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# RISK ASSESSMENT OF TRACE METALS DEPOSITION AND GROWTH OF *Abelmochus esculentus* L. on INDUSTRIALLY POLLUTED SOILS OF FAISALABAD, PAKISTAN

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Vegetables grown in metal-contaminated soil are a potential hazard to human live. Trace metals are introduced into grown vegetables through irrigation of unsanity, industrially polluted water in the field. A soil clean-up analysis of seven industrially polluted sites of Faisalabad district to determine trace metal accumulation in okra. The experiment was based on a completely randomized design where soil from each sample site was placed in a small lysimeter with variation in water capacity (75, 100, and 125% field capacity) with three replicates. After 45 days of germination, the okra plants were harvested and further divided into root and shoot components. The results showed that soil from different locations in Faisalabad and different levels of available water capacity (AWC) significantly affected the root morphological characteristics and total fresh biomass of okra. Contamination of soil with trace metals significantly affected the physical properties of soil and thus resulted to minimization of root morphology and fresh and dry biomass of okra. In the current study, soils irrigated with industrially contaminated with chromium 0.63-2.53 ppm, lead 0.008-0.6 ppm, zinc 3.16-12.24 ppm and cadmium 0.007-1.65 ppm, which pose a potential risk to the health of domestic people. The results indicated a potential human exposure mechanism for gradual poisoning by trace metals related to indirect use of crops grown on soils contaminated with heavy metals and irrigated by contaminated water sources. The current study concluded that Faisalabad industrial wastes have been identified as the source of trace metal contamination in the surrounding agricultural soils of Faisalabad.

**Keyword:** Heavy metals deposition, industrial soil, okra, health risk, assessments.

## INTRODUCTION

*Abelmochus esculentus* L. (okra) is the most important perishable vegetable crop in Pakistan with an area under cultivation of 1.05-2.21 hectares and annual production of 1.06-2.86 tonnes (Hafeez *et al.,* 2020). The major export markets of okra include Japan, the United States, and Europe, exporting about 320.3 million tonnes annually around the world (Balkhair and Ashraf, 2016). Deep frying is a common method used for consumption of okra (Khan and Rab, 2019). Blanching and boiling are other common techniques for cooking okra (Arlai *et al.,* 2010). The difficulties in maintaining and adapting okra production are controlled by proper management of nutrients (Ullah *et al.,* 2012). The optimum plant nutrients used to maintain the desired production level of the crop depend on the supplementation of synthetic inorganic fertilizers with inoculation of bacteria and organic manure to improve okra productivity and maintain soil fertility (Tasrina *et al.,* 2015; Iqbal *et al.,* 2016). The conservative stimulants and organic growth regulators can accelerate the development and growth of perishable products (Karak *et al.,* 2011).

Due to industrialization, the quality and nutrients value of sewage soil has become a challenging threat (Farooq *et al.,* 2008; Haider *et al.,* 2021). The industrialization by-products comprises of smelting, mining, trace metals, pesticide residues, synthetic fertilizers, fossil fuel fire, and disposal of industrial and urban waste that significantly contaminated the ecosystem and agricultural soils (Haroon *et al.,* 2019; Rahman *et al.,* 2019). Trace metals are not produced naturally, such as metals aluminum (Al), cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), arsenic (As), mercury (Hg), iron (Fe and zinc (Zn) (Haider *et al.,* 2021). The release of trace metals into the environment may be accidental, systematic, and well-controlled, such as industrial emissions and essentially inevitable (spills/ chemicals), (Dar *et al.,* 2019). Bioavailability and the capacity for trace metal biomagnifications and bioaccumulation are large in all food web components (Croteau *et al.,* 2005), and dietary intake is the primary source for the induction of trace metals into the food web (Farooq *et al.,* 2008; Olafisoye *et al*., 2020). The toxic pollutants will cycle in between the plants and soils due to the application of various water-enriched soil pollutants to the soil (Tasrina *et al.,* 2015). Hence toxic pollutants enter the food chain and bring changes in the health of animals and human beings (Najam *et al.*, 2015; Rahman *et al.*, 2019). The variation in pH of soil contaminated with trace metals varies to less acidic or neutral depends on industrial waste (Balkhair and Ashraf, 2016). The trace metal mobilization in pollutant sites may relate or not to related to various environmental conditions (Noureen *et al.*, 2015). Moreover, the analysis of total trace metals from heavy pollutant/contaminated soil are very difficult due to complex system contamination of trace-metal (Alker *et al.*, 2000; Haroon *et al.,* 2019). Soil treated with industrialization wastewater is a good source of organic matter and some other nutrients i.e., nitrogen and phosphorous, however, there is a serious issue about the contamination of potential trace metals i.e., Cd, Pb, Cr, As, and Cu, etc. (Balkhair and Ashraf, 2016). Trace metals can also accumulate at harmful levels in the soil during long-termed application of industry wastewater (Haider *et al.*, 2021). Vegetables cultivated in trace metals accumulated soil are usually enriched with trace metals which are serious hazards to human health and the food web (Hamid *et al.*, 2017; Rehman *et al.*, 2020). Yet, the negative effects of Faisalabad industry wastewater on the surrounding agricultural soils and the crop are sketchy. Therefore the current was conducted to assess the accumulation of trace metals and the consequent responses of okra plants cultivated in soil irrigated with industrial wastewater. In this study, we hypothesized that soil contaminated with a higher concentration of trace metals has an adverse effect on the morphological traits and overall biomass of okra.

## MATERIALS AND METHODS

A lysimeter experiment was conducted under closed glasshouse conditions at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad during the month of March-April, 2016. Twenty-one treatments were arranged in the completely randomized design with three replications. The complete design of treatments plan is mentioned in Table 1.

***Collection and preparation of lysimeter experiment*:** A lysimeter consists of 0.30 m long and about 1.22 m wide and it looks like a tube shape. The lysimeter bottom contains holes for aeration and it filled with cotton to avoid discharge of water as well as filled with one-kilogram contaminated soil in each tube from seven different sites of the Faisalabad region (Figure 1) and irrigated with three water levels to check the available water capacity of these contaminated soils included (75%, 100%, and 125% AWC). The experiment was conducted from last week of February 2016 to first week of April 2016 under optimum weather condition for maximum plant growth. The okra variety (sabzperi) has been collected from Ayub Research. It was placed under a closed glasshouse condition especially in a used closed environment because okra required optimum temperature for germination i.e., 25 °C (Khan and Rab, 2019).

**Table 1. Treatment plan for lysimeter experiment of okra.**

| Sr. | Treatment Names | Sr. | Treatment Names |
|---|---|---|---|
| $T_0$ | Control Treatment | $T_{11}$ | Chak 228 JB soil + 100% AWC |
| $T_1$ | Chak 123 JB soil + 75% AWC | $T_{12}$ | Chak 228 JB soil + 125% AWC |
| $T_2$ | Chak 123 JB soil + 100% AWC | $T_{13}$ | Chak 224 JB soil + 75% AWC |
| $T_3$ | Chak 123 JB soil + 125% AWC | $T_{14}$ | Chak 224 JB soil + 100% AWC |
| $T_4$ | Rasool Nagar soil + 75% AWC | $T_{15}$ | Chak 224 JB soil + 125% AWC |
| $T_5$ | Rasool Nagar soil + 100% AWC | $T_{16}$ | Chak 100 JB soil + 75% AWC |
| $T_6$ | Rasool Nagar soil + 125% AWC | $T_{17}$ | Chak 100 JB soil + 100% AWC |
| $T_7$ | Thaliyawala soil + 75% AWC | $T_{18}$ | Chak 100 JB soil + 125% AWC |
| $T_8$ | Thaliyawala soil + 100% AWC | $T_{19}$ | Chakira Soil + 75% AWC |
| $T_9$ | Thaliyawala soil + 125% AWC | $T_{20}$ | Chakira Soil + 100% AWC |
| $T_{10}$ | Chak 228 JB soil + 75% AWC | $T_{21}$ | Chakira Soil + 125% AWC |

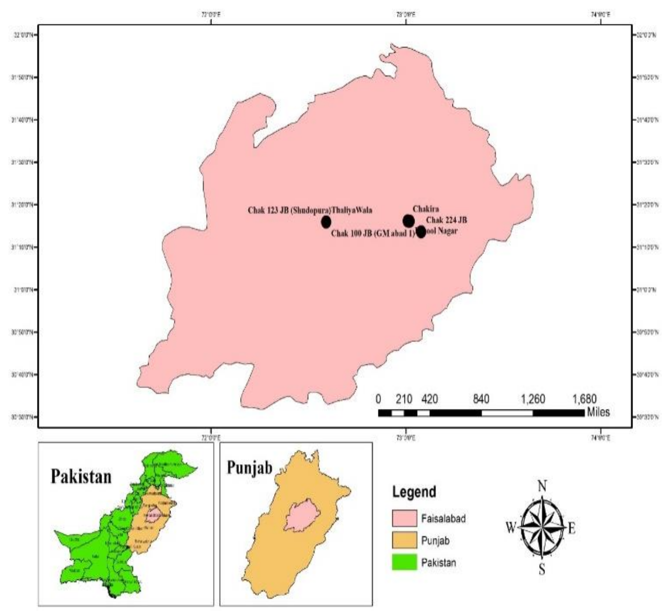


**Figure 1. Sampling Sites in of the study.**

***Root and Shoot parameter analysis*:** Root parameters which include its length, area, diameter, volume and length density were measured by the root synapse software (formula of root volume = root length × root area) and (formula of root length density = root length / root volume). The shoot length was measured by meter rod, shoot fresh and dry weight by electrical balance available in the lab.

***Soil analysis***

***Field capacity (FC) and permanent wilting point:*** Pressure membrane apparatus was used to assess, the soil samples' field capacity, according to the followed by Dane *et al.*, (2002). To extract the water from soil 0.3, 0.6, and 0.9 bar pressure, were inserted by a high pressure chamber on soil placed on ceramic plates. After that sample was weighed and placed in the oven overnight at 105°C and again oven-dried samples were placed at 0.3, 0.6, and 0.9 bar pressure and observed the weight difference before and after the drying of soil in the oven. After weighing the samples were again were pressed at 1.2 and 1.5 bar pressure and again observed the difference in weight of soil before and after the drying in the oven to represents moisture level in the soil at permanent wilting point (PWP).

***Available Water Capacity (AWC)*:** Available water capacity reflects the amount of water distributed and stored in the rhizosphere of soil for the utilization of plant and recorded by computing the difference between water at field capacity and soil water content of permanent wilting point (PWP)

AWC= soil water at field capacity (FC) – soil water content at (PWP)

***pH and EC*:** The pH of soil was measured in the following ways firstly made two buffered solutionsof pH (4 and 7) then weigh 50 g air-dry soil (< 2 mm) into 100 ml flask then added 50 ml distilled water and mixed well by using a glass rod and allowed to stay for 30 minutes. After a one-hour stirring put the electrode into solution above the beaker which has a solution surface and reading was recorded by using pH meter (Andrew and Franson, 2005).

Electrical Conductivity (EC) of the soil samples were measured through following procedure firstly prepared 1:1 (soil:water) suspension then filtered the suspension by using suction with the help of Whatman No. 42 filter paper. Opened the suction and added suction to funnel then transferred to clean filter paper into a 50 ml flask to immerse the conductivity cell in the solution and took the reading and removed the conductivity cell from the filtrate rinsed properly with distilled water (Andrew and Franson, 2005).

***Total Dissolved Solids (TDS)(mg $L^{-1}$):*** Total dissolved solids (TDS) was measured by a combined instrument named Hanna Instrument, Model 8519, Italy, which showed a combined analysis of the both TDS and pH both according to the procedure mentioned by (CPCB, 2007).

***Measurement of Chemical Parameters:***

***Nitrate contents (mg $kg^{-1}$):*** It was measured by the spectrophotometric method of (CPCB, 2007) by using chromo trophic acid. The reagents were used during analysis included copper sulfate solution ($CuSO_4 . 5H_2O$) was prepared by adding 2.5-grams copper sulfate in 1 liter DW and chromo tropic acid solution ($C_{10}H_6Na_2O_8S_2 .2H_2O$) was prepared by dissolved 0.368-gram chromo tropic acids in 200 ml concentrated sulfuric acid etc. Firstly weigh the 10 g air-dry soil and add 50 ml copper sulfate then shake it and filtered the solution then took 3 ml from filtered solution. Add 1 ml chromo tropic acid drop by drop and kept for cooling few minutes then add 5 ml $H_2SO_4$ again kept cooled at room temperature. Made six standards included 1, 2, 4, 8, 16, 32 ppm respectively, by dissolving 3.6092-gram potassium nitrate in 500 ml (stock solution) and diluted the 10 ml stock solution to 200 ml final volume by adding 0.02 N copper sulfate solution (sub stock solution) and draw the standard curve by calculating formula as below

The formula of $NO_3$-N (ppm) = ppm $NO_3$ – N (calibration curve) × $A/W_t$ ×10/V

Where, A = Total volume of the extract (mL), V = Volume of extract used for measurement (3 mL), $W_t$ = Weight of air-dry soil (g)

***Total Organic Carbon (TOC)*:** Soil organic matter was determined following the method described by Nelson and Sommers (1996). Weighed 0.5-gram soils in 250 ml flask then added 5 ml potassium dichromate with 10 ml concentrated sulfuric acid and allowed for cooling about 30 minutes. Added 100 ml distilled water with 5 ml concentrated Orthophosphoric acid again allowed cooling for a few minutes. Added 5-10 drops of indicator (diphenylamine) developed bluish color by using pipette finally titrated with potassium dichromate with the endpoint of greenish color. The formula used to determine TOC as below:

$$TOC = (V_B – V_S) \times 0.3 \times M / \text{weight of soil}$$

Where, M = Molarity of ferrous ammonium sulfate solution(approx. 0.5 M), $V_{blank}$ = Volume of ferrous ammonium sulfate solution required to titrate the blank (mL), $V_{sample}$ = Volume of ferrous ammonium sulfate solution required to titrate the sample (mL), $W_t$ = Weight of air-dry soil (g), 0.3 = 3 × $10^{-3}$ × 100, where 3 is the equivalent weight of C.

***Trace metals*:** The samples of trace metals i.e., Cd, Zn, Cr, and Pb were prepared under the principles followed by AOAC (1990) by using the atomic absorption spectrophotometer instrument model (Hitachi Polarized Zeeman AAS, Z-8200, Japan).

***Statistical analysis*:** The collected data was analyzed statistically the following software statistics 8.1 and comparisons of treatments means were recorded by using LSD (least significant difference) test followed by Steel *et al.* (1997).

## RESULTS AND DISCUSSION

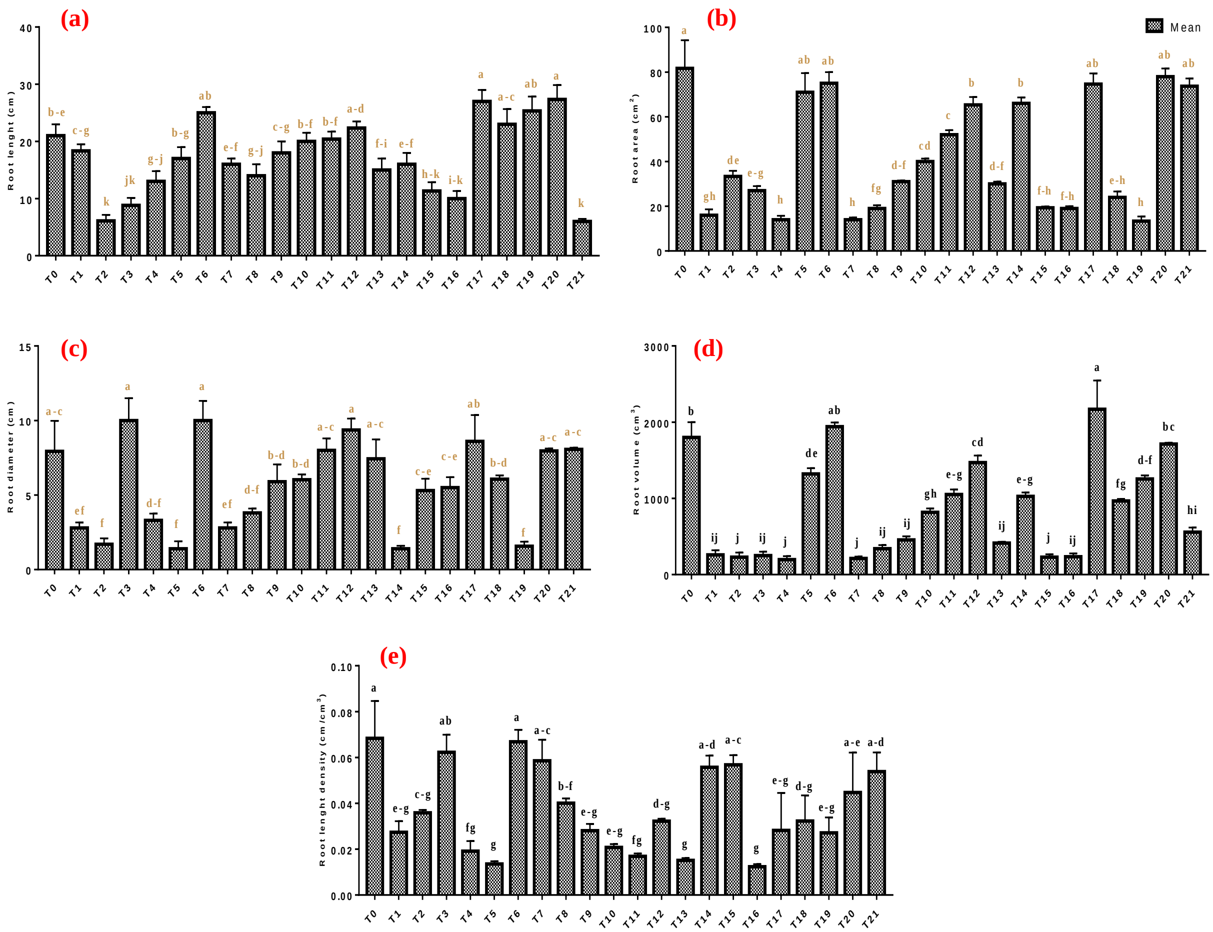


**Figure 2. Effect of contaminated soils and irrigation levels on root length (a), root area (b), root diameter (c), root density (d), and root length density (e) of okra. Error bars represents the standard deviation from the means. Sharing a figure with the same case letter is not significantly different at $p<0.05$%.**

***Root morphological traits*:** The results indicated that soil from various Faisalabad sites and different levels of available water capacity (AWC) significantly influenced root length, root area, root diameter, root volume, and root length density of okra than control (Figure 2). The highest root length of okra was recorded in ($T_{20}$) Chakira soil with 100% AWC by 30.14% than the control that was statistically at par with ($T_{17}$) Chak 100 JB soil with 100% AWC by 28.57% than control and least root length was observed in ($T_{21}$) Chakira soil with 125% AWC having mean value 6 cm that was 71.42% minimum compared to ($T_0$) control i.e., (21 cm) (Figure 2.a).

Correspondingly data regarding root area of okra, highest root area was observed in control i.e., (81.67 $cm^2$) that was statistically followed by ($T_{20}$) Chakira soil with 100% AWC with a mean value of (78.00 $cm^2$) and least root area i.e., (13.33 $cm^2$) was recorded in ($T_{19}$) Chakira soil with 75% AWC (Figure 2.b). Concerning the root diameter of okra maximum root diameter was recorded in ($T_3$) Chak 123 JB soil with 125% AWC and ($T_6$) Rasool Nagar soil with 125% AWC by 26.58% than the control and least root diameter was documented in ($T_5$) Rasool Nagar soil with 100% AWC and ($T_{14}$) Chak 224JB soil with 100% AWC with a mean value of 1.4 cm that was by 82.27% minimum than the control (Figure 2.c). Similarly, maximum root volume was recorded in ($T_{17}$) Chak 100 JB soil with 100% AWC by 20.5% than the control and least was recorded in ($T_7$) Thaliyawala soil with 75% AWC with a mean of 212 $cm^3$ that was 88.22% lower than the control (Figure 2.d). The highest root length density was documented in control i.e., (0.068 $cm/cm^3$) that was statistically at par with ($T_6$) Rasool Nagar soil with 125% AWC with a mean value of (0.067 $cm/cm^3$) and lowered root length density was recorded in ($T_{16}$) Chak 100 JB soil with 75% AWC with a mean value of (0.012 $cm/cm^3$) that was 82.35% than the control (Figure 2.e).

The Faisalabad is an industrial city and nearby villages of Faisalabad are irrigated by industrial polluted water (Najam

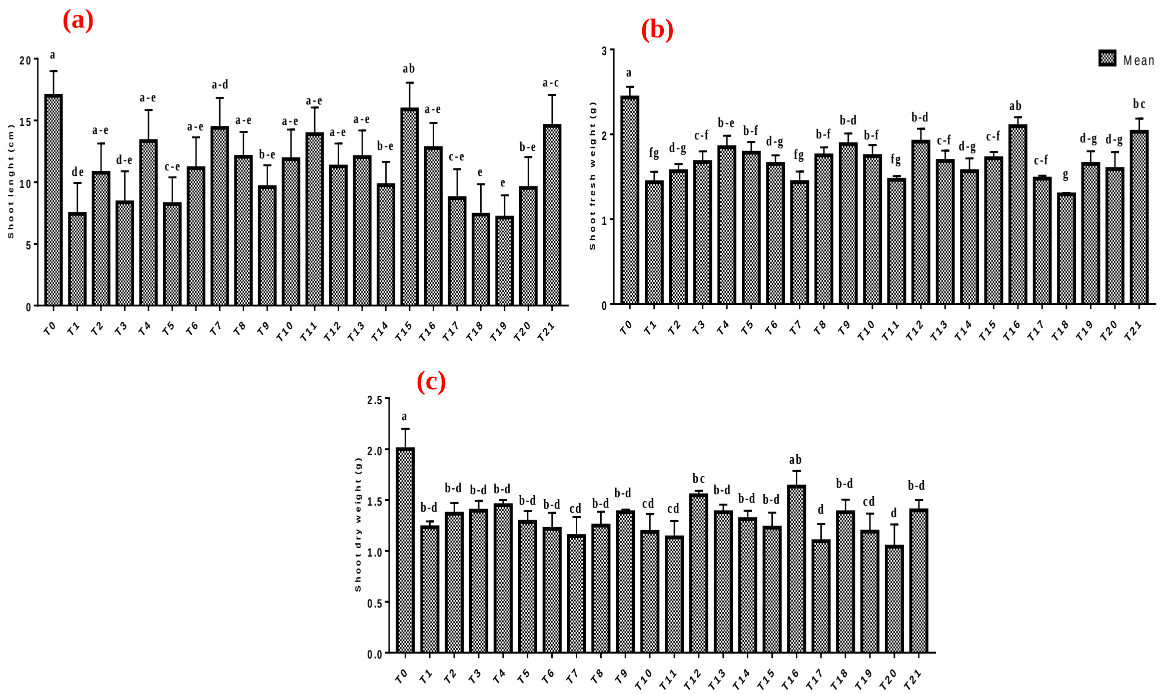


**Figure 3. Effect of contaminated soils and irrigation levels on shoot length (a), shoot fresh biomass (b), and shoot dry biomass(c) of okra. Error bars represents the standard deviation from the means. Sharing a figure with the same case letter is not significantly different at $p<0.05\%$.**

*et al.*, 2015; Hamid *et al.*, 2017). Contamination of trace metals in soil results to minimized crop growth (Balkhair and Ashraf, 2016). Soil having less toxic trace-metal in soil results to enhance the plant root length and root area for proper absorption of water and minerals (Ullah *et al.*, 2012), as it was recorded in Chakira soil and Chak 100 JB soil. Moreover, the higher available water capacity in the root zone of okra plants having tap root system results to minimize the root length of the plant due to anaerobic condition and insufficient supply of oxygen in soil (Farooq *et al.*, 2008), as it was observed in an experiment that available water capacity more 125% in Chakira soil results to minimized the root length of okra than 75% and 100% AWC respectively. Optimum nutrient management results to improve the root area of okra (Balkhair and Ashraf, 2016). The control treatment was stuffed with optimum nutrient and available water contents that results to record maximum root area and root length density of okra than the remaining irrigated Faisalabad soils.

***Plant shoot length, fresh and dry weight*****:** The results indicated that soil from various Faisalabad sites and different levels of available water capacity (AWC) significantly influenced shoot length, shoot fresh, and dry of okra (Figure 3). The highest shoot length of okra was recorded in control with the mean value of (17 cm) and least was recorded in ($T_{19}$) Chakira soil with 75% AWC that was 58.06% minimum than the control (Figure 3.a). Correspondingly, the maximum fresh weight of okra was recorded in control with the mean value of (2.43 g) and the minimum was observed in ($T_{18}$) Chak 100 JB soil with 125% AWC that was 46.91% least than control (Figure 3.b). Similarly, the highest dry weight of okra was documented in control with the mean value of (2 g) and least was recorded in ($T_{20}$) Chakira soil with 100% AWC that was 48.00% than the control as shown in (Figure 3.c). The growth and development of okra are best under sandy loam soil enriched with organic matter (Iqbal *et al.*, 2016; Vongdala *et al.*, 2019). Moreover, the plant has more root area and root length density help to absorb maximum nutrients and water for proper growth of the plant that results in increased the shoot length dry and fresh weight of okra as recorded in control than the remaining collected soils (Ullah *et al.*, 2012; Hamid *et al.*, 2013). The remaining collected soils are irrigated with industrially contaminated water that are spiked with trace metals and results in minimized the plant shoot length, dry, and fresh weight of vegetables.

***Soil properties*****:** The experiment results indicate that soil from various Faisalabad sites and different levels of available water capacity (AWC) significantly influenced the pH, electrical conductivity, water retention, total organic carbon%, nitrate, and total dissolved solid of soil (Figure 4). Correspondingly, high pH of the soil was recorded in ($T_{14}$)

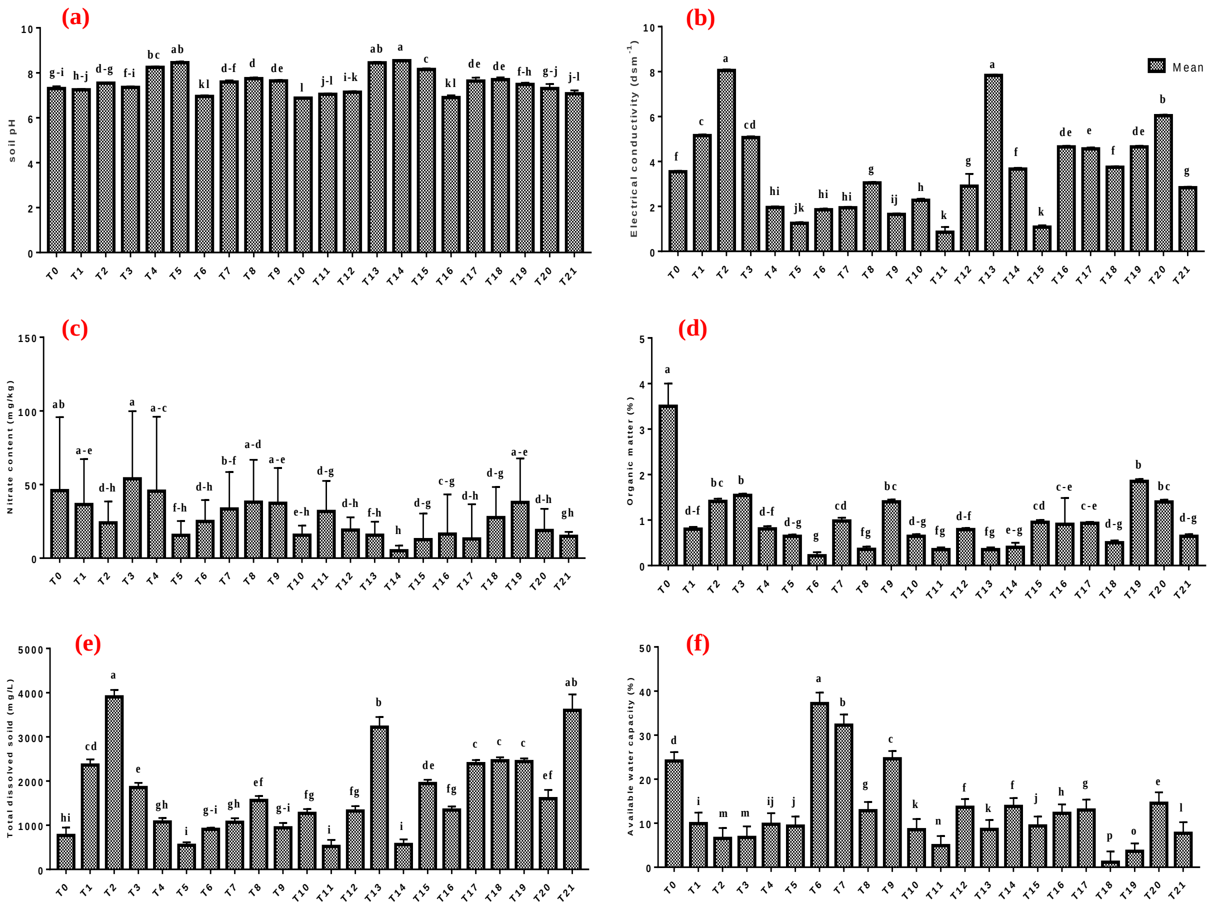


**Figure 4. Effect of contaminated soils and irrigation levels on soil pH (a), electrical conductivity (b), soil organic matter (c), soil nitrate content (d), total dissolved soilds (e), and available water capacity (f) of different okra cultivated soil. Error bars represents the standard deviation from the means. Sharing a figure with the same case letter is not significantly different at p<0.05%.**

Chak 224 JB soil with 100% AWC with a mean value of (8.53) that was 16.85 was high than control, that was statistically followed by ($T_{13}$) Chak 224 JB soil with 75% AWC and least was observed in ($T_{10}$) Chak 228 JB soil with 75% AWC by 5.89% less than the control (Figure 4.a). The higher electrical conductivity of soil was documented in ($T_2$) Chak 123 JB soil with 100% AWC with a mean value of 8.05 $dSm^{-1}$ that was statistically followed by Chak 224 JB soil with 75% AWC with a mean value of 7.83 $dSm^{-1}$ and least was observed in ($T_{11}$) Chak 228 JB soil with 100% AWC with a mean value of 0.85 $dSm^{-1}$ (Figure 4.b). Similarly, the nitrate content in soil was high in ($T_3$) Chak 123 JB soil with 125% AWC by 17.44% higher than the control and low nitrate content was observed in ($T_{14}$) Chak 224 JB soil with 100% AWC by 88.92% lower than control (Figure 4.c). Similarly, higher total organic carbon % was observed in control with the mean value of 3.5%, and low total organic carbon% was observed in ($T_6$) Rasool Nagar soil with 125% AWC with the mean value of 0.22% (Figure 4.d). The total dissolved solid was highly recorded in ($T_2$) Chak 123 JB soil with 100% AWC with the mean value of 3905.67 mg/L and least was observed in ($T_{11}$) Chak 228 JB soil with 100% AWC with the mean value of 520 mg/L (Figure 4.e). Soil samples from collection sites are alkaline and it is not recognized as an acceptable criterion due to varied trends of buffed soil (Balkhair and Ashraf, 2016). Similarly, EC of collected soil revealed that soils are not contaminated with salinity (Iqbal *et al.*, 2016). The application of industrial waste and sewage water in different sites varied that results to bring changes in soil pH, EC, water retention, total organic carbon %, nitrate, and total dissolved solids of soil (Haroon *et al.*, 2019), as compared with control.

***Heavy metal contents in okra*:** The results of the experiment indicate that soil from various Faisalabad sites and different levels of available water capacity (AWC) significantly influenced the heavy metal concentrations i.e., Cr, Zn, Pb, and Cd in okra than the control (Figure 5). The higher Cr

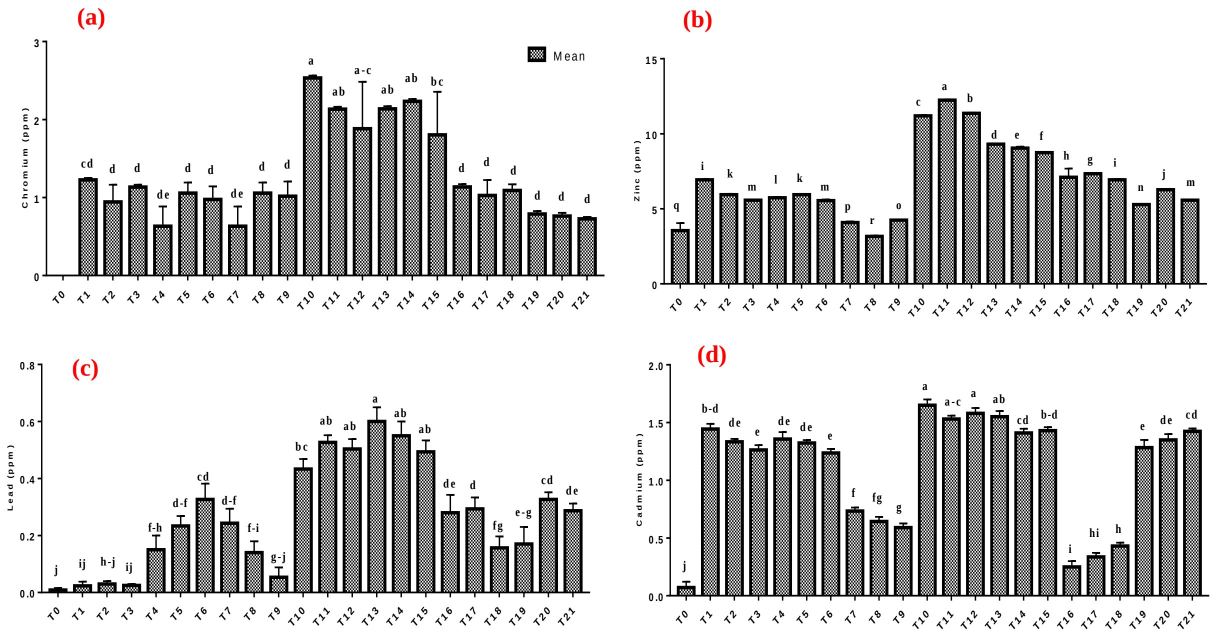


**Figure 5. Effect of contaminated soils and irrigation levels on concentration of chromium (Cr) (a), zinc (Zn) (b), lead (Pb) (c), and cadmium (Cd) (d) in okra shoot. Error bars represents the standard deviation from the means. Sharing a figure with the same case letter is not significantly different at p<0.05%.**

contents were recorded in ($T_{10}$) Chak 228 JB soil with 75% AWC with a mean value of 2.53 ppm and least was recorded in ($T_4$) Rasool Nagar soil with 75% AWC and ($T_7$) Thaliyawala soil with 75% AWC with a mean value 0.63 ppm while in control the Cr contents were zero (Figure 5.a). Maximum Zn concentration was documented in ($T_{11}$) Chak 228 JB soil with 100% AWC with the mean value of 12.25 ppm and least was observed in ($T_8$) Thaliyawala soil with 100% AWC with the a mean value of 3.16 ppm (Figure 5.b). Similarly, higher accumulation of Pb was documented in ($T_{13}$) Chak 224 JB soil with 75% AWC with the mean value of 0.6 ppm and least was documented in control with the a mean value of 0.008 ppm (Figure 5.c). The higher Cd contents were recorded in ($T_{10}$) Chak 228 JB soil with 75% AWC with the mean value of 1.65 ppm and low Cd was recorded in control with the a mean value of 0.071 ppm (Figure 5.d). Various studies reported that soil near the industrial zones is polluted with trace metals (Tasrina *et al.,* 2015). The concentration of trace metals in soil increased with the longer application of industrial and sewage wastewater in nearby villages of an industrial zone (Najam *et al.,* 2015). These trace metals are adsorbing by the plants and become part of the food chain of human beings and animals (Farooq *et al.,* 2008; Ullah *et al.,* 2012).

The accumulation of trace metals in soil is less than the accumulation of trace metals in plants. Furthermore, to the above mentioned supply of trace metals in the soil, the addition of synthetic phosphate fertilizers is a dominant cultural practice and considered an important foundation of Cd accumulation in the agricultural soil (Balkhair and Ashraf, 2016). Cadmium is present as an impurity in rocks phosphate and transferred to the plants due to high mobility in the plants' roots (Nakishore, 2014; Haider *et al.*, 2021). The mobility of trace metals from the soil to the plants depends on the vegetable species and the chemical and physical features of soil (Noureen *et al.,* 2015; Moryani *et al.,* 2020). The higher concentration of trace metals in soils is due to the application of sludge, agrochemicals, combustion of solid waste, exhaustion of vehicles, and industrial wastewater irrigation (Tasrina *et al.,* 2015; Haroon *et al.*, 2019).

***Conclusion*****:** Due to limited water resources, the use of wastewater for irrigation has gained importance worldwide. Faisalabad city is considered as Manchester of Pakistan due to heavy industrialization and the current study revealed that the major source of soil contamination is untreated waste and chemical effluents and irrigation with polluted wastewater enriched with trace metals contributes to increase in concentration of metals in soil and vegetables grown with polluted water. In vegetables, metal concentrations will provide the evidence, and extensive sampling is needed across the country to measure the results. To prevent pollutants from entering the food chain, urban and agricultural pollution should not be discharged uncontrolled into waterways and farmland. Alternative methods of cleaning up already polluted substrates are needed when dealing with wastewater that reaches the fields. Continuous monitoring of the quality of soil, water and plants and the prevention of metals entering the food supply are prerequisites for avoiding possible hazards to human health.

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