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INFLUENCE OF LEAD AND CHROMIUM LOADED SEWAGE WASTE WATER ON PHYSIOLOGICAL, BIOCHEMICAL AND ANATOMICAL TRAITS OF DIFFERENT CROPS

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ABSTRACT

Prolonged irrigation of untreated sewage water results in the accumulation of heavy metals in soil, vegetables, and crops. Sewage water irrigated vegetables and crop samples were collected from the peri-urban area of Faisalabad, "Uchkara". Physiological, biochemical, and anatomical characteristics of vegetables (spinach, brinjal, cauliflower, tomato, pepper) and crops (tobacco, maize) were examined to determine the toxic effects of lead (Pb) and chromium (Cr). Diverse behaviors were observed in the concentrations of chlorophyll, proline, amino acids, protein, soluble sugars, and heavy metals. Higher chlorophyll contents were found in spinach and cauliflower as compared to maize and brinjal. Spinach was found to be a low accumulator of amino acids, and maize was the highest accumulator. A high protein content was observed in spinach, compared to other crops that have low protein content. Soluble sugar contents were found to be higher in all vegetables and crop samples. Tobacco and maize accumulated low Cr concentrations, unlike all other samples. Anatomical analysis revealed a higher number of vascular bundles in the leaf, stem, and root, with thinner epidermis observed in the root and stem. Leaf, root, and stem revealed less cortical cell area in cauliflower and higher meta-xylem area in brinjal. Sewage water irrigation is a necessity of the time, particularly in circumstances of surface water shortage. The present study aims to investigate the toxicity of heavy metals (Pb and Cr) on the physiological, biochemical, and anatomical characteristics of vegetables and crops.

Keywords: Structural variation, Heavy metal pollution, Vegetables, Environment

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1. INTRODUCTION

Water is important for the survival of all organisms. About 90% of available water is used for the agriculture sector, 3% for industrial purposes, and 6% for domestic activities. Water shortage is a critical problem for crop production worldwide, although Pakistan has the most extensive canal irrigation network. A canal water shortage results in increased sewage water demand, which is a rich source of nutrients and organic content, and has a positive impact on plant productivity (Onakpa et al., 2018). Farmers prefer nutrient-rich sewage water, as it reduces their fertilizer costs. At the same time, higher heavy metal concentrations, sodium adsorption ratio (SAR), residual sodium carbonate (RSC), and electrical conductivity (EC) resulted in several drawbacks (Rajagopal et al., 2019). In Pakistan, industrial and domestic sewage waste is drained into canals, and this heavily metal-contaminated water is directly used for crop irrigation (Riaz et al., 2018). Sewage water treatment facilities are inadequate due to high costs, and therefore unaffordable for poor farmer communities in Pakistan. In Islamabad, sewage waste was discharged directly into the Swan River, and a 32,500-hectare area was irrigated with this water (Ensink et al., 2004).

Industries discharge heavy metal contaminants into the environment, leading to significant environmental problems (Dahiya et al., 2022). Tanning industries are main source for heavy metals, such as copper (Cu), chromium (Cr), lead (Pb), boron (B), cobalt (Co), zinc (Zn), manganese (Mn), molybdenum (Mo), and arsenic (As). Some trace metals are essential for plant growth and human beings, but some are non-essential and even minute amount are toxic. These toxic heavy metals accumulate in the soil, get transferred into the food chain through plants, and cause several diseases (Malla et al., 2007).

Metal toxicity affects crop growth and yields, resulting in symptoms such as chlorosis, premature leaf fall, poor and retarded growth, enzyme imbalance, and nutritional diseases commonly observed in crops like cabbage, tomatoes,

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rice, chilies, and many others. Cr is the seventh abundant element on Earth and is present in the soil, air, and water. Cr (III) is less toxic than Cr (IV) because Cr (III) needs a diffusion process, and Cr (IV) enters the cell without any carrier (Chunillal et al., 2005). Inorganic Cr adsorption rate is less than 0.4 to 3%, and Cr toxicity resulted in leaf chlorosis, necrosis, seed germination, root inhibition, and finally decreased biomass. It also affected biological processes in wheat, maize, cauliflower, barley, and vegetables (Ghani, 2011).

Lead (Pb) is a non-biodegradable substance with low water solubility (WHO, 2006). The primary Pb sources are industrial gases and dust that contaminate soil, air, and plants, resulting in toxic effects on the biological system, particularly human health (Verma et al., 2023). However, its accumulation varied among species. Pb reduces root and plant growth even at low concentrations. Likewise, it enters the food chain and affects the human brain, bones, nervous system, and kidneys (Pehlivan et al., 2009).

Vegetables are the primary source of minerals, dietary fiber, vitamins, and potassium (Zaheer et al., 2022). In Pakistan, vegetable production increased by 13-21% from 2007 to 2008, and in Punjab, it increased by 18-23% (Rehman et al., 2015). The Cruciferae family, which includes cauliflower, cabbage, kale, radish, turnip, mustard, rocket, and horseradish, provides glucosinolates in large amounts. The Alliaceae family, which includes garlic, onion, Welsh onion, and leek, is a rich source of thiosulfides, which help minimize the risk of many chronic diseases. Vegetables and fruits are rich sources of folic acid, thiamine, pyridoxine, vitamin C, and niacin, and are essential for human health (Wargovich, 2000). Vegetables and crops have been shown to prevent 20% of various types of cancers, including stomach, esophageal, bladder, pancreatic, and cervical cancers (Hashmat et al., 2021).

Irrigation with sewage water crops results in the accumulation of heavy metals in both edible and non-edible parts of vegetables, posing several health risks to both humans and wildlife. Likewise, the consumption of metal-contaminated vegetables leads to a reduction in essential nutrients in the human body, membrane damage, impairment of physiological functions, and growth retardation. Some of the other primary diseases include food hair loss, fever, poisoning, typhoid, dysentery, cholera, vomiting, pneumonia, lung cancer, diarrhea, dermatitis, and low blood pressure (Husaini et al., 2010). The present study was conducted to determine the negative impacts of sewage water irrigation on the physiological, biochemical, and anatomical structure of crops and vegetables.

2. MATERIALS AND METHODS

Cauliflower, spinach, tomato, pepper, brinjal (vegetables), maize, and tobacco (crops) samples were collected in triplicate from the peri-urban area of Faisalabad, specifically "Uchkara," which is irrigated with sewage water. The waste used for the irrigation of these vegetables was analyzed for chromium (Cr) and lead (Pb) using an atomic absorption spectrophotometer (Table 1). The physicochemical properties of the soil under cultivation at Uchkara are also presented in Table 2 (Shameem et al., 2015). Collected samples were preserved in a 70% ethanol solution. Physiological, biochemical and anatomical parameters were analyzed at the anatomical laboratory of the Government College Women University, Faisalabad.

2.1. Physiological Analysis

Photosynthetic pigments (Chlorophyll and carotenoids) were extracted from fresh leaves following the protocol devised by Arnon (1949).

2.2. Biochemical Analysis

Fresh leaf samples of wastewater-irrigated vegetables and crops were analyzed for proline content estimation using the protocol devised by Bates et al. (1973). Total soluble sugars (Yemm & Willis, 1954), protein concentration (Bradford, 1976), and total free amino acid (Hamilton & Van Slyke, 1943) were determined.

2.2.1. **Heavy Metal Concentration:** Lead and Cr concentrations were determined by sun-drying the collected vegetable and crop samples for 3- 4 days, followed by oven drying at 80°C (Abdullahi et al., 2007). Sample digestion was accomplished by adding 5mL of nitric acid and 5mL of perchloric acid to each sample and allowing to sit overnight. Another 5mL of nitric acid was added the next day and digested on a hot plate until brown vapors converted to colorless fumes. Distilled water was added to the colorless samples to bring the volume up to 50mL (Miller, 1998; Singh et al., 2012). Heavy metal contents (Pb, Cr) were determined through an atomic absorption spectrophotometer (Singh et al., 1999).

2.3. Anatomical Analysis

2.3.1. Dissection and Staining: Leaf, stem, and root transverse sections obtained by cutting the radial plane of a cylindrical portion, with the help of a potato using a 7 o'clock blade. Dissections were kept in a watch glass along with water. Fine, selected sections (cortex, pith, and vascular bundles) of leaf, stem, and root were stained with two different staining dyes. Dissected sections were periodically immersed in 30, 50, and 70% alcohol every 5-

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10min, followed by three-minute dipping in safranin and washing with 90% alcohol. Finally, the slides were observed under a microscope to study structural variation. Data analysed through M-STAT to observe significance (Steel & Torrie, 1960).

2.4. Data Analysis

All experimental data were analyzed using MSTAT-C software following the procedure of Steel and Torrie (1960). Data for physiological (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids), biochemical (proline, total soluble sugars, protein, and free amino acids), anatomical traits (epidermal thickness, cortical cell area, vascular bundle size, metaxylem area), and heavy metal concentrations (Pb and Cr) were subjected to one-way analysis of variance (ANOVA) to determine the significance of differences among crop and vegetable species irrigated with sewage water. The significance of the treatment means was tested using Fisher's Least Significant Difference (LSD) test at $P<0.05$. Results are presented as mean±SD. Graphical illustrations were prepared using Microsoft Excel.

3. RESULTS

3.1. Irrigated Wastewater and Soil Analysis

Analyses of Uchkara wastewater revealed higher concentrations of Pb and Cr, two heavy metals, in the observed samples. These heavy metals in wastewater were found to be at levels manyfold higher than the safe limits (Table 1). Uchkara soil samples were analyzed to determine the levels of Pb and Cr heavy metal concentrations, as well as to assess other soil properties (Table 2).

Table 1: Mean values of heavy metal concentrations in Uchkara wastewater (Shameem et al., 2015) used for irrigation of brinjal, cauliflower, pepper maize, tomato, spinach, and tobacco

Sources	Cr	Pb
Uchkara wastewater	9.0 (0.10 mg L^{-1})	1.5 (0.5 mg L^{-1})
Uchkara soil (0–20 cm)	0.35 (100ppm)	1.32 (5ppm)
Uchkara soil (20–40 cm)	0.33	0.68

WHO permissible limits of heavy metals in water are given in parentheses Denneman and Robberse (1990) and Chiroma et al. (2012)

Table 2: Physical and chemical properties of soil samples, collected from Uchkara (Shameem et al., 2015)

Soil parameters	Upper soil sample (0–20 cm)	Lower soil sample (20–40 cm)
pH	7.68	7.52
CEC (cmol$_c$ kg^{-1})	9.38	6.98
Organic matter (%)	1.27	1.63
Iron (mg kg^{-1})	10.45	12.80
Manganese (mg kg^{-1})	8.59	3.75
Calcium carbonate (%)	7.50	7.50
EC (dS m^{-1})	4.6	4.2
Soil texture	Loam	Loam
Bulk density (Mg m^{-3})	1.41	1.44
Porosity (%)	47	45

3.1.1. Physiological Parameters: As indicated from Fig. 1, cultivation of vegetable crops with untreated wastewater had a significant negative effect on the photosynthetic pigments of all vegetable crops. The negative consequences of wastewater irrigation on photosynthetic pigments were non-significantly differ in all studied vegetables except spinach (Fig. 1). The detail analysis of all vegetables crop's leaves exhibited that there was a significant reduction in chlorophyll a and b irrigated with wastewater except spinach (Fig. 1). The concentrations of Chl. a and b and total chlorophyll were significantly higher in spinach as compared to brinjal, cauliflower, pepper, maize, tomato, and tobacco.

3.1.2. Biochemical Parameters: Heavy metal contaminated sewage water irrigated vegetables and crop samples showed significant differences for proline and soluble sugars, contrary to protein/amino acids levels (Fig. 2). Maximum proline concentration was observed in cauliflower, pepper, and tobacco, while minimum in maize and spinach, or very low in brinjal and tomato. Maize, pepper, and tomato exhibited a higher soluble sugar concentration compared to spinach, tobacco, cauliflower, and brinjal (Fig. 2).

3.2. Anatomical Parameters

3.2.1. Root Anatomy: All sewage water irrigated samples showed significant variation for root anatomy (Fig. 6). The thickest epidermis was noted in spinach, cauliflower, and brinjal, while maize only revealed slight thickness. Variable levels of thickness were observed in tomatoes, peppers, and tobacco. Increased epidermal cell area was found in brinjal, pepper, and tobacco (Fig. 3). Sclerenchyma and parenchyma cell thickness was greater in maize, tomato, and brinjal in comparison to samples from the remaining vegetables and crops (Fig. 3).

Large cortical cells were noted in pepper. Maximum endodermis thickness was observed in tomato, spinach, and pepper (Fig. 3). However, the thickness was decreased in brinjal, maize, tobacco, and cauliflower under heavy metal stress (Fig. 3). Endodermis cell area was higher in pepper only (Fig. 4). The maximum metaxylem area was observed in brinjal, cauliflower, pepper, and tobacco. However, variability for this trait was observed in tomato and spinach (Fig. 4, 6).

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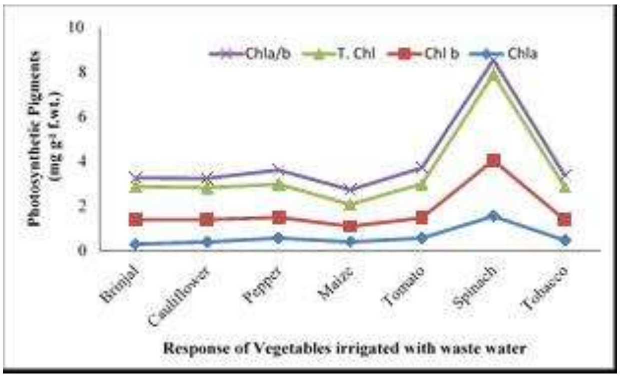


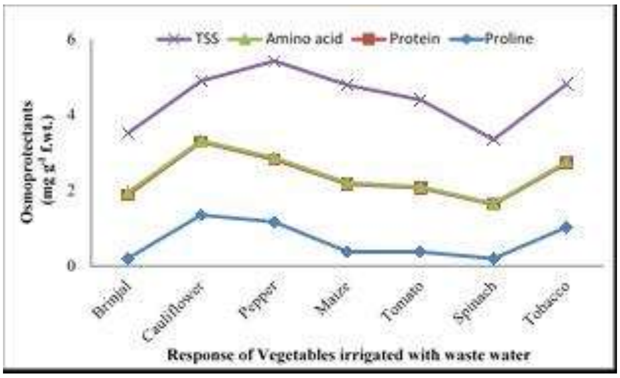
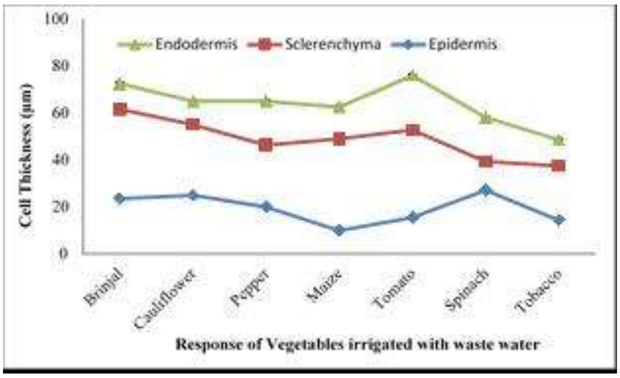


Fig. 1: Effect of wastewater on the performance of photosynthetic pigments of many vegetable crops. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). One-way ANOVA was performed and means differences were tested by HSD (P<0.05).

Fig. 2: Effect of wastewater on the performance of total soluble sugars (TSS), amino acid, protein and proline contents of many vegetable crops. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). One-way ANOVA was performed and means differences were tested by HSD (P<0.05).



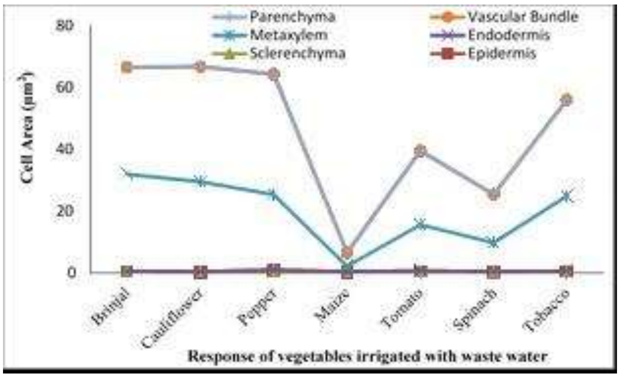


Fig. 3: Effect of wastewater on the thickness of epidermis, sclerenchyma and endodermis cell among many vegetable crops. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). One-way ANOVA was performed and means differences were tested by HSD (P<0.05).

Fig. 4: Effect of wastewater on the area of parenchyma, vascular bundle, metaxylem, endodermis, sclerenchyma and epidermal cell among many vegetable crops. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). One-way ANOVA was performed and means differences were tested by HSD (P<0.05).

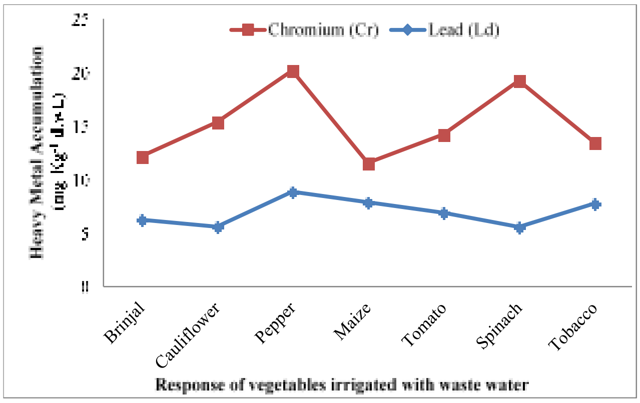


Fig. 5: Effect of wastewater on the accumulation of chromium (Cr) and lead (Ld) many vegetable crops. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). One-way ANOVA was performed and means differences were tested by HSD (P<0.05).

3.2.2. Stem Anatomy: Significant variation was observed in stem anatomy in the vegetable and crop samples irrigated with heavy metal (Pb, Cr) loaded sewage water (Fig. 7). Thick stem epidermis was recorded in spinach, tomato, cauliflower, and tobacco compared to brinjal, pepper, and maize. However, epidermis cell size in brinjal was greater than that of spinach and maize. The lowest cell size was found in cauliflower, pepper, tomato, and tobacco.

Increased cortical cell area and sclerenchyma were found in brinjal and cauliflower, respectively. The highest and lowest parenchyma cell sizes were identified in tobacco and pepper, respectively. An increase in the metaxylem area was indicated in brinjal, pepper, and tomato. A higher vascular bundle area was observed in brinjal, pepper, and spinach.

3.2.3. Leaf Anatomy: Vegetable and crop samples grown with sewage water revealed significant

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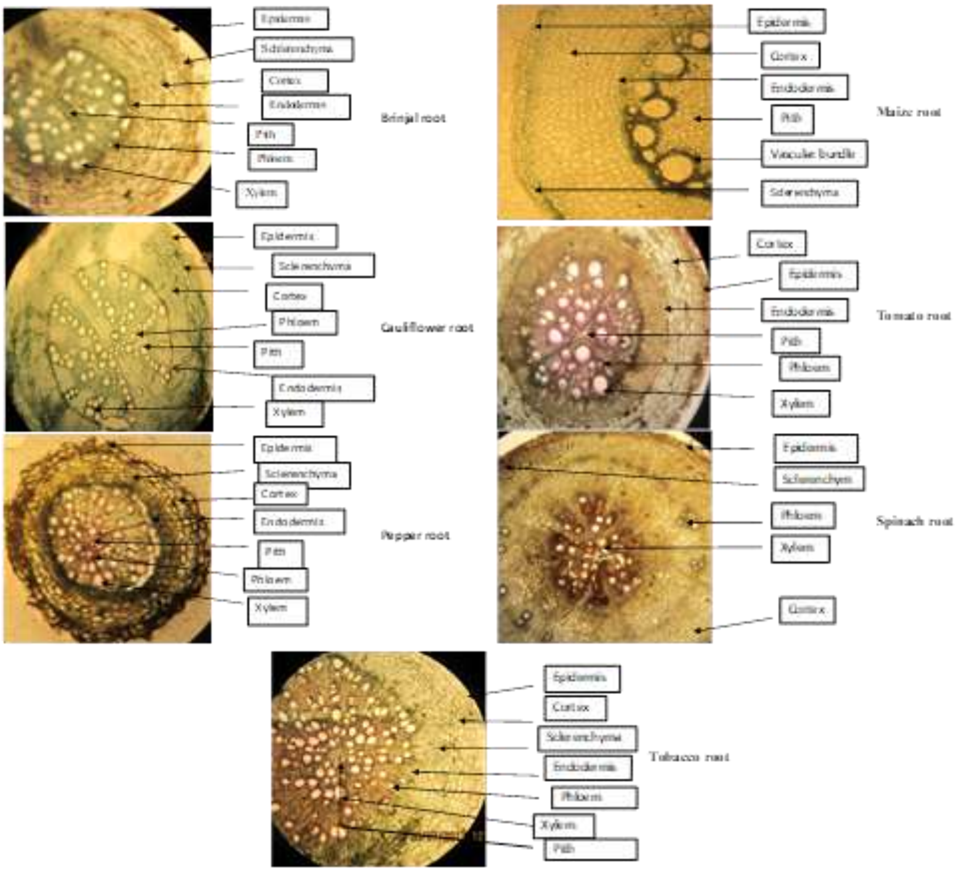

Fig. 6: Micrographs of root cross sections of brijal, cauliflower, pepper, maize, tomato, spinach, and tobacco irrigated with wastewater containing Cr and Pb from the peri-urban area of Faisalabad, "Uchkara.

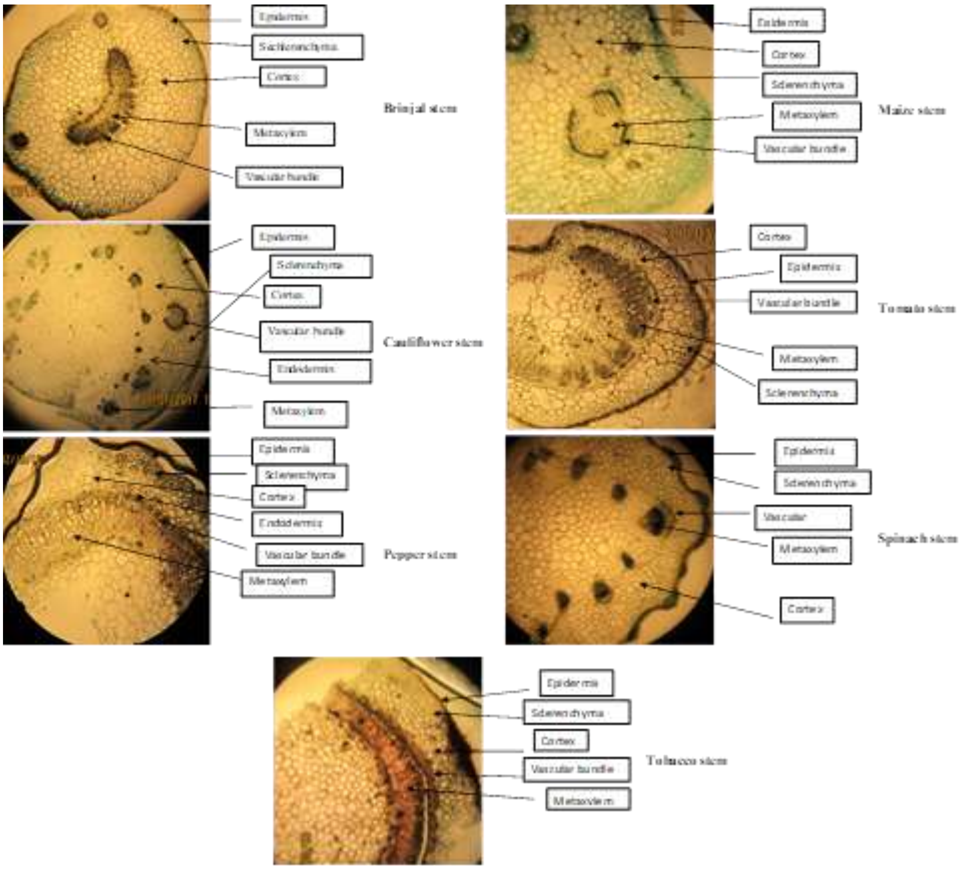

Fig. 7: Micrographs of stem cross sections of brijal, cauliflower, pepper, maize, tomato, spinach and tobacco irrigated with wastewater containing Cr and Pb from the peri-urban area of Faisalabad "Uchkara

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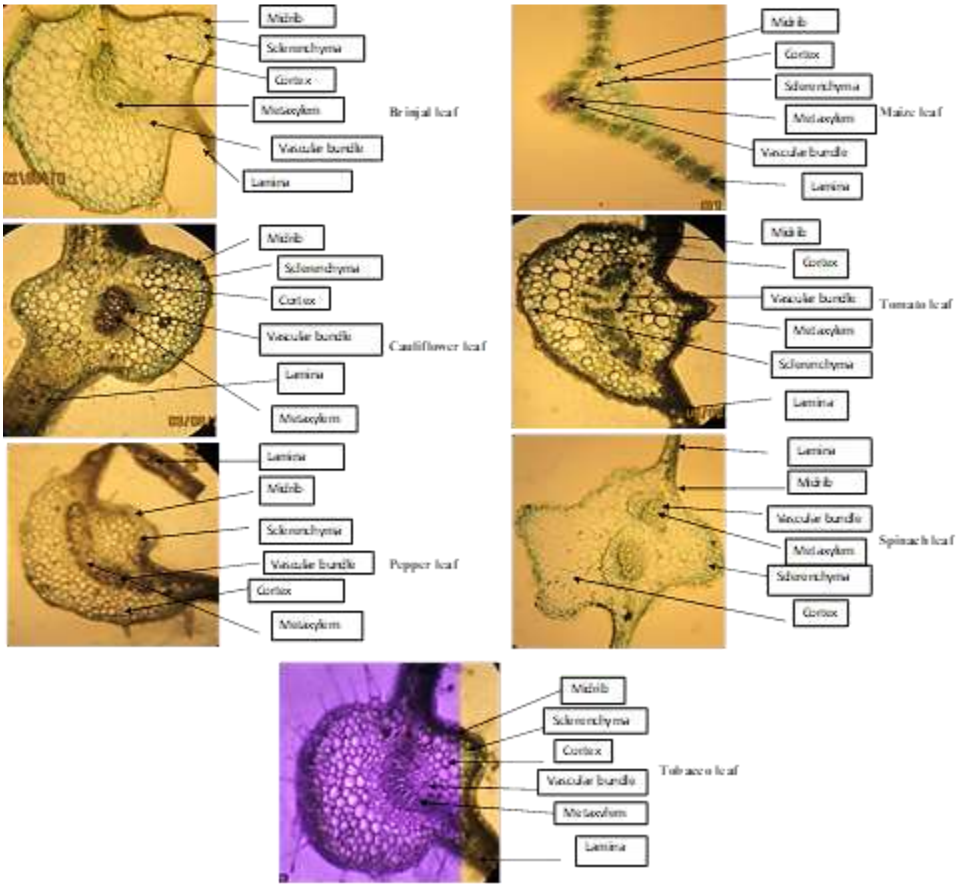

Fig. 8: Micrographs of leaf cross sections of brijal, cauliflower, pepper, maize, tomato, spinach and tobacco irrigated with wastewater containing Cr and Pb from the peri-urban area of Faisalabad "Uchkara

variability for leaf anatomy. The midrib thickness of leaf was greatest in the cauliflower, brinjal, pepper, tomato, spinach, and tobacco compared to maize (Fig. 8). The tobacco sample illustrated the maximum thickness of lamina while cauliflower, tomato, spinach, maize, brinjal, and pepper did not produce sufficiently thick lamina (Fig. 8). Sclerenchyma thickness was observed to be more in tobacco, maize, and spinach. Brinjal and tomato showed higher cell size in cortical cells. Maximum vascular bundles were found in brinjal, cauliflower, pepper, and tobacco. In spinach, tomato, and maize, the metaxylem area was noted to be the minimum (Fig. 8).

3.3. Heavy Metals (Pb, Cr) Concentration in Samples

Significant results were found among observed vegetables and crop samples exposed to heavy metal-contaminated sewage water (Pb, Cr) (Table 1). A higher concentration of Pb was observed in pepper, maize, tomato, and tobacco. The descending order of Pb concentration observed in vegetable samples was pepper > maize > tobacco > tomato > brinjal > spinach > cauliflower. Spinach revealed maximum Cr concentration, and the descending order of Cr concentration observed in vegetable samples was spinach > pepper > cauliflower > tomato > brinjal > tobacco > maize. Fig. Figs. 10 and 11 show graphical illustrations indicating Pb and Cr concentrations in crop samples (Fig. 5).

4. DISCUSSION

The presence of heavy metals has a significant impact on vegetable production (Kumar et al., 2013). Elevated levels of Pb had been reported in edible parts of sugar beet, whereas leafy vegetables showed higher concentrations of Zn, Pb, Cd, Ni, and Fe (Smirnova et al., 2006). The aerial parts of Pelargonium and *Brassica pekinens* were reported to translocate maximum concentration of Pb without damaging their metabolic functions (Xiong et al., 2006; Liu et al., 2008; Zulfiqar et al., 2023). Chlorophyll and carotenoids have been recognized as considered as potential indicators of environmental stress and can be associated with heavy metal toxicity in higher plants. Chlorophylls are the major chloroplast pigments that capture solar radiation for conversion into chemical energy during photosynthetic reactions in NADPH and ATP form (Paulus et al., 2010; Taiz & Zeiger, 2013). Carotenoids are considered important for acting as pro-vitamin factors, sunscreen, and secondary pigments, which remove free radicals from damaged tissue

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during photosynthesis reactions (Pandey et al., 2010; Zafar et al., 2025). Heavy metals substitute themselves for the activation sites of enzymes involved in chlorophyll synthesis, thereby affecting carboxylation and photochemical reactions (Gautum et al*.,* 2011; Ahmad et al., 2011). In this study, Pb decreased the activity of δ-aminolevulinic dehydrogenase enzyme (chief enzyme for chlorophyll synthesis) in brinjal, causing a decline in chlorophyll concentration (Gupta & Kalra 2006). Pb toxicity was also reported to reduce chlorophyll in wheat and rice samples (Li et al., 2010; Bhatti et al., 2013; Hansa et al., 2023). Previous studies have confirmed that chlorophyll content is more negatively affected by heavy metal stress than carotenoids, which exhibit variable behavior in many plant species under different environmental stresses (Lau et al*.,* 2006; Bakshe et al., 2023).

Proline accumulation is one of the effective adaptive behaviors manifested by various plant species (*Vigna radiata, Brassica juncea, Triticum aestivum*) exposed to metal toxicity (Dhir et al., 2004). Hydrophytes (Ceratophyllum, Wolffia, and Hydrilla), however, accumulate less proline when exposed to heavy metal stress. The involvement of amino acids reduces free radicals as they form an integral component of antioxidants (Ashraf & Foolad, 2007; Gill & Tuteja, 2010; Rennenberg & Herschbach, 2014). They have an active role in secondary metabolism and stress signaling in plants (Heidarpour et al., 2007). Under Pb stress, and amino acids like proline increase (Qadir et al., 2010).

Variations in plant protein content indicate changes in some physiological metabolism (Singh & Tewari, 2003). Stress-induced protein synthesis helps reduce the toxicity caused by heavy metals (Doganlar et al., 2012). Proteins have been important components of the cell that are affected in stress conditions (Wu et al., 2010). Under heavy metal stress, higher protein concentrations may be attributed to the production of stress proteins, such as phytochelatin and glutathione, as well as the synthesis of heat shock proteins and enzymes involved in the Krebs cycle. In cadmium (Cd) and Pb contaminated soya beans and Pb-polluted rice, increased levels of protein content have been observed (Mishra et al., 2006; Ahmad et al., 2011).

Soluble sugars are important for photosynthesis. Soluble sugar contents decreased in agricultural crops due to metal effects (Rafique et al., 2010; Maranho & Gomes, 2024). Cu toxicity in *Rosmarinus officinalis* increased sugar contents. Elevated sugar content helps plants combat multiple stresses, such as heavy metals, waterlogging, salinity, frost, and osmotic stress, by preserving biological membranes and molecules (Rattan et al., 2001; Irannejad & Shahbazian, 2004).

The study of anatomical features of stressed plants is critical. Metal accumulation in the root might have resulted in structural variation with functional consequences in the plant. This study reported a decrease in the cortical cell area of different vegetables and crops. The metal toxicity-induced decrease in cell size of the root, stem, and leaf may be attributed to a reduced elasticity of the cell wall (Sharma et al., 2007; Shaari et al., 2022). Cd and Cu toxicity led to reduced midrib thickness and xylem cell area in Sorghum bicolor (Kaushik et al., 2005).

In roots, thicker phloem tissue is favorable for increased phloem transport. However, metal stress significantly reduced the thickness of mesophyll cells, resulting in a thinner leaf blade. Leaf-blade thickness in *Vallisneria spiralis* was reduced due to the parenchyma and epidermal cell size (Sridhar et al., 2005; Melo et al., 2007). The decreased vascular bundle is directly related to the reduction in the xylem vessel area. Heavy metals have been reported to decrease the size of xylem vessels and conducting elements (Rahman et al., 2024). In wheat, Cu and Cr toxicity reduced the thickness and diameter of the vascular bundle in roots (Sandalio et al., 2001; Ceccoli et al., 2011).

5. CONCLUSION

Water scarcity has compelled the farming community to use sewage water for irrigating vegetables and other crops. Lack of resources is a major limiting factor for treating sewage water before it is applied to fields. Therefore, farmers use untreated sewage water for irrigation. Sewage water contains rich loads of heavy metals, which gradually accumulate in the food chain and pose serious hazards to human health. It was concluded that vegetables exhibiting increased anatomical and physiological features can be further investigated for improved adaptive characteristics.

DECLARATIONS

Conflicts of Interest: The authors declare that they have no conflict of interest.

Data Availability: All data are available within the article.

Author's Contribution: Idea and supervision: HS, and SR, Write-up and editing: FF, and MZK, Visualization: KJ, Validation: HS and SR.

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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