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ORIGINAL PAPER

# Trace Metals Accumulated in Pea Plant (*Pisum sativum* L.) as a Result of Irrigation with Wastewater

Dalia Fahmy Slima[1] · Dalia Abd El-Azeem Ahmed[2]

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**Abstract**
Trace metals (TMs) that accumulated in different crops irrigated with untreated wastewater are public hazard problem. The present work aimed to evaluate the impact of utilizing untreated agricultural water in the irrigation system of the peas crop (*Pisum sativum* L.) until seed production on soil and plant characteristics. It also aimed to assess the effect of the applied wastewater on nutrients and TM contents in both soil and different plant parts (root, shoot ,and seed), and it extended to study TMs' bioaccumulation and translocation ability of pea plant. Three composite soil and water samples were collected from the agricultural field (30°32'N and 31° 0′E) and agricultural wastewater effluent (30°34.5'N and 31°00.42′E) in Shebin El-Kom city used in irrigation of different crops. A pot experiment (each pot of 15 × 20 cm) was carried out in the botanic garden of Faculty of Science, Menoufia University, Egypt. TMs and nutrients were investigated for soil and plant samples. There was a significant increase in trace metal contents: cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) for soil and pea plants as a result of irrigation with wastewater. Also, it caused a significant decrease in soil nitrogen (N), phosphorous (P), and Potassium (K) and plant (N, P, K, carbohydrates, and proteins) nutrients. A significant decline in pea growth parameters and the content of chlorophyll a and b and carotenoid in pea leaves were recorded. A high significant in TMs content for seeds (edible parts) was recorded (Cd = 75.6, Cu = 9.7, Cr = 29.8, Fe = 1123.6, Mn = 82.2, Ni = 26.8, Pb = 131.4 and Zn = 35.7 mg $kg^{-1}$). The concentration of Cd, Cr, Fe, and Pb in seeds was in the phytotoxic range. Irrigation with wastewater shows a negative impact on the soil and plant characteristics. Also, it causes a significant decline in soil and plant nutrients. At the same time, it increases the concentration of TMs for soil and all plant organs. The concentration of Cd, Cr, Fe and Pb reached to the phytotoxic range in its seeds. Authors recommended that pea cultivated in soil and/or irrigated with untreated wastewater was not safe for human and animal consumption.

**Keywords** Bioaccumulation · Hyperaccumulator · Plant nutrients · Soil characteristics · Translocation factor · Trace elements

## 1 Introduction

Water is one of the foremost critical normal assets to create life. The quality of water is of vital concern for humanity since it's directly linked with human welfare. Developing countries suffer from an increasing population, so they face tremendous pressure on shrinking of natural resources to meet their ever-growing demand. Expanding the population leads to increase water utilization, as increasing water requirements for industry and irrigation, which resulted in decreasing of available water resources (Naddafi et al. 2005). Africa, Asia, and Latin America contain developing countries that suffer from a shortage of water and use untreated wastewater in agricultural production. At the same time, there are also middle-income countries that use treated wastewater for many purposes (Faruqui et al. 2004). Agriculture uses about 70% of water extraction. By time and in regions suffering from water shortage, planters ordinarily turn to utilize domestic or urban wastewater in irrigation (FAO 2010). One of the resources of wastewater is untreated agricultural water. Irrigation with wastewater had become prevailing, especially in the arid and semiarid regions due to a shortage of freshwater, which had a terrible impact on the soil and human health (Jhamaria et al. 2015).

The contaminated water utilized in a water irrigation system led to adding a few plant supplements to the soil, so increasing the fertility of the soil to some extent, but it contains

✉ Dalia Abd El-Azeem Ahmed
dalia.ahmed@science.tanta.edu.eg; drdalia1080@yahoo.com

[1] Botany and Microbiology Department, Faculty of Science, Menoufia University, Menoufia, Egypt

[2] Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

toxicants that alter soil properties and micro-flora (Eltaher et al. 2019). One of the genuine natural environmental issues is the contamination of water by TMs because of their resistance and toxic impacts (Khalil et al. 2007). If TMs contaminated wastewater is utilized in agriculture irrigation, it will lead to a significant and substantial amount of toxic TMs within the food chain, which had poisonous effects on human beings, animals, and plants (Majid et al. 2012). High TMs content influences both plant growth and metabolism. These TMs even in trace quantities annihilate enzymes of living cells and result in harmful impacts on the quality and crop yield because Cd, As, Cr, and Hg are incredibly poisonous (Zhuo et al. 2019).

Trace metals influence human beings' health and ecosystem equilibrium (Ayangbenro and Babalola 2017; Zhang et al. 2018). The utilization of polluted soil or water for crop cultivation mainly diminishes productivity and results in producing contaminated food grains and vegetables, which had a harmful influence on human health (Galal and Shehata 2015a). Crops and vegetables grew in contaminated soil with TMs enduring from a more prominent accumulation of TMs than those grown in uncontaminated soil. The common TMs toxicity effect in plants is the production of reactive oxygen species (ROS) in high amounts. ROS production causes oxidative stress for the cells (Chen et al. 2012). Plants have developed different defense mechanisms to convert ROS into less toxic products and help plants to sustain the cellular redox state and moderate the damage caused by oxidative stress (Àlvarez et al. 2012; Tang et al. 2010).

Trace metals disclose duality in plants at lower concentrations as they can enhance plant growth and production, but when concentrations increase, they have lethal impacts and can harm plant structure and cell physiology. Plants adapt themselves to withstand these TMs through collecting and/or compartmentalization of them in specific cell organelles (Eltaher et al. 2019). Different crops follow different mechanisms to uptake and transport TMs from wastewater, and this phenomenon is known as hyper-accumulator (phytoremediation). The utilization of wastewater in irrigation systems causes contamination of soil with TMs, leading to soil corruption, and influences on food value and safety, especially when the cultivated plant could translocate these TMs to their edible parts (Ahmed and Slima 2018).

Pea (*Pisum sativum* L.) is one of the preeminent essential crops belonging to the family Leguminosae (Fabaceae) and is grown around the world for human and animal utilization. Pea has long been considered as a cheap crop and readily accessible source of protein; complex carbohydrates; fiber; vitamins A, B6, and C; and potassium, magnesium, copper, phosphorus, zinc, iron, and lutein (Eid et al. 2019). Garden or green pea is picked before the seed ripening for the fresh or fresh-pack shop (Elzebroek and Wind 2008). Pea is an imperative nutritious vegetable because it contains 1535% protein and high contents of the essential amino acids: tryptophan and lysine (Elzebroek and Wind 2008). Carbohydrates (fiber and starch) are considered the main pea components, and their average was 20 and 46%, respectively, of dry matter of seeds (Tzitzikas et al. 2006). The total area grown with pea was about 4326.9 ha (ha), and its total yield was 14 t/ha of green legumes in Egypt (FAO 2014).

Many researchers (e.g., Mojiri and Amirossadat 2011; Mojiri et al. 2013; Pandey et al. 2009; Pandita and Malaviya 2018; Shrestha 2000; Shrestha and Niroula 2003) had studied the effect of irrigation with untreated industrial or sewage wastewater on different crops even pea. They only consider the impact of irrigation with wastewater at the vegetative stage. There are no studies on the effects of using untreated agricultural water in the irrigation of crops, especially pea. Besides, this study shows the impact of irrigation with untreated agricultural water on the seeds as the seeds are an edible part for humans. Because of such points, the current work was conducted to evaluate the impact of utilizing untreated agricultural water in the irrigation system of the pea crop until seed production on soil and plant characteristics. This study also aimed to assess the effect of the applied wastewater on nutrients and TMs contents in both soil and different plant parts (root, shoot, and seed). It also extended to study TMs bioaccumulation and translocation ability of pea plant.

## 2 Material and Methods

### 2.1 Soil and Water Samples

The soil used in pot experiment was collected from the agricultural field from Shebin El-Kom city (30°32'N and 31° 0′E). Composite soil samples of about 8 kg were collected from the surface layer at a depth of approximately 0–50 cm using a shovel. The sampled soil was collected in plastic bags and transported to the laboratory to distribute in pots used in the experiment. Also, three composite water samples were gathered in plastic bottles from the water effluent in Shebin El-Kom city (30°34.5'N and 31°00.42′E). It receives raw agricultural wastewater and it is used for irrigation of different fields in Shebin El-Kom city. The salinity (EC) and pH of the water samples were measured using pH/electric conductivity meter (914 pH/Conductometer- Metrohm AG). Then water samples were acidified directly with nitric acid (1 ml $HNO_3$/l) for the determination of Cd, Cu, Cr, Fe, Ni, Mn, Pb, and Zn (APHA 1999). The wastewater requirements of relative oxygen were determined by measuring the chemical and biological oxygen demand (COD and BOD, respectively); COD was measured by titrimetric analysis (Pitwell 1983), while BOD by 5-day biochemical oxygen demand method (BOD5) (Delzer and Mckenzie 2003).

## 2.2 Cultivation of Peas

Pea seeds were brought from the Center of Agricultural Research in Egypt. The seeds were superficially disinfected by 3.5% sodium hypochloride for 20 min and washed several times with distilled water. A pot experiment was carried out at the botanic garden of Botany and Microbiology Department, Faculty of Science, Menoufia University, Egypt, during the winter season of 2018; the temperature was ranged between 18 and 22 °C. The soil sample was spread in 6 plastic pots of 20 cm in diameter. Pots were prepared in a complete randomized design with three replicates. In each pot, five seeds were sown at 1.5–2-cm depth. Each pot was irrigated with 500 ml of water every 3 days for 3 weeks. Then it increased to 800 ml until the end of the experiment. The irrigation process was carried out in the morning at 8 o'clock. Irrigation was carried out with the tap water as a control and raw agricultural water as a treatment. The germination percentage of the pea seedlings was recorded in sterilized Petri dishes after 1 week of cultivation.

Finally, after 2 months, each pot was harvested carefully to keep the root system complete as can as possible. The plant samples were cleaned by washing with tap water to expel debris and squander and then rinsed with distilled water; after that they separated into parts (root, shoot, and fruits). Fresh and dry weights of shoot and root; shoot and root lengths; the number of lateral branches, leaves, flowers, and legumes per individual; the number of seeds per legume; and the fresh and dry weights of seeds per legume were recorded. The dry weights of different parts of the study plant were determined by drying at 40 °C for 3 days (Allen et al. 1986).

## 2.3 Soil Analysis

After plant harvesting, three soil samples of each treatment were collected from the surface layer as a profile of 0–25 cm, spread over sheets of paper for air drying, and passed through a sieve (2 mm) to get rid of gravels and debris. Soil water extract at 1:5 (*w*/*v*) is prepared for the determination of electrical conductivity (EC) and soil pH by using pH/electric conductivity meter (Jenway3540) (Jackson 1962).

For determining nutrients (N, P, and K) and TMs (Cd, Cu, Cr, Fe, Mn, Ni, Pb, and Zn) content in the collected soil samples, the soil was digested using the acid digestion method described by Wade et al. (1993), using digestion with tri-acid mixture of $HNO_3/H_2SO_4/HClO_4$ (5:1:1, *v*/v/v) for 8 h at 80 °C method and then stored for analysis. Concentrations of total Cd, Cu, Cr, Fe, Ni, Mn, Pb, and Zn in soil samples were determined using an Atomic Absorption Spectrophotometer (DW-AA320N). The total soluble nitrogen was measured by the Kjeldahl method (Pirie 1955) and P was determined by molybdenum blue method using a spectrophotometer (UNICO Vis Model 1200, USA) and set at 660 nm in case of N and 700 nm in case of P. Also, K was determined using Flame Photometer (DW-FP 640). All these analyses follow Allen (1989). The loaded value of each TM in soil was assessed using pollution load index (PLI; PLI = Cp/Cn), where Cp and Cn denote the concentration of TM in the soil irrigated with untreated agricultural wastewater and soil irrigated with tap water as control, respectively (Liu et al. 2005).

## 2.4 Plant Analysis

Photosynthetic pigments analysis was assessed according to Hu et al. (2013) in the pea leaves. A definite weight of fresh leaves (0.5 g) of pea plants was extracted with 100% methanol and stored overnight in the fridge to allow the complete extract of pigments (until total discoloration). Each extract was prepared in 3 replicates, and then the absorbance of pigment extracts was measured by using a spectrophotometer for chlorophyll a, chlorophyll b, and carotenoids. For assessing the constituents of photosynthetic pigment (mg/g dry weight), the following equations were applied (Allen 1989):Chl. a = 10.3 $E_{663}$ − 0.918 $E_{644}$; Chl. b = 19.7 $E_{644}$ − 3.87 $E_{663;}$ and Carotenoids = 4.2 $E_{452}$ − (0.0264 Chl. a + 0.426 Chl. b) where E is the absorbance at the definite wavelength (nm).

For nutrients and TM analysis, three composite dry samples of roots, shoots, and seeds (1 g) were digested by a mixed-acid digestion method (Lu 2000). The concentration of Cd, Pb, Cu, Cr, Fe, Zn, Ni, and Mn in plant samples was determined by Atomic Absorption Spectrophotometer (DW-AA320N)., while nutrients (P, N, and K) were determined as mentioned in Section 2.3. The bioaccumulation factor (BF), measuring the plant's ability to accumulate a specific TM to its concentration in the soil, was calculated as follows: BF = $C_{root}$ / $C_{soil}$, where $C_{root}$ and $C_{soil}$ denote the concentrations of TM in the root and soil. The translocation factor (TF) indicated the relative transference of TM from root to shoot of the plant and was calculated as TF = $C_{shoot}/C_{root}$, where $C_{shoot}$ and $C_{root}$ denote concentrations of the TM in the plant shoot and root (Farahat et al. 2017).

Total soluble proteins and carbohydrates were measured spectrophotometrically (PASCO- PS6200) by using Bio-Rad protein assay and the anthrone-sulfuric acid methods, respectively (Lowry et al. 1951; Umbriet et al. 1959, respectively).

## 2.5 Statistic Analysis

The dissimilarities among soil, water, and plant variables were assessed using a paired-sample *t* test. Besides, one-way analysis of variance (ANOVA-1) was used to evaluate the significant variation in nutrients and TMs between the different parts of the plant using the SPSS program (SPSS 2006).

## 3 Results

### 3.1 Soil and Water Analysis

As appeared from Table 1, irrigation with untreated agricultural wastewater had a significant negative effect on the chemical characters of the soil. The pH value, salinity (expressed as EC: 5.8 μS $cm^{-1}$), and TM concentrations had a significant ($P < 0.001$ and $P < 0.01$) increase for soil irrigated with wastewater. In contrast, the nutrients (N, P, and K) had significantly decreased ($P < 0.001$) as a result of irrigation with wastewater. The studied soil had a neutral pH (pH = 7.4) and reasonable salinity (2.6 μS $cm^{-1}$); using of wastewater in irrigation turned the soil to be alkaline (pH = 8.6) and more salinized (EC = 5.8 μS $cm^{-1}$) (Table 1).

Generally, all nutrients exhibited a significant decrease in the soil irrigated with wastewater compared with control soil (irrigated with tap water). Concentrations of N, P, and K decreased from 272.8, 18.1 and 455.3 mg $kg^{-1}$ in the control soil to 59.6, 5.9, and 34.5 mg $kg^{-1}$ in soil irrigated with wastewater (Table 1).

At the same time, the concentration of all studied TMs was significantly increased as a result of irrigation with wastewater. The percentages of cumulative were as follows: Pb, about 100-fold, Cd, about fourfold; Cr, about 373-fold; Cu and Mn, 22.5-fold; Ni about fivefold; Fe about 38-fold; and Zn, about 63-fold. The order of cumulative of TM concentrations was Fe > Cr > Mn > Pb > Zn > Cu > Ni > Cd in the wastewater-irrigated soil (Table 1).

Also, the PLI indicated that soil irrigated with wastewater was loaded with a high concentration of TMs; Cr had a higher loading percent (373.3) followed by Pb (105.5), then Fe (38), and Cu (22.7). The concentration of all TMs except for Cu and Ni in the soil irrigated with wastewater is above the tolerant level, according to WHO (1996) (Table 1).

Data in Table 2 indicated that the wastewater was slightly alkaline (pH = 7.8) and salty (EC = 5.8 μS $cm^{-1}$) and had a high amount of chemical and biological oxygen demand (COD = 647 mg $L^{-1}$) and (BOD = 316 mg $L^{-1}$), respectively, compared with tap water. In addition, there were high significant values of TM concentrations than that of tap water, and all the studied TM concentrations were above the tolerant level, according to WHO (1996) and Environmental Protection Agency (US-EPA 2006) (Table 2).

### 3.2 Growth Parameters

The data listed in Table 3 showed that plants irrigated with wastewater had a highly significant reduction in all its growth parameters. The percentage of decrement was as follows: germination percentage, 30%; average no. of lateral branches per individual, 21.6%; no. of leaves per individual, 19.5%; no. of flowers per individual, 75%; no. of fruits per individual, 32%; no. of seeds per legume, 68.8%; root length, 10%; root fresh weight, 50%; root dry weight, 40%; shoot length, 19.6%; shoot fresh weight, 37.1%; shoot dry weight, 65.2%; weight of fruit per plant, 55.6%; weight of fresh seeds per legume, 79.6%; and dry seeds weight per legume, 70.2%, in plants irrigated with wastewater compared with control (plants irrigated with tap water).

**Table 1** Soil characteristics and pollution load index (PLI) of soil irrigated with untreated agricultural wastewater. SE: standard error, *t*-values are provided. Tolerable limits according to the World Health Organization (1996). *$P < 0.01$ and **$P < 0.001$

| Soil characters | Soil irrigated with tap water (control) | | Soil irrigated with waste water | | *t-value* | Tolerable Limits | PLI |
|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | | | |
| PH | 7.40 | ± 0.005 | 8.60 | ± 0.02 | 37.90** | – | – |
| EC μS $cm^{-1}$ | 2.60 | ± 0.01 | 5.80 | ± 0.03 | 78.60** | – | – |
| N (mg $kg^{-1}$) | 272.80 | ± 0.10 | 59.60 | ± 0.90 | 2332.00** | – | – |
| P | 18.1 | ± 0.02 | 5.90 | ± 0.10 | 125.90** | – | – |
| K | 455.30 | ± 0.8 | 34.50 | ± 0.5 | 532.30** | – | – |
| Cd | 0.32 | ± 0.00 | 1.40 | ± 0.012 | 14.60* | 0.02–0.7 | 4.38 |
| Cu | 2.60 | ± 0.03 | 58.90 | ± 0.35 | 45.30** | 0.27–100 | 22.7 |
| Cr | 0.21 | ± 0.005 | 78.40 | ± 0.012 | 23.90* | 5–30 | 373.3 |
| Fe | 4.50 | ± 0.012 | 171.0 | ± 1.5 | 121.20** | 0.15–7 | 38 |
| Mn | 3.40 | ± 0.12 | 76.0 | ± 0.52 | 56.20** | 20.0 | 22.4 |
| Ni | 0.54 | ± 0.006 | 2.60 | ± 0.005 | 12.10* | 5.0 | 4.8 |
| Pb | 0.67 | ± 0.006 | 70.70 | ± 0.866 | 56.80** | 0.01–50 | 105.5 |
| Zn | 0.00 | ± 0.006 | 63.0 | ± 2.54 | 19.30* | 10–50 | – |

PLI = Cp / Cn, where Cp and Cn represent the trace metal concentrations in the soil irrigated with untreated agricultural wastewater and soil irrigated with tap 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 (US-EPA 2006). *$P < 0.01$ and **$P < 0.001$

| Water Character | Tap water (control) | Wastewater | | *t-value* | Tolerable limits |
|---|---|---|---|---|---|
| pH | 7.3 ± 0.017 | 7.8 | ± 0.01 | 12.5* | – |
| EC (μS cm$^{-1}$) | 2.01 ± 0.13 | 5.8 | ± 0.17 | 124.6** | – |
| COD mg L$^{-1}$ | 6.1 ± 0.17 | 647 | ± 0.0005 | 113.8** | – |
| BOD | 2.76 ± 0.04 | 316 | ± 0.03 | 17.4* | – |
| Cd | 0.00 | 2.6 | ± 0.02 | 57.3** | 0.01 |
| Cu | 0.00 | 32.1 | ± 0.20 | 71.1** | 2.0 |
| Cr | 0.00 | 48.2 | ± 0.40 | 32,9** | 0.1 |
| Fe | 0.01 ± 0.005 | 88.3 | ± 0.6 | 68.7** | 0.3–1.0 |
| Mn | 0.03 ± 0.002 | 29.9 | ± 0.3 | 26.5** | 0.05 |
| Ni | 0.00 | 0.89 | ± 0.002 | 12.6* | 0.025 |
| Pb | 0.00 | 55.6 | ± 0.5 | 75.3** | 0.05 |
| Zn | 0.00 | 39.1 | ± 0.33 | 18.6* | 5.0 |

## 3.3 Plant Analysis

### 3.3.1 Photosynthetic Pigments

The photosynthetic pigments analysis of pea leaves shows that there was a significant reduction in chlorophyll a and b and there was a non-significant reduction in carotenoids for the plant leaves irrigated with wastewater (Fig. 1). Chlorophyll a was declined by 40.9%, chlorophyll b by 36.4%, and carotenoids by 40.5%.

### 3.3.2 Plant Nutrients

In pea roots, the percentage of N and P was decreased from 2.8 to 1.8% and from 1.8 to 0.9% for plants irrigated with wastewater, respectively (Fig. 2a). In addition, K was decreased in roots of plants irrigated with wastewater by 36.4% (Fig. 2b). In pea shoots, the percentage of N and P was decrement from 3.3 to 2.18% and from 2.3 to 1.8% for plants irrigated with wastewater, respectively (Fig. 2a), and the shoot concentration of K decreased by 7.9% (Fig. 2b).

As shown in the results presented in Fig. 2c, the concentration of carbohydrates and proteins of the roots and shoots of pea plants irrigated with raw wastewater was significantly decreased compared with the control plants. In pea root, the concentration of carbohydrates decreased by 31.9%, while proteins decreased by 14.6%. In pea shoot, the concentration of carbohydrates was reduced by 19.9%, while proteins reduced by 15.5% (Fig. 2c).

### 3.3.3 Trace Metals

From results in Table 4, irrigation with untreated wastewater caused a highly significant increase ($P < 0.001$) in all studied

**Table 3** Growth parameters (mean ± SE) of *Pisum sativum* L. irrigated with tap water (control) and untreated agricultural wastewater (treatment). ns: not significant (i.e., $P > 0.05$), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$

| Growth parameters | Control plant | | Wastewater irrigated plant | | *t*-value |
|---|---|---|---|---|---|
| Germination (%) | 85 | | 55 | | 147.7$^{***}$ |
| No. of lateral branches (No. ind$^{-1}$) | 9.25 | ± 0.69 | 7.25 | ±0.50 | 34.6$^{***}$ |
| No. of leaves per plant (No. ind$^{-1}$) | 10.25 | ±1.50 | 8.25 | ±0.70 | 25.5$^{ns}$ |
| No. of flowers per plant (No. ind$^{-1}$) | 4.0 | ±0.55 | 1.0± | ±0.01 | 3.4$^{ns}$ |
| No. of fruits per plant (No. ind$^{-1}$) | 2.50 | ±0.42 | 1.7 | ±0.38 | 23$^{**}$ |
| No. of seed per legume (No. legume $^{-1}$) | 5.25 | ±0.53 | 1.64 | ±0.5 | 180.4$^{***}$ |
| Root length (cm ind. $^{-1}$) | 7.50 | ±0.20 | 6.75 | ±0.34 | 27.7$^{**}$ |
| Root fresh wt. (gm ind. $^{-1}$) | 0.18 | ±0.36 | 0.09 | ±0.05 | 5.9$^{ns}$ |
| Root dry wt. (gm ind. $^{-1}$) | 0.05 | ±0.011 | 0.03 | ±0.01 | 7.0$^{ns}$ |
| Shoot length (cm ind. $^{-1}$) | 37.0 | ±0.67 | 29.75 | ±1.32 | 122.0$^{***}$ |
| Shoot fresh wt. (gm ind. $^{-1}$) | 3.40 | ±0.30 | 2.14 | ±0.36 | 43.0$^{**}$ |
| Shoot dry wt. (gm ind. $^{-1}$) | 1.12 | ±0.19 | 0.39 | ±0.05 | 124.7$^{***}$ |
| Fruit wt. (gm ind. $^{-1}$) | 4.12 | ±0.42 | 1.83 | ±0.40 | 88.1$^{***}$ |
| Fresh seed wt. per legume (gm legume $^{-1}$) | 2.45 | ±0.081 | 0.50 | ±0.04 | 65.8$^{***}$ |
| Dry seed wt. per legume (gm legume $^{-1}$) | 0.57 | ±0.042 | 0.17 | ±0.03 | 18.1$^{**}$ |

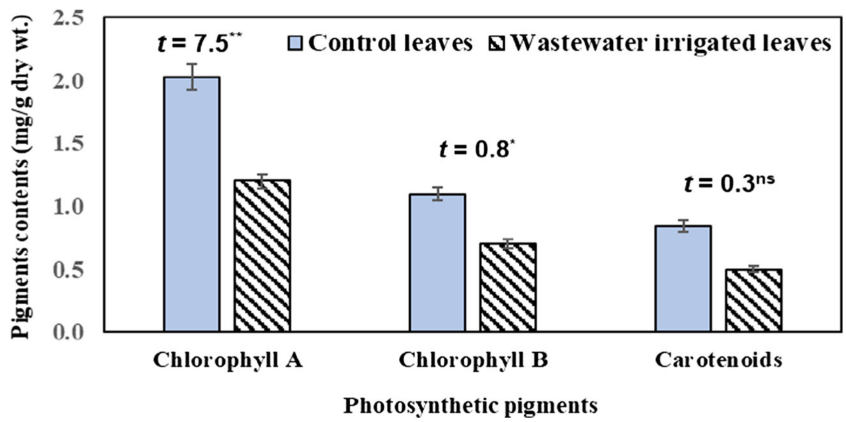


**Fig. 1** Photosynthetic pigments analysis of the leaves of *Pisum sativum* L. irrigated with tap water as a control and untreated agricultural wastewater. Values are significant at * $P < 0.05$ and ** $P < 0.01$ and ns: not significant using a paired-sample *t* test

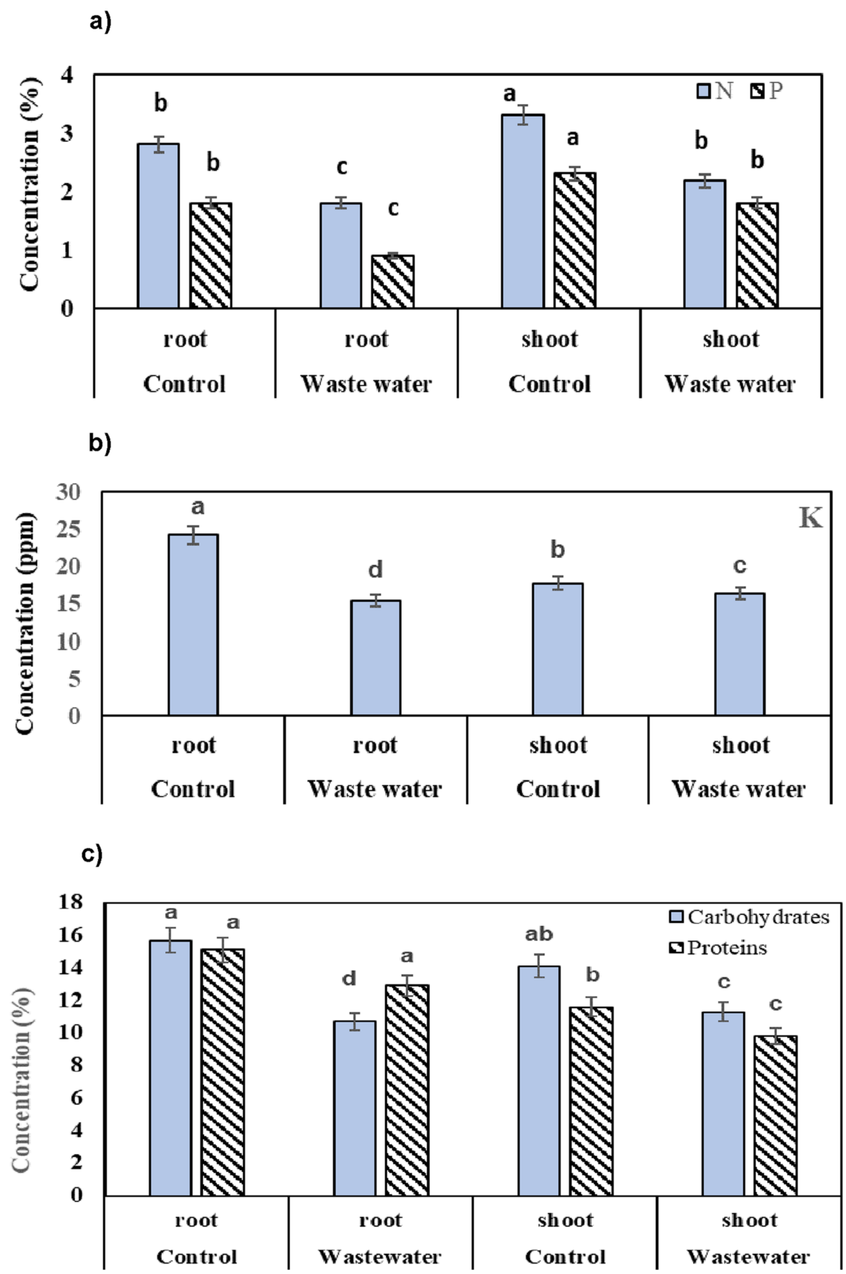


**Fig. 2** Nitrogen (N), phosphorous (P), potassium (K), and organic nutrient (total carbohydrates and protein) concentrations in the root and shoot of *Pisum sativum* L. irrigated with tap water as a control and untreated agricultural wastewater. Means with the same letters are not significant according to Duncan's multiple range tests. Bars represent standard errors

TM concentrations in pea roots and shoots compared with control. Compared with the pea roots, the concentrations increase as follows: Pb, 66.1-fold; Cd, 59.1-fold; Cr, 23.7-fold; Cu, 32.1-fold; Ni, 4.1-fold; Fe, 25.1-fold; Mn, 21.7-fold; and Zn, 81.1-fold (Table 4). Compared with the pea shoot, TM concentrations increased as follows: Pb, 90.1-fold; Cd, 260-fold; Cr, 69-fold; Cu, 78.2-fold; Ni, 43-fold; Fe, 33.1-fold; Mn, 6.7-fold; and Zn, 122-fold (Table 4).

In addition, irrigation with untreated wastewater caused a highly significant increase ($P < 0.001$) in all determined TM concentrations in pea seeds (the edible part) compared with plants irrigated with tap water (Table 5). The results showed that Fe was the highest TM accumulated in the seeds of the plant irrigated with wastewater, followed by Pb, Cd, Mn, Zn, Cr, Ni, and Cu. It was a worth notice that the concentration of Cd, Cr, Fe, and Pb was in the phytotoxic range, according to Kabata-Pendias (2010).

### 3.4 Trace Metal Bioaccumulation and Translocation

Generally, pea plants irrigated with untreated wastewater had TMs BF values greater than one except for Cr and Cu, while TF values were higher than one except for Mn and Zn (Table 6). The results showed that Cd had the highest BF (74.93) in plants irrigated with wastewater, followed by Ni (11.45) and Fe (8.46). The highest TF value was recorded in Cd (1.76), followed by Cr (1.46), then Fe and Ni (each of 1.45), and finally Cu (1.34) and Pb (1.25).

## 4 Discussion

Trace metals are non-biodegradable and accumulated in the soil to the lethal levels and persistent in the environment by enduring use (Zhuo et al. 2019). In the present study, the harmful effect of irrigation with wastewater was manifested on soil and plant characteristics. Irrigation with wastewater significantly increases soil salinity (EC), pH, and loading soil with TMs (Table 1). These findings were in agreement with Ahmed and Slima (2018) and Mojiri (2011) who reported that the application of wastewater in irrigation often increases salinity, soil hydrogen reaction (pH), and the concentration of TMs that may cause environmental and human risks for the long term. High soil pH decreases TM solubility in soil solution (Shehata and Galal 2020). In the present study, wastewater irrigated soil had PLI higher than 1 with the highest value (373.3 and 105) for Cr and Pb, respectively. Li et al. (2015) indicated that PLI values higher than one are an indication for contamination, while PLI values smaller than one indicate that TMs loading is close to secure levels. Also, a PLI value greater than 100, meaning heavily contaminated soil, which needs rapid interference to be safe (Shehata and Galal 2020). The TMs concentration in soil irrigated with wastewater (except Cu and Ni), and untreated agricultural wastewater was above the secure level, according to US-EPA (2006) and WHO (1996).

The maximum limit of BOD and COD in wastewater used for irrigation was 125 mg $O_2$/L, according to the Romanian Law (2005). In the present study, the analysis of wastewater used in irrigation revealed that it contains high values of BOD and COD (316 and 647, respectively), which shows high organic load and it becomes unsuitable for irrigation, drinking, or any other human uses. According to Abdel Wahaab (2015) and AbuZeid and Elrawady (2014) this effluent water belonged to Grade D (BOD ≤ 350). This code is prohibited from utilizing treated wastewater in watering any food crops (vegetables, field crops, all sorts of fruits, and medicinal plants).

On the other side, a significant decrease in soil nutrients was detected in the present study. Many studies reported that there was a significant decrease in the content of N, P, and K of soil irrigated with wastewater (Farahat et al. 2017; Galal 2016; Galal and Shehata 2015a; Galal et al. 2018). Singh and Kalamdhad (2011) reported that TMs cause changes in the activity of the soil microbial community, which led to a reduction in soil nutrients and organic matter by decreasing the

**Table 4** Trace metal concentrations (mean ± SE) in the shoot and roots of *Pisum sativum* L. irrigated with tap water as a control and untreated agricultural wastewater

| Trace metal | | Control plant | | | | Wastewater irrigated plant | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoot | | Root | | Shoot | | Root | |
| Cd | (mg kg$^{-1}$) | 0.72 | ± 0.08 c | 1.80 | ± 0.17 c | 187.30 | ± 2.25 a | 106.3 | ± 1.70 b |
| Cu | | 0.45 | ± 0.08 c | 0.82 | ± 0.11 c | 35.20 | ± 4.20 a | 26.3 | ± 1.60 b |
| Cr | | 0.60 | ± 0.03 d | 1.20 | ± 0.17 c | 41.40 | ± 4.70 a | 28.40 | ± 3.60 b |
| Fe | | 63.5 | ± 20.4 c | 57.50 | ± 12.3 d | 2101.30 | ±72.50 a | 1444.80 | ± 28.80 b |
| Mn | | 11.4 | ± 0.52 c | 10.00 | ± 2.5 ac | 76.70 | ± 2.9 b | 216.5 | ± 1.20 a |
| Ni | | 1.0 | ± 0.023 d | 7.20 | ± 1.3 c | 43.10 | ± 0.75 a | 29.80 | ± 2.80 b |
| Pb | | 1.90 | ± 1.04 c | 2.10 | ± 0.23 d | 172.70 | ± 5.60 a | 138.70 | ± 6.10 b |
| Zn | | 0.40 | ± 0.70 c | 1.10 | ± 0.40 d | 48.80 | ± 2.50 b | 89.20 | ± 1.80 a |

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

**Table 5** Trace Metal concentrations (mg $kg^{-1}$) in seeds (edible part) of *Pisum sativum* L. irrigated with tap water as control and untreated agricultural wastewater. **P* < 0.01 and ***P* < 0.001. Phytotoxic range according to Kabata-Pendias (2010)

| Trace metals | Control seed | | Wastewater irrigated seed | | *t*-value | Phytotoxic range |
|---|---|---|---|---|---|---|
| Cd | 0.53 | ± 0.09 | 95.6 | ±1.2 | 232.9** | 5–30 |
| Cu | 0.35 | ± 0.04 | 9.73 | ±0.52 | 65.54** | 20–100 |
| Cr | 0.76 | ± 0.22 | 29.8 | ±0.64 | 48.4** | 10–100 |
| Fe | 423.5 | ± 25.4 | 1123.6 | ±19.8 | 37.8** | > 500 |
| Mn | 8.4 | ± 0.96 | 82.2 | ±0.81 | 44.3** | > 500 |
| Ni | 0.81 | ± 0.18 | 26.82 | ±0.63 | 38.7** | 40–246 |
| Pb | 6.9 | ± 1.12 | 131.4 | ±1.32 | 78.710** | 30–300 |
| Zn | 2.0 | ± 0.92 | 35.7 | ±1.42 | 8.3* | 100–400 |

number and activity of soil microorganisms. Also TMs have an indirect effect on soil enzymatic activities by their toxic impact on soil biota, which synthesizes soil enzymes (Shun-hong et al. 2009).

Soil pH affects not only TM bioavailability but also its uptake by the roots of the plant (Galal and Shehata 2015b). Lasat (2000) reported that Cd and Zn became more available for the plant uptake than other TMs in high soil salinity and pH. In the present study, peas irrigated with untreated agricultural wastewater had high concentrations of all studied TMs in its root, shoot, and seeds (edible part). This result is reinforced by the results obtained by Ahmed and Hanafy (2017), who reported a significant increase of Zn, Cd, Ni, and Cu in pea plants irrigated with wastewater, and this is possibly due to the high levels of these TMs in the wastewater.

In the present study, the germination percentage was decreased in seeds irrigated with wastewater (Table 3). Shrestha (2000) stated that the presence of different substances in the wastewater as a result of pollutions as acids, alkali, TM salts, phenolics, fluorides, and others inducing in an adverse matter on the seed germination. Moreover, during water imbibition, the dissolved toxicants enter the seed, affecting some physiological processes in the germination of seeds and may decrease osmotic entry of water as a result of high TMs concentration and other solutes (Timsina 1988). Sethy and Ghosh (2013) reported that Ni decreases amylase, protease, and ribonuclease enzyme activity, thus delaying the seed germination and growth of many crops. Also, Cu causes oxidative stress by the generation of ROS, Cd causes also delaying in seed germination, and Pb strongly affects the morphology and physiology of seeds. This finding is supported by many studies (Ahmed and Slima 2018; Eissa and Negim 2018; Khalilzadeh et al. 2020; Shehata and Galal 2020).

Trace metals are toxic for plants resulting in weak plant growth and yield reduction (Singh and Kalamdhad 2011). In the present study, a significant decrease in growth parameters was recorded (Table 3). The negative effect of TMs on root length was due to the reduction in water absorption ability (Farid et al. 2013). Also, it may be related to TMs-inhibition of root protein synthesis (Drazkiewicz and Baszyński 2005). Hassanein et al. (2013) recorded a significant reduction in the growth parameters as leaf area, root, and shoot fresh and dry weights of lettuce (*Lactuca sativa* L.) and turnip (*Brassica napus* L.) due to the irrigation with wastewater. Bini et al. (2012) reported that wastewater containing TMs had a toxic effect on the shoot and fresh root weights, which may be attributed to the lessening of water uptake and inhibition uptake of nutrients, which cause inhibition of normal plant

**Table 6** Bioaccumulation (BF) and translocation (TF) factors for trace metals in *Pisum sativum* L. irrigated with tap water as control and untreated agricultural wastewater

| Trace metal | Bioaccumulation (BF) and translocation (TF) | | | |
|---|---|---|---|---|
| | Tap water irrigated plant | | Untreated agricultural wastewater Plant | |
| | BF | TF | BF | TF |
| Cd | 5.63 | 0.40 | 74.93 | 1.76 |
| Cu | 0.32 | 0.60 | 0.45 | 1.34 |
| Cr | 5.7 | 0.50 | 0.36 | 1.46 |
| Fe | 12.77 | 1.10 | 8.46 | 1.45 |
| Mn | 2.9 | 1.14 | 2.85 | 0.35 |
| Ni | 13.3 | 0.14 | 11.45 | 1.45 |
| Pb | 3.1 | 0.91 | 1.96 | 1.25 |
| Zn | – | 0.36 | 1.42 | 0.55 |

BF = $C_{root}$ / $C_{soil,}$ where $C_{root}$ and $C_{soil}$ represent the trace metal concentrations in the root and soil and TF = $C_{shoot}$ / $C_{root,}$ that $C_{shoot}$ and $C_{root}$ denote the trace metal concentrations in the plant shoot and root growth. Wastewater causes adverse effects on plant growth by several causes as osmotic injury (Briccoli et al. 1994) and toxicity of TMs (Zeid and Abou El Ghate 2007).

Photosynthetic pigments (chlorophylls and carotenoids) had an essential role in plants. They are responsible for photosynthesis. The biosynthesis of photosynthetic pigments may be inclined to several biotic and abiotic stresses, including TM stress (Myśliwa-Kurdziel and Strzałka 2002). The presence of TMs in the wastewater used in the irrigation of plants causes terrible damage to the chloroplast machinery of the plant leaves (Ahmed and Hanafy 2017). This trend was observed in the present study by a decline in the content of chlorophyll a and b and carotenoids. These results are armored by the results of Maleva et al. (2012), who reported that Mn and Cu caused the high reduction of chlorophyll content, while Cu caused the most significant decline in carotenoids. A high concentration of Zn in wastewater was the main reason for the decrease in chlorophyll (Hajihashemi et al. 2020). This trend is supported by several studies (Ahmed and Slima 2018; Farahat et al. 2017; Galal and Shehata 2015a; Khalilzadeh et al. 2020; Shehata and Galal 2020).

The presence of a high concentration of TMs in wastewater may compete with the absorption of essential nutrients leading to their deficiency in the plant tissues. The plant inorganic (N, P, and K) nutrients estimated in the roots and shoots in the present study were significantly decreased. These results are armored by the results of Shukry (2001), who reported a decrease in total N and protein-N in *Triticum aestivum* L. and *Vicia faba* L. plants irrigated with contaminated wastewater (industrial effluents) which contaminated the Nile River. Also, Zafar et al. (2016) noted a significant decrease in the total N and total protein in *Brassica oleracea* and *Spinacia oleracea* crops irrigated with untreated wastewater. Singh and Kalamdhad (2011) reported that TMs are toxic for plants resulting in reduced nutrient absorption and the ability to fix nitrogen in leguminous plants.

Trace metals accumulated in plant tissues may control their carbohydrates content (Ahmed and Slima 2018). Galal (2016) reported a decrease in carbohydrate contents of *Cucurbita pepo* L. cultivated in polluted soil as a result of TM accumulated in their tissues. Similarly, TMs accumulated in plant tissues also led to stop or decrease the synthesis of some proteins (John et al. 2008). The present study recorded decreases in the contents of carbohydrates and proteins in the tissues of wastewater irrigated peas plants. The decline in protein content may be as a result of increased protein breaking down as a result of the additional activity of protease enzyme (Palma et al. 2002), which increases under stress conditions. Farahat et al. (2017) indicated that irrigation with trace metals contaminated wastewater led to a significant decrease in both organic (carbohydrates and proteins) and inorganic nutrients (N, P, and K) in roots and leaves of *Triticum aestivum* and *Zea mays*.

Different plant species appear different ability to uptake and accumulate TMs. This ability is ascribed to distinctive soil characteristics (soil TM concentrations and soil pH) and physiology of plant promoting the TMs transference from soil to plant and their translocation to shoot parts (Eltaher et al. 2019). TMs translocation from root to shoot was a vital physiological process influence on plants consumption in eating (Galal and Shehata 2016). Two biological indices were used to evaluate the TMs accumulation capacity of Pea (Galal and Shehata 2015b). The BF assessed the plants capability growing in polluted soil to gather TMs in their roots, and the TF assessed plants ability to transfer TMs from roots to shoots (Galal and Shehata 2016).

The difference in the obtained BF and TF values influenced by the TMs-binding capacity with roots, TMs accessibility, interactions between physicochemical parameters and the plant grown in these soils, which might affect the uptake efficiency of TMs (Mahmoud and Ghoneim 2016). Plant species with (BF values $> 1$ and TF values $< 1$) can be used in phytostabilization (Yoon et al. 2006). In the current study, the BF values are $> 1$ for Mn and Zn, while their TF values are $< 1$, indicating that pea accumulated high concentrations of these TMs in their roots and did not translocate them to the shoots, and this may lead to the reduction in root growth. Plants with TF values $> 1$ and BF values $< 1$ translocate metals from their roots to shoots (Eltaher et al. 2019); hence pea translocates Cu and Cr to their shoots including seeds (edible part). Also, Pb, Cd, Ni, and Fe showed BF and TF values higher than one, indicating that pea was efficiently conveying these TMs from roots to shoots (Mganga 2014). It is worth to notice that the concentrations of Pb, Cd, Cr, and Fe are within the phytotoxic range (Kabata-Pendias 2010) in seeds (edible part), so it is not safe for human consumption.

## 5 Conclusion

This study presents a dangerous alarm for using untreated agricultural wastewater in irrigation of pea crops. It causes a decrease in soil nitrogen (N), phosphorous (P), and Potassium (K) and plant nutrients (N, P, K, carbohydrates, and protein) and at the same time causes accumulation of trace metals cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) in both soil and plant. Pea seeds (edible part) had a phytotoxic level of Pb, Cd, Cr, and Fe. Authors recommended that people should not eat pea seeds cultivated in fields irrigated with untreated agricultural wastewater, because it is not safe for human consumers or using for animal feeding.

### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

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