

# Anatomical Study of Four Wild Plant Species Growing Along Contaminated Water Channel in Khokhar Town, Bund Road, Lahore, Pakistan

Nadeem-Ullah\*, Ashraf W, Shafiq-ur-Rehman, Qurat-ul-ain

Department of Botany, University of Central Punjab, Lahore, GCU Lahore, Pakistan

## ABSTRACT

The present study described the effect of waste water used for irrigation purpose on the anatomical characters of plants. This waste water comprises of municipal, industrial and dairy waste containing organic matter, Ca<sup>++</sup>, Mg<sup>++</sup>, Nitrogen, Phosphorus and Potassium. Anatomy of four wild plants i.e. *Trianthema portulacastrum* L., *Amaranthes viridis* L., *Portulaca oleracea* L. and *Persicaria barbata* L. was studied. It was found that *Trianthema portulacastrum* L. and *Portulaca oleracea* L. were directly affected and the size of vascular tissues and cortex reduced many times as compared to control, while on the other hand *Amaranthes viridis* L. and *Persicaria barbata* L. showed high growth than control.

**Keywords:** *Trianthema portulacastrum* L.; *Amaranthes viridis* L.; *Portulaca oleracea* L.; *Persicaria barbata* L.

## INTRODUCTION

Three fourth cities of Asia, Latin America and Africa are suffering from municipal and industrial waste water irrigation. Individually and synergistically, the toxic substances released from industries are badly affecting the growth of plants. Sewage waste containing metal and industrial effluents can cause the pollution of agricultural soil and crops. The concentration, type of pollutants as well as plant species in industrialized regions affect crop yield. The major source due to which river plants are affected, due to pollution, are wastes from town and cities [1-8].

During the present age of industrialization, heavy metal toxicity cause great damage to crops in some important agricultural areas. It was reported by [9], that concentration of heavy metals is increasing day by day and if proper steps are not taken it will impossible to cultivate agricultural areas. It is due their use in large number of industries such as in engineering of alloys, batteries, electroplated metal parts, textile dyes, pesticides and steel. [10-16].

Khokhar village is located on bund road in north of Lahore near premises of Badami bagh Lahore. Badami bagh is an industrial area where large steel and plastic industries are located. The waste water of these mills is directly or indirectly disposed to water which is used for irrigation purposes.

Water used for irrigation in experimental site contain large

amount of heavy metals in it including chromium. Industries disposed their waste water and garbage into water channel which is used in irrigation via open or covered routes. Similarly, people in this village established their Dairy farms along the water body and they use to throw dung and other waste material in it. The site which is taken as control is botanic garden, GCU Lahore which is located at Mall road Lahore. Botanic garden of GCU is established in 1912. The water used there for irrigating the plants is fresh water and was taken as control. The effect of different heavy metals i.e. Iron, Lead, Copper, Nickel and Chromium was studied on four wild plant species *Persicaria barbata* L. from Polygonaceae, *Trianthema portulacastrum* L. from Aizoaceae, *Amaranthus viridis* L. from Amaranthaceae and *Portulaca oleracea* L. from Portulacaceae.

## MATERIALS AND METHODS

### Location of study area (khokhar village bund road lahore) assava plants and growth conditions

Soil samples were collected from polluted (Khokhar village) (Figure 1) and Botanic garden. The soil from Khokhar village was considered as experimental and soil from Botanic Garden was considered as control. For the analysis of soil procedure described by Richard, Allen and Saeed was adopted [16-19]. By using these methods pH, Electrical conductivity, Calcium, Magnesium,

**Correspondence to:** Nadeem-Ullah, FOS, Department of Botany, University of Central Punjab, Lahore, GCU Lahore, Pakistan, E-mail: nadeem.ullah@ucp.edu.pk

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Bicarbonates, Chlorides, Phosphorus, Soil Carbon, Organic matter, Soil Moisture and Nitrogen were determined. Similarly, soil texture was measured by adopting the method described by Bouyoucos (1962) [20].



Figure 1: Location of study area (Khokhar Village Bund Road Lahore).

### Water analysis

For water analysis the method described by Gregg (1989) was used [21]. This method was also adopted by environmental protection agency (EPA) Pakistan. The water samples were collected from sewage canal khokhar village used for irrigation of plants and other water sample was collected from tube well of botanic garden, GC university Lahore. This water is used for irrigation of plants in botanic garden and it is also used for drinking purposes. This water sample is taken as control.

### Metal analysis

A total of five metallic elements were determined in the pre-treated samples of water using Atomic Absorption Spectrophotometry as described by Gregg (1989) [21]. These include, Iron, lead, copper, nickel and chromium.

### Anatomical studies

Four wild plants were collected from polluted and non-polluted sites. The polluted site was khokhar village near bund road, where sewage and industrial water, which was drained in nearby water body, was used for irrigation purposes. Botanic garden, GCU Lahore was taken as controlled site. The four wild plant species used for anatomical investigation were i.e. *Portulaca oleracea* L., *Amaranthus viridis* L., *Trianthema portulacastrum* L., *Persicaria barbata* L.

The standard method for section cutting was followed. Cylindrical parts of plants like stem and root were used for study of internal structure. Free hand sections were cut mostly with the help of good sharp razor (safety razors were also used to cut sections). The method of double staining and mounting of slide [22,23] was followed. These sections were observed under low and high magnification power of compound microscope. The method

of micrometry was followed by mehmood et al., [24]. Size of vascular bundle, size of cortex, size of pith and size of epidermis were measured both for control and experimental plants and compared.

### RESULTS

According to this, pH of control soil was 7.5 and of experimental 7.20. Electrical conductivity showed that experimental soil is slightly alkaline. The amount of organic matter showed that the experimental soil had slightly well for agriculture. The % moisture showed that experimental soil had maximum water holding capacity, while the texture of soil is silt loam in control and silt in experimental soil. Number of carbonates was same in both, while the amount of bicarbonates was greater in control soil, i.e. 2.25 in control and 1.54 meq/L in experimental soil. Amount of Ca<sup>++</sup> ions in experimental soil was 7 and in control it was 2.4. Amount of Mg<sup>++</sup> in control is 10 and experimental is 8.15. The amount of nitrogen in control is 0.035% while in experimental is 0.112%. The amount of phosphorus in control soil was 6 ppm while in experimental soil it was 5.35 ppm. The amount of potassium in control is 21 ppm while in experimental is 32 ppm. Table 1 showed physicochemical properties of soil.

Table 1: Soil analysis of experimental and control samples.

Sr. No.	Parameter	Controlled	Experimental
1	pH	7.5	7.2
2	EC dSm-1	1.75	3.544
3	% Organic matter	3	2.12
4	% moisture	11.8	23.46
5	Soil texture	Silt Loam	Silt
6	CO <sub>3</sub> - (meq/l)	1.08	1.08
7	HCO <sub>3</sub> - (meq/l)	0	1.54
8	Ca <sup>++</sup> (meq/l)	7	2.4
9	Mg <sup>++</sup> (meq/l)	10	8.15
10	Nitrogen (N) %	0.035	0.112
11	Phosphorus (P) ppm	6	5.35
12	Potassium (K) ppm	21 ppm	32 ppm
*Control is botanic garden		**Experimental is khokhar village	

Table 2 explained the heavy metal concentration in the waste water samples which were collected from Khokhar village. It was found that concentration of Iron, Lead and copper was 1.2314, 0.0980 and 0.1194 respectively. But on the other hand, Nickel and chromium was not found in waste water. The concentration of copper is much greater than NEQS value i.e. 0.05 mg/L.

Table 2: Water analysis of control and experimental samples.

Metal	Experimental mg/l	Control mg/l	NEQS
Iron	1.2314	BDL	2
Lead	0.098	BDL	0.05
Copper	0.1194	BDL	0.05
Nickel	BDL	BDL	
Chromium	BDL	BDL	0.05
*BDL stands for Below Detection Limit. **control is botanic garden ***experimental is khokhar village			

Figure 2 showed that the size of epidermis, cortex, Vascular bundle and pith in stem of *Trianthema portulacastrum* L. was 16.6, 89.33, 68 and 135  $\mu\text{m}$  in control plant respectively but on the other hand size of same tissues in experimental plant was 10, 40, 16 and 110  $\mu\text{m}$  respectively. The relationship showed that controlled plant shows better growth than experimental. Growth of epidermis is 6.6  $\mu\text{m}$ , growth of cortex is 49  $\mu\text{m}$ , growth of vascular bundle is 52  $\mu\text{m}$  and growth of pith is 25  $\mu\text{m}$  greater than experimental.

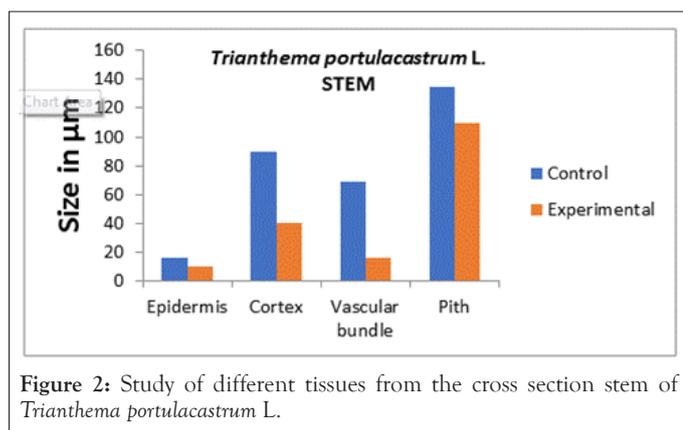


Figure 2: Study of different tissues from the cross section stem of *Trianthema portulacastrum* L.

Figure 3 explains that the size of epidermis, cortex, vascular bundle and pith in root of *Trianthema portulacastrum* L. was 9, 45, 92 and 47  $\mu\text{m}$  in control plant respectively and the size of same tissue in experimental plant was 7, 16, 92 and 38  $\mu\text{m}$  respectively. This relationship showed that roots of controlled plant shows maximum growth than experimental. Growth of epidermis is 2  $\mu\text{m}$ , growth of cortex is 29  $\mu\text{m}$ , Growth of vascular bundle is 11  $\mu\text{m}$  and growth of pith is 9  $\mu\text{m}$  greater in control.

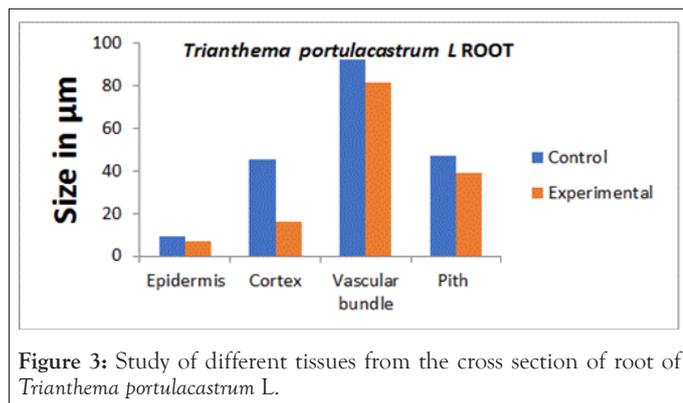


Figure 3: Study of different tissues from the cross section of root of *Trianthema portulacastrum* L.

While studying the anatomy of plants morphological characters were also noticed. Those plants were selected which were of equal size and healthy. In *Trianthema portulacastrum* L. control, the epidermis is one celled thick from which hairs were originated. Cortex is 5 to 6 celled thick which had many sclerenchymatous cells. Endodermis was very thick and casparian strips were not well developed. Vascular bundles were less developed but Pith was well developed and it had very less amount of parenchyma cells. In experimental plants epidermis was one celled thick and

also had trichomes. This epidermis was not well developed and sometimes even not clearly seen under light microscope. The cortex was many celled thick but cells were smaller in size and had less diameter. The endodermis was again well developed and seemed like a circle but cells were smaller in size and not clearly seen under light microscope. Vascular bundles were of unequal size, some were larger and well developed while other were not well developed. Again pith was well developed and larger cells were found.

Similar case was observed in the root of same plant. Root was woody and branched. Epidermis was one celled thick but was damaged. Cortex was not well developed and longitudinal cells were observed. Endodermis was clearly seen at some places and sometimes we were unable to observe it. A helical structure was observed throughout the root of plant. Number of sclerenchyma cells was less but number of parenchyma cells was larger. Sometimes small air spaces was also found, but in experimental plant, anatomical studies revealed that epidermis was also one cell thick but cell were larger than control and breakage was not clearly seen. Cortex was few celled thick and cells were rectangular and 4-5 layered. Endodermis was clearly seen in experimental helical structure was not prominent here. Air spaces were present more in experimental as compared to control. Longitudinal cells were present beneath the epidermis with small partitions in them. In the center of root in pith circular cells were present and results showed that the growth of cells was more healthy in experimental as compared to control.

Figure 4 showed that the size of epidermis, cortex and vascular bundle in stem of *Amaranthus viridis* L. in experimental is 13, 45 and 76  $\mu\text{m}$  in respectively and the size of same tissues in control 8, 12 and 19  $\mu\text{m}$  respectively. Results explained that experimental plant shows maximum growth. Growth of epidermis in experimental plant 5  $\mu\text{m}$ , growth of cortex is 33  $\mu\text{m}$ , growth of vascular bundle is 57  $\mu\text{m}$  greater than control.

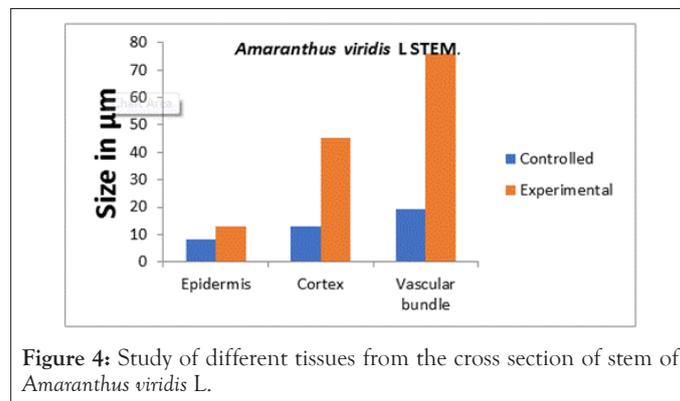


Figure 4: Study of different tissues from the cross section of stem of *Amaranthus viridis* L.

Figure 5 showed that size of epidermis, cortex, vascular bundle and pith of root of *Amaranthus viridis* L. in control is 18, 68, 54, 34  $\mu\text{m}$  respectively while the size of same tissues in experimental plant is 46, 77, 87 and 50  $\mu\text{m}$ . It was observed that root of experimental plant showed better growth than control plant. Growth of epidermis in experimental is 28  $\mu\text{m}$ , growth of cortex

is 9  $\mu\text{m}$ , Growth of vascular bundle is 33  $\mu\text{m}$  and growth of pith is 16  $\mu\text{m}$  greater than control.

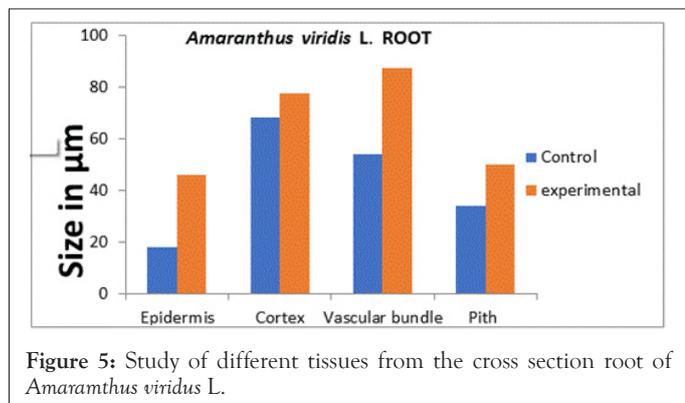


Figure 5: Study of different tissues from the cross section root of *Amaranthus viridis* L.

Anatomical studies of *Amaranthus viridis* L. control plants revealed that trace material was found in cells of control plant. Epidermis was one celled thick which are oval or polygonal in shape. Cortex was 6 to 7 cell layered thick and shape was from polygonal to oval in shape. Beneath the cortex endodermis was clearly seen along with casparian strips. Vascular bundles were in the form of ring and were of variable size. Metaxylem was clearly seen outside and small cells were seen inside. Pith was present in the center of the cell. Pith cells were comparatively larger than other cells. Pith consists of sclerenchyma cells but anatomical studies of experimental plants showed that epidermis is 2 to 3 celled thick. While cortex is 8 to 9 layered thick and shape of cells were from oval to polygonal and cells were much larger than control. Deposition of trace material was more clearly visible in experimental especially present on intercellular spaces. Vascular bundles were more prominent in experimental and were present in more number as compared to control. Metaxylem was present on inner side and protoxylem was present on outer side. Metaxylem and casparian strips were not clearly visible in them. Endodermis was much more clearly seen in experimental and 4 to 5 celled thick. Pith was more prominent in experimental and cells were larger in size in experimental as compared to control.

Similar kinds of results were seen in root. It showed better growth in control plant than experimental one. The root was 2 to 3 celled thick and cortex was smaller as compared to other plants. Endodermis was well developed but cells were compact and boundaries were not visible. In the same way casparian strips were not seen. On the inner side vessels were present in the form of rays. Xylem is endarch i.e., protoxylem was on the inner side and metaxylem on outer side. Phloem was present on the outer side of xylem. Pith was present on the inner side but a star shaped cluster of sclerenchyma cells were seen in the center of plant. On the other hand in experimental plant the epidermis is 2 to 3 layered structure and cortex was many celled layer structure. All the cells were cuboidal in shape and endodermis was not visible. Vascular bundle was in the form of a circle and small bundles. Here the number of sclerenchyma cells was less. Pith was large and covered the maximum space of root. In the center of root

bundle of sclerenchyma cells were present which were arranged in four corners.

Figure 6 showed the size of epidermis, cortex, vascular bundle and pith of stem of *Portulaca oleracea* L. in control is 17, 168, 104 and 220  $\mu\text{m}$  respectively, while the size of same tissue in experimental is 16, 60, 54 and 186  $\mu\text{m}$ . Results clearly showed that control plant shows maximum growth than experimental one. Growth of epidermis of stem in experimental plant is 1  $\mu\text{m}$  less than control. Growth of cortex is 108  $\mu\text{m}$ , growth of vascular bundle is 50  $\mu\text{m}$  and growth of pith is 34  $\mu\text{m}$  greater than experimental.

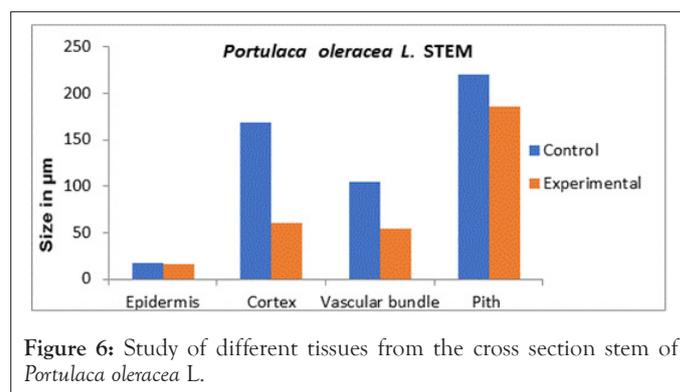


Figure 6: Study of different tissues from the cross section stem of *Portulaca oleracea* L.

Figure 7 showed the size of epidermis, cortex, vascular bundle and pith in root of *Portulaca oleracea* L. in control plant, which is 20, 30, 202, 130  $\mu\text{m}$  respectively while the size of same tissue in experimental is 10, 18, 145, 44  $\mu\text{m}$ . Result depicts that control plant show better growth than experimental plant. Growth of epidermis of control is 10  $\mu\text{m}$ , growth of cortex is 12  $\mu\text{m}$ , growth of vascular bundle is 57  $\mu\text{m}$  and growth of pith is 86  $\mu\text{m}$  greater than experimental.

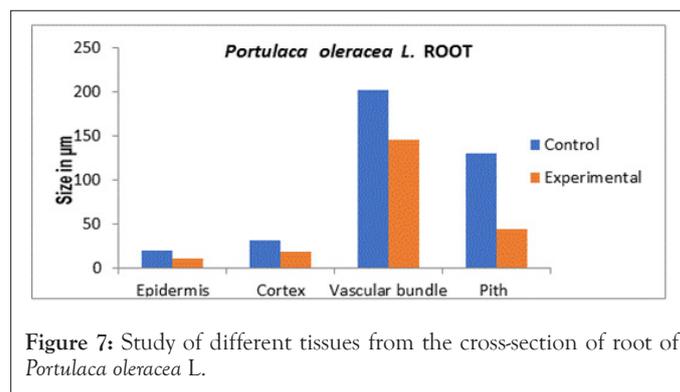


Figure 7: Study of different tissues from the cross-section of root of *Portulaca oleracea* L.

Anatomical studies of *Portulaca oleracea* L. experimental showed that epidermis was one celled thick surrounded by red colored layer; cells were thin and rectangular in shape. Beneath the epidermis cortex was present and shape of cells was oval or polygonal. Cells were thick walled and 9 to 10 layers of cells in cortex were present. Under the cortex, endodermis was present it was also one layered thick. Vascular bundles were present in the form of ring. Under the endodermis metaxylem can also be clearly

distinguished. Pith was present in the center. Pith cells were thick walled and rounded in shape. But in control plants epidermis was 2 layered thick and a prominent red layer was present outside the epidermis. Beneath the epidermis cortex was present, which was 9 to 10 layered thick. Under the cortex, 3-celled thick endodermis was present. Vascular bundles were present in the form of ring. Protoxylem and metaxylem were visible in vascular bundles. Pith was present in the center of the stem which was more thick and large than control. This showed that anatomy of control was best than experimental. The same behavior was observed in roots. Roots of control plant showed that epidermis was many celled thick and all the cells were cuboidal in shape. Cortical cells were not prominent but tissue was easily recognized similar case was with endodermis. Vascular bundles were arranged in a circle and in radial fashion. Vessels were very small in their diameter and pith cells were larger and polygonal in their shape. Number of sclerenchyma cells was smaller while number of parenchyma cells was much greater. In experimental plant, root showed much reduced growth. Epidermis was clear but very much damaged. Similar results were observed in cortex, in which large intercellular spaces were present. Again vascular bundles were in the form of radial rays but these were not prominent as was in control plant. The diameter of pith cells was much reduced and their shape was from oval to polygonal.

Figure 8 showed that the size of epidermis, cortex and vascular bundle in stem of *Perscaria barbata* L. control plant is 12, 21 and 76  $\mu\text{m}$  respectively while the size of same tissue in experimental is 20, 110 and 88  $\mu\text{m}$  respectively. Results showed that the growth was best in experimental plant. Growth of the epidermis of experimental plant is 8  $\mu\text{m}$ , cortex is 89  $\mu\text{m}$  and vascular bundle is 12  $\mu\text{m}$  greater than control.

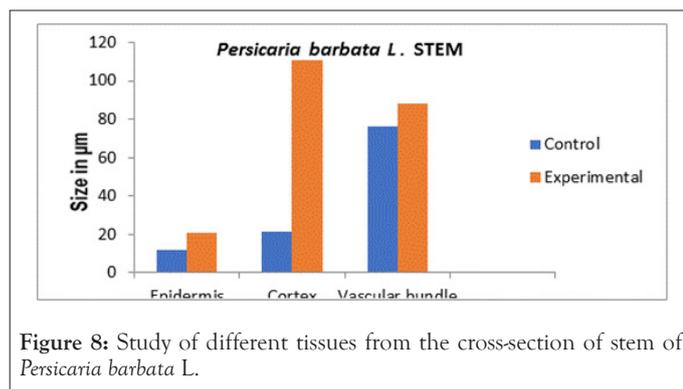


Figure 8: Study of different tissues from the cross-section of stem of *Perscaria barbata* L.

Figure 9 showed that the size of epidermis, cortex, vascular bundle and pith in root of *Perscaria barbata* L. control plant is 24, 28, 40 and 116  $\mu\text{m}$  respectively while the size of same tissue in experimental is 28, 96, 96 and 144  $\mu\text{m}$  respectively. Results showed that the growth was better in experimental plant than control plant. Growth of epidermis of experimental plant is 4  $\mu\text{m}$ , growth of cortex is 68  $\mu\text{m}$ , growth of vascular bundle is 56  $\mu\text{m}$  and pith is 28  $\mu\text{m}$  greater than control.

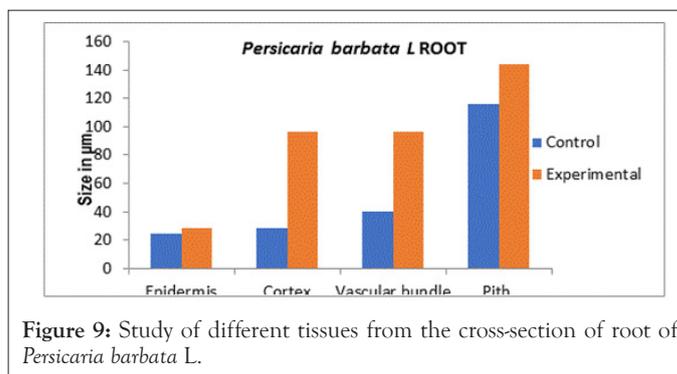


Figure 9: Study of different tissues from the cross-section of root of *Perscaria barbata* L.

## DISCUSSION

Anatomical studies of *Perscaria barbata* L. experimental showed that epidermis was 4-5 layered thick with small rounded cells beneath the cortex 10 to 12 layered of cortical cells were present. Cells were thick walled and usually rounded in shape. Under the cortex endodermis was present. Casparian strips were also present in endodermis, which was easily recognized. Vascular bundles were present in center of stem and protoxylem and metaxylem can be easily distinguished and central portion is hollow. But in control plant epidermis was only 2 layered thick and cortex was much reduced in control as compared to experimental. Cortical cell size and number was much reduced in control. Vascular bundles were clearly visible in them. Metaxylem was much more prominent in them. Endodermis was present but casparian strips were not clearly visible. Stem was also hollow from the center like experimental one.

From the present study it was concluded that those plants show better growth than controlled one, which were growing away from the waste irrigated water because they absorb low concentration of water but on the other hand those plants which were in direct contact with waste water showed less growth. As there were several factors which directly reduces the growth of plants like concentration of copper, concentration of Phosphorus and Potassium, electrical conductivity etc. Similar results were reported by Tamousidis et al., [25]. According to them treated urban waste water of municipality is suitable for the irrigation of crops this may be due to nutritive value of waste water. But on the other hand Khan et al., reported that yield of plants gradually decreased as the concentration of waste water increased [26-29].

## CONCLUSION

In the current study, increased concentration of waste water also raise the soil pH, EC, and SAR value and soil turned to saline which reduces the plant ability to absorb nutrients needed for vegetative growth. Experimentally proved that there is higher concentration of heavy metals in waste water which are potent to retard plant growth and development and adversely affect the yield. Moreover, by applying the wastewater the pH, EC as well as SAR values of the soil increases which change the characteristics of soil and turned it into saline one and also retard the plant growth. Similar findings have also been described in canola.

Similarly, determined that Fe<sup>+2</sup> and Cu<sup>+2</sup> cause oxidative stress.

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