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Long-Term Irrigation with Treated Municipal Wastewater from the Wadi-Musa Region: Soil Heavy Metal Accumulation, Uptake and Partitioning in Olive Trees

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Abstract: Utilization of treated wastewater (TWW) for agricultural purposes has grown over the past few years because of limited available water resources. This study was performed to assess the long-term irrigation of treated wastewater from the Wadi-Musa region on the accumulation of heavy metals in soil and their uptake and translocation to various parts of olive trees. Fifteen year old trees that had been grown and irrigated with treated wastewater resources since their establishment were used in this study. Irrigation water, soil, and plant samples (root, stem bark, leaves, fruits) were collected and chemically analyzed for their heavy metal content. Accumulation of heavy metals in irrigation water and soil were found to be within the acceptable range for the safe use of treated wastewater according to the standards of the WHO. However, long-term and continuous irrigation with TWW resulted in significant accumulation of heavy metals in plant parts when compared to their levels in irrigation water and soil. Uptake of metals was consistent among plant parts with the highest concentrations for Fe, Mn, Pb and Zn, and the lowest concentrations for Ni, Cr and Cd. Assessment of the bioaccumulation factor (BFC) and translocation factors (TF) of heavy metals into different plant parts indicated selective absorption and partitioning of these heavy metals into different plant parts. High BCF values were observed for Fe, Cu and Ni in roots and fruits, and Fe, Mn, Cd and Pb in leaves. Translocation factors of metal ions were variable among plant parts. Fruits had the highest TF for Cu, Cd and Zn metals, and the lowest for Mn and Fe, while leaves have the highest TF for Fe, Zn and Mn and the lowest for Cd and Pb. The results of this study indicate that olive trees are heavy metal accumulators, caution should be considered in long-term use of TWW and periodic assessment of possible hazards, especially on fruits and oil quality is required.

Keywords: bioconcentration factor; heavy metals; irrigation; *Olea europaea* L.; translocation factor; treated wastewater

1. Introduction

Jordan is considered one of the countries most affected by climate change, with a marked decrease in precipitation over the past few decades [1]. This has increased the negative impact of drought and water shortage on crop growth and productivity of major agronomic crops [2,3]. Jordan is considered the fourth poorest country in the world in terms of water resources [4]. Overuse of water resources due to increased population growth and the influx of refugees has resulted in a large depletion over the last few decades. The water deficit is estimated to increase from about 160 million m^3 in the year 2015 to 490 million m^3

by the year 2025 [5]. Climate change and changing precipitation patterns will aggravate the problem.

Sustainable utilization of water resources necessitates the utilization of alternative water resources such as treated wastewater, especially for irrigation purposes to minimize the damage of depleting freshwater resources [6]. Supplementary irrigation has been considered a major factor for increasing crop productivity in arid and semiarid environments [7]. Farmers have developed techniques for the use of marginal water, such as treated wastewater (TWW), as a supplementary source of irrigation water. Department of statistics records indicate that about 160 million m^3 of TWW was used in 2018, mainly for irrigation purposes, which represents about 14% of total water consumption [8]. Whereas TWW can be used at reasonable rates to improve plant growth and production under drought conditions [9], the main challenge of using TWW is the high risk of soil pollution and phytotoxicity due to the hazardous accumulation of salts and heavy metals [10]. Heavy metal contamination has been reported in soils irrigated with TWW effluents in different regions across Jordan regardless of the low heavy metal content present in the effluents [11,12]. Contradicting results have been reported about increased levels of soil Pb, Cd, Mn and Cu [13], and low levels to moderate levels of Pb, Cu, Zn and Cr [14] along the Zarqa river basin in response to irrigation with TWW effluents.

There is a growing interest in the control of heavy metal accumulation in TWW and in the soil to prevent their uptake by plants and, therefore, prevent their transfer into the food chain and humans [15]. Heavy metals are highly toxic, and their presence in the atmosphere, soil and water, and their accumulation in the food chain, is considered dangerous to human health [16–18]. The olive tree (*Olea europaea* L.) is the most cultivated fruit tree in the Mediterranean basin. Jordan is among the top ten countries producing olives in the Mediterranean region, where 220 thousand tons of olive yield are harvested from 15 million trees in Jordan annually [19,20]. In Jordan, olives are primarily grown under rainfed conditions without supplemental irrigation; however, due to the increased impact of climate change through the increased incidence of drought episodes and increased temperatures, there is a growing need for the use of supplemental irrigation for maintaining crop productivity [21]. Olive trees are considered tolerant to adverse climatic conditions, characterized by their ability to withstand drought [22]. However, an increase in yield is usually obtained when supplementary irrigation is used [23]. Due to the limited freshwater resources in Jordan, olive farmers have been looking for alternative water resources that sustain crop requirements and yet have no negative impact on the environment [24]. Utilization of treated wastewater resources on various crop species, including olive trees, has been evaluated worldwide [25–29]. Plants differ in their absorption, translocation and accumulation of heavy metals in their tissues and organs. Accumulation of trace elements in various organs at concentrations higher than those present in the soil might indicate biohazardous accumulation of these metals, especially in edible parts [30–32]. Environmental pollution with heavy metals is a serious global concern. Uptake of heavy metals by plants from polluted water or soil might contaminate the food chain for both humans and animals. Heavy metals such as Cu, Cd, Cr, As, Pb and Ni were reported in olive oil in Cyprus at concentrations that can cause significant health hazards [33]. Furthermore, Spanish table olives were reported to contain significant levels of Cd, Co, Cr, Li, Ni, Pb and Sr [34]. In this perspective, olive trees are considered important heavy metal bioaccumulators in different plant parts for Cu, Pb and Zn [31]. These high levels of heavy metals such as Cd, Pb, Cr, Hg, and As were reported to have adverse effects on increased oxidative stress and cellular damage to both plants and animals [35]. Further negative effects on human health, such as kidney failure, diabetes, cardiovascular disease, neurotoxic effects, cancers and many other developmental disorders, were reported [36].

Heavy metal content in soils irrigated with TWW has been studied in several locations across Jordan [21,32]. However, less information is available about the irrigation of plants in the Wadi- Musa region. Furthermore, the fate of heavy metals in soils, and their absorption and bioaccumulation in olive plants in response to long-term irrigation with TWW effluents, has not been studied before in this region. Therefore, the present study aimed to explore the effects of long-term irrigation with TWW on the accumulation of heavy metals in soils, and of uptake and partitioning of selected heavy metals in olive trees.

2. Materials and Methods

2.1. Study Area, Soil, and Water Characteristics

This study was carried out at the Wadi-Musa Wastewater Treatment Plant (WMTP), 31 km to the north of the city of Maan (latitude 30°10′ N and longitude 35°47′ E with an elevation of 1069 m) near the ancient city of Petra. The Wadi Musa treatment plant handles all wastewater flows from four communities adjacent to the Petra Archaeological Park, with four pump stations located, respectively, in the communities of Wadi-Musa, Tayyibeh, Beidha and Umm Sayhoun [37].

The demonstration farm was established near the WMTP on an area of seven hectares, and an area of ~100 hectares is used for farming by local tribal communities. The demonstration area is cropped with fodder crops and fruit trees (almond, apricot, lemon, olives, pistachios, and date palm). The location is characterized by a typical eastern Mediterranean climate with low precipitation (annual rainfall average: 170 mm) and a hot dry summer with an average maximum temperature that exceeds 30 °C from June to September (Figure 1). The soil of the experimental site was classified as a typical sandy loam with pH and EC values of 7.5 and 1.75 dS m^{-1}, respectively, and 13.5 g kg^{-1} organic matter (OM) and 15 g kg^{-1} $CaCO_3$ (Table 1).

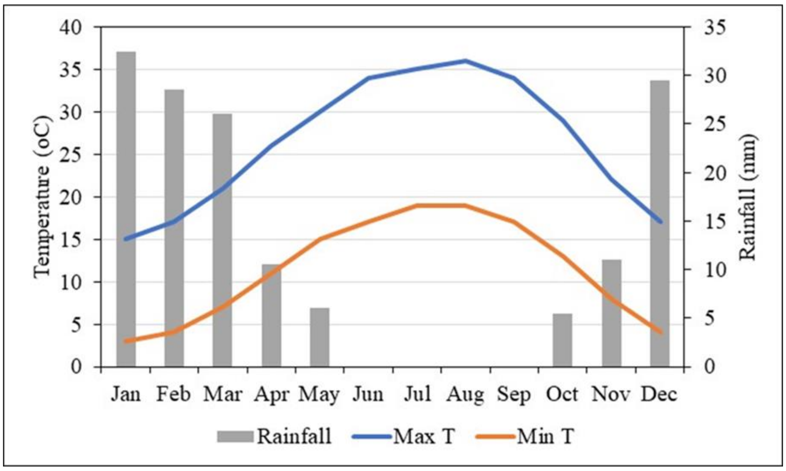

Figure 1. Long-term average monthly minimum and maximum temperatures and average monthly rainfall at the Wadi-Musa Wastewater Treatment Plant experimental site.

Table 1. Main physical and chemical properties of soil at the Wadi-Musa Wastewater Treatment Plant experimental site. The data are averages ± SD (n = 3).

Soil Analysis	Unit	Measurement
Sand	20–200 µm	78.4 ± 6.1
Silt	2–20 µm	12.7 ± 0.90
Clay	<2 µm	8.9 ± 0.71
Texture		Loamy-sand
EC	(dS m^{-1})	
pH		8.2 ± 0.52
OM	(g kg^{-1})	13.5 ± 0.97
$CaCO_3$	(g kg^{-1})	15.0 ± 1.04

The study was conducted on 15-year-old olive trees (*O. europaea* L. cv. 'Nabali Baladi') cultivated with a density of 420 trees/ha. Trees were irrigated continuously over the past 15 years with treated wastewater effluents from the treatment plant. The trees are irrigated twice a week using a drip irrigation system with an average total water supply of 1300 m^3 ha^{-1} $year^{-1}$. Samples of treated wastewater effluents used for irrigating olive trees during 2020 growing season were collected and their average physiochemical analysis is shown in Table 2. Analysis of effluents indicated that irrigation water pH, EC and biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), sodium adsorption ratio (SAR) and total coliforms count (MPN/100 mL) lie within the standards of Jordan Standards and Metrology Organization [38], and the safe use of treated wastewater for irrigation by the World Health Organization [39]. Bacterial load in treated wastewater was below the levels that restrict their use for irrigation purposes [38,39].

Table 2. Physiochemical analysis of treated municipal wastewater effluents at the Wadi-Musa Wastewater Treatment Plant during the growing season of 2020.

Parameter	TWW	JISM [1]	WHO [2]
pH	7.78	6.0–9.0	6.5–8.0
EC (dS m^{-1})	1.81	1.0–3.0	0.7–3.0
BOD (mg L^{-1})	10	60	300
COD (mg L^{-1})	28.3	120	500
TDS (mg L^{-1})	826.3	<2000	450–2000
SAR (ratio)	8.3	9	<13
Total Coliforms (MPN/100 mL)	<1.8	<10	<9

[1] JISM, Jordan Institution for Standard and Metrology; [2] WHO, World Health Organization. MPN, most probable number.

2.2. Sampling and Analysis

Random samples of the olive plant, soil and irrigation water were collected and prepared as described below for the analysis of the heavy metals iron, manganese, copper, zinc, cadmium, lead, chromium, and nickel.

2.2.1. Plant Sampling

Olive trees were sampled for their roots, stem bark, leaves and fruits in November 2020. The roots of each olive tree were sampled using a soil corer (five cm in diameter). The samples were taken 50–75 cm from the stems to a depth of about 0–50 cm. Roots around two 2 mm in diameter were washed under running tap water and dried at 60 °C for 48 h. Stem bark samples were also collected from the outer 3 mm of the olive tree trunk at 1.5 m above the ground level using a sharp knife. Collected stem bark materials were dried at 60 °C for 48 h. Fully mature leaf samples were collected from the upper 5 cm of one-year old twigs selected from different locations in the canopy. Collected leaf samples were dried at 60 °C for 48 h. Samples of dried roots, stem bark and leaves were ground using LFJ-20B Hammer grinder (Guangzhou Duoshun Machinery Co., Ltd., Guangzhou, China). The samples were sieved to a 2 mm mesh size and stored for chemical analysis. Fully ripened fruit samples were collected at maturity harvest time (green to purple stage). Fruits were dried at 75 °C to a fixed weight and crushed with an Abencor (MC2, Ingenieria Y Systems, Seville, Spain) hummer crusher. Prepared root, stem bark, leaves, and fruit samples were used for the analysis of Fe, Mn, Cu, Zn, Cd, Pb, Cr and Ni.

2.2.2. Irrigation Water and Soil Sampling

Irrigation water was sampled three times from June to November 2020 during the growing season. Irrigation water samples were collected from the drip irrigation lines nearest to the tree. Collected samples were filtrated before adding a few drops of concentrated HNO_3 and stored at 4 °C for chemical analysis. Random soil sampling was also collected at the experimental site at the same time as sampling plant parts. Samples were collected from the upper 0–30 cm using a soil corer (5 cm in diameter). The samples were taken

50–100 cm away from the main trunk. Soils were sieved through a 2 mm sieve and dried at 75 °C for 48 h before analyses of heavy metals according to [40].

2.2.3. Heavy Metals Analysis of Plant, Soil and Water

Soil, root, bark, and leaf samples were analyzed for Fe, Mn, Cu, Zn, Cd, Pb, Cr and Ni using the procedure of Estefan et al. [41]. Samples (0.5 g each of soil, bark, leaves and fruits) were digested in a mixture of four ml of HNO_3 (70%) and one ml of $HClO_4$ (62%) using a Microwave Labstation System model ETHOS 9000 equipped with a control unit terminal 240 [41]. Then, the digested samples were filtered through a 45-µm filter, and the final solutions were filled to 25 mL in volumetric flasks with 1% (v/v) HNO_3 solution. Subsequent, analysis of heavy metals was performed with an atomic absorption spectrophotometer (Analyst 200, PerkinElmer, Waltham, MA, USA). Wastewater samples were filtered through a 0.45 µm filter and acidified with HNO_3 (70%) and analyzed according to the methods of the soil, plant and water analysis manual by Estefan et al. [41].

2.2.4. Bioconcentration and Translocation Factors

The bioconcentration factor (BCF) and the translocation factor (TF) were used to determine uptake and translocation of various heavy metals into various olive tree organs as described by Mellem [42]:

$$\mathbf{BCF} = \frac{\textbf{concentration in organ}}{\textbf{concentration in soil}}$$

$$\mathbf{TF} = \frac{\textbf{BCF of bark or leaf or fruit}}{\textbf{BCF of root}}$$

2.3. Statistical Analysis

A completely randomized design with three replicates for each collected sample was used. A composite sample was prepared from four collected subsamples of plant, soil and water. The uptake and accumulation of various heavy metals in different parts of olive trees in response to long-term irrigation with treated wastewater were subjected to analysis of variance (type I ANOVA) using the Statistical Analysis System (SAS; Version 9.3 for Windows; SAS Institute, Cary, NC, USA), and standard deviations were used for means separation.

3. Results and Discussion

3.1. Chemical Analysis of Irrigation Water and Soil

The composition of heavy metals in irrigation water and soil samples are shown in Tables 3 and 4, respectively. No significant amounts of copper and manganese were detected in the TWW used for irrigation (Table 3). On the other hand, relatively high amounts of iron, lead and zinc were detected compared to cadmium, chromium and nickel. Based on JISM [38] and WHO [39] standards, the concentration of various ions in the irrigation water was within the acceptable range for the safe use of TWW. Results of the chemical analysis of TWW match those of other studied sites in Jordan, especially that in which municipal water is used and where no industrial effluents are being disposed [25,32,43].

The chemical analysis of soil samples showed that the contents of N, P, K, Na and Cl were considerably high suggesting some fertilizing effects in response to irrigation with TWW (Table 4). Such increase in ions content could be attributed to increased solubility and organic matter adsorption resulting from TWW irrigation [44–46]. For instance, the availability of P and K was correlated with higher organic matter content in TWW [45]. Such increases in nutrient availability in the soil is beneficial for crop growth and productivity [21,25], which might reduce the need for the application of commercial fertilizers. However, a noticeable accumulation of both Na and Cl in the TWW-irrigated soil was observed in this study (Table 4). Concentrations reached 345 and 749 mg·kg^{-1} for both Na and Cl, respectively. The high Na and Cl concentrations in the soil solution could be attributed to the high content of these ions in the TWW, and the antagonistic activity of other ions such as K that limit Na adsorption to the soil particles [44,47]. On the other hand, comparatively low soil heavy metals levels might suggest the possibility of higher plant uptake of these elements to the extent that their levels in soils may have been significantly lowered [48]. On the contrary, the higher levels of Fe, Mn, and Pb in the soil might suggest that crop uptake of these elements is not as high as others, or that TWW causes a buildup of soil heavy metal in cultivated soils [48].

Table 3. Chemical analysis of various nutrient and metal ions in TWW used to irrigate olive trees at the Wadi-Musa Wastewater Treatment Plant compared with allowable Jordanian (JISM) and WHO standard limits for irrigation water. The data are averages ± SD (n = 3).

Element	TWW	JISM [1]	WHO [2]
N (mg L^{-1})	11.9 ± 1.49	50	5–50
PO_4 (mg L^{-1})	15.2 ± 1.21	30	30
NO_3 (mg L^{-1})	38.9 ± 3.74	45	50
Ca (mg L^{-1})	81.2 ± 4.44	400	230
Mg (mg L^{-1})	14.6 ± 1.32	60	60
K (mg L^{-1})	34.2 ± 3.29	80	80
Na (mg L^{-1})	94.2 ± 5.31	230	69–207
Cl (mg L^{-1})	115.4 ± 8.36	400	140–350
Cr (mg L^{-1})	0.01 ± 0.002	-	0.02
Cu (mg L^{-1})	ND	-	0.2
Mn (mg L^{-1})	ND	-	0.2
Ni (mg L^{-1})	0.02 ± 0.002	-	0.2
Zn (mg L^{-1})	0.76 ± 0.14	2	<2.0
Fe (mg L^{-1})	3.79 ± 0.18	5	0.1–1.5
Cd (mg L^{-1})	0.002 ± 0.001	0.01	<0.01
Pb (mg L^{-1})	3.03 ± 0.2	5	<5.0

[1] JISM, Jordan Institution for Standard and Metrology; [2] WHO, World Health Organization; ND, not detected in samples.

Table 4. Soil chemical analysis of various nutrient and metal ions at the Wadi-Musa Wastewater Treatment Plant experimental site. The data are averages ± SD (n = 3).

Element	Measurement
N total (g kg^{-1})	13.3 ± 0.3
P (mg kg^{-1})	93.7 ± 7.03
K (mg L^{-1})	710 ± 44.0
Na (mg L^{-1})	345 ± 39.2
Cl (mg L^{-1})	749 ± 45.5
Cd (mg L^{-1})	0.15 ± 0.05
Cr (mg L^{-1})	0.18 ± 0.05
Cu (mg L^{-1})	0.28 ± 0.01
Fe (mg L^{-1})	2.22 ± 1.20
Mn (mg L^{-1})	3.45 ± 1.54
Ni (mg L^{-1})	0.16 ± 0.03
Pb (mg L^{-1})	1.56 ± 0.19
Zn (mg L^{-1})	0.78 ± 0.1

3.2. Effects of Irrigation with TWW on Heavy Metals Content in Olive Trees

Heavy metals analysis of leaves, roots, stem bark and fruits indicated noteworthy variations in response to continuous irrigation with TWW (Figure 2). Irrigation with TWW resulted in a significant accumulation of heavy metals in different parts of the olive tree. Variable ion contents were observed among different organs, and the highest concentration of Mn, Cd and Pb (Figure 2C,G,H) were observed in the leaves compared to other organs, while Cr content (Figure 2E) was the highest in the stem bark. Manganese content ranged from 177% to 641% of that in roots and fruits, respectively (Figure 2C). A similar trend was observed with Pb but with values ranging from 131–191% of that in the roots and fruits, respectively (Figure 2H). On the other hand, Cd was the lowest in roots and fruits compared to other parts (Figure 2G). The highest concentration of Cr was found in the stem bark (3.97 mg kg^{-1}) and was the lowest in the leaves (1.9 mg kg^{-1}) (Figure 2E). Roots were also found to have high Fe concentrations, which were higher by 183% than that in leaves and by 636% than that in fruits (Figure 2B). Fruits were also found to have the highest concentrations of Zn, Cu and Ni compared to other plant organs (Figure 2A,D,F). Moreover, Cd followed by Cr, Mn and Ni had the lowest concentration in fruits. In all plant parts, Cd, Ni and Cr had the lowest concentrations.

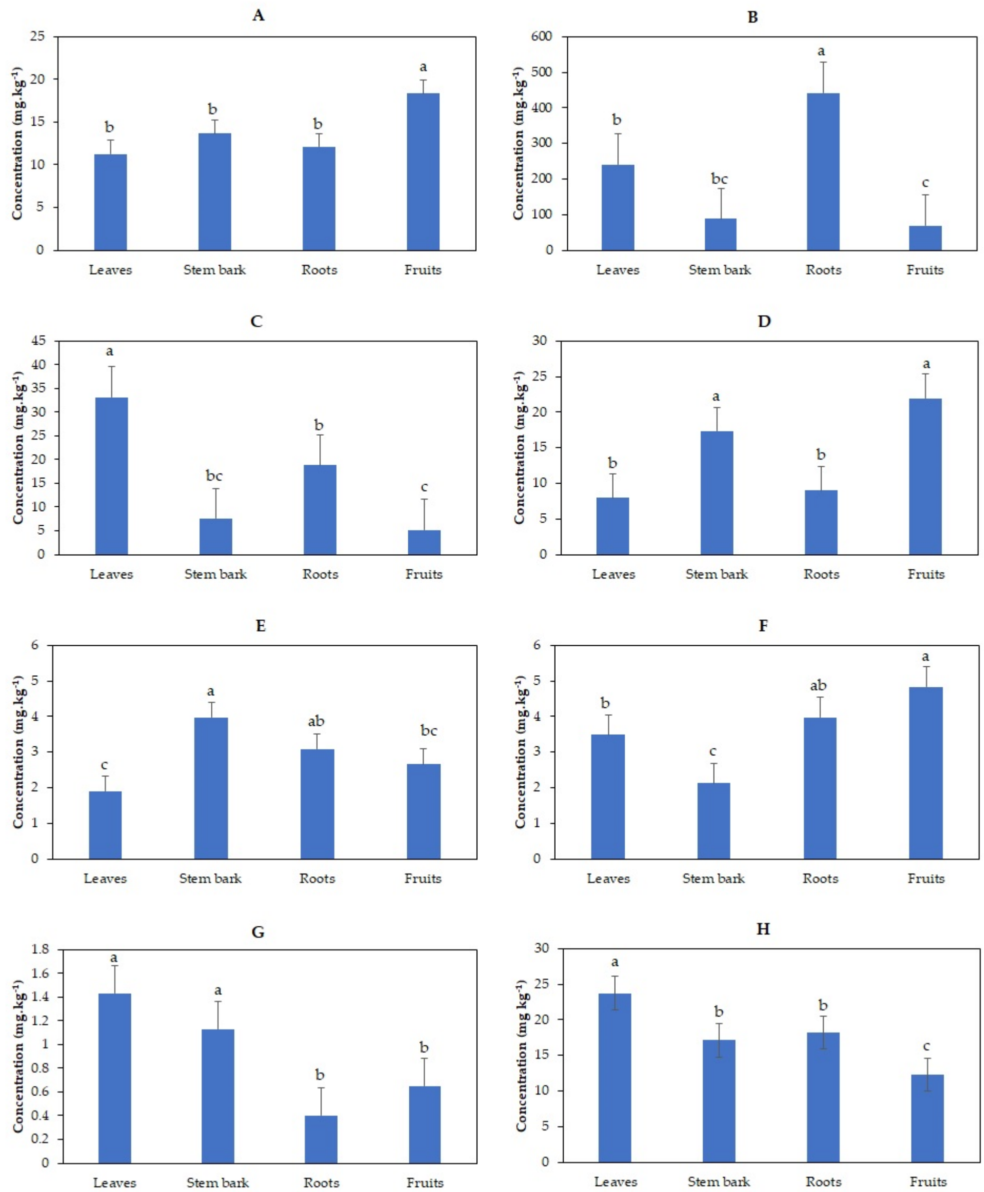

Figure 2. Concentration (mg kg^{-1}) of Zn (**A**), Fe (**B**), Mn (**C**), Cu (**D**), Cr (**E**), Ni (**F**), Cd (**G**), Pb (**H**) in the leaves, stem bark, roots and fruits of olive trees irrigated continuously with TWW at the Wadi-Musa Wastewater Treatment Plant experimental site. Bars represent mean ± SE. Different letters indicate a significant difference between plant parts by LSD ($p \leq 0.05$).

Irrigating crops with TWW usually raises concern about increased heavy metals uptake by plants [49]. The presence of heavy metals in TWW, especially in that of industrial effluent, tends to accumulate in the soils, where they are potentially bioavailable for crops [46,49,50]. Although the concentrations of heavy metal ions were found to be within local and international standards [38,39], a noticeable accumulation of these elements in soil and plants was found in response to long-term irrigation with treated wastewater in this study. The results of this study agree with those found by Al-Lahham et al. [51], who reported increased levels of Fe, Mn, Zn, Cu and Ni contents of TWW-irrigated tomato, which was associated with the increase in the quantity of municipal effluent used in irrigation.

The accumulation of various heavy metals in different plant parts of olive trees in the present study in response to irrigation with treated wastewater is typical for olives grown under such conditions [21,52]. Wilson and Pyatt [31] considered olive trees as important bioaccumulators of Cu, Pb, and Zn in their tissues and organs when grown in an area contaminated with these metals. Previous studies indicated increased Mn contents of Celosia in response to irrigation with treated effluents having high Mn levels [53].

3.3. Effects of Irrigation with TWW on Bioconcentration Factor of Heavy Metals in Olive Trees

The values of the BCF of heavy metals in different olive tree organs are shown in Figure 3. Bioconcentration factors of heavy metals greater than 1 were found for all elements in all studied organs. The BCF values of heavy metals in the roots were ranked as follows: Fe > Cu > Ni > Cr > Zn > Mn > Cd > Pb, with values ranging between 201.4 1.56 for Pb. Similarly, the accumulation of heavy metals was in the order of Cu > Fe> Cr >Zn > Ni > Pb > Cd > Mn in stem bark with BFC values reaching 40.1 to 61.4 for both Fe and Cu, respectively. It is clear that BCF in leaves was higher than in stem bark except for Zn and Cr. The highest BFC was for Fe (110.5), while the lowest BFC was for Cd (9.6) in olive leaves (Figure 3B,G). Fruits were also found to accumulate various heavy metals at various levels. BCF values ranged from 1.55 for Mn (Figure 3 C) to 77.7 for Fe in fruits flesh (Figure 3B).

BCF values higher than 1 are used as an index to ensure that the plant is an accumulator for the metal from the soil to the plant tissue [54]. The results of this study agree with those of Wilson and Pyatt [31], who found that the flesh of olive fruits accumulated the highest concentration of copper and lead. On the other hand, leaves accumulated more zinc than most other tissues [28]. Similar responses were observed for other species such as Salix trees, which have a high potential for translocating Cd and Zn from soil to their parts [54]. Other tree species such as *Paulownia fortunei* and *Broussonetia papyrifera* exhibit high BCF values in their tissues for metals such as Pb, Zn, and Cd [55].

The average BCFs were variable among different plant parts where higher BCF values of Fe and Cr (201.4, and 17.6, respectively) were observed in roots than in leaves (110.5 and 10.7, respectively) (Figure 3B,E). On the other hand, Pb (Figure 3H), Mn (Figure 3C), and Cd (Figure 3G) had higher BCF values in leaves (15.4, 9.9 and 9.6, respectively) than in roots (1.6, 5.5, 2.7). Fruits also exhibited higher Cu (Figure 3D), Ni (Figure 3F), and Zn (Figure 3A) BCF values (77.7, 30.2 and 23.8, respectively) than leaves, stem bark and roots, whereas Fe (Figure 3B) and Cd (Figure 3G) within fruits exhibited lower BCF values (31.5 and 1.7, respectively) than other organs. Chromium was found to accumulate at higher levels in stem bark with a BCF 13.3 (Figure 3E). Heavy metals with higher BCF values have easy routes with a greater opportunity to translocate to tree fruits [56]. High BCF results were reported results for *Eruca sativa*, *Brassica campestris* and *Triticum aestivum* irrigated with wastewater for Cd, Cr and Pb, that present risks to human beings [57].

3.4. Effects of Irrigation with TWW on Translocation Factor of Heavy Metal in Olive Trees

The translocation factor (TF) was measured to indicate the level of heavy metals transferred from roots to other organs. Figure 4 illustrates the TF of heavy metals in the different parts of olive trees. The translocation factor from roots to stem bark exhibited the following descending trends Pb> Cd > Cu > Cr > Zn > Ni > Mn > Fe. A similar trend was seen in TF and metal mobility from olive roots to leaves, with TF values in descending order as follows: Pb> Cd > Mn > Zn > Cu > Ni > Cr > Fe (Figure 4). Lead (Figure 4H) was most actively translocated to olive tree leaves with a TF value of 10. Cd

(Figure 4G) and Mn (Figure 4C) had TF values of 3.9, 1.8, respectively. On the other hand, lower translocation rates of Zn (Figure 4A), Cu (Figure 4D), Ni (Figure 4F), Cr (Figure 4E) and Fe (Figure 4B) to leaves was observed with TF values less than 1.0. Translocation of heavy metals from roots to fruits was less pronounced than between other parts. Lead (Figure 4H), Cu (Figure 4D), Zn (Figure 4A) and Ni (Figure 4F) were translocated to fruits at high ratios with TF values of 5.25, 2.45, 1.52, 1.23, respectively. By contrast, Cr (Figure 4E), Cd (Figure 4G), Fe (Figure 4B) and Mn (Figure 4C) exhibited lower TF values (<1.0).

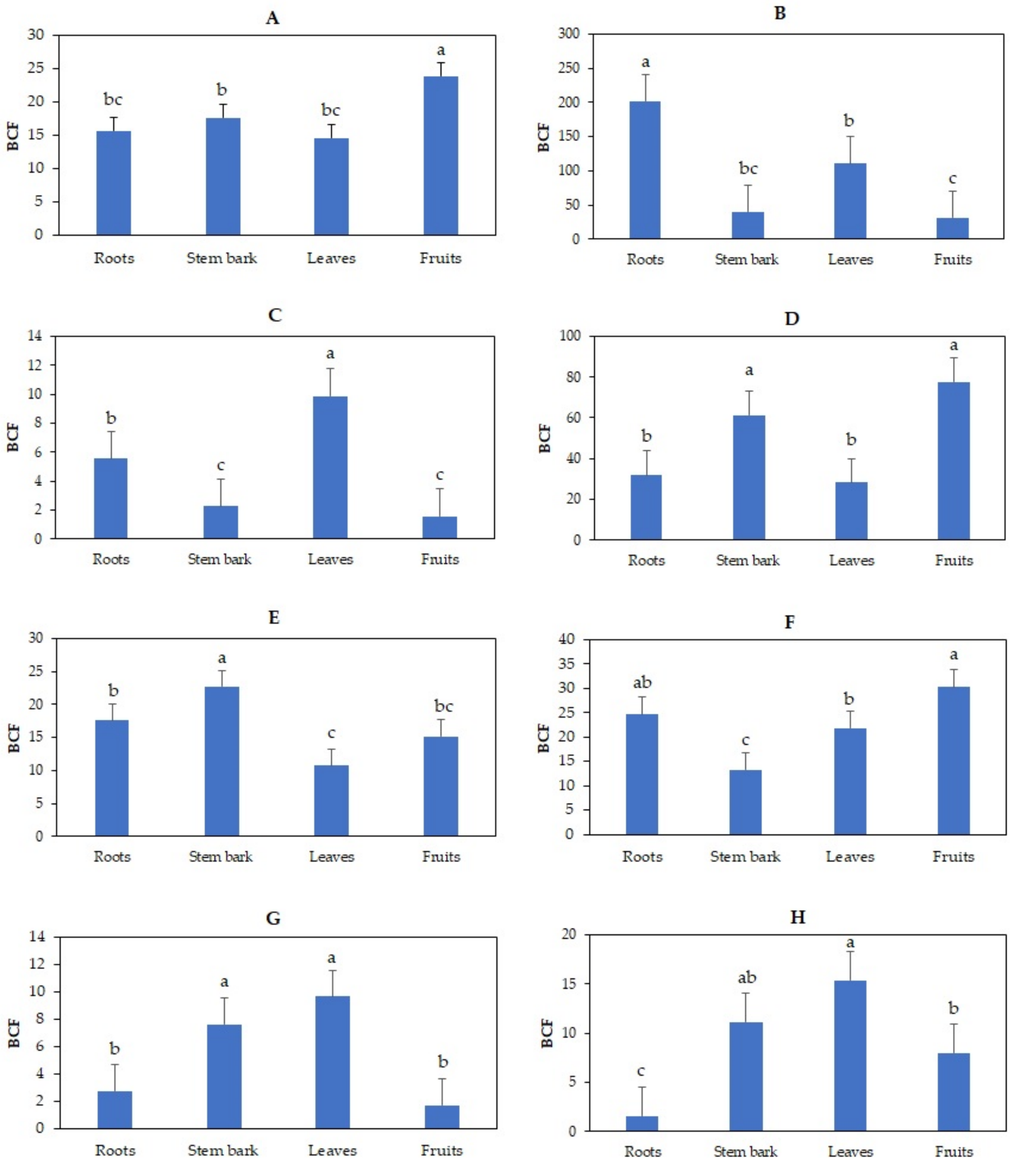

Figure 3. Bioconcentration factor (BCF) of Zn (**A**), Fe (**B**), Mn (**C**), Cu (**D**), Cr (**E**), Ni (**F**), Cd (**G**), Pb (**H**) in different parts of olive trees irrigated with treated wastewater at the Wadi-Musa Wastewater Treatment Plant experimental site. Bars represent mean ± SE. Different letters indicate significant differences between plant parts by LSD ($p \leq 0.05$).

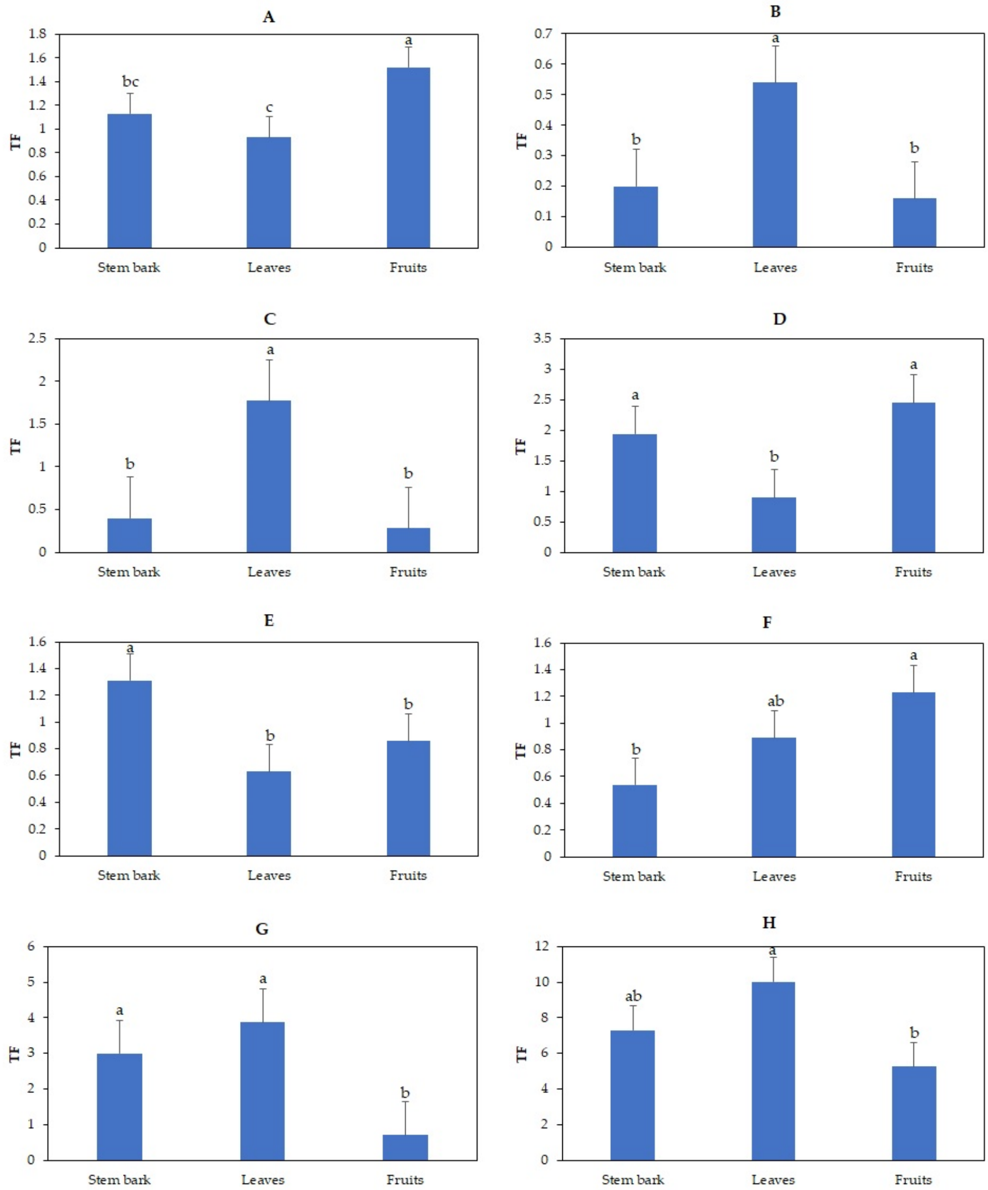

Figure 4. Translocation factors (TFs) for Zn (**A**), Fe (**B**), Mn (**C**), Cu (**D**), Cr (**E**), Ni (**F**), Cd (**G**), Pb (**H**) in the different parts of olive trees irrigated continuously with TWW at the Wadi-Musa Wastewater Treatment Plant experimental site. Bars represent mean ± SE. Different letters indicate significant differences between plant parts by LSD ($p \leq 0.05$).

Higher TF values for olive leaves than stem bark and fruits were found for Fe (Figure 4B), Mn (Figure 4C), Cd (Figure 4G) and Pb (Figure 4 (H) (0.54, 1.77, 3.87 and 5.25, respectively), whereas Cu (Figure 4D) had a lower TF to leaves (0.89) than for bark and fruits (1.94 and 2.45), respectively. Higher translocation rates to fruits than that to bark and leaves were observed for Zn (Figure 4A), Cu (Figure 4D) and Ni (Figure 4F) with TF values of 1.5, 2.5 and 1.2, respectively. Moreover, Cr (Figure 4E) was the only metal that exhibited higher significant translocation to the olive bark than to other parts with a TF value of 1.3. These results are consistent with the results of Mehmood et al. [57] and Lu et al. [58]. Bioconcentration factor and TF are used to determine if a plant is an accumulator or excluder for heavy metals [58,59]. Plants featuring a TF < 1 are considered excluders that can inhibit metal entrance into the plant or restrict root-to-shoot translocation [57].

4. Conclusions

This study investigated the influence of long-term use of treated wastewater on uptake and partitioning of heavy metals in olive trees. Results indicate that long term irrigation of TWW over the past fifteen years resulted in the accumulation of corresponding heavy metals in olive trees. Moreover, the uptake and portioning of various heavy metals by different plant parts (roots, leaves, stem bark, and fruits) were correlated to their concentration in treated wastewater and soil. Higher levels of Zn, Cu, Cr, and Ni were accumulated in olive fruits than in leaves. However, more Mn, Cd and Pb were also found in leaves compared to other parts. Only Fe and Cr were found in higher concentrations in roots and stem bark than in fruits and leaves, respectively. The bioaccumulation factor of heavy metals from the soil to different plant parts indicates selective absorption and partitioning of these heavy metals. High BCF values >1 was found in roots of olive trees with the following descending order: Fe > Cu >Ni > Cr > Zn > Mn > Cd > Pb. A similar trend was observed for fruit BFC values with the following order: Cu > Fe > Ni > Zn > Cr > Pb > Cd > Mn. On the other hand, leaf BFC values indicated higher Fe, Mn, Cd, and Pb than those for other parts. Translocation factors of various heavy metals were variable among plant parts of olive trees. Fruits had the highest TF values for Cu, Cd and Zn, and the lowest for Mn and Fe, while leaves had the highest TF values for Fe, Zn and Mn and the lowest for Cd and Pb. Stem bark was different in its TF, with the highest value for Cr. In conclusion, olive trees are heavy metals accumulators and, therefore, caution should be considered in long-term use of TWW. Periodic assessment of possible hazards, especially on fruits and oil quality, is required.

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