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Anatomical Variations in Leaves of *Pisum sativum* Grown with Wastewater of Different Industries

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Abstract

An experiment was carried out to evaluate the anatomical variations in leaves of Pisum sativum grown in medium irrigated with different industrial wastewaters. The Sukhrawa drain area was selected for the collection of wastewaters of different industries in district Okara, Punjab, Pakistan. The experiment was comprised of six wastewater treatments, including protein farm wastewater, rice mill wastewater, combined wastewater of hospital and oil mill, paper mill wastewater and municipal wastewater. The results of anatomical variations in the epidermis, vascular bundle, palisade, and mesophyll cells indicated that the apical part, center, and base of leaf blade all showed normal structure and healthy cells when irrigated with rice mill wastewater and paper mill wastewater, while the center of leaf blade also showed good results when irrigated with the municipal wastewater. So, the results indicate that pea plants can be grown with wastewater from the rice mill, paper mill and municipal wastewater. In the future, wastewater from rice mills, paper mills and municipal wastewater should be considered to check for possible anatomical variations in other plants.





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Introduction

Environmental pollution has become a global issue that affects severely all organisms. The main cause of environmental pollution is urbanization and industrialization advancement in human lifestyle and their friendly behavior toward technology. Pollution is of several types caused by different anthropogenic activities. Among all types of pollutions, one that directly or indirectly affects the entire ecosystem is water pollution. Water is the most important component of the ecosystem used for drinking, cooking, washing, nurturing cattle's and for agricultural purposes. It is necessary to characterize the sources of heavy metals in the water to determine the risks to human health and food safety [1]. The main source of water pollution includes industrial waste. agricultural waste, human activities, low-quality fuel that is dunked in water bodies used for agricultural purposes, etc. Plants and vegetables that grow in such a contaminated atmosphere show indications of damage that include general weakness and maturation before time. Human actions like development, farming preparation take part in the deprivation of surroundings and openly interrupt water bulks like rivers [2]. Polluted water contains many types of pollutants, including disease-causing agents (bacteria, viruses, protozoa and parasitic worms), heavy metals and oxygen demanding wastes (waste that required oxygenated bacteria for its decomposition), etc. [3]. The use of polluted water is dangerous for humans and plants. For example, heavy metals inhibit the photosynthetic process in plants [4]. In humans, exposure to heavy metals plays a role in fetal diseases like cancer [5]. Nowadays, plants, especially vegetables, are grown using polluted irrigation water, which shows adverse effects on plant growth, marketability, and food safety [6]. Pea (Pisum sativum) is a common small spherical

seed that belongs to the family *Fabaceae*. This plant is of great importance because it is considered a tiny powerhouse of minerals that act as anti-aging agents, blood sugar controllers, and reduced blood cholesterol. It has been reported that irrigation with contaminated water causes anatomical changes in the internal structure of root, stem, and leaf. For example, it was observed in some plants like *Potamogeton* L. that contamination of heavy metals caused the reduction of the thickness of the blade and no. of conducting cells in leaves [7]. Considering the deleterious effects of contaminated wastewater, this study was planned. The main objective of this study was to evaluate the anatomical variations in leaves of pea plants irrigated with wastewater collected from five different industrial outlets.

Materials and Methods

The study pertaining to see the anatomical variations in leaves of pea grown in culture irrigated with different industrial wastewater was conducted in the Environmental Bio Laboratory, University of Okara, Punjab, Pakistan. The area selected for the collection of wastewaters was the disposal sites of different industries at Sukhrawa drain in district Okara, Punjab, Pakistan. Sukhrawa drainage collects wastewater from various industries situated in the Okara district.

Selection of plant and preparation of growth media

The pea plant was selected to evaluate the effect of wastewater on plants. The excellent quality, high viability and fungicide-treated seeds of the pea plant were purchased from a local market. The plant growth medium was prepared by mixing a field soil with 7% clay and 3% sand thoroughly to get a homogeneous mixture of soil.

Collection of wastewaters of different industries

Water for irrigation of the experimental crop was collected from five different sites of the Sukhrawa drain near Nemat Banaspati Ghee Factory, Okara. In this drain, several factories dispose of their wastewater. The selected five industries for the wastewater were: (S1) protein farm wastewater; (S2) rice mill wastewater; (S3) combined wastewater of Nemat Banaspati Ghee Factory and Fazal Din Hospital; (S4) paper mill wastewater and (S5) municipal wastewater, which was 500 meters away from other sites where all the wastewater mixed. After collection, all samples were brought to the Environmental Laboratory, University of Okara, Renala Khurd, District Okara. As a control treatment, tap water (S0) was used.

Experimental setup

The plastic pots were used and filled with 70 g of prepared soil medium. In each pot, five seeds were sowed approx. 1 inch below the surface and irrigated with collected wastewater from selected five sites and tap water as control. The germination of seeds and all the changes in plants were noted carefully and regularly. The experiment was comprised of five replicates. On the first day of the experiment, pots were irrigated with 20 ml of wastewater, after that 15 ml of irrigation treatments were given regularly until the 20th day. At the end of the experiment, leaf samples were collected.

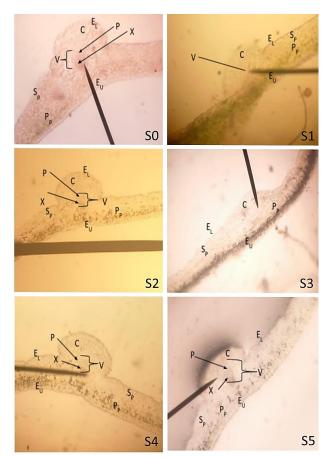
Anatomical analysis

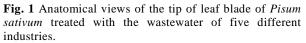
At the end of the experiment, leaves were collected from each treated plant, cut into thin sections and stained by the double staining method. Stained sections of leaves were studied under a microscope and photographed. In addition, anatomical changes were noted. For analysis, three different leaf points; base of the leaf blade, center of the leaf blade and tip of the leaf blade was selected.

Results and Discussion

Anatomical view of the apical part of leaf blade of pea plant treated with wastewater of different industries

The anatomy of apical part of leaf blade of pea plant treated with wastewater of different industries was observed. The anatomy of control (S0) was found normal having healthy and normal cells of the epidermis, vascular bundle and differentiated palisade and spongy mesophyll cells. The anatomy of S1 showed damaged upper epidermis, palisade mesophyll and cortical cells, and shriveled spongy mesophyll tissue, while the vascular bundle was not visible (Fig. 1). In S2 treatment, plants had normal epidermis, mesophyll tissue and vascular bundle, while shriveled cortical cells were observed. In S3, plant's leaf had thin and distorted cells, reduced cells of spongy and palisade mesophyll, while xylem and phloem were absent and distorted cortex was present. In S4, the anatomy of leaf showed normal and healthy epidermis and mesophyll tissues and shriveled xylem but still visible, while phloem cells were under development (Fig. 1). In S5, the upper epidermis and mesophyll cells were healthy and normal, while the lower epidermis was damaged. The xylem and phloem cells of S5 were larger and reduced cortical cells were observed (Table S1). These results are in accordance with Chandra et al. [8] who reported that Cr, which is a component of industrial wastewater, has a negative impact on the leaf anatomy of Vigna species and caused distortion of epidermis and stele.





EL= lower epidermis; C = cortex; V = vascular bundles; EU = upper epidermis; X = xylem; P = phloem; Sp = spongy parenchyma; PP = palisade parenchyma

Anatomical view of the center of leaf blade of pea plant treated with wastewater of different industries

The anatomy of central part of leaf blade of pea plant treated with wastewater of different industries was observed and the anatomy of control (S0) was found normal having healthy and normal cells of the epidermis, vascular bundle and differentiated palisade and spongy mesophyll cells (Fig. 2). In S1, anatomy of leaf showed distortion in the epidermis, palisade mesophyll and cortical cells. While spongy mesophyll has healthy and shriveled cells and vascular bundles were normal. In S2, normal cells of epidermis, mesophyll and cortex were observed, while the xylem and phloem cells were shriveled. In S3, thin, reduced, and distorted epidermis was observed, while mesophyll cells, vascular bundles and cortical cells were

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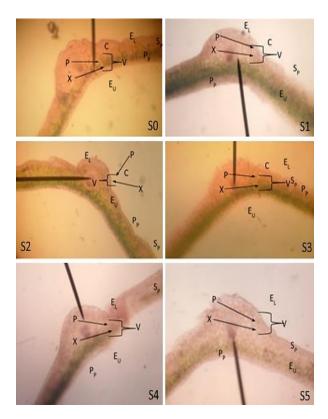


Fig. 2 Anatomical views of the center of leaf blade of *Pisum sativum* treated with the wastewater of five different industries.

EL= lower epidermis; C = cortex; V = vascular bundles; EU = upper epidermis; X = xylem; P = phloem; Sp = spongy parenchyma; PP = palisade parenchyma

reduced and some of those were not clear. In S4, healthy epidermis, mesophyll and cortical cells were observed, while the vascular bundle mainly xylem was shriveled, and phloem was underdeveloped (Table S2). In S5, normal epidermal and mesophyll cells were observed, while the xylem and phloem had larger cells and reduced cortical cells were observed. These results are in accordance with the observations of Sun et al. [9] who observed a reduction in xylem due to wastewater treatment. These results are also in accordance with Hilmi et al. [10] who examined anatomical responses of *Reutealis trisperma* at different concentrations of gold-mine tailings. The treatment with 100% tailing caused a reduction in the diameter of vascular bundle and upper and lower epidermis thickness.

The anatomy of basal part of leaf blade of pea plant treated with wastewater of different industries was observed. The anatomy of S_0 was normal having healthy and normal cells of the epidermis, vascular bundle and differentiated

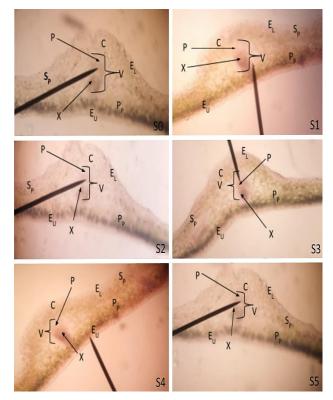


Fig. 2 Anatomical views of the base of leaf blade of *Pisum* sativum treated with the wastewater of five different industries.

EL= lower epidermis; C = cortex; V = vascular bundles; EU = upper epidermis; X = xylem; P = phloem; Sp = spongy parenchyma; PP = palisade parenchyma

palisade and spongy mesophyll cells (Fig. 3). In S1, the anatomy of leaf showed the damaged epidermis, distorted palisade mesophyll cells, shriveled spongy mesophyll cells and distorted cortical cells, while the vascular bundle was normal. In S2, normal and healthy cells were observed in the epidermis, mesophyll, and vascular bundle, while shriveled cortical cells were observed. In S3, thin and reduced epidermis, mesophyll and cortical cells ware observed. In S4, normal cells and reduced area of cortex were observed. In S5, normal and thick upper epidermis was present, while thin and damaged lower epidermis, larger xylem, phloem cells were observed (Table S3). These results are in accordance with the observations of Hu et al. [11] who reported a reduction in mesophyll and vascular bundle in response to wastewater irrigation. These results are also in accordance with Samira and Okkiah [12] who carried out two pot trials to see the impact of sewage water on the growth, yield, and anatomy of faba bean plants. All

concentrations of sewage water affected anatomy, but 100% sewage water gave the lowest value of the diameter of xylem vessel and cortical tissues.

Conclusion

Based on the findings of this study, it may be concluded that the apical part of leaf blade of pea plant was better in S2 and S4 in which pea were grown with wastewater from rice mills and paper mills. The center of leaf blade of pea plant showed better results for S2, S4 and to some extend for S5 in which pea was grown with wastewater from the rice mill, paper mill and municipal wastewater. The base of leaf blade of pea plant showed better results for S2 and S4 in which pea were grown with wastewater from rice mill and paper mill. These results could help in the future safe use of industrial wastewater.

Conflict of Interest

The authors declare that they have no conflict of interest

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