

## PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES, HEAVY METAL ACCUMULATION AND HUMAN HEALTH RISK ASSESSMENT OF VEGETABLE CROPS IRRIGATED WITH WASTEWATER

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### Abstract

#### Keywords

District Kohat, APX activity, Chromium, Garlic, Okra, Tomato, Wastewater, Irrigation.

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The present study was conducted to evaluate the physiological and biochemical responses of vegetables (garlic, tomato and okra) grown at Jurma, Canal Road, Hangu Road and Kharmatu, District Kohat under wastewater irrigation. Crops at Jurma were irrigated with tube-well water, whereas those at Canal Road, Hangu Road and Kharmatu were irrigated with wastewater. Vegetable samples were analyzed for heavy metal accumulation, antioxidant enzyme activities and morphological attributes using RCBD. The results revealed that the highest Pb concentrations were recorded in Kharmatu for garlic (1.97 mg kg<sup>-1</sup>), tomato (1.31 mg kg<sup>-1</sup>) and okra (1.21 mg kg<sup>-1</sup>). Similarly, the maximum Cd concentration was observed in garlic (0.107 mg kg<sup>-1</sup>) at Kharmatu, while tomato (0.095 mg kg<sup>-1</sup>) and okra (0.087 mg kg<sup>-1</sup>) showed the highest Cd levels at Hangu Road. Chromium concentration was highest in garlic (2.40 mg kg<sup>-1</sup>) at Hangu Road, whereas tomato (2.52 mg kg<sup>-1</sup>) and okra (2.37 mg kg<sup>-1</sup>) exhibited maximum Cr accumulation at Kharmatu. In contrast, Jarma consistently showed the lowest heavy metal concentrations. Antioxidant enzyme activities increased markedly in wastewater-irrigated sites, with the highest APX activity recorded in Kharmatu for garlic (2.25 min<sup>-1</sup> g<sup>-1</sup> F. wt), tomato (3.77 min<sup>-1</sup> g<sup>-1</sup> F. wt) and okra (2.34 min<sup>-1</sup> g<sup>-1</sup> F. wt). The highest CAT activity was observed in garlic (32.40 min<sup>-1</sup> g<sup>-1</sup> F. wt) and tomato (69.95 min<sup>-1</sup> g<sup>-1</sup> F. wt) at Kharmatu and in okra (54.14 min<sup>-1</sup> g<sup>-1</sup> F. wt) at Hangu Road. Likewise, maximum POD activity was noted in garlic (19.06 min<sup>-1</sup> g<sup>-1</sup> F. wt) and okra (25.40 min<sup>-1</sup> g<sup>-1</sup> F. wt) at Hangu Road and in tomato (30.49 min<sup>-1</sup> g<sup>-1</sup> F. wt) at Kharmatu, while the highest SOD activity was recorded in garlic (64.03 min<sup>-1</sup> g<sup>-1</sup> F. wt), tomato (97.35 min<sup>-1</sup> g<sup>-1</sup> F. wt) and okra (89.39 min<sup>-1</sup> g<sup>-1</sup> F. wt) at Kharmatu. Overall, the lowest antioxidant enzyme activities were observed in Jarma. Morphological assessment showed superior growth performance under tube-well irrigation, where the highest fresh weight of garlic (149 g), tomato (721 g) and okra (444 g), as well as the highest dry weight of garlic (42 g), tomato (135 g)

and okra (94 g) were recorded at Jarma. Similarly, maximum root length of garlic (8.13 cm), tomato (25 cm) and okra (22 cm) along with the highest shoot length of garlic (62 cm), tomato (63 cm) and okra (75 cm) were observed at Jarma, whereas the lowest values were recorded at Kharmatu and Hangu Road. The study revealed that wastewater irrigation, particularly at Kharmatu and Hangu Road, significantly increased heavy metal accumulation and induced oxidative stress in vegetables, leading to reduced growth and biomass. Therefore, alternative irrigation sources should be adopted to minimize heavy metal contamination and ensure the production of safe and healthy vegetables.

## INTRODUCTION

Vegetables are considered as a composite of carbohydrates, macro and micro nutrients, vitamins, proteins, fiber and basic cancer preventive catalysts that are thought to lessen the impact of harmful substances (Bean *et al.*, 2010). Vegetables assume critical part in meeting the nutrients need of individuals around the world, since they are imperative suppliers of different fundamental requirements of the body i.e. minerals, vitamins and dietary fibers (Ogle *et al.*, 2001; Mukerji, 2004). They are additionally potential supplements of iron, calcium and protein which have obvious health benefits (Arai, 2002). Utilization of various vegetables are extremely effective in prevention of a variety of medical issues since their leaves, fruits or roots are rich source of vitamins and minerals like Beta Carotene, B6, Ascorbic acid, vitamin E, Ca, Folate, copper and dietary fiber (Hirai and Matsumoto, 2007). So different vegetables can be utilized to cure different malady including whooping congestion, digestive inconvenience, tumor, congestion, dyspepsia, liver issues, constipation, joint pain, gallstones, gall bladder diseases, kidney stones and intestinal parasites (Mukerji, 2004).

The volume of wastewater discharged by residential, mechanical and business sources has expanded with populace, urbanization, improved living conditions and monetary advancement (Qadir *et al.*, 2008). In urban ranges of several developing nations, Peri urban and urban horticulture depend on used water as a source of irrigation for their crops. The nature and conditions under which this water is utilized, change incredibly. In underdeveloped countries, this water in certain situation may transform into

raw sewage, regardless of the possibility that this can be viewed as illicit (Huibers *et al.*, 2004). However, the nature of the wastewater utilized and the way of its utilization differ immensely, both between and inside nations (Faruqui *et al.*, 2004). It may provide growers with irrigation water enriched with nutrients (Ullah *et al.*, 2011; Gosh *et al.*, 2012) and may offer the population a dependable and inexpensive framework for utilized water disposal and treatment (Feigin *et al.*, 1991). In such conditions, growers prefer used water even in the availability of fresh water, because of the fact that they gain higher returns utilizing wastewater. Used water can be extra dependabl e source as far as its accessibility and volume are concerned, beside fresh water supply or precipitation or from irrigation system. In such situations, it additionally enables farmers to produce multiple crops per year (Ensink *et al.*, 2004).

Developing countries like Pakistan produce huge volume of industrial and domestic untreated water that makes its way to other water channels. On one hand, the wastewater being rich in plant nutrients and organic matter enhances plant growth and development, hence, utilized by poor growers for watering that prevent them spending extra money on buying synthetic fertilizers. On the other hand, the untreated wastewater contains heavy metals (Cu, Zn, Pb, Cd, Cr, Ni) which are poisonous to human being as well as contaminates the nature (Al-Enezi, 2004).

Metals are integral part of the earth crust, but today soil pollution with enormous metals is an alarming issue on a worldwide scale and it is being highly focused as the industrialization is getting increased (Salvatore *et al.*, 2009). Metals can be accumulated in the ground at dangerous levels

because of continuous use of waste water (Bohn *et al.*, 1985). In numerous zones, these discharges are utilized by local growers for watering their crops, hence supplying these contaminants to the plants (Warning *et al.*, 1996). Toxic heavy metal accumulation may induce stress conditions in plant system and may interfere with plant physiological and biochemical processes. Heavy metals are known for damaging plasma membrane and structural disruption of cell organelles that ultimately result in disability in the plant physiological working and cause hindrance in development (Kimbrough *et al.*, 1999; Chien and Kao, 2000; Long *et al.*, 2003; Zhang *et al.*, 2002). The present study aims to evaluate the impact of wastewater irrigation on selected vegetable crops by determining the accumulation of heavy metals in edible plant tissues and assessing their physiological and biochemical responses to such irrigation practices. Specifically, the research will quantify the concentrations of various heavy metals accumulated in vegetables irrigated with wastewater and investigate the associated changes in physiological and biochemical characteristics of the plants. The findings will contribute to a better understanding of the potential risks of wastewater irrigation to crop productivity, food safety, and environmental sustainability in Pakistan.

## MATERIALS AND METHODS

The experiment “Physiological and biochemical responses of vegetables irrigated with waste water” was conducted at Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat, Pakistan. Vegetables samples of garlic, tomato and okra were collected from four different locations such as Kharmatu (KM), Canal Road (CR), Hangu Road (HR) and Jarma (JR) of district Kohat. The collected plant samples were evaluated for physiological traits (antioxidant enzyme activity), morphological characteristics (fresh and dry weights, root and shoot lengths), and biochemical properties (heavy metal accumulation).

### Sampling:

For sample collection, four major locations were selected in the district Kohat i.e. Hangu Road, Canal Road, Kharmatu and Jarma. Among these

four locations, crops at Hangu Road, Canal Road and Kharmatu areas were irrigated with waste water and crops at Jarma were irrigated with tube well water. Vegetable, soil and irrigation water samples were collected from the selected locations and subjected to laboratory analyses. Soil and water samples were examined for heavy metal concentrations and the corresponding results are presented in Table 1. In addition, selected morphological, physiological and biochemical parameters of the vegetables were measured and evaluated using standard analytical procedures described below.

### Heavy metals analysis of plants

Vegetables samples were placed in an oven for 72 hrs at 70°C for drying. After complete drying the sample was grind to obtain powder. Each sample was analyzed for heavy metals through atomic absorption spectrophotometer using wet digestion (Jones, 1991).

### Fresh weight and Dry weight (g)

The fresh weight of randomly selected plants from each treatment and replication was recorded using a digital balance and the mean value was calculated. For dry weight determination, plant samples were oven-dried at 70°C for 72 hours until a constant weight was achieved, after which their weights were measured using a digital balance and the average dry weight was computed.

### Shoot length and Root length (cm)

Shoot and root lengths (cm) of randomly selected plants from each treatment and replication were measured using a measuring tape and the average values were calculated.

### Determination of antioxidant enzymatic activity

Fresh plant sample (500 mg) was utilized for antioxidant enzymatic activity. The fresh sample was crumpled in 10 ml pre-cooled phosphate buffer ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$   $0.6663\text{gL}^{-1}$ ,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$   $16.385\text{gL}^{-1}$ ) with motor. Centrifugation was done at 4°C for about 20 min at 20000 rpm. The resulting antioxidant enzymes were examined.

### Catalase activity

Catalase activity was studied by following method of Aebi *et al* (1984). A mixture of 3 ml was

arranged having 25 mM (2.8 ml) potassium phosphate buffer, 100 µl enzyme extract, 30mM (100 µl) H<sub>2</sub>O<sub>2</sub> and catalase activity was determined by placing the prepared mixture in spectrophotometer at 240 nm.

**Peroxidase activity**

Peroxidase activity was determined by the technique described by Kumar and Khan (1982). About 3 ml of mixture was arranged and then 0.1 ml of (1.5%) guaiacol, 2.7 ml potassium phosphate buffer, 0.1 ml enzyme extract and 0.1 ml of (0.4%) H<sub>2</sub>O<sub>2</sub> was added. For response, wait about 2 min and enzyme activity was calculated at 470 nm. Distilled water 0.1 ml was taken as free rather than enzyme extract.

**Ascorbate peroxidase activity**

Ascorbate peroxidase activity was determined by following the technique of Nankano and Asada (1981). A mixture was prepared that was comprising of 100 mM phosphate buffer (pH 7.0), 0.3 mM of ascorbic acid, 0.1 mM sodium EDTA, 0.06 mM of H<sub>2</sub>O<sub>2</sub> and 100 µL of enzyme extract. Enzyme activity was calculated at 290 nm in spectrophotometer.

**Superoxide dismutase**

SOD was analyzed by using the method of Beauchamp and Fridovich (1971). For substrate composition NBT 15.5 mg, Riboflavin 0.2 mg, Na EDTA 100 mg and methionine 485 mg was taken and mixed in a reagent bottle and by the addition of distilled water to make the final volume of 250 ml. For determination, a reaction of 3 ml was prepared in beaker containing 2.725 ml reaction substrate, 0.25 ml H<sub>2</sub>O and 0.025 ml enzyme extract and placed under light condition at 4000 lux for 20 minutes. The control sample was also placed under dark and in light condition for 20 minutes and then absorbance was measured at 560 nm for analyzing the activity.

**Statistical Analysis**

The data collected was subjected to one-way Analysis of Variance (ANOVA) technique using RCB design. All the results were the means ± SD of three replications. Means were separated by LSD test at 1% level of significance. For calculating ANOVA and LSD value computer software Statistix (8.1) was used (Jan et al., 2009).

**Table 1: Heavy metals concentration in the soil as well as in irrigated water of different selected Locations.**

Locations	Soil (mg kg <sup>-1</sup> )			Water (mg L <sup>-1</sup> )		
	Lead	Cadmium	Chromium	Lead	Cadmium	Chromium
Jarma	0.92	0.75	0.06	0.15	0.04	0.020
Canal Road	1.59	1.76	0.29	0.54	0.06	0.063
Hangu Road	3.42	2.21	2.14	1.18	0.82	0.128
Kharmatu	3.54	2.66	1.91	1.39	1.09	0.115

**RESULTS AND DISCUSSION**

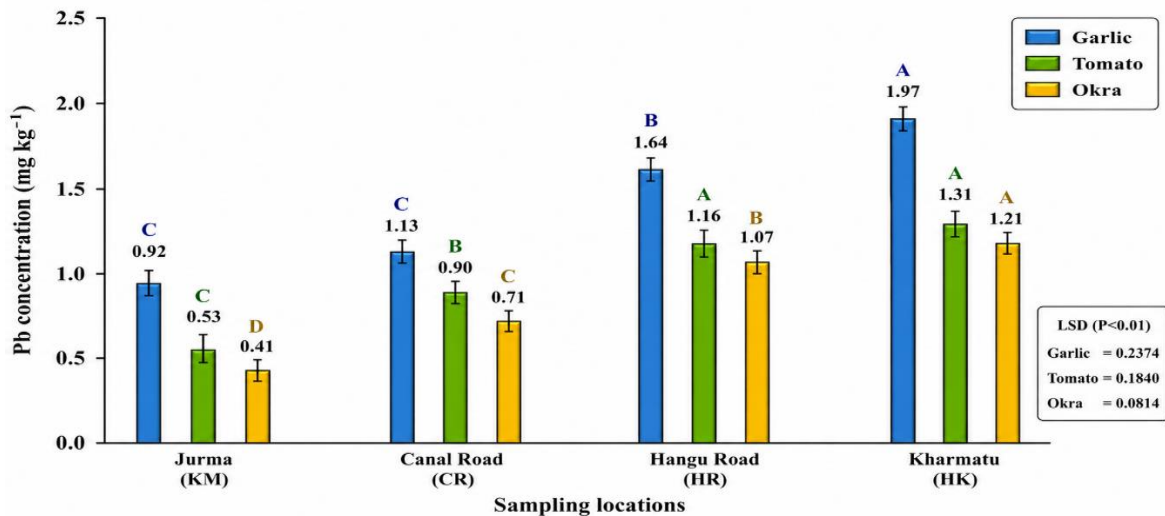
**Lead Concentration (mg kg<sup>-1</sup>) in Garlic, Tomato and Okra**

The analysis revealed that Pb concentration in garlic samples varied from 0.92 to 1.97 mg kg<sup>-1</sup> among the selected locations. The highest mean value for lead concentration (1.97 mg kg<sup>-1</sup>) in garlic bulb was observed in sample collected from Kharmatu, while lowest lead concentration (0.92 mg kg<sup>-1</sup>) was observed in sample collected from Jarma. Highest mean value for lead concentration (1.31 mg kg<sup>-1</sup>) was observed at tomato fruit of KM, area while lowest lead concentration (0.53 mg kg<sup>-1</sup>) was recorded in JR. On the other hand, lead

concentration in okra fruit samples were in the range of 0.41 mg kg<sup>-1</sup> to 1.21 mg kg<sup>-1</sup> with the highest concentration (1.21 mg kg<sup>-1</sup>) in KM area, while lowest concentration (0.41 mg kg<sup>-1</sup>) in JR area. Statistical analysis revealed significant (P ≤ 0.01) differences in lead concentration among garlic, tomato, and okra samples collected from different locations. Heavy metal accumulation, including lead, cadmium, and chromium, was significantly influenced by wastewater irrigation. Continuous use of polluted water can lead to the accumulation of toxic metals in soil and crops, posing serious risks to food safety and human health through their entry into the food chain

(Rattan et al., 2005; Khan et al., 2008). The present findings are consistent with those of Farooq et al. (2008), who reported that wastewater

irrigation is a major source of heavy metal accumulation in vegetables and other crops, facilitating their entry into the food chain.



Error bars represent  $\pm$ SE of mean ( $n = 3$ ). Means followed by similar letters are statistically similar using LSD test ( $P < 0.01$ ).

JR: Hangu Road, CR: Canal Road, HR: Kharmatu, KM: Jarma

Maximum permissible limit for Pb concentration = 0.1 mg kg<sup>-1</sup> (FAO/WHO 2011).

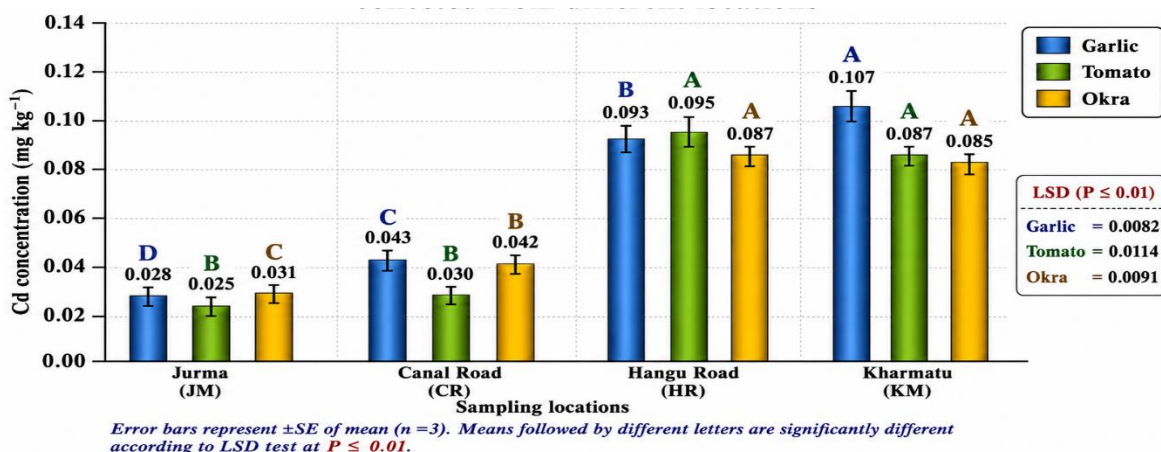
Fig 1. Lead conc. (mg kg<sup>-1</sup>) in garlic, tomato and okra collected from different locations.

### Cadmium Concentration (mg kg<sup>-1</sup>) in Garlic, Tomato and Okra

Cadmium concentration in garlic samples were in the range of 0.028 mg kg<sup>-1</sup> to 0.107 mg kg<sup>-1</sup>. The highest mean value for cadmium concentration (0.107 mg kg<sup>-1</sup>) in garlic bulb was observed in sample collected from Kharmatu while lowest cadmium concentration (0.028 mg kg<sup>-1</sup>) was observed in sample collected from Jarma area. Similarly, cadmium concentration in tomato fruit were in the range of 0.025 mg kg<sup>-1</sup> to 0.095 mg kg<sup>-1</sup>, Highest mean value for cadmium concentration (0.095 mg kg<sup>-1</sup>) was observed in tomato fruit of HR area, while lowest cadmium concentration (0.025 mg kg<sup>-1</sup>) was recorded in JR. On the other hand, cadmium concentration in okra fruit samples collected were in the limit of 0.031 mg kg<sup>-1</sup> to 0.087 mg kg<sup>-1</sup> with the highest concentration (0.087 mg kg<sup>-1</sup>) in HR area, while lowest concentration (0.031 mg kg<sup>-1</sup>) in JR area. The data on statistical analysis showed that lead concentration in garlic, tomato and okra samples

were significantly ( $P \leq 0.01$ ) different in different locations of the study area.

Previous studies have also presented the same result but there were differences in Cd accumulation in different plant parts and types of vegetables Sharma and Agrawal (2006), Islam et al. (2007). The concentrations of heavy metals like cadmium, lead and chromium in the three vegetables were the lowest at site Jarma, whereas the highest concentrations for all the considered heavy metals were noted at waste water sites Kharmatu and HR. The accumulation of elevated heavy metals in various vegetables might be because of the usage of waste waters which carries those heavy metals (Rattan et al., 2005). These vegetables are considered unhygienic for human health as it contains heavy metals in large amounts which are painstaking risk for human health due to its entrance in the food chain (Khan et al., 2008).



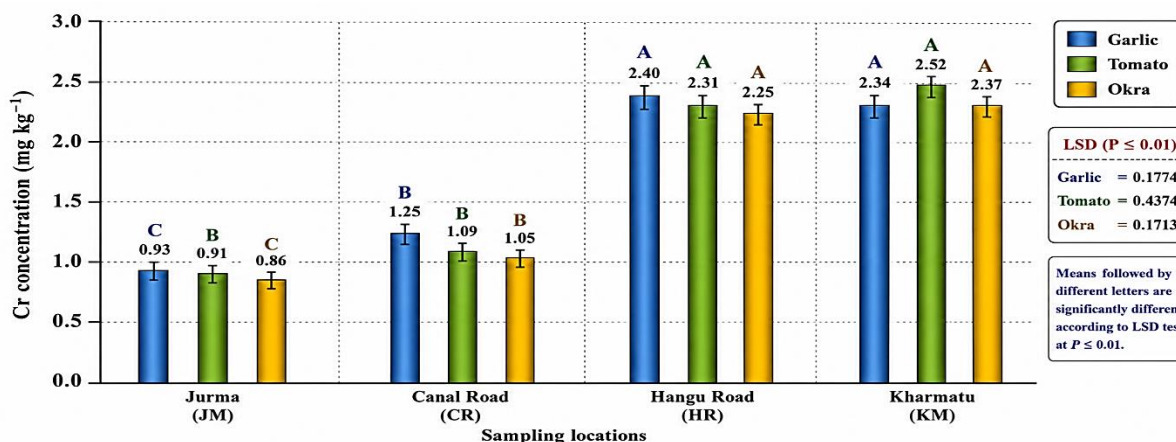
Maximum permissible limit for cadmium concentration = 0.05 mg kg<sup>-1</sup> (FAO/WHO 2011).  
 Fig 2. Cadmium conc. (mg kg<sup>-1</sup>) in garlic, tomato and okra collected from different locations.

### Chromium Concentration (mg kg<sup>-1</sup>) in Garlic, Tomato and Okra

The data analysis revealed that chromium concentration in garlic samples were in the range of 0.93 mg kg<sup>-1</sup> to 2.40 mg kg<sup>-1</sup>. The highest mean value for chromium concentration (2.40 mg kg<sup>-1</sup>) in garlic bulb was observed in samples collected from Hangu Road (HR), while lowest chromium concentration (0.93 mg kg<sup>-1</sup>) was observed in sample collected from Jarma (JM) area. Chromium concentration in tomato fruit was in the range of 0.91 mg kg<sup>-1</sup> to 2.52 mg kg<sup>-1</sup>. Highest mean value for chromium concentration (2.52 mg kg<sup>-1</sup>) was observed in tomato fruit of KM area, while lowest chromium concentration (0.91 mg kg<sup>-1</sup>) was recorded in JR. Likewise, chromium concentration in okra fruit samples collected were in the range of 0.86 mg kg<sup>-1</sup> to 2.37 mg kg<sup>-1</sup> with the highest concentration (2.37 mg kg<sup>-1</sup>) in KM area, while the lowest concentration (0.86 mg kg<sup>-1</sup>) in JR area. The data on statistical analysis showed that lead concentration in garlic, tomato and okra samples were significantly ( $P \leq 0.01$ ) different in different locations of the study area.

The present findings are in agreement with those reported by Bakhsh, (2005), who observed that wastewater irrigation may induce growth-stage-

dependent sensitivity in vegetable crops. Similarly, Sandalio *et al.* (2001) suggested that heavy metal toxicity adversely affects plant growth through reduced nutrient uptake, disruption of enzymatic activities, and the induction of oxidative stress. The elevated concentrations of heavy metals detected in vegetable fruits in the present study indicate their accumulation from contaminated soils subjected to wastewater irrigation. These results are consistent with the findings of Dahdoh *et al.* (1996), who reported that extractable Pb concentrations in both cultivated and uncultivated soils increased with increasing Pb additions. Furthermore, Badawy and El-Motaium, (2003) demonstrated that the concentrations of bioavailable heavy metals, including Cd, Pb and Cu in soil significantly increased with higher rates of sludge application. Collectively, these studies support the observed accumulation of heavy metals in vegetables grown under wastewater-irrigated conditions and highlight the potential risks associated with long-term contamination of agricultural soils.



Error bars represent ±SE of mean (n = 3). Means followed by different letters are significantly different according to LSD test at P ≤ 0.01.

Maximum permissible limit for chromium = 2.3 mg kg<sup>-1</sup> (FAO/WHO 2011).

Fig 3. Chromium conc. (mg kg<sup>-1</sup>) in garlic, tomato and okra collected from different locations.

### Ascorbate Peroxidase Activity (min<sup>-1</sup> g<sup>-1</sup> F. wt.) of Garlic, Tomato and Okra

Ascorbate peroxidase (APX) activity was found variable for garlic, tomato and okra samples collected from JR, CR, HR and KM. The activity of APX for garlic was found in the range of (0.93 to 2.25 min<sup>-1</sup> g<sup>-1</sup> F. wt.) The highest activity (2.25 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found collected from KM, while the lowest activity (0.93) was found for JR. The activity of APX was found for tomato samples collected from JR, CR, HR and KM in the range of 1.69 to 3.77 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest concentration (3.77 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found in samples collected from KM, while the lowest concentration (1.69 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for JR.

The APX activity for okra samples collected from JR, CR, HR and KM was in the range of 1.10 to 2.34 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest concentration

(2.34 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found in samples collected from KM, while lowest concentration 1.10 min<sup>-1</sup> g<sup>-1</sup> F. wt. was found for JR.

Similar activity was observed in tomato plants (*Lycopersicon esculentum* L.) ameliorated oxidative injury induced by metal and salt stress (Wang *et al.*, 2014). The up regulation of APX has been observed in plants subjected to both abiotic Boguszewska *et al.* (2010) and biotic stresses Torres, (2010), Świątek *et al.* (2010). Overexpression of APX in transgenic plants resulted in higher metal, salt or drought tolerance (Badawi *et al.*, 2004). Thus, SODs have a critical role in the survival of plants under environmental stresses. An increase in APX activity under diverse abiotic stresses has been shown in several plants Gill & Tuteja (2010). In extracts obtained from potato cultivars, three activity bands of ascorbate peroxidase (APX) were observed Boguszewska *et al.* (2010).

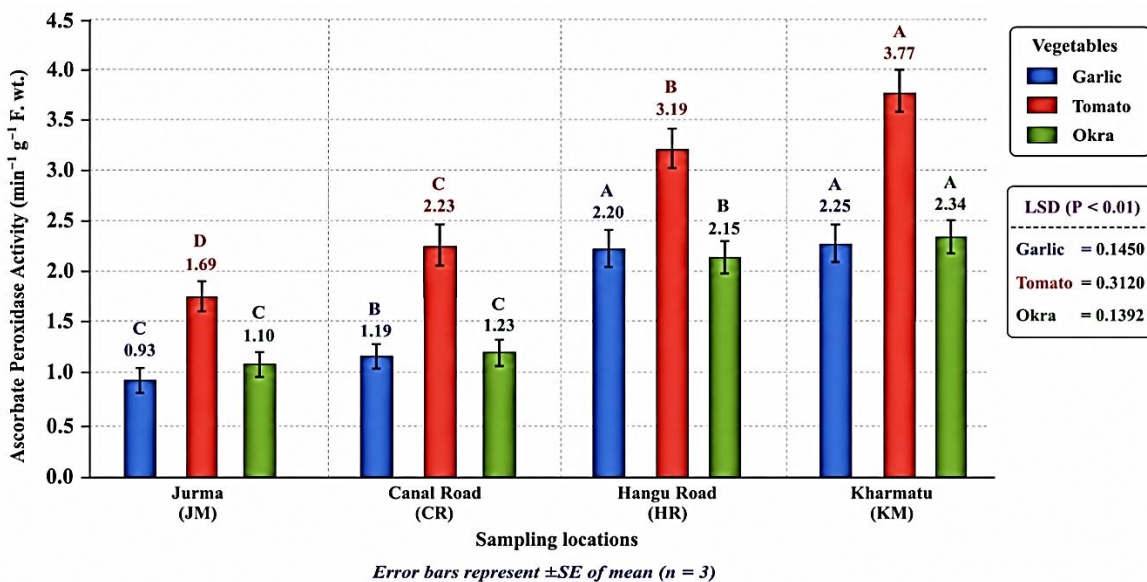


Fig 4. Ascorbate peroxidase Activity (min<sup>-1</sup> g<sup>-1</sup> F. wt.) of garlic, tomato and okra collected from different locations.

**Catalase Activity (min<sup>-1</sup> g<sup>-1</sup> F. wt.) of Garlic, Tomato and Okra.**

Catalase (CAT) activity was found variable for garlic, tomato and okra samples collected from JR, CR, HR and KM. The activity of CAT for garlic was found in the range of 24.55 to 32.40 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest activity (32.40 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found in plants collected from KM, while lowest activity (24.55 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for CR.

The CAT activity was found for tomato samples collected from JR, CR, HR and KM in range of 44.26 to 69.95 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest activity (69.95 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found collected from KM, while lowest activity (44.26 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for JR. The CAT activity for okra samples collected from JR, CR, HR and KM was in the range of 41.39 to 54.14 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest concentration (54.14 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found collected from HR, while the lowest

concentration (41.39 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for CR.

The activities of CAT, SOD, POD, and APX are induced in various plant species by accumulation of heavy metals Noctor and Foyer, (1998). ROS have potential to interact with many cellular components causing significant damage to membranes and other cellular structure. However, an elaborate and highly plant ROS network composed of antioxidant enzymes and antioxidants is responsible for maintaining the levels of ROS under tight control. In plant cells antioxidant enzymes like SOD, POD, CAT have been considered as a defensive mechanism whose combined purpose to protect plant from oxidative damage (Foyer and Noctor, 2005). The increased activity of catalase in plants of heavy metal polluted areas indicate that plant is under stress and increased activity to cope with this stress to ensure its survival.

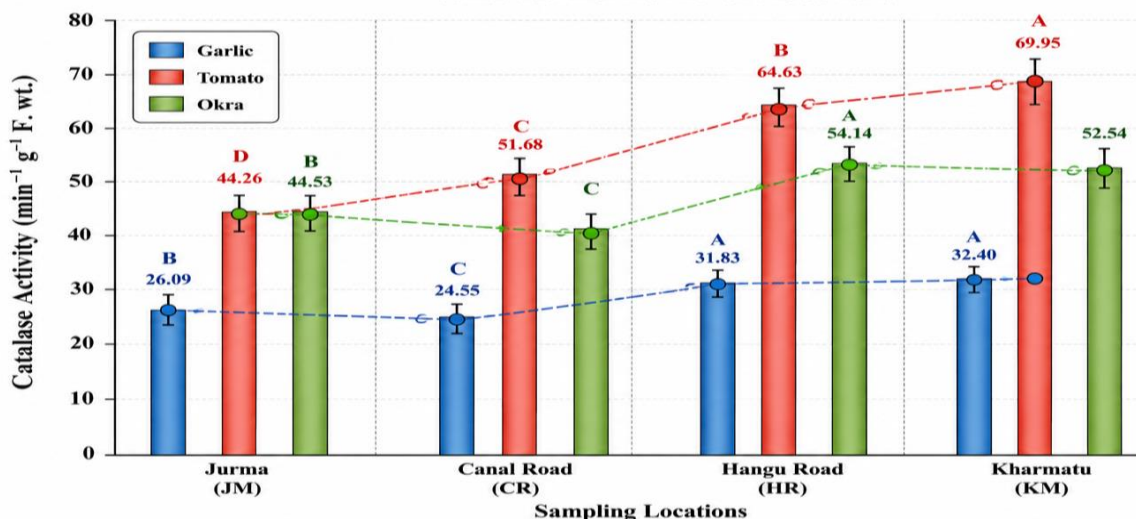


Fig 5. Catalase Activity (min<sup>-1</sup> g<sup>-1</sup> F. wt.) of garlic, tomato and okra collected from different locations.

### Peroxidase Activity (min<sup>-1</sup> g<sup>-1</sup> F. wt.) of Garlic, Tomato and Okra

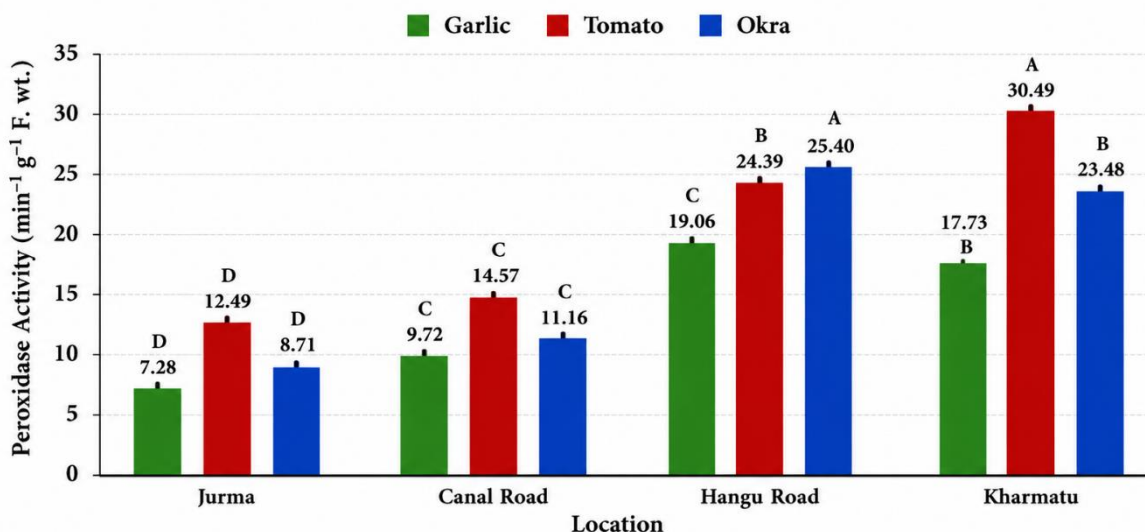
Peroxidase (POD) activity was found variable for garlic, tomato and okra samples collected from JR, CR, HR and KM. The activity of POD for garlic was found in the range of (7.28 to 19.06 min<sup>-1</sup> g<sup>-1</sup> F. wt.). The highest activity (19.06 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found collected from HR while lowest c activity (7.28 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for JR.

The content of POD was found for tomato samples collected from JR, CR, HR and KM in range of 12.49 to 30.49 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest concentration (30.49 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found collected from KM, while the lowest concentration (12.49 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for JR.

The activity of POD for okra samples collected from JR, CR, HR and KM was in the range of 8.71 to 25.40 min<sup>-1</sup> g<sup>-1</sup> F. wt. The highest activity (25.40 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found collected from KM,

while the lowest activity (8.71 min<sup>-1</sup> g<sup>-1</sup> F. wt.) was found for JR.

Usually, the plants encountered oxidative damage when exposed to lead and other heavy metals (Dixit *et al.*, 2001; Macfarlane, 2003; Erdei *et al.*, 2008). In plants, toxic heavy metals cause the production of oxidative stress by the generation of reactive oxygen group namely; hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH<sup>•</sup>) and superoxide radical (O<sup>2•-</sup>) (Devi and Prasad, 1998). ROS rapidly hit and cause damage to all type of bio-molecules including nucleic acids, proteins and lipids. In contrary, plants possess antioxidant enzymes, counting SOD, POD, APX and CAT to fight against oxidative damage under stressful situation (Li *et al.*, 2010).



Means followed by similar letters are statistically similar using LSD ( $P \leq 0.01$ )

LSD (1%) Garlic = 0.6145; LSD (1%) Tomato = 2.0494; LSD (1%) Okra = 1.4419

Fig 6. Peroxidase Activity ( $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) of garlic, tomato and okra collected from different locations.

#### Superoxide Dismutase Activity ( $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) of Garlic, Tomato and Okra

Superoxide dismutase (SOD) activity was found variable for garlic, tomato and okra samples collected from JR, CR, HR and KM. The activity of SOD for garlic was found in the range of 41.08 to 64.03  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$  The highest activity (64.03  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) was found collected from HR, while lowest activity (41.08  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) was found for JR.

The activity of SOD was found for tomato samples collected from JR, CR, HR and KM in range of 65.35 to 97.35  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$  The highest activity (97.35  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) was found collected from KM, while lowest activity (12.49  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) was found for JR. The activity of SOD for okra samples collected from JR, CR, HR and KM was in the range of 60.16 to 89.39  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$  The highest activity (89.39  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) was found collected from KM, while lowest activity (60.16  $\text{min}^{-1} \text{g}^{-1} \text{F. wt.}$ ) was found for CR.

Environmental stresses such as metal toxicity, drought, and chilling increase the activity of SOD. The plants have the ability to tolerate the oxidative components of these environmental stresses (Boguszewska *et al.*, 2010; Mhamdi *et al.*, 2010). Elevated contamination of heavy metals ions concentrations could also hinder photosynthetic features, collapse of protein synthesis and also disintegrate the anti-oxidative protecting of plants by affecting anti-oxidative enzymes such as SOD, CAT, APX and POD, thus, begin to decrease plant endurance or plant defiance to both abiotic and biotic stresses (Hao *et al.*, 2006). The highest SOD activity at the most polluted area indicates the plants are under stress and hence plant increase activity of SOD and other antioxidant enzyme to cope with that stress.

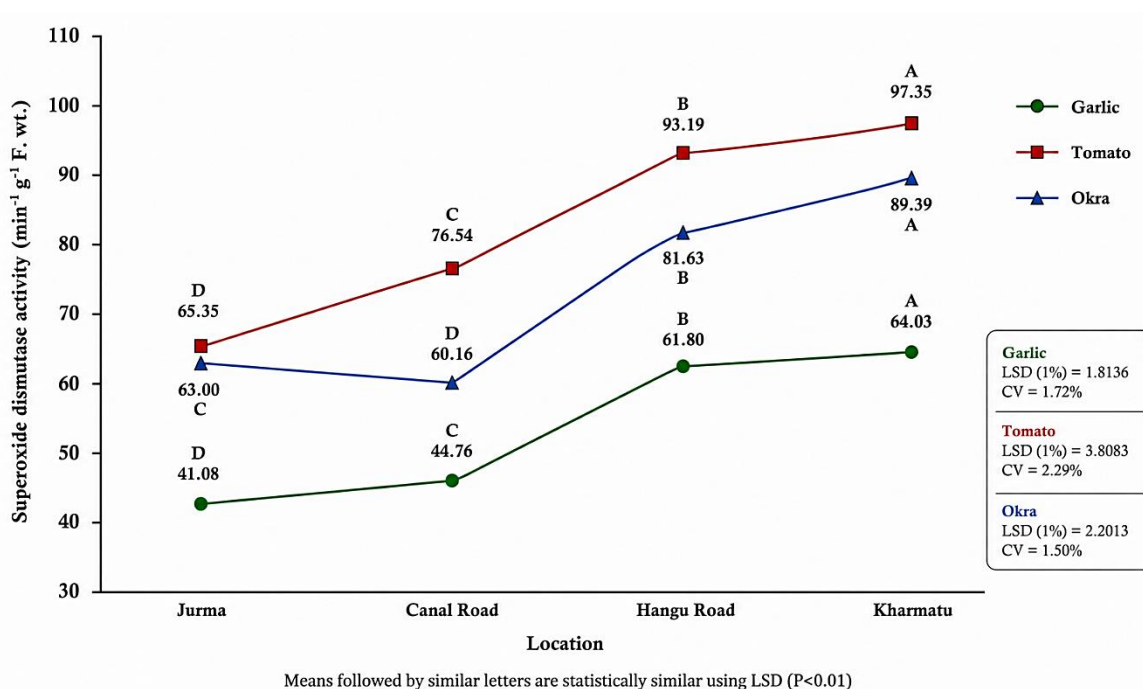


Fig 7. Superoxide dismutase Activity (min<sup>-1</sup> g<sup>-1</sup> F. wt.) of garlic, tomato and okra of different locations.

#### Fresh Weight (g) of Garlic, Tomato and Okra

Data shows that fresh weight of garlic samples ranged from 124 to 149 g with maximum weight (149 g) observed for garlic sample collected from JR area, while minimum weight (124 g) was recorded for samples collected from KM. Fresh weight of tomato samples showed a range of 640 to 721 g with maximum weight of 721 g for sample collected from JR area and minimum weight of 640 g was noted for samples collected from HR local of district Kohat. On the other hand, fresh weight of okra samples collected were in the range of 394 to 444 g with maximum weight noted for samples collected from JR area and minimum weight was noted for okra samples collected from KM. Statistical analysis of data showed that fresh weight of garlic, tomato and okra samples collected from different location were significantly (P<0.01) different from each other.

These results are in close proximity to those of Saeed and Ahmed, (2009) who investigated that

biomass is significantly declined by the use of 20 to 30 % polluted water to tomato plants. In wheat reduction in fresh weight was also perceived when irrigated by waste water (Kakar *et al.*, 2010). Reduction in growth and height of plants was realized in (*Leucaena leucocephala* L.) when irrigated with polluted or sewage water Hassan and Ali, (2013). The reduction in growth in the studied vegetables due to waste water treatments might be because of the accretion of heavy metals like Cd, Pb, and Ni in large quantities in the soil Scoccianti *et al.* (2006) and Bini *et al.* (2012), who stated that the negative impact of contaminated water on fresh weight of plants roots and shoots may perhaps be due to these metals which decrease the root water uptake and cause damage to root of plants which blocks nutrient uptake and finally resulted in constraining plant growth.

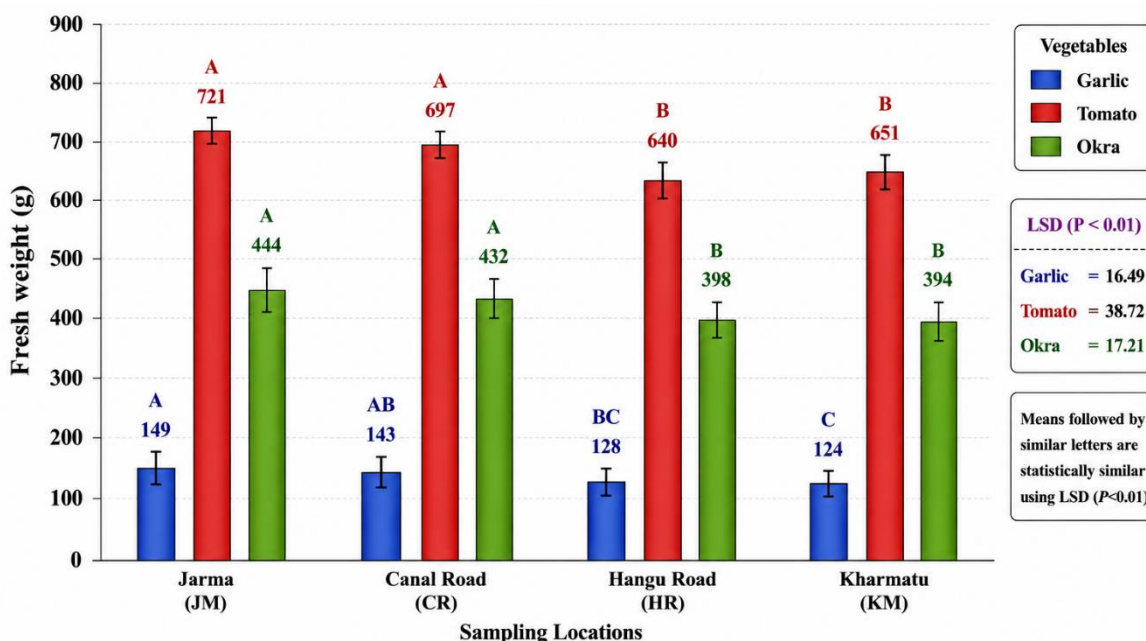


Fig 8. Fresh weight (g) of garlic, tomato and okra collected from different locations.

**Dry Weight (g) of garlic, Tomato and okra collected from different locations**

Data shows that dry weight of garlic samples ranged from 35 to 42 g with maximum weight (42 g) observed for garlic sample collected from JR area while minimum weight 35 g was recorded for sample collected from KM. Dry weight of tomato samples showed a range of 120 to 135 g with maximum weight of 135 g for sample collected from JR area and minimum weight of 120 g was noted for samples collected from HR of district Kohat. On the other hand, dry weight of okra samples collected were in the range of 83 to 94 g with maximum weight 94 g noted for samples collected from JR area and minimum weight 83 g was noted for okra samples collected from KM. Statistical analysis of data showed that dry weight of garlic, tomato and okra samples collected from different locations were significantly ( $P \geq 0.01$ ) different from each other.

Dry weight of such vegetables is significantly affected by different waste water treatments sites.

Maximum dry weight for garlic, tomato and okra were noticed at site JR, while lowest dry weight were pragmatic at KM for two vegetables except tomato whose lowest dry weight was noticed at HR. This might be due to the accumulation of toxic substances and metals in various parts of the plants, disturbing the physiological and biochemical activities in plant that ultimately bring reduction in the growth and biomass of the plants Sing and Agrwal, (2007). Solicitation of waste water reduced both fresh and dry weight of different vegetables like okra, tomato and pumpkin. These results are in close proximity to those of Saeed and Ahmed, (2009) who investigated that biomass is significantly declined by the use of 20 to 30 % polluted water to tomato plants. In wheat reduction in dry weight was also perceived when irrigated by waste water (Kakar *et al.*, 2010).

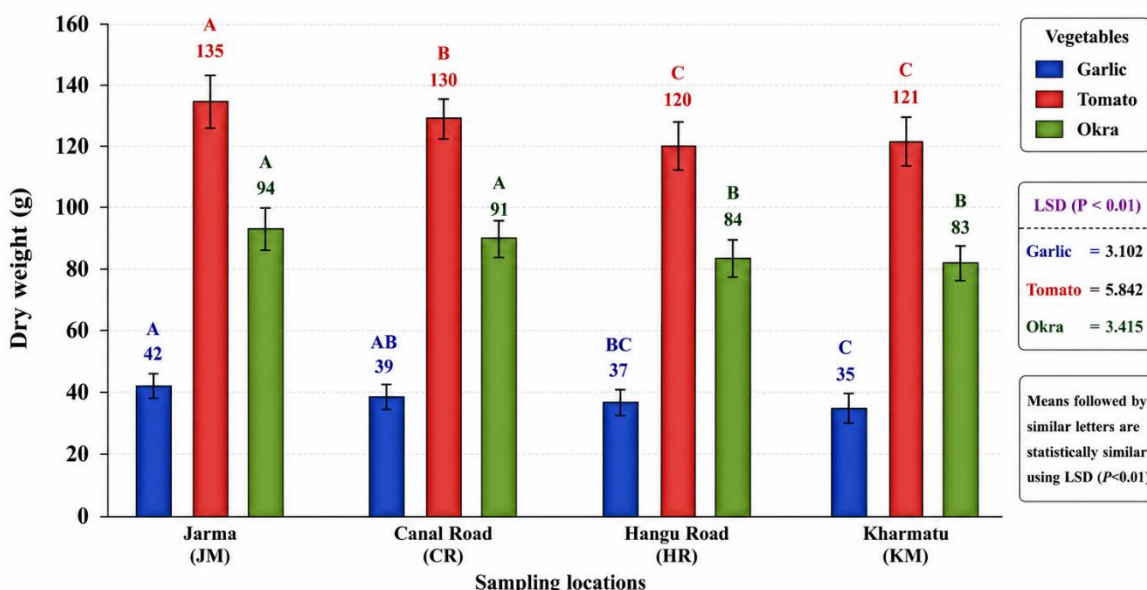


Fig 9. Dry weight of garlic, tomato and okra (g) collected from different locations.

**Root length (cm) of Garlic, Tomato and Okra**

Root length of garlic plants samples were ranging from a minimum of 7.20 cm in KM area to a maximum length 8.13 cm in JR area, while root length of garlic plants collected from CR and HR area were 7.93 cm and 7.40 cm, respectively. Similarly mean data table shows that the value for root length of tomato plants samples were within the range 20 cm to 25 cm, Maximum mean value 25 cm for root length of tomato plant was observed in samples collected from JR area, while minimum mean value 20 cm of root length were noted of both KM and HR plant samples. Result also shows that root length of okra plant samples was ranging from a minimum of 17 cm in KM site of district Kohat to a maximum of 22 cm in JR area, while root length of garlic plants samples from CR and HR area were 21 cm and 18 cm, respectively. Statistical analysis witnessed that root length of garlic, tomato and okra plants were significantly different in different sites of the study area.

Growth inhibition due to the metal toxicity is found to reduce biomass accumulation (Chaoui *et al.*, 1997; Quariti *et al.*, 1997). Excessive Cd accumulation in soil resulted into reduction in root growth causing reduced mineral absorption, altered carbohydrate metabolism leading to reduced fresh and dry matter production in palak Singh and Agrawal, (2007) and rice Moya *et al.* (1993). Peters *et al.* (2000) reported that most heavy metals are toxic to plants and that the first result of toxicity is reduction in plant growth, which later leads to reduction in yield due to resulting adverse effects on its reproductive capabilities. It is therefore reasonable to say that the level of these heavy metals may be responsible for the non-germination of seeds, flowering and fruiting of the okra and tomato irrigated with waste water containing higher concentrations of Cd, Pb, and Cr.

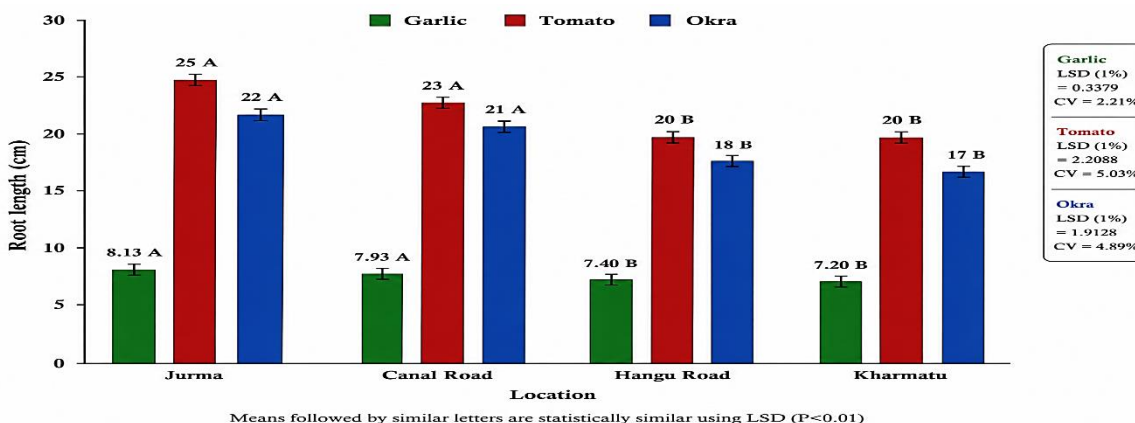


Fig 10. Root length (cm) of garlic, tomato and okra collected from different locations.  
Shoot length (cm) of Garlic, Tomato and Okra

Shoot length of garlic plants samples were ranging from a minimum of 53 cm in KM area to a maximum length 62 cm in JR area, while shoot length of garlic plants collected from CR and HR area were 60 cm and 54 cm respectively, Similarly the mean value for shoot length of tomato plants samples were within the range of 51 cm to 63 cm. Maximum mean value 63 cm for shoot length of tomato plant was observed in samples collected from JR area. Figure 11 also shows that shoot length of okra plant samples was ranging from a minimum of (67 cm) in KM site of district Kohat to a maximum of (75 cm) in JR area, while shoot length of garlic plants samples from CR and HR area were 73 cm and 68 cm, respectively. Statistical analysis witnessed that shoot length of garlic, tomato and okra plants were significantly different in different sites of the study area.

Peters *et al.* (2000) reported that most heavy metals are toxic to plants and that the first result of toxicity is reduction in plant growth, which later leads to reduction in yield due to adverse effects on its reproductive capabilities. It is therefore, reasonable to say that the level of these heavy metals may be responsible for the non-germination of seeds, flowering and fruiting of the okra and tomato irrigated with waste water containing higher concentrations of Cd, Pb, and Cr. Growth inhibition due to the metal toxicity is found to reduce biomass accumulation (Chaoui *et al.*, 1997; Quariti *et al.*, 1997). Excessive Cd accumulation in soil resulted into reduction in root growth causing reduced mineral absorption, altered carbohydrate metabolism leading to reduced fresh and dry matter production in palak Singh and Agrawal, (2007) and rice Moya *et al.* (1993).

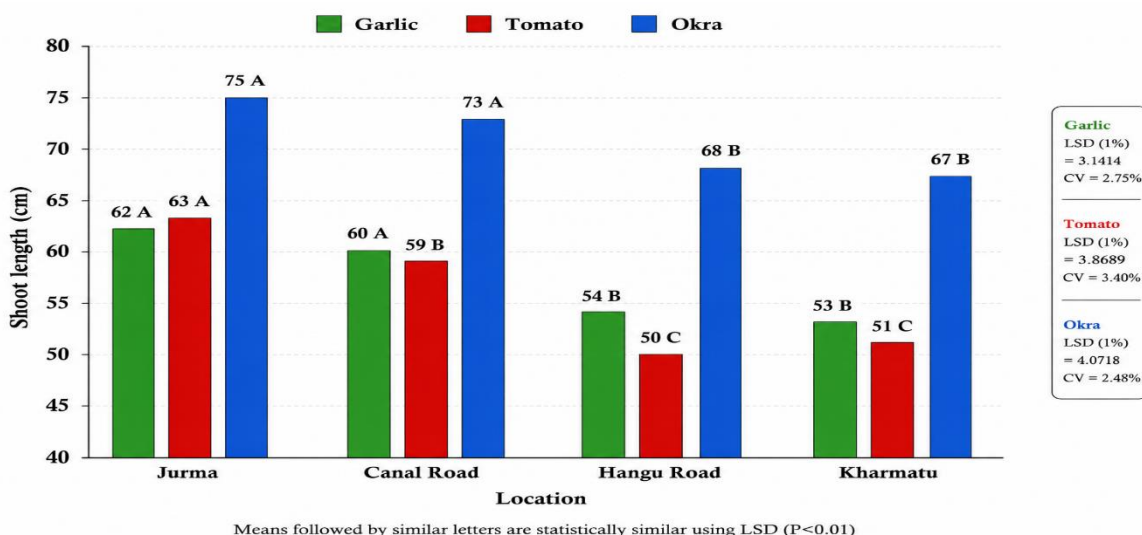


Fig 11. Shoot length (cm) of garlic, tomato and okra collected from different locations.

**CONCLUSION**

The results of the present study revealed that garlic, tomato, and okra samples collected from the wastewater-irrigated areas of Kharmatu and Hangu Road accumulated significantly higher concentrations of lead (Pb), cadmium (Cd) and chromium (Cr) compared to Jarma site. The levels of these heavy metals exceeded the maximum permissible limits recommended by WHO/FAO, indicating potential environmental and food safety concerns. Furthermore, elevated heavy metal accumulation adversely affected the morphological, physiological and biochemical characteristics of the studied vegetables, resulting in reduced growth performance and altered antioxidant enzyme activities. These findings suggest that prolonged irrigation with untreated wastewater poses a serious threat to crop quality, agricultural sustainability and human health.

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