Accumulation and Translocation of Eight Trace Metals by the Different Tissues of Abelmoschus Esculentus Moench. Irrigated With Untreated Wastewater

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Abstract

Usage of wastewater to irrigate crops increases in Egypt and the whole world as a result of water shortage. This study is conducted to explore the potential of *Abelmoschus esculentus* Moench. (Okra plant) to accumulate and translocate eight trace metals: lead: Pb, cadmium: Cd, chromium: Cr, copper: Cu, iron: Fe, manganese: Mn, nickel: Ni and zinc: Zn in its different tissues due to irrigation with untreated industrial wastewater. It extended to assess the effect of the irrigation with wastewater on the growth parameters, nutrients, pigments and organic contents of the cultivated okra plants. Two studied sites at South of Cairo was conducted, the first site (29°42'31.17" N and 31°15'11.56" E); represented by five cultivated fields irrigated with Nile water (control) and the second site (29°42'37.87" N and 31°17'14.53" E); fields irrigated with effluent receive untreated industrial wastewater. Three composite soil and irrigated water samples were collected from each site. A significant decrease in nutrients: nitrogen (N), Potassium (K) and Phosphorous (P) in soil and Plants were resulted due to irrigation with wastewater. Also, there was a significant increase in trace metals concentration in both soil and plants irrigated with wastewater. A significant decrement in okra growth parameters and leaves photosynthetic pigments (chlorophyll a and b and carotenoids) due to irrigation with wastewater. Iron was the highest accumulated metal in the plant's fruits (edible part) irrigated with wastewater. Also, the concentration of Cd, Cu, Fe, Mn, Ni and Zn (42.57, 140,67, 2756.67, 1293.33, 1326.67 and 877.83 mg kg^{-1}, respectively) was in the phytotoxic range. Wastewater irrigated okras accumulate all of the studied trace metals in their roots (Bioaccumulation factor: BF > 1). In contrast, okra plants had no accumulated trace metals strategy in their shoot, as translocation factor values were less than one. Authors recommended avoiding consuming okra plants cultivated in fields irrigated with wastewater due to high trace metals concentration in their edible part.

1. Introduction

Food production increases continuously to meet the demand of increasing populations, which creates a severe challenge to the agricultural community to increase food production by more than 70% (Kannan and Anandhi 2020). Sustainable food production required sustainable resource availability, such as water and energy. The utilization of untreated wastewater in agricultural irrigation is prevailing globally, especially in developing countries, due to water scarcity (Malakar et al. 2019). Low wastewater quality was the source of polluted food crops and led to soil and freshwater contamination. Using industrial, municipal, or agricultural contaminated wastewater in crop irrigation changes the soil quality and hence, trace metals concentration in soil and crops (Malakar et al. 2019).

The industrial wastes and sewage water are drained to agricultural fields where they are used to irrigate different vegetable crops. These industrial sewage effluents are considered the main source of nutrients and organic matter, but they are also a source of many trace metals (e.g. Cu, Cr, Cd, Co, Fe, Mn, Ni and Zn), which accumulate in the soil, then transfer to plants growing in these polluted soils (Balkhair and Ashraf 2016). Different crop plants have various capacities to remove and accumulate trace metals in their tissue parts (Slima and Ahmed 2020). Due to the continuous application of wastewater in agricultural irrigation, the accumulated trace metals increase to toxic levels in soil and enter the food chain due to uptake and accumulation of these trace metals by growing crops (Ahmed and Slima 2018). The accumulation of trace metals in plants may affect the quality of vegetable crops grown on polluted soils, and the accumulation of these metals in vegetable crops can cause harmful effects on local inhabitants health (Dan et al. 2017). The pathway of trace metals entrance into the food web is the uptake and accumulation of these metals by crops grown in polluted soils (Christou et al. 2017).

Vegetables are one of the essential components of human nutrition. They are a source of carbohydrates, proteins, vitamins, minerals and fibers. Trace metals polluted vegetables are due to using untreated industrial wastewater and chemical and organic substances to enhance plant growth (Khan et al. 2019). Okra or lady's finger (*Abelmoschus esculentus* Moench.) is a fruit crop. It belongs to the family Malvaceae, which is used for its edible immature fruit. Other parts of Okra can also be used, including stems, leaves, flowers, and seeds (Petropoulos et al. 2017). *A. esculentus* Moench. is an economically important vegetable crop in tropical and sub-tropical parts of the world. Its centers of origin are North Asia and East Africa. Okra seeds are used for oil production and non-caffeinated substitute for coffee (Petropoulos et al. 2017). Okra plant contains a high content of fibers, which helps in stabilization of blood sugar (Ngoc et al. 2008), and it treats digestive disorders and colon health (Messing et al. 2014), promotes healthy pregnancy (Zaharuddin et al. 2014), improves heart health and controls body cholesterol level (Gemede et al. 2015). In

Egypt, Okra is cultivated for its immature fruit as human food. The total area cultivated is about 17 thousand feddans, with an average production of 88.8 tonnes (Ministry of Agriculture and Land Reclamation 2019).

Accumulation of heavy metal in plants depends on plant species and the different plant efficacy in metals absorption. The absorption ability was assessed by transfer factors (i.e. bioaccumulation and translocation factor) (Eltaher et al. 2019). The bioaccumulation ability of heavy metals in the underground organs acts as a self-protective mechanism to protect plant shoots from harmful toxic trace metals (Ahmed et al. 2021). Accumulation of trace metals in plants' edible parts resulted from crop cultivation on polluted soil, leading to dangerous risks to human and animal health (Shehata and Galal 2020). Hence, the present study aims to evaluate the potential of *Abelmoschus esculentus* Moench. (Okra plant) to accumulate and translocate eight trace metals (Pb, Cd, Cu, Cr, Fe, Mn, Ni and Zn) in its different tissues due to irrigation with untreated industrial wastewater.

2. Material And Methods

2.1. Soil sampling and analysis:

The soil samples that support the growth of okra were collected from the agriculture fields at south cairo in summer 2018. From El-Attef city (29°42'31.17" N and 31°15'11.56" E) as control (filed irrigated with Nile water and Ekhsas Gharb city (29°42'37.87" N and 31°17'14.53" E) as treatment (field irrigated with effluent receives untreated industrial wastewater) at a depth of 0-50 cm below the soil surface. The soil samples were air-dried and sieved by 2-mm sieve to remove gravel and plant debris. The soil water extract (1:5 w/v) was prepared to determine soil reaction using pH meter (Model 9107 BNORION type) according to **Brower and Zar (1984)** and electrical conductivity (EC) as an indicator for soil salinity using an electrical conductivity meter (60 Sensor Operating Instruction Corning) according to **Roweel (1994)**. Soil was digested using the acid digestion method adopted by **Wade et al. (1993)** to determine the soil nutrients (N, P and K) and eight trace metals (Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn). The total soluble nitrogen (N) was determined according to **Piper (1947)** using the Kjeldahl method,the molybdenum blue method was used to determine P content in soil samples using a spectrophotometer at 660 nm in case of N and 700 nm in case of P (UNICO Vis Model 1200, USA). At the same time, the potassium (K) content was measured at the Flame Photometer (CORNING M410). All the mentioned procedures were according to **Allen (1989)**. The concentration of the investigated trace metals were determined according to **APHA (1999)** using a Perkin-Elmer 3100 Atomic Absorption Spectrophotometer.

2.2. Water Sampling and analysis:

Three composite water samples were collected from both the River Nile surface (control) and wastewater effluent (29°42'37.87" N and 31°17'14.53" E) receives untreated industrial wastes from pottery and faience clay brick factories and its water reaches to different agricultural canals. The water samples were collected in plastic bottles and used in irrigation of okra crop in the laboratory. Collected water samples were also acidified immediately after collection with nitric acid (1ml HNO_3/l) to determine the concentration of different heavy metals as Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn (**APHA 1999**). Water pH and TDS were measured using a dual pH/electrical conductivity meter (60 Sensor Operating Instruction Corning), according to **Roweel (1994)**. The relative oxygen requirements for water samples were determined by measuring chemical and biological oxygen demand (COD and BOD, respectively). COD was measured as described by **Pitwell (1983)** using the titrimetric analyses method, while BOD was measured according to **Delzer and Mckenzie (2003)** by using the 5-day biochemical oxygen demand method.

2.3. Sampling of *Okra plants*:

The samples of okra plants were collected from five fields (each about one acre) distributed equally in botth study sites; El-Attef city (29°42'31.17" N and 31°15'11.56" E) as control and Ekhsas Gharb city (29°42'37.87" N and 31°17'14.53" E) as treatment under natural conditions. At each cultivated field 10 quadrats of area 4 m^2 were chosen randomly, to sample okra plants. The first study site, cultivated fields were irrigated using River Nile water while the second sites fields were irrigated with wastewater from the effluent which receive untreatment industrial wastes. The plants were harvested carefully and different growth parameters were measured (number of individuals per m^2, stem length, root length, number of leaves per individual), then the collected samples were divided into root, shoot and fruits (edible part) for determining fresh weights of leaves and stems per m^2, dry weights for leaves and stems per m^2, fresh and dry biomass and productivity kg per acre). Dry weight for leaves and stems was carried out at 40⬚C for 3 days, according to **Allen et al. (1986)**.

2.4. Plant Analyses

The plant samples were separated into roots, shoots and fruits. Air-dried and ground using the electrical blinder to determine the concentration of different heavy metals (Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn) and plant nutrients (N, P, and K), proteins and carbohydrates. One gram for each plant sample (from control and polluted samples) was digested using the acid-mixture digestion method (**Lu 2000**). The concentration of different heavy metals was measured using atomic absorption spectrophotometer (DW-AA320N). The plant nutrients (N, P and K) were determined as methods described in the soil section. Total proteins were determined by using Bio-Rad protein assay according to **Lowry et al. (1950)**, while total soluble carbohydrates were determined according to **Umbriet et al. (1959)** using the anthrone-sulfuric acid method.

For determination of leave pigments (chlorophyll a, b and carotenoids); 2g of fresh leaves of okra plant was extracted with 50% acetone (v/v) in complete darkness (as light causes degradation of chlorophyll) and kept at 4⬚C overnight, after that the samples were measured spectrophotomically (UNICO Vis Model 1200, USA) according to **Metzner et al. (1965)**. Each pigment was measured as following: Chl. a = $10.3 E_{663} - 0.918 E_{644}$, Chl. b = $19.7 E_{644} - 3.87 E_{663}$ and Carotenoids = $4.2 E_{453} - (0.0264$ chl.a + 0.426 chl.b). where E is the absorbance at the definite wavelength (nm).

2.5. Data Analyses

The differences between the studied variables for soil, water and plant tissues in the studied sites were tested using a paired-sample *t*-test. The significance of variations of trace metals in the different plant organs was tested using one-way analyses of variance (ANOVA) according to SPSS software. Duncan's test was used in the mean multiple comparisons and then the data for normality and homogeneity of variance was tested (**SPSS 2006**). The plant's ability to store a specific metal in relation to its soil concentration is referred to as the bioaccumulation factor (BF). It is calculated as follows: BF = C_{root} / C_{soil}, where C_{root} and C_{soil} represent the heavy metal concentration in the root and soil respectively. The relative translocation of metal from root to shoot of the plant referred to the translocation factor (TF) and is calculated as follows: TF = C_{shoot} / C_{root}, where C_{shoot} and C_{root} represent the trace metal concentration in the plant shoot and root, respectively (**Galal et al. 2021a**). PLI referred to the pollution load index for soil irrigated with wastewater and was calculated as follows: PLI = Cp / Cn, where Cp and Cn represent the trace metal concentrations in the soil irrigated with untreated industrial wastewater and soil irrigated with Nile water, respectively (**Slima and Ahmed 2020**).

3. Results

3.1. Soil and water properties

Untreated industrial wastewater irrigation causes a significant adverse effect on soil chemical properties (**Table 1**). The pH, TDS and trace metals concentrations were significantly increased (P < 0.01 and P < 0.05) in soil irrigated with untreated wastewater. At the same time, the concentration of soil nutrients (N, P, K) was significantly decreased (P < 0.01) as a result of untreated wastewater irrigation. Soil irrigated with Nile water had a neutral pH (pH = 7) and acceptable EC (1.84 µS cm⁻¹), while irrigation with wastewater led to raising pH to be more alkaline (pH = 7.85) and salinized (EC = 5.70 µS cm⁻¹).

The concentration of all trace metals (Pb, Cd, Cr, Cu, Fe, Mn, Ni, and Zn) was increased significantly as a result of using untreated industrial wastewater in irrigation, while they are still in the safe tolerable limits according to WHO (1996) except Fe, Mn and Zn which exceeds the tolerable limits (**Table 1**). In addition, the PLI showed that soil irrigated with untreated industrial wastewater was loaded with a high concentration of trace metals; Zn had the higher PLI value (21), followed by Pb (16.92) and Fe (15.46).

Analyses of irrigation waters indicated that; industrial wastewater was slightly alkaline (pH = 7.8), salinized (TDS = 2339 µS cm⁻¹), and had high values of biological and chemical oxygen demands (BOD = 312 mg L⁻¹) and (COD = 639 mg L⁻¹), respectively compared with Nile water (control). Also, all studied trace metals were significantly increased (P < 0.001 and P < 0.01) and were above the tolerable limits, except for Cr and Cu were in the range of tolerable limits (**Table 2**).

3.2. Growth Parameters

Okra plants irrigated with untreated industrial wastewater had a significant decrease (P < 0.01 and P < 0.05) in most of its growth parameters (**Table 3 and Fig. 1**). The percentage of decrease was as follows: number of individuals /m^2: 33.5%, stem length: 20.3%, root length: 51.2%, number of leaves per individual: 26.8%, leaves fresh weight (g m^{-2}): 61.4%, stem fresh weight (g m^{-2}): 66.9%, leaves dry weight (g m^{-2}): 89.2%, stem dry weight (g m^{-2}): 72.9%, fresh biomass (kg acre^{-1}): 66.1%, dry biomass (kg acre^{-1}): 71.4% and productivity (kg acre^{-1}): 59.3% for okra plants irroigated with untreated industrial wastewater compared to okra plants irrigated with Nile water (control).

3.3. Plant analyses

3.3.1. Photosynthetic pigments

Okra leaves indicate a significant decrement (P < 0.05 and P < 0.01) in photosynthetic pigments (chlorophyll a and b) for plants irrigated with untreated industrial wastewater compared to control. At the same time, carotenoids show a significant increase (P < 0.05) in leaves of okra plants irrigated with untreated industrial wastewater (**Fig. 2**). The percentage of decrement in chlorophyll a was 16.2% and in chlorophyll b was 61.9%, while the rate of increase in carotenoids was 80%.

3.3.2. Nutrients and trace metals

In roots of okra, the percentage of N and P was declined from 2.95% and 2.54% to 1,97% and 2.17%, respectively, for plants irrigated with untreated industrial wastewater (**Table** 4). Also, K was declined in roots of plants irrigated with untreated industrial wastewater from 18.71 to 12.95 ppm compared to control. In Okra shoots, the percentage of N and P was declined from 3.65% and 2.83% to 2.22% and 2.33%, respectively for plants irrigated with untreated industrial wastewater compared to control, and the concentration of K was declined from 21.23 to 15.05 ppm (**Table 4**). The decrement of the concentration of carbohydrates and proteins in the Okra shoots and roots as a result of irrigation with untreated industrial wastewater was indicated in **figure 3.** In Okra roots, the concentration of carbohydrates declined by 37.9%, and in proteins, by 33.3%.

A significant increase (_P_ < 0.01) in all studied metals concentrations for Okra roots and shoots irrigated with untreated industrial wastewater compared to control (**Table 4**). Compared to Okra shoot, an increase in the concentration of Pb: 26-fold, Cd: 36.9-fold, Cr: 8.6-fold, Cu: 8.4-fold, Fe: 3.5-fold, Mn: 2.9- fold, Ni: 24-fold and Zn: 4.6-fold. Compared to Okra root, trace metals increased as follows: Pb: 2.4-fold Cd: 192.7-fold, Cr: 64.3-fold, Cu: 1.7-fold, Fe: 3.5-fold, Mn: 2.9-fold, Ni: 24.5-fold, and Zn: 5.2-fold (**Table 4**). Besides, fruits of Okra plants irrigated with untreated industrial wastewater had a highly significant increase (_P_ < 0.001) in all studied trace metals concentrations compared with control (**Table 5**).

The results showed that Fe was the highest metal accumulated in the plant's fruits irrigated with untreated industrial wastewater, followed by Ni, Mn, Zn, Pb, Cu, Cd, and Cr. It is dangerous to notice that the concentration of Cd, Cu, Fe, Mn, Ni and Zn was in the phytotoxic range, according to **Slima and Ahmed (2020)**.

3.4. Trace metal Bioaccumulation and Translocation

Generally, okra plants irrigated with untreated industrial wastewater had metal BF values higher than one, while TF values were less than one (**Table 6**). The results indicated that okra plants are hyper-accumulate for Ni and Cd in their root; Ni had BF (9477.88) and Cd had BF (2478.89) for plants irrigated with untreated industrial wastewater. Also, it accumulate other studied metals in its roots; that Cr had BF (140.07) followed by Mn (32,67), Fe (18.65), Zn (13.05), Pb (11.81), and Cu (10.44). Okra plants had no accumulated trace metals strategy in their shoot, as TF values were less than one.

4. Discussion

4.1. Soil and water properties

The pH and the TDS of soil is the influential factor influencing the availability, mobility and transport of heavy metals in the soil and the mobility of heavy metals increase with the increase of soil pH and TDS (Salman et al. 2019). In the present study, the measured soil pH and TDS indicated that pH value turned to be slightly alkaline (pH = 7.85) and more saline (TDS = 5.7 μS cm^{-1}) in soil irrigated with untreated industrial wastewater (**Table 1**). This may be attributed to the formation of insoluble organic

complexes or the precipitation of carbonates and hydroxides (Slima and Ahmed 2020). This notice is dangerous for agricultural fields because crops were known to uptake and accumulate trace metals from polluted soil in their edible parts (**Ahmed and Slima 2018**; **Shehata and Galal 2020**; **Slima and Ahmed 2020**; **Galal et al. 2021b**). Simultaneously, the increase of trace metals concentration in soil affects the activity (growth and metabolism) of soil microbial communities. Soil microorganisms are necessary for the decomposition of soil organic matter and availability of soil nutrients; that any reduction in the soil microorganisms may affect plants' nutrient availability (Xie et al. 2016). In the present study, the soil nutrient concentrations are declined in soil contaminated with trace metals due to irrigation with wastewater.

The result of the present study indicated that the concentration of trace metals (Cu, Fe, Mn, and Zn) in soil watered with untreated industrial wastewater increase the safe limits displayed by the World Health Organization (**WHO 1996**) and Environmental Quality Standards for soil samples (**US-EPA 2012**) and overloaded with those trace metals (Zn, Pb, Fe, Cu and Mn). The long-term application of watering with trace metals contaminated wastewater led to the accumulation of these trace metals at toxic levels (**Ahmed et al. 2021**). According to **WHO (1996)** and the Environmental protection agency (**US-EPA 2006**), the trace metals concentration in wastewater is above safe limits, while Nile water is free from most studied trace metals.

4.2 Growth parameter

Okra plants irrigated with untreated industrial wastewater had a significant decrease (P < 0.01 and P < 0.05) in most of its growth parameters (**Table 3**). The accumulation of heavy metals in plant tissues causing changes in physiological and biochemical characters (**Singh and Singh 1981; Fisher et al. 1981**). **Batool et al. (2014)** mentioned that heavy metals influence morphological and physiological characters and causing a reduction in plant growth parameters. In the present study, all plant growth measurements, including the number of individuals /m^2, stem length, root length, number of leaves per individual, leaves fresh weight, stem fresh weight, leaves dry weight and stem dry weight showed a significant decrease (**Table 3**) and this is in agreement with **Mami et al. (2011)** who recorded reduction in growth parameters of tomato plant irrigated with water containing high percentage of heavy metals especially Fe, Pb and Cu. Also, **Galal et al. (2021a)** recorded a reduction in growth parameters of *Cyperus alopecuroides* as a result of the high concentration of heavy metals present in irrigation water which responsible for several metabolic alterations. Similar results were reported by **Islam et al. (2006)** on rice plant and found reduction in plant height and the number of tillers per pot and shoot dry matter weights as a result of irrigation with contaminated water. Also, many studies report the reduction of plant growth as result of irrigation with untreated wastewater as: **Farahat et al. (2017)** on wheat and maize, **Ahmed and Slima (2018)** on *Corchorus olitorius* L., **Galal et al. (2018)** on cabbage, **Slima and Ahmed(2020)** on *Pisum sativum* L., **Shehata and Galal (2020)** on cucumber (*Cucumis sativus* L.) and **Galal et al. (2021b)** on *Ricinus communis* L. **Ahmed and Slima (2018)** reported that *Corchorus olitorius* irrigated with untreated industrial wastewater, had reduced plant growth parameters due to less uptake of water and nutrients, or it may be due to inhibition of root protein synthesis (**Drazkiewicz and Baszyński 2005**). In present study, fresh and dry biomass and productivity of okra plants irroigated with untreated industrial wastewater are reduced in compared to okra plants irrigated with Nile water (control). This reduction may be due to ecophysiological conditions as the water availability or other unfavorable conditions as pollution with trace metals (**Galal and Shehata 2016**). **Galal et al. (2021a)** recorded reduction in the biomass and production of *Cyperus alopecuroides* when irrigated with contaminated water due to the presence of heavy metals in high concentrations. **Batool et al. (2014)** mentioned that when Ni and Cd present in high concentration causing a decrease in the productivity and biomass of plants.

4.3 Plant analysis:

4.3.1 Photosynthetic pigments:

Okra leaves indicate a significant decrement in photosynthetic pigments (chlorophyll a and b) for plants irrigated with untreated industrial wastewater compared to control; this may be due to inhibition of the activity of enzymes leading to inhibition of metabolic processes (**Zengin and Munzuroglu 2005; Shakya et al. 2008**), especially enzymes of chlorophyll biosynthesis **Żurek et al. (2014)**. Similar results were obtained by **Saini et al. (2014)** in spinach, **Muradoglu et al. (2015)** in strawberry, **Ahmed and Slima (2018)** in *Corchorus olitorius* and **Slima and Ahmed (2020)** in *Pisum sativum*. Accumulation of heavy metals in wastewater used in irrigation of crops damage the sensitivity of the chloroplast machine of plant leaves (**Ahmed and Hanafy 2017**). Maleva et al. (2012) found that Mn and Cu causing a decrease in the plant content of chlorophyll a and b, while Zn high concentration in wastewater also decreasing the content of plant chlorophyll (**Hajihashemi et al. 2020**).

4.3.2 Nutrients and heavy metals:

In the present study, there is a significant decline in the concentration of N, P and K in different plant organs (root and shoot) as a result of irrigation with untreated industrial wastewater. This result agreed with **Begum et al. (2011)** who study the effect of using textile industrial wastewater in irrigation of rice crop and this might be due to the presence of high content of Pb in irrigation water. Also, **Osawa and Tazuke (1990)** found the decrease of N content resulted from increasing in Cu, which had an antagonistic effect with N content. In the present study, polluted water used in irrigation of okra containing Pb and Cu above the permissible limit reduces the N content of okra. Also, **Singh and Kalamdhad (2011)** mentioned that trace metals reducing nutrient absorption and affecting the ability of legume plants to fix nitrogen. The decline in the content of P and K of the crop may be a result of the presence of a high amount of Pb, Zn and Cu in the wastewater, which had an antagonistic effect with P and K (**Muchrimsyah and Mercado 1990**). The result of present study agreed with **Shukry (2001)**, **Begum et al. (2011)**, **Ahmed and Slima (2018)** and **Slima and Ahmed (2020)**.

There is also a decline in the concentration of carbohydrates and proteins in the present study due to photosynthesis inhibition which agreed with the results obtained by **Galal (2016)** on *Cucurbita pepo* L. and **Farahat et al. (2017)** on maize and wheat irrigated with wastewater. The decrease in protein content of plant tissues may result from protein breaking down by the increasing the activity of protease enzyme as a result of the presence of the plant under stress conditions (**Palma et al. 2002**).

There is a significant increase in all studied metals concentrations for Okra roots and shoots irrigated with untreated industrial wastewater compared to control, so okra plant considers a hyperaccumulator for all heavy metals determined in the study except Cr. The results indicated that all estimated heavy metals (Pb, Cd, Cu, Fe, Mn, Ni and Zn) are found more than the permissible levels (except Cr) and exceeding the phytotoxic range in different plant tissues (root, shoot and fruits). All these results coincide with the results obtained by **Ahmed and Slima (2018)** on *Corchorus olitorius* irrigated with wastewater, **Slima and Ahmed (2020)** on pea plant.

4.4. Trace metal Bioaccumulation and Translocation

The mobility of trace metals from soil to plant and from plant root to plant shoot depends on the plant itself (**Slima and Ahmed 2020**) and can be determined by calculating BF and TF, respectively. BFs value greater than one means that plants prefer to store metals inside their roots than transfer them to their shoot tissues, while TFs value greater than one tells that plants prefer to transfer and store metals in their shoot tissues (**Ahmed et al. 2021**). The present study indicated that okra plants accumulate trace metals in their root tissues than in shoot tissues (**Table 6**), despite the high level of studied trace metals in okra plant shoots and fruits (edible part) irrigated with untreated industrial wastewater. Still, okra plants cannot accumulate trace metals in their shoot tissues as TFs values of all studied trace metals are less than one.

Conclusion

This study seeks to draw attention to the risks posed by irrigation with untreated industrial wastewater for okra plants. It led to decrement in soil nutrient (N, P, and K) and plant nutrients (N, P, K, carbohydrates and proteins). Also, it causes decrement in different growth parameters (No. of individual, root and stem length, No. of leaves and fresh and dry weight of stem and leaves) and crop productivity (decreased by 59.3%). At the same time, irrigation with wastewater causes trace metal (Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn) accumulation in both soil and plant. Okra fruit (edible part) had a phytotoxic levels of Cd, Cu, Fe, Mn, Ni and Zn. Authors recommended that okra crop irrigated with untreated industrial wastewater should not be consumed neither by people or as animal food.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: Please contact the authors for data requests.

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Tables

Table 1 Soil characteristics and pollution load index (PLI) of soil irrigated with wastewater. SE: standard error, *t*-values are provided. Tolerable limits according to the World Health Organization (1996) and Environmental Quality Standards for soil samples (USEPA 2012). *P < 0.05 and **P < 0.01

Soil characters	Soil irrigated with Nile water (control)		Soil irrigated with waste water		*t-value*	Tolerable Limits	PLI
	Mean	SE	Mean	SE			
PH	7.00	± 0.01	7.85	± 0.08	46.90**	-	-
TDS µS cm^{-1}	1.84	± 0.01	5.70	± 0.05	76.60**	-	-
N (mg kg^{-1})	224.17	± 0.76	51.60	± 2.00	2342.00**	-	-
P	21.5	± 0.02	11.72	± 0.11	141.90**	-	-
K	486.33	± 0.58	53.90	± 1.58	642.30**	-	-
Pb	1.81	± 0.01	30.63	± 1.18	17.60*	0.01 - 50	**16.92**
Cd	0.51	± 0.00	0.15	± 0.01	75.30**	0.02-0.7	0.294
Cr	1.00	± 0.02	0.45	± 0.02	13.70*	5 - 30	0.45
Cu	2.90	± 0.02	16.20	± 0. 21	132.20**	0.27 - 100	5.59
Fe	9.81	± 0.01	151.67	± 3.06	46.20**	0.15-7	**15.46**
Mn	15.59	± 0.04	38.27	± 0.64	16.10*	20.0	2.46
Ni	0.40	± 0.002	0.19	± 0.005	76.80**	5.0	0.48
Zn	4.19	± 0.01	88.0	± 2.65	23.40*	10 - 50	**21.00**

PLI = Cp / Cn, where Cp and Cn represent the trace metal concentrations in the soil irrigated with industrial untreated wastewater and soil irrigated with Nile water.

Table 2 Irrigation water characteristics (mean ± SE). COD and BOD are the chemical and biological oxygen demand, respectively. Tolerable limits are according to the World Health Organization (WHO 1996) and Environmental protection agency (EPA 2006). *P < 0.01 and **P < 0.001.

Water Character	Nile water (control)	Wastewater		*t-value*	Tolerable limits
pH	7.5 ± 0.05	7.8	± 0.02	10.5*	-
TDS (µS cm^{-1})	8.56 ± 0.04	2339	± 1.54	224.6**	-
COD mg L^{-1}	5.11 ± 0.12	639	± 0.05	143.8**	-
BOD	2.33 ± 0.03	312	± 0.05	13.4*	-
Pb	0.00	0.33	± 0.5	65.3**	0.05
Cd	0.00	0.55	± 0.02	27.2**	0.01
Cr	0.00	0.87	± 0.40	12.7**	0.1
Cu	0.00	1.44	± 0.20	51.6**	2.0
Fe	0.05 ± 0.003	2.1	± 0.6	52.7**	0.3 – 1.0
Mn	0.04 ± 0.001	0.27	± 0.3	29.3**	0.05
Ni	0.00	0.45	± 0.002	10.6*	0.025
Zn	0.00	1.54	± 0.33	17.7*	5.0

Table 3 Growth parameters (mean ± SE) of okra plant (*Abelmoschus esculentus* Moench.) irrigated with Nile water (control) and untreated industrial wastewater. ns: not significant (i.e., $P > 0.05$), *$P < 0.05$, and **$P < 0.01$.

Plant parameter	Control Plant		Wastewater irrigated plant		*t*-test
Number of individuals m^{-2}	2.00	±0.0	1.33	±0.58	2^{ns}
Stem length (cm)	128.33	±7.37	102.33	±4.51	5.97*
Root length (cm)	14.33	±0.58	7.00	±1.0	8.32*
Number of leaves (No. ind^{-1})	16.17	±1.26	11.83	±0.76	4.67*
Leaves fresh weight (g m^{-2})	425.33	±38.44	164.00	±62.86	4.50*
Stem fresh weight (g m^{-2})	5365.33	±212.05	1778.67	±957.85	6.26*
Leaves dry weight (g m^{-2})	144.73	±193.36	15.67	±4.63	1.14*
Stem dry weight (g m^{-2})	588.27	±20.33	159.20	±87.34	8.89*

Table 4 Elements concentrations (mean ± SE) in the shoot and roots of okra plant (*Abelmoschus esculentus* Moench.) irrigated with Nile water as a control and untreated industrial wastewater.

Element		Control plant				Wastewater irrigated plant			
		Shoot		Root		Shoot		Root	
Inorganic Nutrients									
N		3.56	±0.08c	2.95	±0.09b	2.22	±0.37a	1.97	±0.30a
P	%	2.83	±0.13b	2.54	±0.22ab	2.33	±0.22a	2.17	±0.23a
K		21.23	±0.42d	18.71	±0.18c	15.06	±0.63b	12.95	±0.07a
Trace metals									
Pb		12.33	± 2.24 a	12.75	± 2.14 a	320.33	± 5.67 b	361.83	± 5.55 b
Cd	(mg kg^{-1})	1.28	± 0.05 a	1.93	± 0.07 a	47.28	± 3.71 a	371.83	± 28.79 b
Cr		0.52	± 0.04 a	0.97	± 0.04 a	4.48	± 0.08 a	62.33	± 8.13 b
Cu		16.92	± 4.25 a	98.50	± 9.05 a	142.17	± 3.01 ab	169.17	± 8.23 ab
Fe		740.17	± 12.95 a	810.00	± 21.36 a	2613.33	± 47.26 b	2828.33	± 41.63 c
Mn		371.83	± 16.51 a	426.25	± 8.75 a	1095.00	± 52.92 b	1250.00	± 67.27 c
Ni		64.17	± 6.29 a	72.92	± 11.88 a	1541.67	± 41.63 b	1785.00	± 61.44 c
Zn		201.50	± 12.07 a	221.83	± 3.51 a	931.67	± 52.04 b	1148.33	± 73.71 c

Means with the same letters in a row are not significant according to Duncan's multiple range tests.

All values are significant at (P < 0.01).

Table 5 Trace Metal concentrations (mg kg^{-1}) in fruits (edible part) of okra plant (*Abelmoschus esculentus* Moench.) irrigated with Nile water as control and untreated industrial wastewater. *P < 0.001. Phytotoxic range according to Slima and Ahmed (2020).

Trace metals	Control seed		Wastewater irrigated seed		t-value	Phytotoxic range
Pb	6.3	± 0.07	404.17	± 4.16	167.692*	30-300
Cd	1.13	± 0.01	42.57	± 0.16	439.0*	5 - 30
Cu	13.59	± 0.14	140.67	± 0.29	571.88*	20-100
Cr	0.31	± 0.01	4.48	± 0.03	300.12*	10-100
Fe	205.3	± 0.8	2756.67	± 2.9	1281.2*	> 500
Mn	261.2	± 0.96	1203.33	± 7.64	92.67*	> 500
Ni	47.5	± 0.25	1326.67	± 2.89	713.3*	40-246
Zn	105.3	± 0.25	877.83	± 0.29	3504.11*	100-400

Table 6 Bioaccumulation (BF) and translocation (TF) factors for trace metals in okra plant (*Abelmoschus esculentus* Moench.) irrigated with Nile water as control and untreated industrial wastewater.

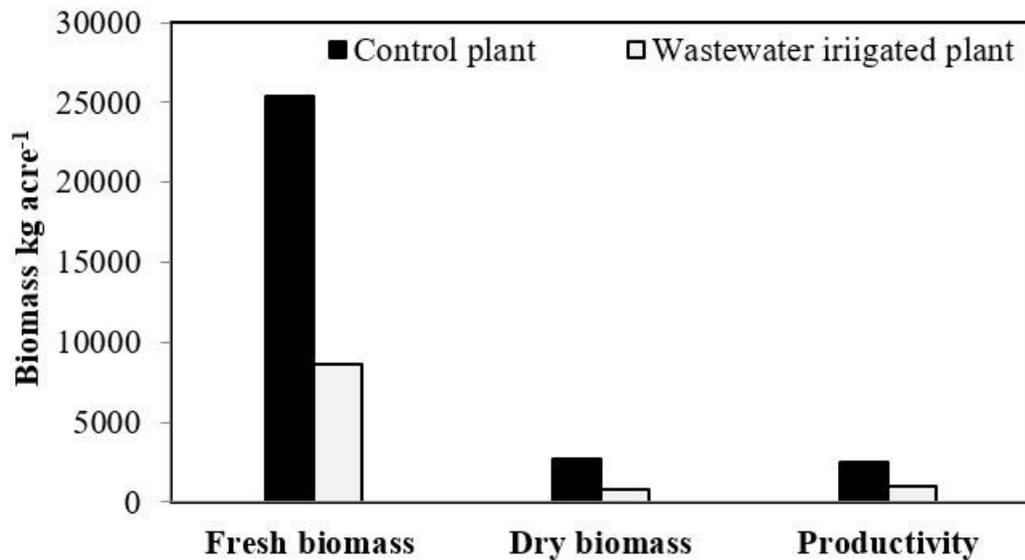| Heavy metal	Control Plant		Wastewater irrigated plant	
	BF	TF	BF	TF
Pb	7.06	0.97	11.81	0.89
Cd	3.76	0.66	2478.89	0.13
Cu	33.93	0.17	10.44	0.84
Cr	0.96	0.53	140.07	0.07
Fe	82.57	0.91	18.65	0.92
Mn	27.34	0.87	32.67	0.88
Ni	180.93	0.88	9477.88	0.86
Zn	52.92	0.91	13.05	0.81

Figures

Figure 1

Fresh and Dry biomass and productivity of okra plant (Abelmoschus esculentus Moench.) irrigated with Nile water (control) and untreated industrial wastewater.
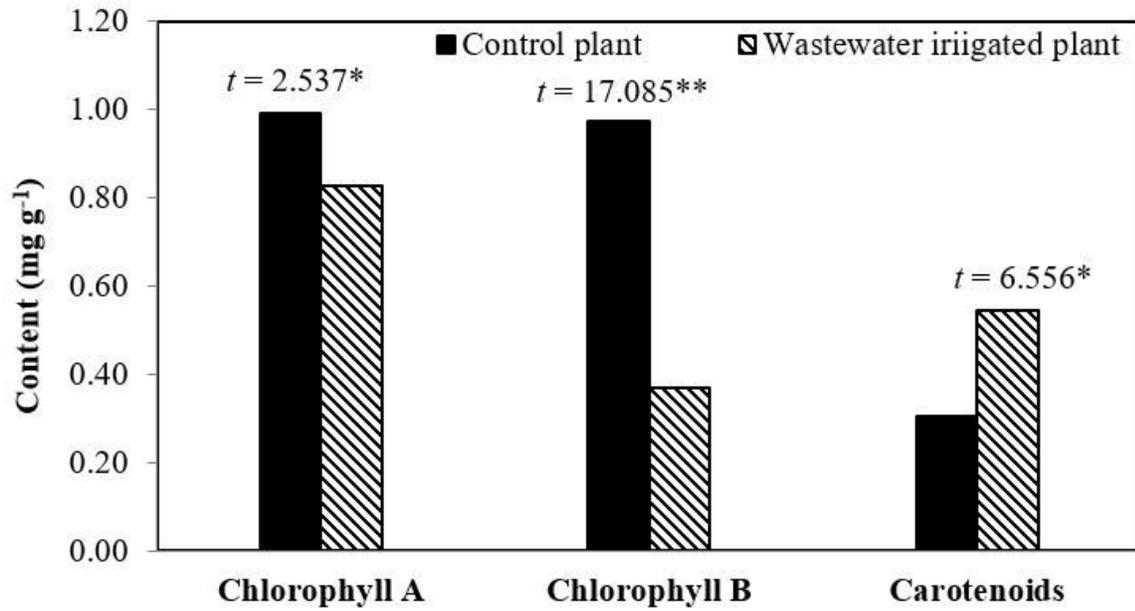
Figure 2

Pigment contents (mg/g dry wt.) in leaves of okra (Abelmoschus esculentus Moench.) irrigated with Nile water (control) and untreated industrial wastewater. Values are significant at * P < 0.05 and ** P < 0.01 using a paired-sample t test.

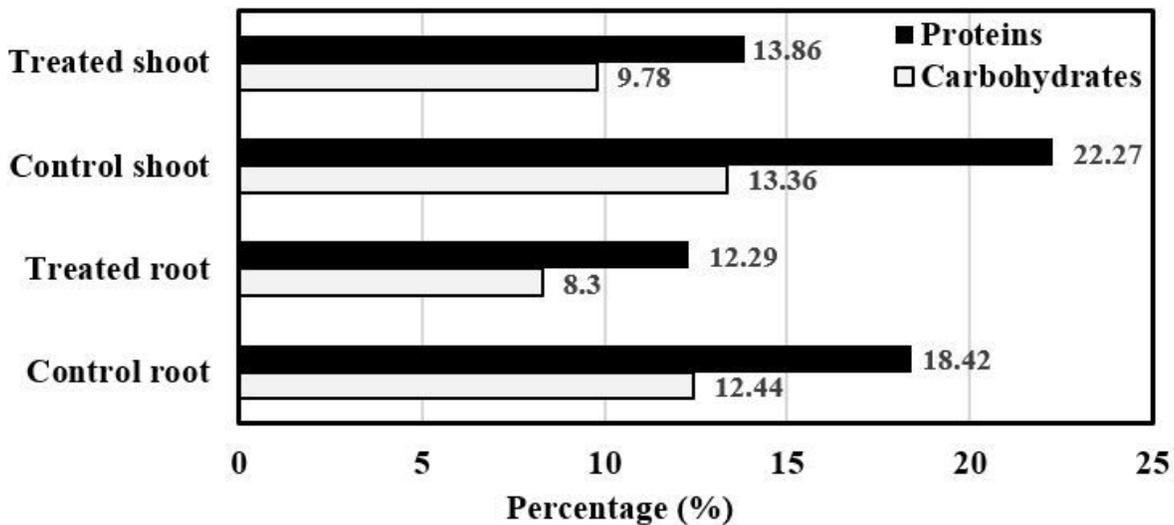**Figure 3**

Proteins and caerbohydrates contents of okra (Abelmoschus esculentus Moench.) irrigated with Nile water (control) and untreated industrial wastewater (Treated). The variation in concentration of proteins and carbohydrates in the roots and shoot of plants were statically significant at P < 0.01.