physiological effect of the pollutants. Khan et al. (2017) has showed the presence of hooks on both the epidermal surfaces of *Phalaris minor* Retz. While Namrata (2017) and Uka et al. (2017) both have stated increase in abundance of trichomes for plants collected from polluted sites as a possible defense mechanism against the exposure to pollutants, absence of spines as seen in our study may be the result of a pure physiological/biochemical alteration due to the contact of leaf with harmful pollutants as presence of spines is a deterrence against herbivory and not exposure to pollutants.

Conclusion

The present study revealed the major anatomical and morphometric variations observed in both *Triticum aestivum* L. and*Phalaris minor* Retz. growing in polluted and non-polluted study sites. Reduction in morphometric parameters for both the specimens growing in polluted study sites indicated a negative effect of pollutants on the growth of these pooideae members. Not only that, changes in the anatomy of the polluted site specimens, both in the culm and leaf anatomy indicated in some cases, an adaptive response on the part of the grasses to counter the effect of the pollutants. While SEM images of the abaxial and adaxial surface of the leaves showed micro-morphological changes, with stomatal size and number showing a reduction in case of *Triticum aestivum* L. for the polluted site specimens while the size of stomata showed an increase in the case of *Phalaris minor* Retz. growing in polluted study sites. Similarly, absence of spines in polluted study site specimens further corroborated changes in micro-morphological changes. These variations along with similarities shown by these two grasses when exposed to high pollution levels highlighted the unique response of these two pooideae members to difficult environmental conditions. Changing climatic conditions along with the rise in pollution will significantly impact the growth and development of these important economical grasses in the long run. Thus, further studies are required to analyze a detailed response of these pooideae members in terms of their biochemical, physiological and genetic makeup in events of extreme environmental conditions both natural and anthropogenic.

Declarations

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Author contributions: Mudassir Ahmad Bhat and Aditya Singh contributed to the study conception and design. Experimental work, data collection and analysis were performed by Aditya Singh, Raveena Akhter, Damini supolia, Farida Akhter and Marukh Irshad helped in data analysis. The first draft of the manuscript was written by Aditya Singh and Damini supolia. Ashok Kumar and Mudasir Nazir Bhat helped in SEM analysis. Prof. Brijmohan Singh Bhau and Mudassir Ahmad Bhat supervised the work. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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Figures

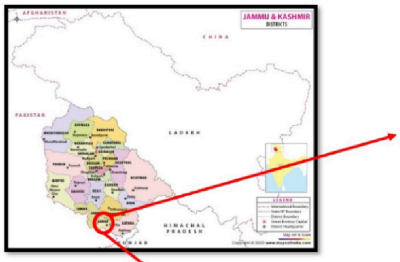


Fig 1 A: Map of J&K showing the districts of Jammu division. Red circle shows the location of Samba district.

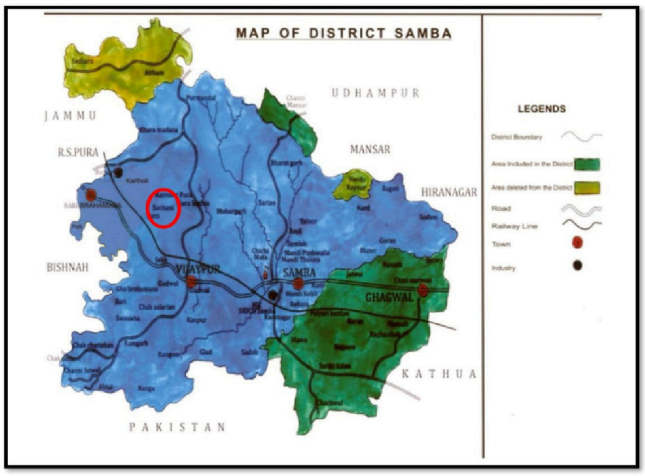


Fig. 1B : Red circle showing the location of Rahya-Suchani area in district Samba, J&K.

Figure 1

A: Map of J&K showing the districts of Jammu division. Red circle shows the location of Samba district.

B: Red circle showing the location of Rahya-Suchani area in district Samba, J&K.

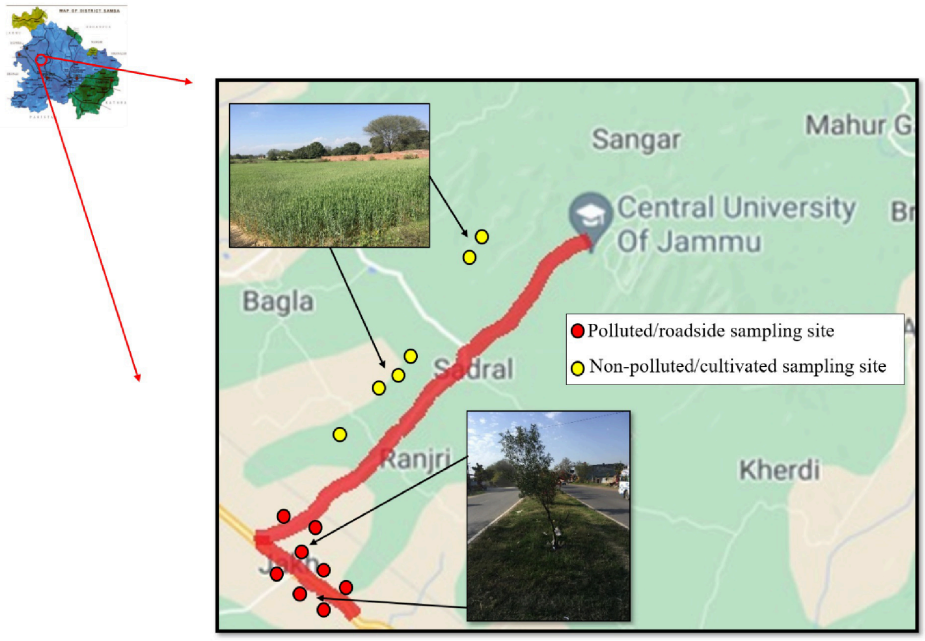


Figure 2

Actual sampling sites at Rahya-Suchani. Red path indicates the major road while red dot and yellow dots represent polluted and non-polluted sampling sites respectively.

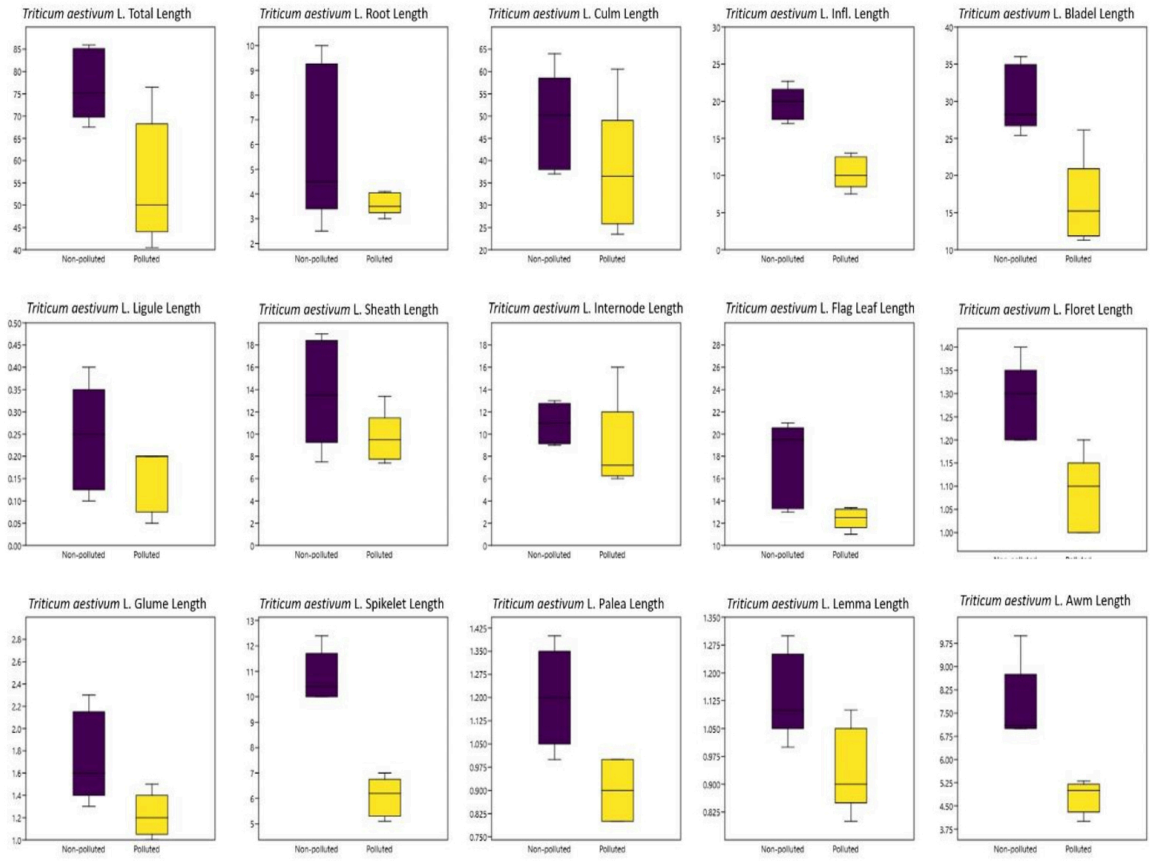


Figure 3

Box-plots of morphometric data for *Triticum aestivum* L. showing the variation in size distribution for samples collected from non-polluted and polluted sites.

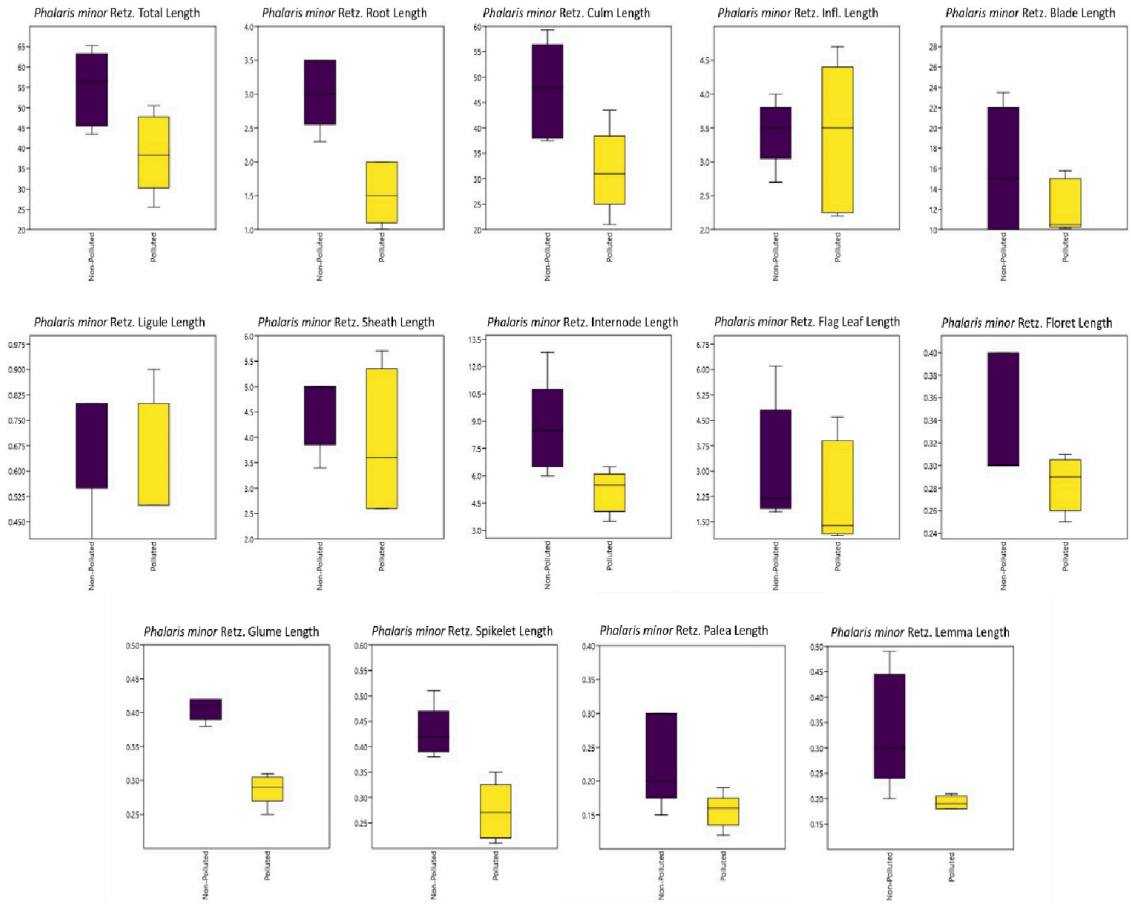


Figure 4

Box-plots of morphometric data for *Phalaris minor* Retz. showing the variation in size distribution for samples collected from non-polluted and polluted sites.

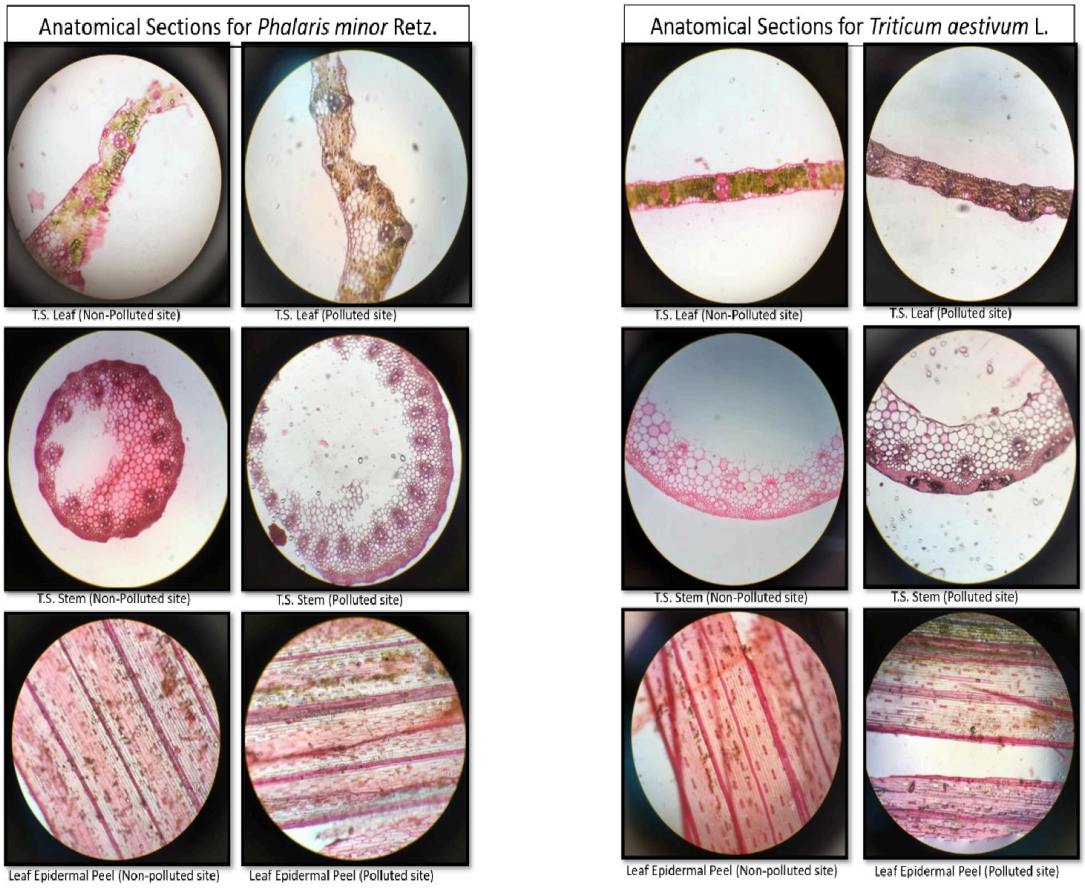


Figure 5

Anatomical sections (T.S of leaf, T.S. of culm) and epidermal peel for both *Triticum aestivum* L. and *Phalaris minor* Retz. collected from non-polluted and polluted sites.

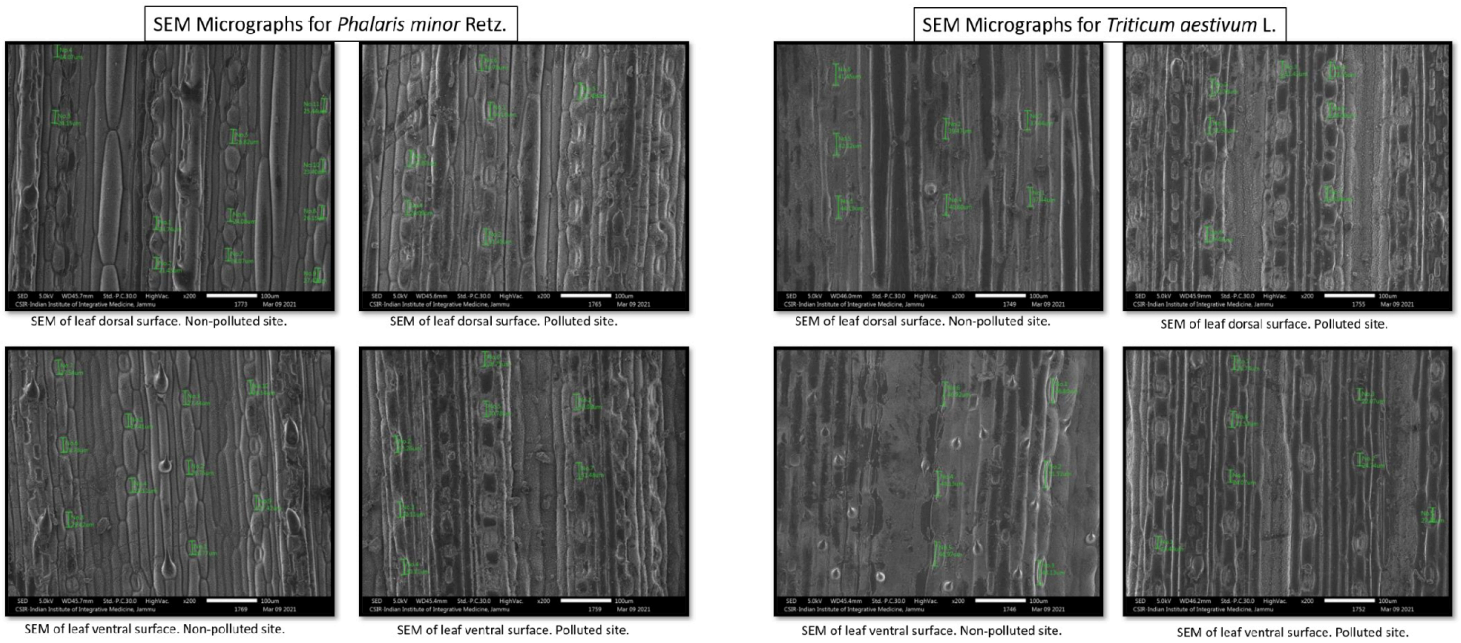


Figure 6

SEM images of abaxial and adaxial surface of leaves of *Phalaris minor* Retz. and *Triticum aestivum* L. growing in both polluted and non-polluted sites.

Supplementary Files

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- GraphicalAbstract.jpg