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# Phytodiversity and morpho-anatomical modification of the wild plants irrigated by tannery effluents

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## Abstract

**Background** With the rapid increase in industrialization, irrigation through waste or sewage water is a common practice due to low and scarce freshwater supply. Plants are exposed to hazardous metals that accumulate in plant tissues and negatively affect them. Therefore, current study aimed to investigate the phytodiversity and morpho-anatomical modification of wild plants irrigated by tannery effluents. Six wild plant species were collected from different sites of Multan near tannery industries through the quadrat method.

**Results** Results indicated that *Cynodon dactylon* L. had the highest relative cover, relative frequency, relative density, and important index value, i.e., 35, 10, 30, and 75% followed by other species. The morpho-anatomical modifications showed a reduction in leaf characteristics in *C. dactylon*, *Eragrostis barrelieri* Daveau, *Polypogon monspeliensis* L., *Solanum nigrum* L., *Triticum aestivum* L., except in *Asparagus adscendens* Roxb. A decrease in leaf length was observed in all species with maximum reduction of 55% in *T. aestivum* followed by *P. monspeliensis*. Similarly, reduction in metaxylem area was observed in *T. aestivum*, whereas sclerenchymatous thickness found to increase in *P. monspeliensis* by 15%. *Asparagus adscendens* showed decrease by 70% in comparison to other species.

**Conclusion** Variation in the trend of the morpho-anatomical parameters as well as phytodiversity helps to understand the tolerance potential of the wild plants under wastewater irrigation released by tannery industries and that adverse developmental features in wild plants could be used as bioindicators of toxic heavy metal pollution.

**Keywords** Plant adaptations, Anatomical changes, Tannery wastewater, Water pollution, Environmental stress, Bioindicators

## 1 Introduction

Water pollution is one of the major environmental concerns all over the world [1]. It is defined as the contamination of the water due to rapid industrialization, excessive exhaustion, and waste, mainly garbage [2]. One

of the major industries, tannery, deals in treating many skins and hides of animals to produce leather [3]. Data collected from different countries showed that each year, about three hundred million bits of cowhides are handled and processed to make leather and consequently these industries release about three hundred million tons of wastewater [4].

Leather industry in Pakistan plays a crucial role in the economy, ranking as the second-largest export-earning sector and contributing approximately 5% to the national GDP [5]. Employing over 500,000 people [6], the industry has experienced steady growth, with export earnings reaching 700 million US\$ in 2013 [7] and rising to 724 million US\$ in 2014–15 [6]. The major

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leather production hubs are located in Karachi, Sialkot, Kasur, Lahore, Gujranwala, Multan, and Peshawar [8]. The sector is broadly divided into six subindustries: leather tanning, footwear, garments, gloves, shoe uppers, and other leather goods. Internationally, Pakistan is recognized for its superior-quality leather products, ranking 21st in the global market and contributing 6.17% to leather garments, 1.12% to hides and skins, 10.76% to leather gloves, 0.28% to leather footwear, and 0.21% to other leather products [5]. In 2014, the country's leather exports totaled 0.7 billion US\$, with gloves leading the market at 0.3 billion US\$, securing Pakistan's position as the world's top exporter of leather gloves [6]. Despite its economic contributions, the leather industry is also one of the most water-intensive sectors. Tanning a single ton of leather requires up to 4,000 L of water, and the wastewater generated during this process is often discharged untreated into natural water bodies [6]. This effluent, laden with toxic chemicals, severely contaminates groundwater sources, which serve as a primary source of drinking water. A single tannery has the potential to pollute groundwater within a 7–8 km radius, posing serious environmental and health risks to humans, animals, and aquatic ecosystems [6]. With Pakistan's growing prominence in the international leather market, the challenge lies in balancing industrial expansion with sustainable environmental practices.

Irrigation through waste or sewage water is a common practice due to low and scarce freshwater supply [9]. The tannery industry releases wastewater that majorly includes lead, chromium, organic and inorganic compounds, suspended and dissolved solids as well as sulfides [10]. The effluents discharged from leather industries accumulate in plant tissues and reduce biomass, synthesis of chlorophyll, proteins and nitrates [11] that resulting in chlorosis, stunted growth, and death [12]. Industrial pollution in Pakistan has emerged as a significant environmental challenge, driven by the rapid growth of industries such as textiles, tanneries, and chemical manufacturing [13]. Among these, the leather industry is a major contributor, with tannery operations discharging large volumes of untreated wastewater containing toxic substances like chromium, heavy metals, and organic pollutants into surrounding water bodies [14]. This not only compromises water quality, but also leads to soil contamination, adversely affecting both local ecosystems and public health. Furthermore, air pollution from industrial activities exacerbates respiratory problems in nearby communities, while improper waste disposal continues to degrade the environment [15]. The cumulative impact of these pollutants presents a serious threat to both human well-being and biodiversity. Effective mitigation of industrial pollution in Pakistan requires the implementation of

stricter regulations, enhanced waste treatment processes, and the adoption of cleaner, more sustainable industrial practices to safeguard both the environment and public health.

Wheat or bread wheat is encouraged to be cultivated across Pakistan as a main staple food [16]. Irrigation with wastewater causes the accumulation of heavy metals in plant tissues reducing the yield [17], growth [18], and morpho-physiological as well as anatomical reduction [19]. The heavy metal-contaminated grain affects the human body and leads to ulcer, lung cancer, and fatal liver diseases [20]. Nightshade plant or Mako is a perennial or annual weed that has the ability to grow fast [21]. Various parameters have been experimented by growing *S. nigrum* under heavy metal stresses that showed alteration in seedlings [22] as well as cell wall components [23]. *Eragrostis barrelieri* is an annual herb. It contains fertile spikelets. It is commonly called love grass or cane grass. Bermuda grass is a summer-season species with extraordinary turfs quality like those found in fields, parks, gardens as well as golf courses [24]. *Polypogon monspeliensis* or rabbit-foot grass is an intrusive monocot grass that is hypothetically valuable for foodstuff, decoration, and fodder [25].

Heavy metal adaptations in plants involve various morphological, physiological, and anatomical modifications to mitigate metal toxicity. Heavy metal adaptations in *T. aestivum* and *S. nigrum* involve various morphological, physiological, and anatomical modifications to mitigate cadmium (Cd) toxicity [26, 27]. In wheat, exposure to different Cd concentrations (0, 50, 100, 200, and 400  $\mu\text{M}$ ) resulted in Cd accumulation in different plant parts, leading to reductions in growth and development [27]. However, wheat plants managed lower stress levels, indicating a degree of tolerance. In tomato, Cd exposure restricted both fresh and dry mass of leaves and internodes, with a notable reduction in internode length, diameter, and leaf area. Morphological changes included altered stem morphogenesis, cellular division disorders, and abnormal enlargement of cortical and medullary cells. High Cd levels (100  $\mu\text{M}$   $\text{CdCl}_2$ ) caused a decrease in mesophyll cell size and spongy intercellular spaces, leading to leaf blade thinning. Despite these negative effects, wheat and tomato plants demonstrated adaptive responses such as modifications in stomatal density, tissue structural changes, and adjustments in photosynthetic activity to tolerate Cd stress. Similar under NaCl stress, *C. dactylon* showed thickness in sclerenchymatous cells [28].

Plants show morpho-anatomical adaptations as well as variation in diversity when they are continuously exposed to environmental pollution. The objectives of this study were to investigate the phytodiversity within the selected area and evaluate the effects of pollution on

local plant species. The study aimed to establish a correlation between the morpho-anatomical characteristics of leaves under both normal and polluted conditions. Additionally, the research sought to assess the sensitivity of plant leaves to pollution and compare the responses of various plant species by analyzing their leaf anatomical structures. Through these objectives, the study aimed to provide a comprehensive understanding of how pollution influences plant morphology and anatomy, as well as their adaptive responses to environmental stressors.

## 2 Materials and methods

### 2.1 Study site and sampling

This study was conducted in the industrial zones of Multan, Punjab, Pakistan (30°12' latitude and 71°28' longitude) to assess the phytodiversity and morpho-anatomical adaptations of plant species exposed to tannery effluents. Samples of effluents and soil were collected for heavy metal analysis as depicted in Table 1 [29]. The selection of plant species was based on their prevalence in polluted areas and their ecological significance. These species were commonly at the study site, suggesting natural adaptations to the contaminated environment. Moreover, their potential to exhibit their potential to exhibit morpho-anatomical modifications in response to pollution stress, making them suitable indicators for assessing the impact of tannery effluents on plant physiology. Selection criteria ensured that plants from both sites belonged to the same species and were at a similar developmental stage. Since exact chronological age was unknown, plants were chosen based on comparable morphological attributes. This approach helped standardize comparisons and allowed for an accurate assessment of the impact of tannery effluents on plant growth and structure.

Plant sampling was carried out using the quadrat method [30]. In this, quadrates were randomly placed across the selected sites to identify and collect the most dominant species. A total of six plant species were selected from the polluted site including *A. adscendens* (Asparagus fern, Safed Musli), *C. dactylon* (Bermuda grass, Khabbal),

*E. barrelieri* (Love-grass, Chiri ghas), *P. monpeliensis* (Annual beard grass), *S. nigrum* (Black-nightshade, Mako), and *T. aestivum* (Wheat, Gandam). To ensure a comprehensive analysis, control plants were collected from the unpolluted environment of Cholistan Institute of Desert, Bahawalpur, Pakistan. The following formulae were used for the collection of ecological data:

$$\text{Density} = \frac{\text{Total number of individuals of a species}}{\text{Total area of quadrats}} \times 100$$

$$\text{Relative density of a species} = \frac{\text{Density of a species}}{\text{Total density of all species}} \times 100$$

$$\text{Frequency} = \frac{\text{Total number quadrats in which a species existed}}{\text{Total area of quadrats sampled}} \times 100$$

$$\text{Relative density of a species} = \frac{\text{Frequency of a species}}{\text{Total frequency of all species}} \times 100$$

$$\text{Cover} = \text{Total percent cover of a species}$$

$$\text{Relative cover} = \frac{\text{Percent cover of a species}}{\text{Total percent cover of all species}} \times 100$$

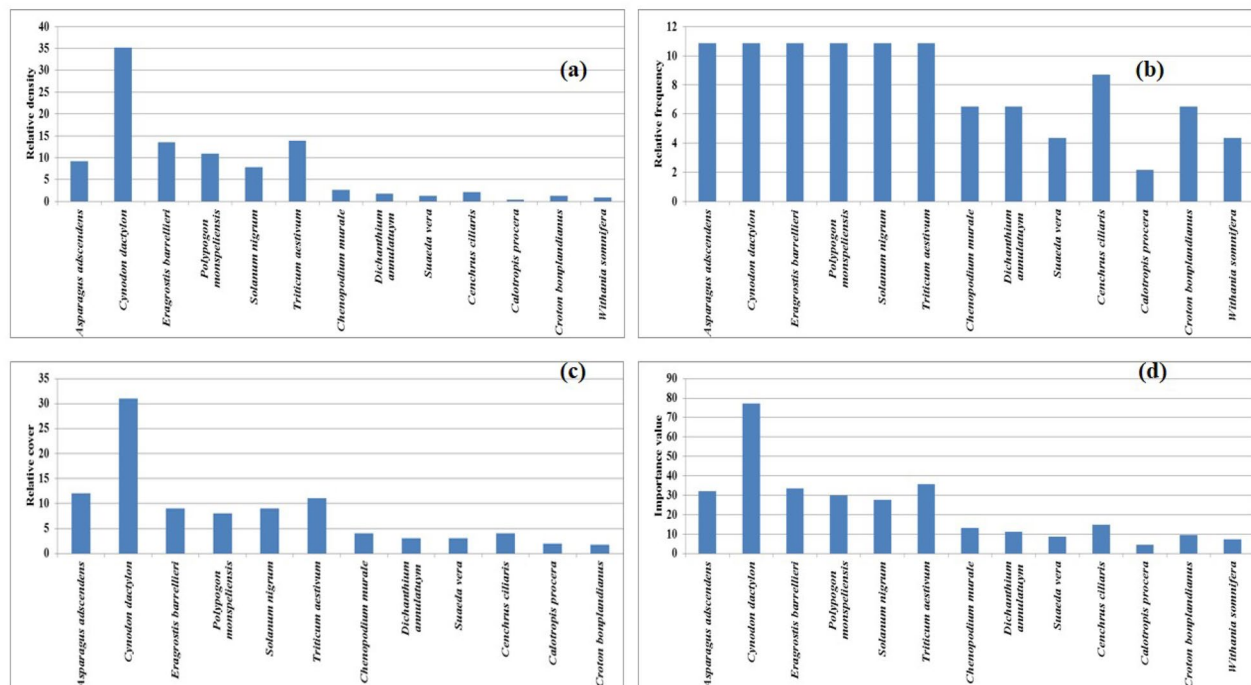
$$\text{Important index value} = \text{Relative density} + \text{Relative frequency} + \text{Relative cover}$$

### 2.2 Determination of morphological attributes

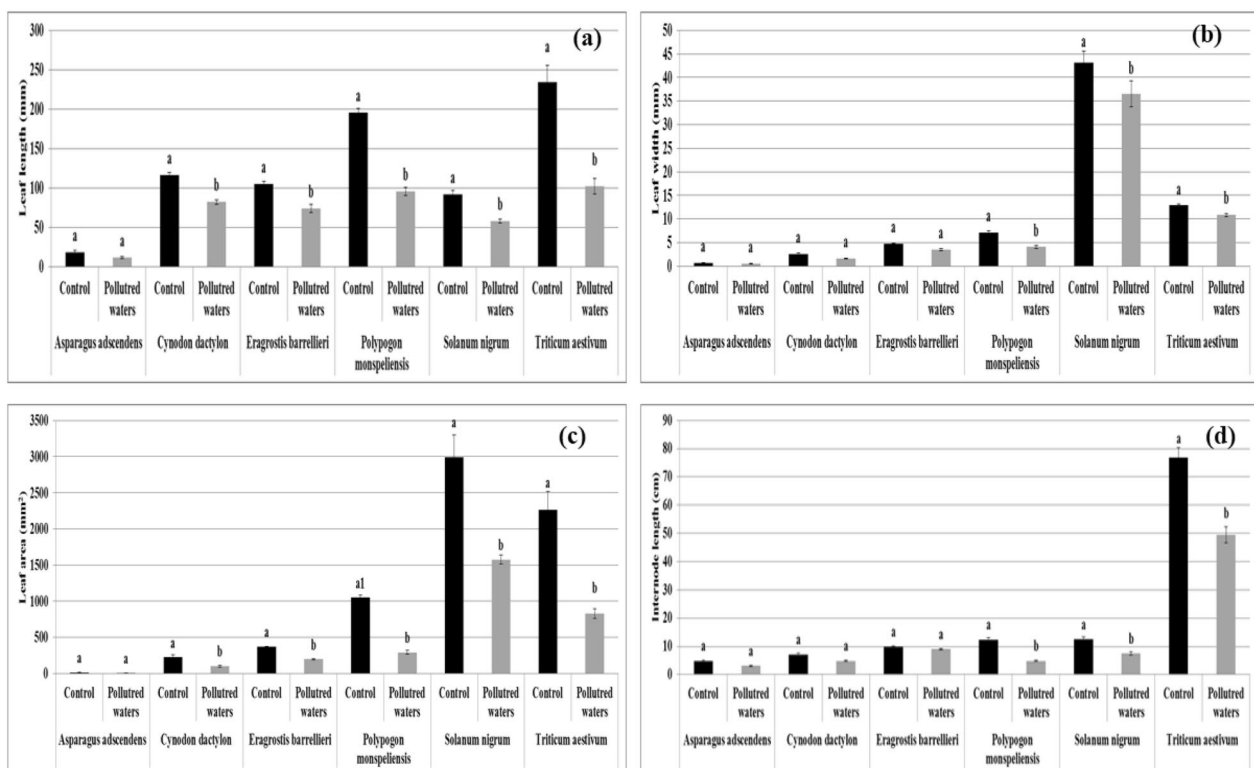
Morphological parameters such as leaf length (mm), leaf width (mm), leaf area (mm<sup>2</sup>), and internode length (cm) were measured before preservation to ensure that the plant samples retained their natural dimensions. These measurements were taken using a ruler and digital calipers, while the leaf area was calculated either by using a formula. Plant samples were preserved by placing the leaves in a plastic bottle containing a fixative solution of formalin, acetic acid, alcohol (FAA), which

**Table 1** Summarized statistical distribution of heavy metals in tannery effluents and soil samples

|                 |         | Na     | K      | Ca     | Mg     | Fe   | Mn   | Cr    | Cd   | Ni   | Pb   |
|-----------------|---------|--------|--------|--------|--------|------|------|-------|------|------|------|
| Effluent (mg/L) | Minimum | 30.2   | 27.1   | 0.30   | 2.96   | 0.07 | 0.02 | 0.21  | 0.01 | 0.08 | 0.10 |
|                 | Maximum | 9,194  | 2,639  | 1,445  | 276    | 16.6 | 2.15 | 1,383 | 0.23 | 45.4 | 2.80 |
|                 | Mean    | 5,494  | 946    | 566    | 110    | 3.35 | 0.44 | 209   | 0.10 | 4.65 | 0.97 |
| Soil (mg/kg)    | Minimum | 6,300  | 82.0   | 555    | 68.0   | 0.12 | 0.31 | 0.55  | 0.09 | 0.48 | 0.50 |
|                 | Maximum | 13,640 | 14,060 | 30,805 | 39,911 | 49.0 | 60.5 | 1,187 | 9.00 | 101  | 58.5 |
|                 | Mean    | 10,021 | 6,796  | 9,272  | 9,957  | 23.5 | 18.4 | 496   | 2.94 | 55.5 | 21.2 |



**Fig. 1** Phytodiversity attributes of wild species collected from industrial polluted waters



**Fig. 2** Morphological characters of six wild species collected from control and industrial polluted waters. Graphs represent the means  $\pm$  SE (n = 3). Different letters above each bar indicate statistically significant differences between groups as determined by the LSD test ( $p \leq 0.05$ )

was prepared by adding 90% ethanol, 5% acetic acid, and 5% formaldehyde. This solution ensured adequate preservation of the plant tissues [31, 32].

### 2.3 Leaf preservation and anatomical examinations

Once the morphological measurements were completed, the plant material was preserved in FAA fixative for anatomical analysis. For anatomical analysis, parameters such as leaf thickness ( $\mu\text{m}$ ), epidermal thickness ( $\mu\text{m}$ ), vascular bundle area ( $\mu\text{m}^2$ ), metaxylem area ( $\mu\text{m}^2$ ), sclerenchymatous thickness ( $\mu\text{m}$ ), stomatal density, and stomatal area ( $\mu\text{m}^2$ ) were examined. Anatomical examinations followed standard histological methods. Fresh samples of leaves, stems, and roots from both tannery effluent-exposed and control sites were fixed in FAA for tissue preservation. The samples were then embedded in paraffin wax, and transverse and longitudinal sections were prepared using a rotary microtome [33]. In cases requiring freehand sections, a razor blade was employed. The sections were stained with safranin and fast green to differentiate tissue types. Finally, the slides were examined under a light microscope, and anatomical variations, such as changes in the epidermis, vascular bundles, and cellular structures, were documented through photomicrographs, focusing on stress-related alterations due to effluent exposure. Magnification of all micrographs is 20X. The plant samples were immersed in the FAA fixative for a minimum of 24 h to ensure adequate tissue preservation, and the fixation time was carefully monitored to prevent over-fixation, which could alter anatomical integrity. After fixation, the specimens were processed for further analysis.

### 2.4 Statistical analysis

The data collected in this study were analyzed using Statistix 8.1 software. The data were subjected to

two-way analysis of variance (ANOVA) to assess the variance among different groups, specifically comparing the control and tannery effluent-exposed plant samples. To further examine the differences between the groups, the least significant difference (LSD) test was applied for pairwise comparison of means. Statistical significance was determined at a p-value of less than 0.05, with each test repeated a minimum of three times to ensure the reliability of the results. This rigorous statistical approach strengthened the validity of the findings of study [34].

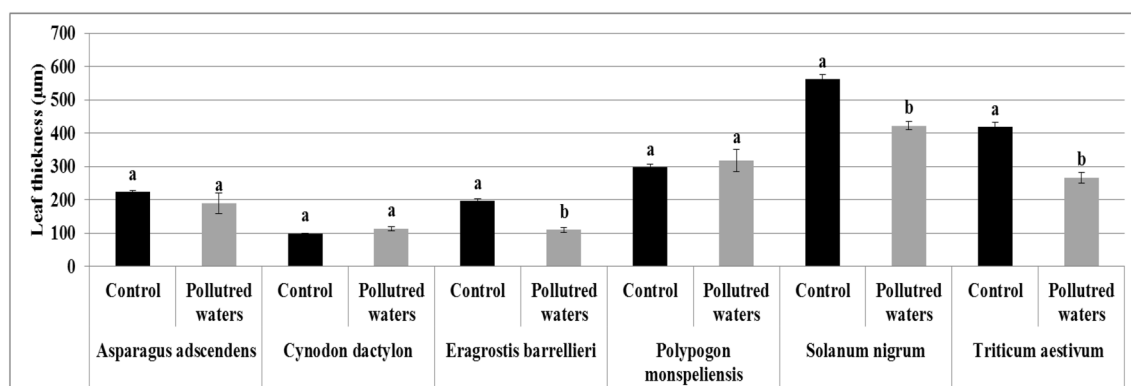
## 3 Results

### 3.1 Phytodiversity

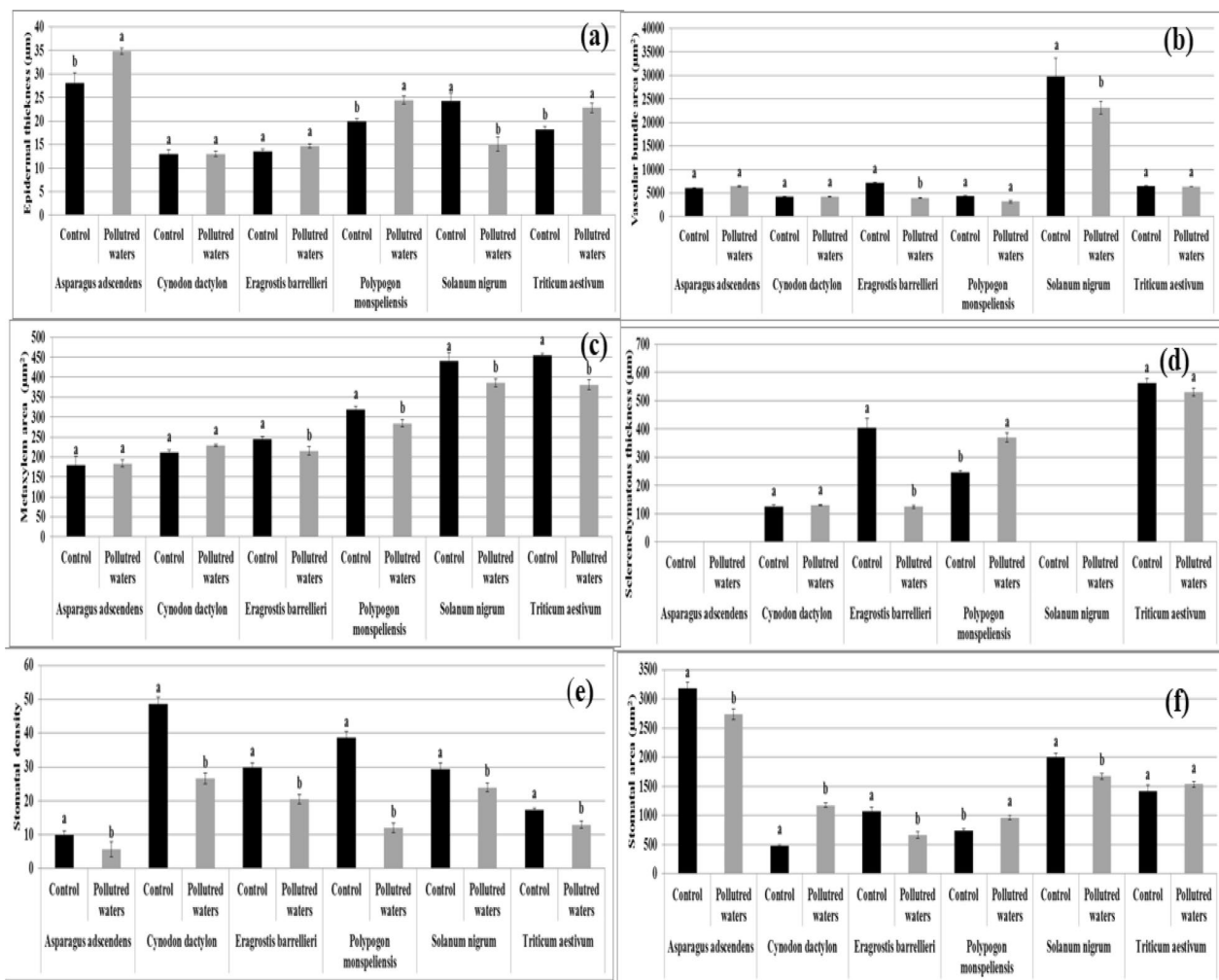
The phytodiversity of the selected area was assessed using the quadrat method. To evaluate plant diversity, relative densities were calculated among various species ( $p \leq 0.05$ ). *Cynodon dactylon* had the highest relative density at 35%, followed by *T. aestivum* at 15% (Fig. 1a). The relative frequencies of *A. adscendens*, *C. dactylon*, *E. barrelieri*, *P. monspeliensis*, *S. nigrum*, and *T. aestivum* were all recorded at 10%, the highest among other wild species (Fig. 1b). The relative cover was also highest for *C. dactylon* at 30%, followed by *A. adscendens* at 10% (Fig. 1c). The highest important index value (IV) was observed in *C. dactylon* at 75%, while *T. aestivum* had an IV of 35% (Fig. 1d).

### 3.2 Morphological characters

The results showed significant interaction between species and treatments ( $p \leq 0.05$ ). All species exhibited a significant decline in leaf length under polluted water conditions (Fig. 2a). The maximum decrease was recorded in *T. aestivum* with a 55% reduction under polluted water. *Polypogon monspeliensis* also showed a 50% decrease in leaf length as compared to the control. *Cynodon dactylon* and *E. barrelieri* exhibited



**Fig. 3** Leaf thickness of six wild species collected from control and industrial polluted waters. Graph represent the means  $\pm$  SE ( $n = 3$ ). Different letters above each bar indicate statistically significant differences between groups as determined by the LSD test ( $p \leq 0.05$ )



**Fig. 4** Comparing anatomical adaptations of six wild species collected from control and industrial polluted waters. Graphs represent the means  $\pm$  SE ( $n=3$ ). Different letters above each bar indicate statistically significant differences between groups as determined by the LSD test ( $p \leq 0.05$ )

approximately a 30% reduction in leaf length in plants collected from the polluted waters. Similarly, all species showed a significant decline in leaf width under polluted water conditions (Fig. 2b). The maximum reduction was recorded in *P. monspeliensis* with a 43% decrease. *Cynodon dactylon* showed a 35% decline in leaf width compared to the control, while *S. nigrum* and *T. aestivum* exhibited a 15% decrease.

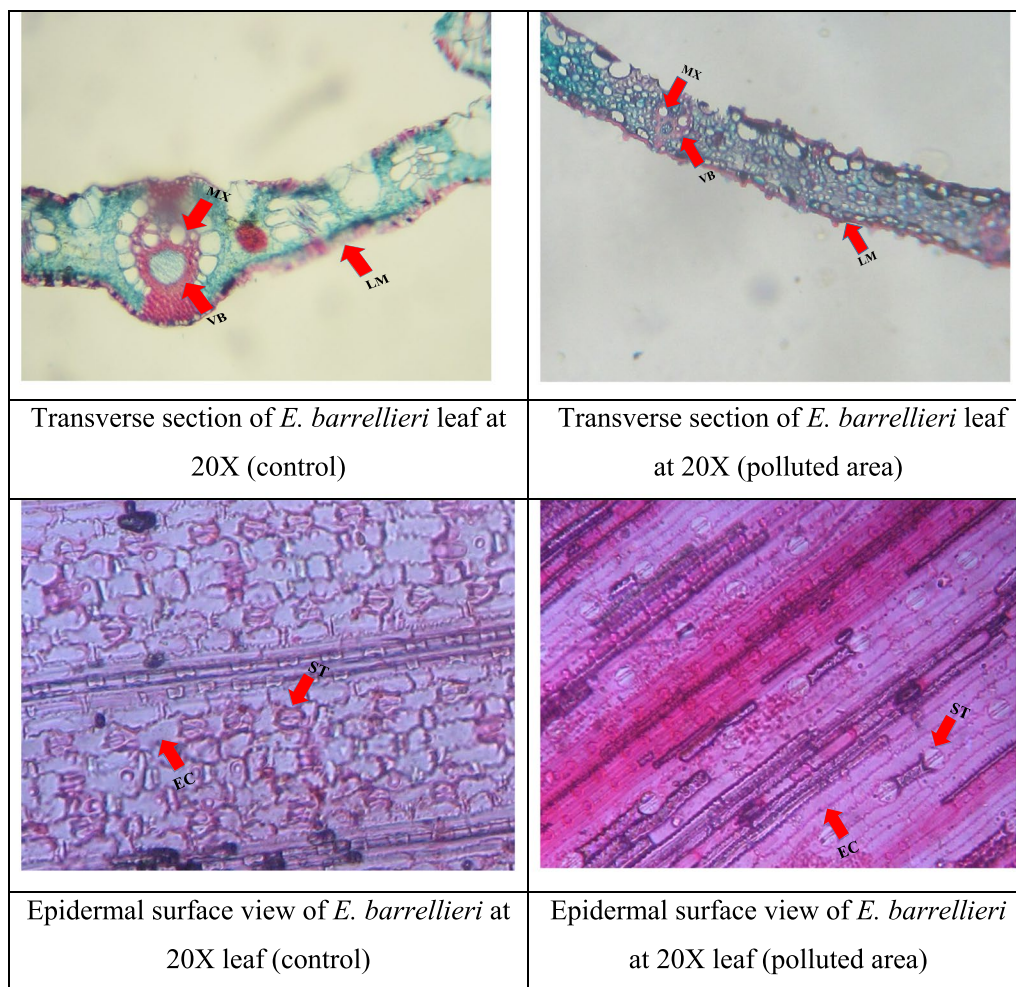
Leaf area also showed a significant decline across all species under polluted water conditions (Fig. 2c). The highest reduction was observed in *P. monspeliensis*, with a 70% decrease. *Triticum aestivum* showed a 60% reduction, while *E. barrelieri* and *S. nigrum* experienced a 45% decrease in leaf area. The results indicated significant effects of species, treatment, and their interaction. All species showed a significant decline in internode length under polluted waters (Fig. 2d). The maximum decrease

was recorded in *P. monspeliensis* with a 60% decrease under polluted waters. *Solanum nigrum* showed a 40% reduction, while *C. dactylon* and *E. barrelieri* exhibited decreases of 30% and 4%, respectively.

### 3.3 Anatomical characters

Figure 3 depicts the distinct pattern of leaf thickness across different species grown under polluted water conditions ( $p \leq 0.05$ ). The greatest reduction was observed in *E. barrelieri*, with a 40% decrease under polluted water. *Asparagus adscendens* showed a 15% reduction in leaf thickness compared to the control. In contrast, *C. dactylon* exhibited a 15% increase, while *P. monspeliensis* showed a slight increase of 5% in leaf thickness under polluted water conditions.

The statistical results revealed significant effects of species treatment and their interaction. All species

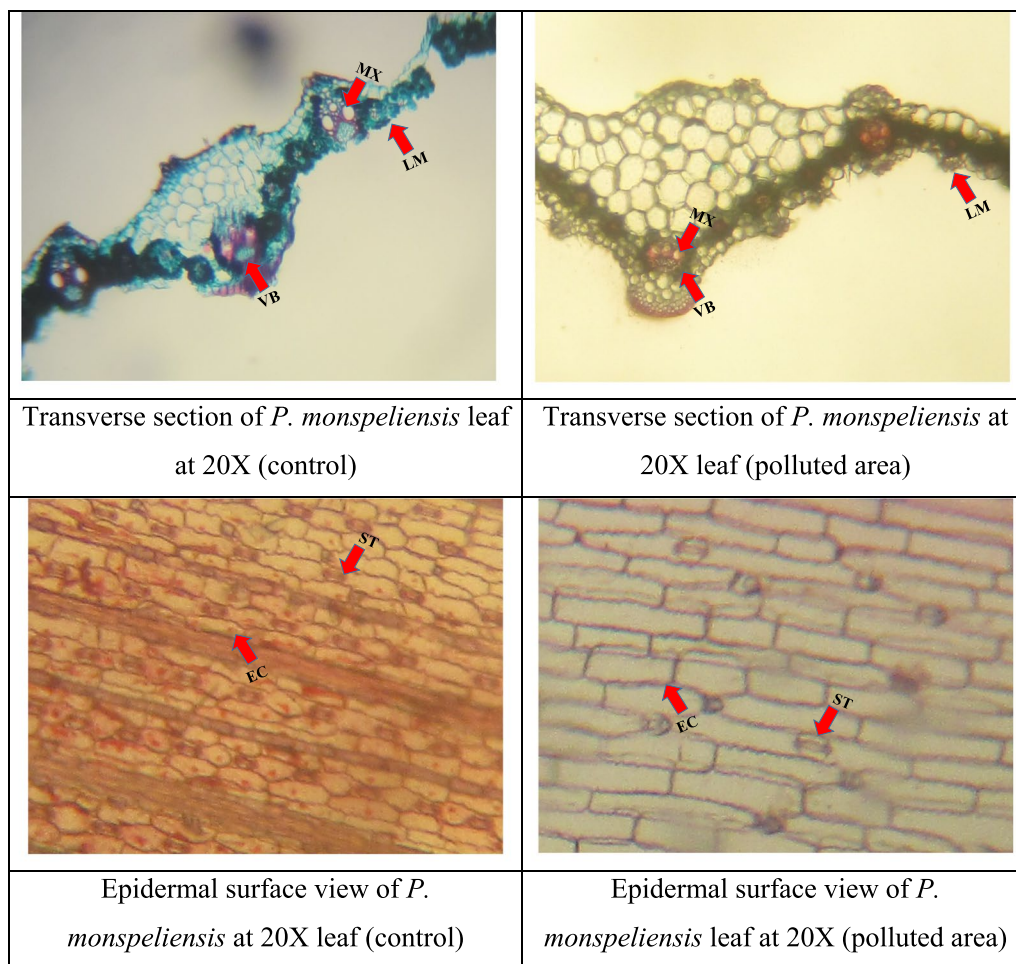


**Fig. 5** Leaf of *S. nigrum* collected from control and tannery affected area where VB-vascular bundles, LM-lamina, MX-metaxylem, ST-stomata, EC-epidermal cell

showed a significant increase or decrease in the epidermal thickness under polluted waters (Fig. 4a). The most substantial decrease was recorded in *S. nigrum* (Fig. 5), with a reduction of over 35%. Conversely, *T. aestivum* showed exhibited a 25% increase in epidermal thickness under polluted waters conditions as compared to the control (Fig. 8). Similarly, *E. barrelieri* and *C. dactylon* showed 10 and 1% increase in epidermal thickness collected from polluted waters as compared to the control (Figs. 6, 7). A similar trend was observed in the vascular bundle area, where all species exhibited significant changes under polluted water (Fig. 4b). The largest decline was recorded in *E. barrelieri* (Fig. 6), with a decrease of over 40%. *Triticum aestivum* showed a slight 1% reduction (Fig. 8), while *A. adscendens* and *C. dactylon* exhibited increases of 5 and 2%, respectively (Fig. 7). The metaxylem area also showed significant variations under polluted water conditions (Fig. 4c). The

highest reduction was recorded in *T. aestivum* (Fig. 8), with a 15% decrease. *Polypogon monspeliensis*, showed about 10% decrease (Fig. 9), whereas *C. dactylon* and *A. adscendens* exhibited increases of 10 and 3%, respectively (Fig. 7).

A significant variation was also observed in sclerenchymatous thickness under polluted water conditions (Fig. 4d). The *E. barrelieri* exhibited the highest reduction, with a 70% decrease (Fig. 6). *Triticum aestivum* showed a minor decrease of 2% (Fig. 8), while *P. monspeliensis* exhibited a substantial 50% increase (Fig. 9), and *C. dactylon* showed a slight increase of 1% in sclerenchymatous thickness collected from polluted waters (Fig. 7). Stomatal density showed a significant decline under polluted water conditions (Fig. 4e). The highest reduction was recorded in *P. monspeliensis* (Fig. 9), with a 70% decrease, followed by *C. dactylon* with a 43% reduction (Fig. 7). *Solanum nigrum* and *T. aestivum* exhibited



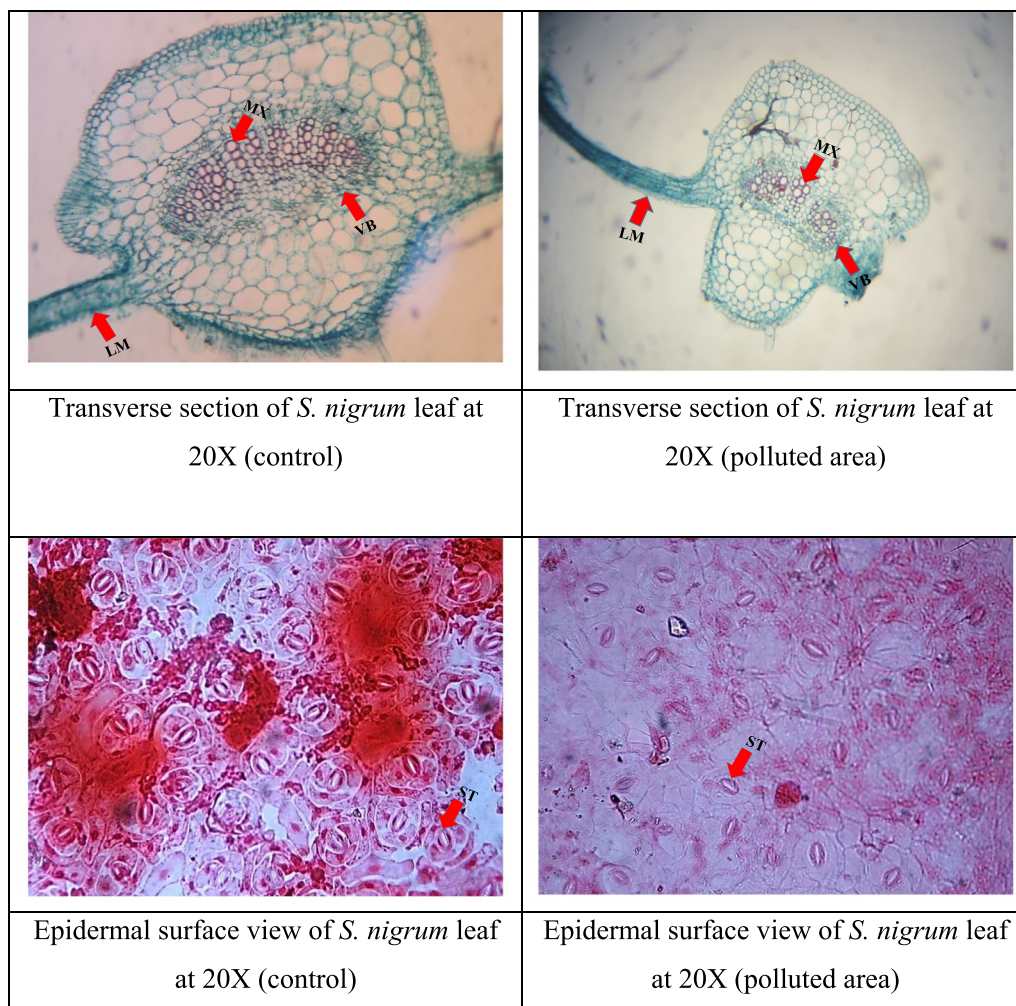
**Fig. 6** Leaf of *E. barrelieri* collected from control and tannery affected area where VB-vascular bundles, LM-lamina, MX-metaxylem, ST-stomata (stomata in all grasses are graminaceous type, guard cells dumbbell-shapes with two subsidiary cells), EC-epidermal cell

decreases of 20 and 25%, respectively, in stomatal density collected from polluted waters (Fig. 5, 8). Results for species treatment and interaction between species and treatments showed significant outcomes. All species showed a significant increase or decrease in the stomatal area under polluted waters (Fig. 4f). The maximum decrease was recorded in *E. barrelieri* of 45% (Fig. 6), followed by *A. adscendens*, displaying 5% decrease in stomatal area. *Cynodon dactylon* showed 150% increase in the stomatal area (Fig. 7), while *T. aestivum* showed 5% increase in the stomatal area collected from polluted water (Fig. 8).

#### 4 Discussion

Metal toxicity is caused by pollution that affects the plant by reducing its growth and development [35]. Phytodiversity of the selected area showed highest relative density, relative frequency, relative cover, and important index value for *C. dactylon*, i.e., 35, 10, 30, and 75% followed by other species. Species like *C. dactylon*,

*E. barrelieri*, *P. monspeliensis*, *S. nigrum*, and *T. aestivum* were very sensitive to stress and showed a huge reduction in leaf characteristics [12]. *Asparagus adscendens* showed least reduction because it has cladodes (modified stem) that camouflage its function like a leaf. Parenchyma cells in its stem store water that helps *A. adscendens* to withstand in stress condition [36]. The reduction in leaf characteristics of plants exposed to tannery effluents is primarily due to the toxic effects of heavy metals, high salinity, and chemical pollutants present in the wastewater [37]. Heavy metals such as Cd and Pb interfere with cellular processes, leading to stunted leaf growth and reduced leaf number [38]. The high salt content in the effluents causes osmotic stress, disrupting water uptake and leading to dehydration, which further limits leaf expansion [39]. Additionally, the presence of toxic compounds creates a nutrient imbalance by hindering the absorption of essential elements like nitrogen, phosphorus, and potassium, affecting overall

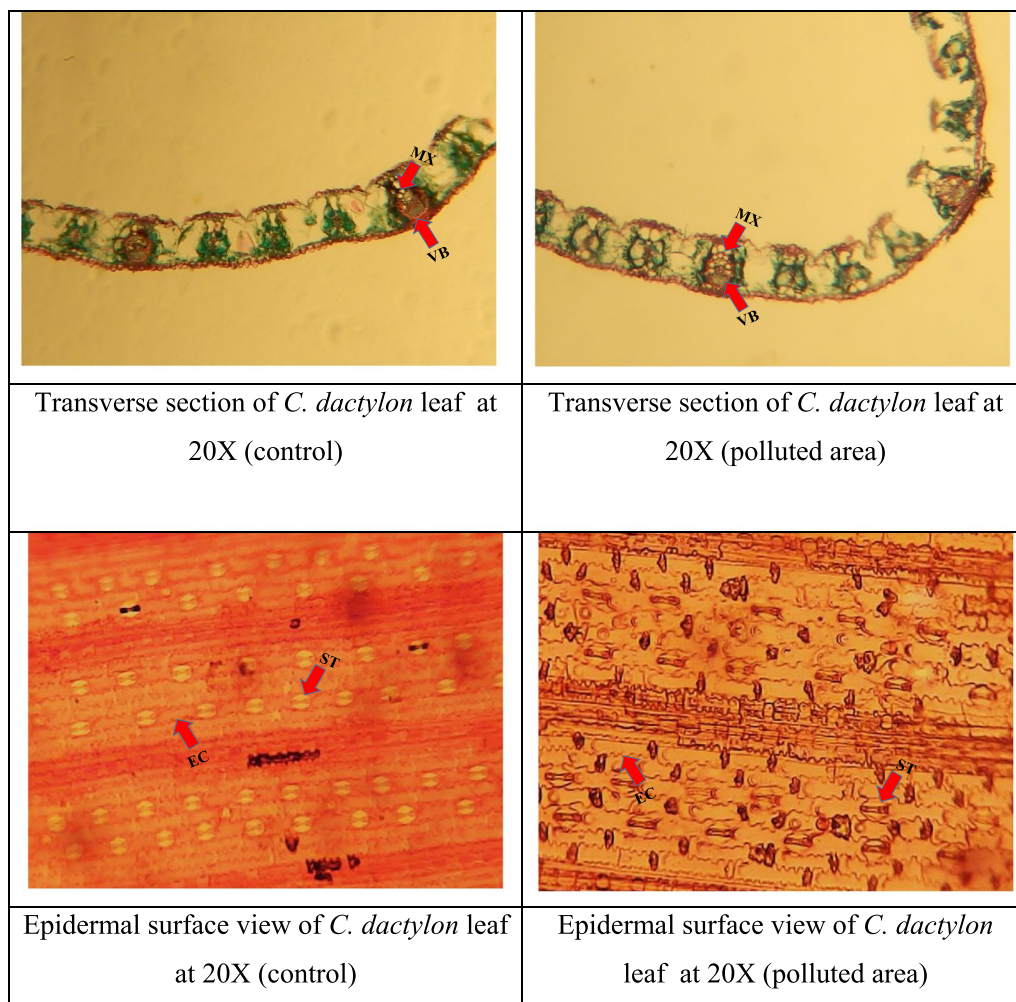


**Fig. 7** Leaf of *C. dactylon* collected from control and tannery affected area where VB-vascular bundles, LM-lamina, MX-metaxylem, ST-stomata (stomata in all grasses are graminaceous type, guard cells dumbbell-shapes with two subsidiary cells), EC-epidermal cell

leaf development [40]. Oxidative stress induced by these pollutants results in excessive production of reactive oxygen species (ROS), causing cell damage and leaf chlorosis [41]. Furthermore, tannery pollutants can impair chlorophyll content and stomatal function, reducing the photosynthetic efficiency and leading to smaller, weaker leaves like in *Datura inoxia* [42, 43]. Some chemicals in the effluents also interfere with plant enzymatic activity, disrupting growth and further inhibiting leaf formation. Such changes in leaf attributes of *Phragmites australis*, *Moringa oleifera* Lam., and *Azadirachta indica* have been reported under tannery effluents [44, 45].

Comparing the anatomical characters, leaf thickness was not much affected. *Eragrostis barrelieri*, *S. nigrum* and *T. aestivum* showed a significant decrease in leaf thickness, however, remained unchanged in *A. adscendens*, *C. dactylon*, and *P. monspeliensis*. The

anatomical comparison showed that these species can tolerate unfavorable conditions [46]. The decrease in leaf thickness correlate with one of the reported study on the aquatic macrophyte exposed to tannery effluent due to reduction in cell size of plant organs under stressed conditions [47]. Additionally, the decrease in leaf thickness under heavy metal stress is closely linked to reduced water supply to the shoot, caused by the inhibition of transpiration through the reduction of intercellular space, lamina thickness, leaf size, and stomatal density [48]. Epidermal thickness is a protective layer covering all over the leaf surface. Only broad-leaved (dicot) species showed a reduction in this characteristic, but all other species are not affected by polluted waters or showed significant increase in plants collected from polluted waters. Vascular bundle area showed less stability under polluted water, only two species *A. adscendens* and *C. dactylon* showed slight



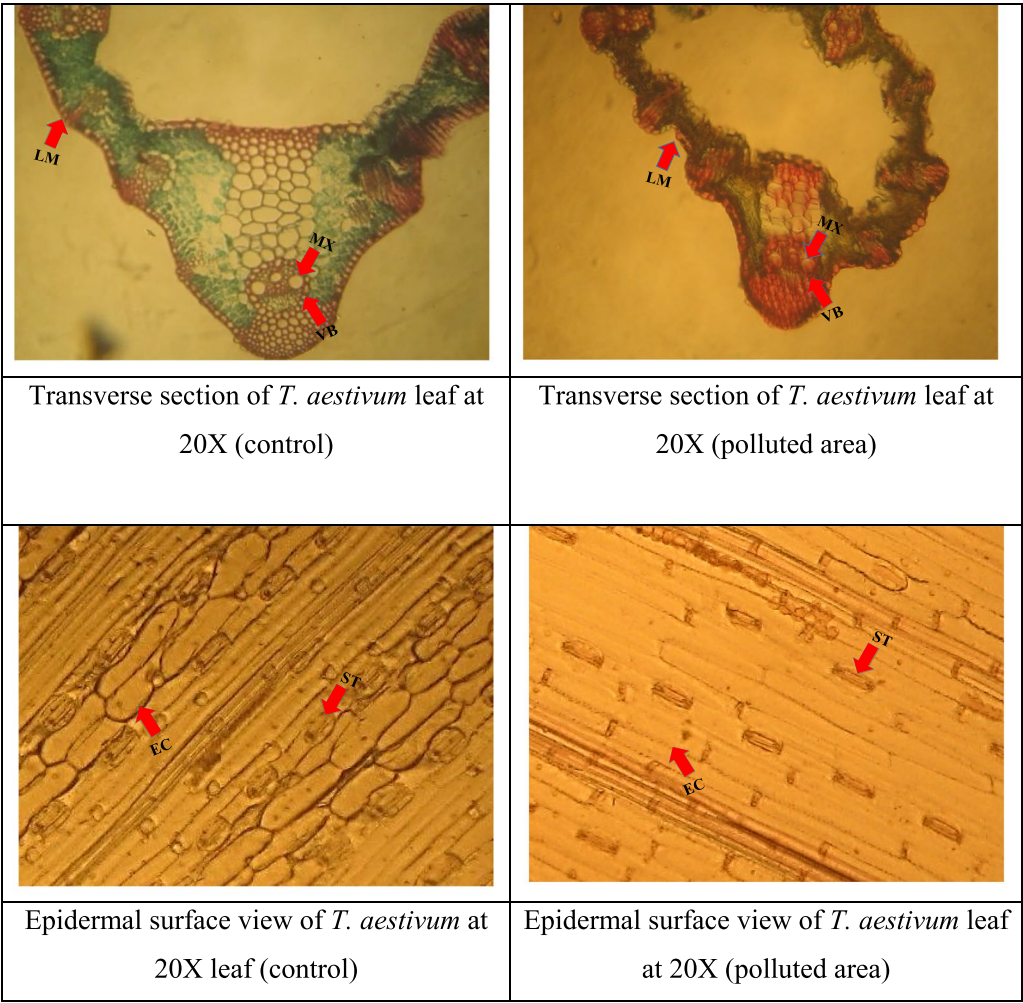
**Fig. 8** Leaf of *T. aestivum* collected from control and tannery affected area where VB-vascular bundles, LM-lamina, MX-metaxylem, ST-stomata, EC-epidermal cell

increase in vascular bundle area. Larger vascular bundles are responsible for better water conduction. The epidermal cells and vascular bundles of the treated plants exhibited intense staining, possibly due to metal deposition as reported in *Azolla pinnata* [49].

Metaxylem area was significantly decreased under polluted waters, while remained stable in two species (*A. adscendens* and *C. dactylon*). *Asparagus adscendens* has root tubers, therefore, it can store additional water. Mechanical tissue (sclerenchyma) is a characteristic of desert species, which provides mechanical strength to leaf tissue and protects them from desiccation. *Polypogon monspeliensis* showed significant increase in sclerenchyma, which may be vital for its successful survival [50]. In contrast, *E. barrelieri* showed a decline in sclerenchymatous thickness, which is a pure desert species. This can affect growth and development of *E. barrelieri* under polluted waters regarding these characteristics, because

this species is less exposed to pollution than other species with broad range of distribution [51].

Reduction of stomatal density has also been reported by several authors under polluted water like *C. dactylon* and *S. nigrum*. Treated tannery effluent was found to be toxic, as it negatively impacted shoot and root length, and caused abnormal root system structures with improper root development. Additionally, stomata and cotyledonary hairs appeared collapsed, and cytological studies revealed the plasma membrane detaching from the cell wall [52]. The stomatal area, however, showed differential response, decreased in three species (*A. adscendens*, *E. barrelieri*, and *S. nigrum*), increased in *C. dactylon* and *P. monspeliensis*, and not affected in *T. aestivum*. This study observed an increase in stomatal density and closure in plants exposed to tannery sludge with high metal concentrations, likely due to reduced leaf area and water stress. These findings align with previous



**Fig. 9** Leaf of *P. monspeliensis* collected from control and tannery affected area where VB-vascular bundles, LM-lamina, MX-metaxylem, ST-stomata (stomata in all grasses are graminaceous type, guard cells dumbbell-shapes with two subsidiary cells), EC-epidermal cell

research showing stomatal closure under metal stress, such as in *Phaseolus vulgaris* exposed to Cd [53] and sunflower leaves [54]. Stomatal closure may be a strategy to reduce water loss through transpiration, as metals disrupt water and solute transport. The closure could also be due to the inhibition of energy systems or changes in  $K^+$  fluxes in guard cells [53].

5 Conclusion

Environmental pollution is a critical issue affecting both public health and ecosystems. Phytodiversity, which represents the variety of plant species in a given area, plays an essential role in assessing the impact of pollution on plant communities. In this study, the quadrature method was used to investigate the plant diversity in an area exposed to tannery effluents. The most dominant species identified were *A. adscendens*, *C. dactylon*, *E. barrelieri*, *P. monspeliensis*, *S. nigrum*, and *T. aestivum*, with *C.*

*dactylon* exhibiting the highest importance index value. Morphological analysis showed a significant reduction in leaf length, leaf width, and leaf area across most species, with *T. aestivum* experiencing the highest decline in leaf length (55%). Similarly, anatomical parameters such as leaf thickness, epidermal thickness, vascular bundle area, metaxylem area, sclerenchymatous thickness, stomatal density, and stomatal area exhibited variable responses, with some increasing and others decreasing depending on the species. Anatomically, *T. aestivum* exhibited a reduction in metaxylem area, while *P. monspeliensis* showed an increase in sclerenchymatous thickness by 15%. *A. adscendens* demonstrated a 70% decrease in certain anatomical features, highlighting species-specific variations in response to pollution stress. Notably, stomatal density was consistently lower in all plants exposed to pollution, highlighting a common response to environmental stress. Overall, the findings suggest that

industrial pollution, particularly from tannery effluents, significantly affects plant morphology and anatomy, ultimately impacting their growth and development.

Despite these findings, this study has certain limitations. It primarily focuses on morphological and anatomical changes without considering physiological and biochemical responses such as metal accumulation, photosynthetic efficiency, and enzymatic activities, which could provide deeper insights into plant adaptation mechanisms. Additionally, the study was restricted to a specific industrial region, limiting the generalizability of findings across different environmental conditions. The implications of this research are significant for environmental monitoring and conservation. The observed morpho-anatomical changes suggest that certain wild plants can serve as bioindicators of heavy metal pollution. These findings emphasize the urgent need for improved wastewater treatment strategies in tannery industries to minimize ecological damage. For future studies, it is recommended to conduct broader investigations across multiple industrial zones to compare pollution impacts. Research on molecular and physiological adaptations of these plants could provide a deeper understanding of their tolerance mechanisms.

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#### Author Contribution

S.N and S.S.Z conducted the investigation. S.N, S.S.Z, and M.H, formally analyzed and validated the data. MH conceptualized the study. S.N, P.O.O, and M.H wrote, reviewed, and edited the manuscript. All others approved the manuscript for submission.

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#### Data Availability

No datasets were generated or analyzed during the current study.

#### Declarations

#### Ethics statement

Not applicable.

#### Consent for publication

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#### Competing Interests

The authors declare no competing interests.

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#### References

- G. I. Edo et al., "Impact of environmental pollution from human activities on water, air quality and climate change," *Ecological Frontiers*, 2024.
- Saxena V (2025) Water Quality, Air Pollution, and Climate Change: Investigating the Environmental Impacts of Industrialization and Urbanization. *Water Air Soil Pollut* 236(2):1–40
- Abdulhusein HS, Kadim BM (2024) The Economic Significance of Animal Products and Methods Used in Leather Protection. *Haya Saudi J Life Sci* 9(8):322–338
- Zhao C, Chen W (2019) A review for tannery wastewater treatment: some thoughts under stricter discharge requirements. *Environ Sci Pollut Res* 26:26102–26111
- Ghafoor A (2015) Determinants of leather exports from Pakistan: a time series analysis. *VFAST Transactions on Education and Social Sciences* 3(1):36–45
- A. Siddiqui, A. Uroos, and F. Nadeem, "Sectoral competitiveness and value chain analysis: leather Gloves value chain analysis in Pakistan," *Trade development authority of Pakistan*, 2016.
- Shahab S, Mahmood MT (2013) Comparative advantage of leather industry in Pakistan with selected Asian economies. *Int J Econ Financ Issues* 3(1):133–139
- Hashmi GJ, Dastageer G, Sajid MS, Ali Z, Malik M, Liaquat I (2017) Leather industry and environment: Pakistan scenario. *International Journal of Applied Biology and Forensics* 1(2):20–25
- W. Sarfraz et al., "Ameliorative role of foliar Zn-lysine application on wheat (*Triticum aestivum* L.) stressed by Tannery Wastewater," *Physiology and Molecular Biology of Plants*, pp. 1–12, 2022.
- Song Z, Williams C, Edyvean R (2004) Treatment of tannery wastewater by chemical coagulation. *Desalination* 164(3):249–259
- McGrath S (1982) The uptake and translocation of tri- and hexa-valent chromium and effects on the growth of oat in flowing nutrient solution and in soil. *New Phytol* 92(3):381–390
- Ouzounidou G, Moustakas M, Eleftheriou E (1997) Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. *Arch Environ Contam Toxicol* 32:154–160
- Ilyas M, Ahmad W, Khan H, Yousaf S, Yasir M, Khan A (2019) Environmental and health impacts of industrial wastewater effluents in Pakistan: a review. *Rev Environ Health* 34(2):171–186
- Monira U, Mostafa M (2023) Leather industrial effluent and environmental concerns: a review. *Sustainable Water Resources Management* 9(6):181
- Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E (2020) Environmental and health impacts of air pollution: a review. *Front Public Health* 8:14
- Rizvi A, Zaidi A, Ameen F, Ahmed B, Alkhatani MD, Khan MS (2020) Heavy metal induced stress on wheat: phytotoxicity and microbiological management. *RSC Adv* 10(63):38379–38403
- S. Stanis, L. Ignjatović, S. Popov, S. Škrivanj, A. Đorđević, and A. Stojić, "Heavy metal accumulation in wheat and barley: The effects of soil presence and liquid manure amendment," 2016.
- Maqbool A et al (2018) Management of tannery wastewater for improving growth attributes and reducing chromium uptake in spinach through citric acid application. *Environ Sci Pollut Res* 25:10848–10856
- Zaheer IE et al (2019) Zinc-lysine prevents chromium-induced morphological, photosynthetic, and oxidative alterations in spinach irrigated with tannery wastewater. *Environ Sci Pollut Res* 26:28951–28961
- Farid M et al (2018) Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. *Ecotoxicol Environ Saf* 151:255–265
- M. Z. ur Rehman et al., "Remediation of heavy metal contaminated soils by using *Solanum nigrum*: a review," *Ecotoxicology and environmental safety*, vol. 143, pp. 236–248, 2017.
- Wang M-Q et al (2019) Transcriptomic analysis of *Verbena bonariensis* roots in response to cadmium stress. *BMC Genomics* 20:1–14
- Wang J et al (2022) Comparative cytology combined with transcriptomic and metabolomic analyses of *Solanum nigrum* L. in response to Cd toxicity. *J Hazard Mater* 423:127168
- M. Licata, D. Farruggia, N. Iacuzzi, C. Leto, T. Tuttolomondo, and G. Di Miceli, "Effect of irrigation with treated wastewater on bermudagrass (*Cynodon dactylon* (L.) Pers.) production and soil characteristics and estimation of plant nutritional input," *Plos one*, vol. 17, no. 7, p. e0271481, 2022.
- Khan A et al (2018) Elemental and nutritional values of wild fodder plants of poaceae in District Bannu, Pakistan. *Int J Agron Agric Res* 13:53–61

26. Djebali W et al (2010) Aspects on growth and anatomy of internodes and leaves of cadmium-treated *Solanum lycopersicum* L. plants. J Biol Res (Thessaloniki) 13:75–84
27. Özyiğit İİ, Baktibekova D, Hocaoglu-özyiğit A, Kurmanbekova G, Chekirov K, Yalçın İE (2021) The effects of cadmium on growth, some anatomical and physiological parameters of wheat (*Triticum aestivum* L.). International Journal of Life Sciences and Biotechnology 4(2):235–253
28. Tufail A et al (2020) Salt toxicity in a natural habitat induces structural and functional modifications and modulate metabolism in Bermuda grass (*Cynodon dactylon* [L.] Pers.) ecotypes. Appl Ecol Environ Res 18(5):6569–6588
29. Tariq SR, Shaheen N, Khaliq A, Shah MH (2010) Distribution, correlation, and source apportionment of selected metals in tannery effluents, related soils, and groundwater—a case study from Multan, Pakistan. Environ Monit Assess 166:303–312
30. Rice EL (1967) A statistical method for determining quadrat size and adequacy of sampling. Ecology 48(6):1047–1049
31. J. E. Simmons, *Fluid preservation: a comprehensive reference*. Rowman & Littlefield, 2014.
32. N. Feder and T. O'Brien, "Plant microtechnique: some principles and new methods," *American journal of Botany*, vol. 55, no. 1, pp. 123–142, 1968.
33. Isaac UE, Oyo-lta E, Igwe NP, Ije EL (2023) Preparation of histology slides and photomicrographs: Indispensable techniques in anatomic education. Anatomy Journal of Africa 12(1):2252–2262
34. R. G. Steel and J. H. Torrie, "Principles and procedures of statistics: a biometrical approach," ed: McGraw-Hill, New York New York, USA, 1980.
35. Al-Obaidi JR, Jamaludin AA, Rahman NA, Ahmad-Kamil E (2024) How plants respond to heavy metal contamination: a narrative review of proteomic studies and phytoremediation applications. Planta 259(5):103
36. E. F. Gilman, "Asparagus densiflorus Myers 1," *University of Florida Cooperative Extension Service Fact Sheet FPS-52*, 1999.
37. G. L. Tadesse, T. K. Guya, and M. Walabu, "Impacts of tannery effluent on environments and human health: a review article," *Advances in Life Science and Technology*, vol. 54, no. 10, 2017.
38. Pålsson A-MB (1989) Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants: a literature review. Water Air Soil Pollut 47:287–319
39. Yadav S, Irfan M, Ahmad A, Hayat S (2011) Causes of salinity and plant manifestations to salt stress: a review. J Environ Biol 32(5):667
40. M. Ahmed, M. Hasanuzzaman, M. A. Raza, A. Malik, and S. Ahmad, "Plant nutrients for crop growth, development and stress tolerance," *Sustainable agriculture in the era of climate change*, pp. 43–92, 2020.
41. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48(12):909–930
42. Mohanty C et al (2024) Ecological implications of chromium-contaminated effluents from Indian tanneries and their phytoremediation: a sustainable approach. Environ Monit Assess 196(10):995
43. Vernay P et al (2008) Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia*. Chemosphere 72(5):763–771
44. Sinha SN, Paul D, Biswas K (2016) Effects of *Moringa oleifera* Lam. and *Azadirachta indica* A. Juss. leaf extract in treatment of tannery effluent. Our Nature 14(1):47–53
45. Calheiros CS, Rangel AO, Castro PM (2008) The effects of tannery wastewater on the development of different plant species and chromium accumulation in *Phragmites australis*. Arch Environ Contam Toxicol 55(3):404–414
46. Hameed M, Ashraf M (2008) Physiological and biochemical adaptations of *Cynodon dactylon* (L.) Pers. from the Salt Range (Pakistan) to salinity stress. Flora-Morphology, Distribution, Functional Ecology of Plants 203(8):683–694
47. Q. M. Qaisar Mahmood, Z. P. Zheng Ping, M. Siddiqi, E. Islam, M. Azim, and Y. H. Yousaf Hayat, "Anatomical studies on water hyacinth (*Eichhornia crassipes* (Mart.) Solms) under the influence of textile waste water," 2005.
48. Naveed M et al (2023) Morphological and structural responses of *Albizia lebbek* to different lead and nickel stress levels. Agriculture 13(7):1302
49. S. G. Sumati Gaumat, K. G. Kiran Gupta, U. B. Usha Bajpai, and K. Mishra, "Ameliorating effect of iron on lead induced phototoxicity in *Azolla pinnata*," 2011.
50. Lendzian KJ (2006) Survival strategies of plants during secondary growth: barrier properties of phellements and lenticels towards water, oxygen, and carbon dioxide. J Exp Bot 57(11):2535–2546
51. Rasheed S et al (2022) Ecological assessment and indicator species analyses of the Cholistan desert using multivariate statistical tools. Pak J Bot 54(2):683–694
52. Nath K, Singh D, Shyam S, Sharma Y (2009) Phytotoxic effects of chromium and tannery effluent on growth and metabolism of *Phaseolus mungo* Roxb. J Environ Biol 30(2):227–234
53. Singh S, Sinha S (2004) Scanning electron microscopic studies and growth response of the plants of *Helianthus annuus* L. grown on tannery sludge amended soil. Environ Int 30(3):389–395
54. F. Bazzaz, R. W. Carlson, and G. Rolfe, "The effect of heavy metals on plants: Part I. Inhibition of gas exchange in sunflower by Pb, Cd, Ni and Tl," *Environmental Pollution* (1970), vol. 7, no. 4, pp. 241–246, 1974.

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