Irrigation with Treated Municipal Wastewater on Artichoke Crop: Assessment of Soil and Yield Heavy Metal Content and Human Risk

Giuseppe Gatta [1,*] [iD]**, Anna Gagliardi** [1] [iD]**, Grazia Disciglio** [1]**, Antonio Lonigro** [2]**,**
Matteo Francavilla [1]**, Emanuele Tarantino** [1] **and Marcella Michela Giuliani** [1] [iD]

[1] Department of Agricultural, Food and Environmental Sciences, University of Foggia, 71122 Foggia, Italy;
    anna.gagliardi@unifg.it (A.G.); grazia.disciglio@unifg.it (G.D.); matteo.francavilla@unifg.it (M.F.);
    emanuele.tarantino@unifg.it (E.T.); marcella.giuliani@unifg.it (M.M.G.)
[2] Department of Agricultural and Environmental Science, University of Bari, 70121 Bari, Italy;
    antonio.lonigro@uniba.it
* Correspondence: giuseppe.gatta@unifg.it; Tel.: +39-0881-589238

Abstract: Industrial and municipal wastewaters are often used for irrigating agricultural fields in arid and semi-arid countries, representing the most attractive option to alleviate pressure on fresh-water resources. However, the wastewater may contain various potentially toxic elements and organic matters with highly harmful effects on human and animal health. During two growing seasons of globe artichoke, the effects of irrigation with secondary (SWW) and tertiary (TWW) municipal wastewater on heavy metal soil and plant content were evaluated, together with the consequent human risk from artichoke head consumption. The heavy metal contents (i.e., Al, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn, and Mn) of the irrigation water, soil, plant, and yield were analyzed. Total and extractable heavy metals were quantified to determine the bioaccumulation factors, and the health risks to adults and children were determined according to hazard indices. The heavy metal contents of the artichoke heads harvested after SWW and TWW irrigation were lower than the international threshold values, and low bioaccumulation factors suggested that these heavy metals did not accumulate in the edible part of the artichoke crop. The hazard indices that were based on the consumption of the artichoke heads remained <1.0 for both adults and children, thus indicating that the health risks involving the different heavy metals are not significant.

Keywords: globe artichoke; treated wastewater re-use; irrigation; heavy metal; risk assessment; bioaccumulation factor

1. Introduction

Water scarcity is the primary reason for the increasing trend toward wastewater re-use in agriculture worldwide. Industrial and municipal treated wastewaters are often used for irrigation of agricultural fields, particularly in arid and semi-arid countries, because this represents the most attractive option for alleviating the pressure on freshwater resources [1–4]. Moreover, wastewaters may represent a significant nutrient source for plants that are grown in low fertility soils [5,6]. However, in addition to plant nutrients, wastewaters may contain various potentially toxic mineral elements and organic matter, and these components can have harmful effects on human and animal health [7]. As for heavy metals, while low concentrations in the soil are often beneficial to the growth and metabolism of plants, they can have undesirable effects at higher concentrations [8,9]. Although the heavy metals content in wastewater that is used for irrigation must comply with the legal limits, continuous use of wastewater can lead to their soil enrichment. Accumulation of toxic heavy metals cause stress in

plants due to interference with the metabolic activities and physiological functioning of the plants [10]. Excessive levels of metals can also result in the degradation of soil quality, reduction in marketable crop yield, and/or poor quality of marketable agricultural products, and these effects can also pose significant hazards to the health of humans, animals, and the ecosystem [9,11].

The leafy parts of vegetables tend to accumulate higher amounts of heavy metals than the fruits [12]. Studies on the plants heavy metals uptake have shown that they can be transported from roots to shoots through the xylem vessels [13], while they generally have poor mobility in the phloem [14]. Plant storage organs, such as fruits and seeds, have low transpiration rates and do not accumulate heavy metals because they are largely phloem-loaded. The accumulation of heavy metals in agricultural soils through wastewater irrigation might not only result in soil contamination, but might also affect food quality and safety [15]. Adverse effects of soil and vegetables heavy metals contamination on human health have been widely reported [9,16,17]. Major damage to health is strongly correlated with heavy metal toxicity, which can cause tissue damage, kidney tubule dysfunction, skeletal damage, osteoporosis, cancer of the blood and lungs, metabolic disorders, anaemia, and hypochromic anaemia [18].

The risks that are related to the uptake of heavy metals by food crops depends more on the increase of their available fraction than on the their total concentration in the soil [19], on the heavy metals speciation and solubility, and on the crops that are cultivated [20].

Globe artichoke (*Cynara scolymus* L.) is an irrigated crop that is widespread in Mediterranean areas, and it is of particular importance in the Mediterranean diet. In areas in which crops are irrigated with wastewater it is relevant to evaluate the accumulation of individual heavy metals in the edible parts in order to estimate potential risks to human health. To the best of the authors' knowledge, no information are available, in the scientific literature, on toxic heavy metal contamination of globe artichoke crop irrigated with treated wastewater neither on the risks to human health that may be associated with such contamination.

The aim of the present study was to evaluate the accumulation of heavy metals in the soil, with specific reference to the exchangeable fraction, and in the edible part of the artichoke crop irrigated with fresh, secondary (SWW), and tertiary (TWW) municipal wastewater. Moreover, it was also evaluated the bioaccumulation factors and then the human risk that is related to the consumption of artichoke heads.

2. Materials and Methods

2.1. Site Description and Agronomic Conditions

The research was conducted in the Apulia region of Italy (Trinitapoli, 41°21′ N, 16°03′ E; altitude 10 m a.s.l.) on globe artichoke (*Cynara cardunculus* (L.), subsp. *scólymus* Hayek), cultivar 'Violetto of Provenza' over two cropping cycles (2012–2013 and 2013–2014). The experimental trial was performed in a loam soil (United States Department of Agriculture classification) with the following physical and chemical characteristics: sand, 45.3%; silt, 30.0%; clay, 24.7%; field capacity (measured by pressure plate apparatus at −0.03 MPa) of 30.7% dry weight (dw); wilting point (measured by pressure plate apparatus at −1.5 MPa) of 15.2% dw, and a bulk density of 1.45 Mg m^{-3}; organic matter, 1.2% (Walkley and Black method); available phosphorus (Olsen method), 114.0 mg kg^{-1}; total potassium, 1.27 g kg^{-1} (determinated by coupled plasma optical emission spectrometer, Agilent, ICP-OES 720); total nitrogen, 0.91‰ (Kjeldahl method).

Three types of irrigation water were applied to the crop: freshwater (FW), secondary municipal wastewater (SWW), and tertiary municipal wastewater (TWW). The FW was obtained from the irrigation network system that is normally used by the farmers in the area for crop irrigation; the SWW and TWW were from secondary and tertiary municipal water recycling plant that was located near the experimental site. A randomized complete block design with three replications has been used. Overall, nine plots of dimensions 16.8 m × 6.0 m were defined. In the first growing season (GS$_1$),

the artichoke plants were planted as offshoots on 15 July 2012 in rows 1.20 m apart and with 1.20 m spacing within the rows to provide a density of 6944 plants ha^{-1}. In the second growing season (GS_2), the plants were re-awakened on 20 July 2013 by applying water in order to establish soil field capacity. A drip irrigation system was used with dripper lines placed along each row. The in-line drippers were located 0.40 m apart; the dripper flow rate was 4 L h^{-1} at an operating pressure of 1.5 bar. Irrigation was performed whenever the water was lost (excluding the useful rainfall) by crop evapotranspiration (ET_c) in the soil layer containing the roots reached a predetermined level (30 mm, i.e., ~50% of available water depletion). The watering volume used restored 100% of the water lost. During the GS_1 and GS_2 growing cycles, the seasonal water irrigation volumes applied were 3300 m^3 ha^{-1} and 3000 m^3 ha^{-1}, respectively. During these two growing seasons, standard agronomic practices for artichoke crops were followed.

2.2. Water, Soil and Plant Sampling

Water samples were collected and analyzed six times during the two growing seasons. In particular, during GS_1 water samples were taken at 5, 71, 128, 191, 247, 300 days after transplanting (S_{W1}–S_{W6}), while during GS_2, they were taken at 2, 58, 113, 185, 245, 303 days after transplanting (S_{W7}–S_{W12}). The samples were taken as three replications using 1000 mL sterile glass bottles, transported in refrigerated bags to the laboratory, and were stored in a refrigerator at 4 °C. To prevent microbial activity, 1 mL concentrated HNO_3 was added to each water sample.

Soil samples were collected randomly from each experimental plot before transplanting time (S_{S1}, for determination of background soil heavy metal content) and at the end of the first (S_{S2}) and second (S_{S3}) artichoke growth cycles. The soil samples were collected at depths of 0–40 cm (H_1) and 40–80 cm (H_2). H_1 represented the high root density zone (i.e., top profile), while H_2 represented the deeper part of the root zone (i.e., bottom profile). Soil samples were air-dried, crushed, and passed through a 2-mm sieve prior to chemical analysis. The full soil sampling comprised one sample per plot × 9 plots (i.e., 3 irrigation treatments × 3 replicates) × 3 sampling dates × 2 soil depths × 2 growing seasons, yielding a total of 108 soil samples.

Sampling of the artichoke heads (i.e., the portion of the plant that is edible to humans) was performed three times (in November, March and May), in each growing season by picking five marketable artichoke heads per experimental plot. At the end of each growth cycle, three randomly selected plants were collected from each plot and divided into leaves and stems. The artichoke plant and head samples were dried in an oven at 70 °C for 24 h, ground into fine powder, sieved through 2-mm mesh, and were stored at room temperature. The full sampling comprised five heads per plot × 9 plots (i.e., 3 irrigation treatments × 3 replicates) × 3 sampling dates × 2 seasons, yielding a total of 270 artichoke heads.

2.3. Determination of Heavy Metals in the Water, Soil and Plant Samples

All of the samples (water, soil, plants, and artichoke heads) were analysed for the trace elements Al, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn, and Mn. In the soil samples, the extractable metal fractions were also determined. The elements analysed are hereinafter referred to as 'heavy metals'. All of the samples were analysed in triplicate to ensure data accuracy, and the means of three instrumental replicates were used in data interpretation. The choice of the heavy metals for analysis in the soil and in the plant marketable yield (i.e., artichoke heads) was based on their concentrations in the three types of irrigation water that were used. Thus, the metals that were below the limit of detection in the irrigation water samples (i.e., Pb and Cd) were not analysed in the soil and in the artichoke plant and head samples.

For the analytical determination of the heavy metal water content, each sample was filtered through a 0.20-μm syringe filter consisting of a cellulose acetate membrane and diluted, if necessary, with ultrapure water (Milli-Q). The levels of the heavy metals were determined using inductively coupled plasma optical emission spectrometer (Agilent, ICP-OES 720). Soil samples (0.5 g dw)

were mineralized in 10 mL 70% (*v/v*) HNO_3 in a microwave oven (CEM-Mars6). After cooling, the digested samples were transferred into 50-mL flasks and brought to the final volume with Milli-Q water [21], and than analyzed by ICP-OES. The extractable metal from soil samples were obtained using diethylenetriaminepentaacetic acid (DTPA) solution, according to ISO 14870 method [22]. About 10 g of each soil sample was extracted with 100 mL 0.005 M DTPA for 30 min, under shaking at 180 oscillations/min. The mixtures obtained were filtred and analyzed by ICP-OES.

For analytical determination of the total heavy metal concentrations in the artichoke plants and heads, the samples (about 0.5 g dw) were mineralized in 10 mL HNO_3/H_2O_2 (3:1; *v/v*) in a microwave oven (CEM-Mars6). After cooling, the digested samples were diluted with Milli-Q water to 50 mL [23] and were analyzed by ICP-OES.

2.4. Quality Assurance and Quality Control

Appropriate quality assurance procedures and precautions were followed to ensure the reliability of the data. All of the reagents used were of analytical grade. Glassware was cleaned thoroughly with detergent and was rinsed several times with deonized water. Milli-Q water was used for all of the dilutions throughout the study. Blank reagent determinations were used to correct instrument readings. To ensure data quality, the samples were analysed in triplicate, and one standard sample was analysed for every three experimental samples. To maintain instrument calibration, blank and drift standards were run after every five determinations.

2.5. Bioaccumulation Factor and Characterization of Human Risk

The bioaccumulation factor (BAF) is an index related to the accumulation in food of a particular metal with respect to its concentration in the soil; here, it was calculated according to Cui et al. (2004) [24], as defined in Equation (1).

$$BAF_M = C_{Mplant}/C_{Msoil} \tag{1}$$

where C_{Mplant} and C_{Msoil} represent the total concentrations of a particular heavy metal in the edible parts of the plant and in the soil, respectively, on dry weight basis. In the present study, BAF was calculated both relative to the total metal concentration (BAF_t) and also relative to the 'extractable' heavy metals concentration (DTPA-extractable metal fraction; BAF_e).

The hazard indices (HIs) used to determine the non-carcinogenic risks to human health were estimated by comparing the daily intake of a particular metal (DI_M) through the edible part of the artichoke crop with the corresponding oral reference dose (RfD_M) [9,24–26]. Thus, the HIs for the consumption of the artichoke heads were calculated using Equation (2) [27].

$$HI = \frac{Ef \times Ed \times DI}{RfD \times BW \times ET} \tag{2}$$

where Ef is the frequency of exposure (the frequency of artichoke consumption) in the experimental area (240 days $year^{-1}$), Ed is the exposure duration (82 years, equivalent to the mean lifespan of males and females in Italy) [28], DI is the daily metal intake (mg $person^{-1}$ day^{-1}), RfD is the amount of the chemical element that can be ingested daily over a lifetime without causing harmful health effects (mg kg^{-1} day^{-1}), BW is the mean body weight of the consumer (kg), and ET is the mean exposure time for non-carcinogens (280 days $year^{-1}$ $\times$ number of exposure years, assuming 50 years in this study). In Equation (2), DI for each particular heavy metal (DI_M) was calculated by multiplying the daily fresh artichoke head consumption (kg $person^{-1}$ day^{-1}) by the average amount of that heavy metal in the artichoke heads (mg kg^{-1} fresh matter). The mean daily vegetable intakes for adults and children were considered to be 0.021 and 0.0105 kg $person^{-1}$ day^{-1} [29], respectively, and the mean adult (18 years of age or over) and child (8 years of age) body weights were considered to be 70.2 kg and 31.9 kg, respectively [30,31]. The RfD for each heavy metal (RfD_M) was derived from the US Environmental Protection Agency [32] guidelines

(http://www.epa.gov). Finally, the additive HIs (AHIs) for the total heavy metal contaminants for both adults and children [32] has been calculated, as reported in Equation (3).

$$AHI = \sum_{i=1}^{n} HI \tag{3}$$

In Equation (3), HI is defined for the individual *n* heavy metals as shown in Equation (2).

2.6. Statistical Analysis

The datasets were tested according to the basic assumptions of analysis of variance (ANOVA). The normal distribution of the experimental error and the common variance of the experimental error were verified through Shapiro-Wilk and Bartlett's tests, respectively. When required, Box-Cox transformations [33] were applied prior to analysis.

For all of the datasets, the ANOVA procedure was performed according to a randomized complete block design with three replicates. For irrigation water data, two-way ANOVA was performed (irrigation water × water sampling date). The irrigation water was considered to be a fixed factor, and the water sampling date was considered a random factor. Each soil heavy metal content was subjected to a three-way ANOVA procedure (irrigation water × soil depth × soil sampling date) when considering the type of irrigation water and the soil depth as fixed factors and the soil sampling date as a random factor. For the heavy metal content of the artichoke plants, combined analysis of the data was performed to determine the mean irrigation water response over the two growing seasons.

Lastly, the data on bioaccumulation factors (BAF_t and BAF_e) and hazard indices (HI and AHI) were subjected to a one-way ANOVA procedure in which the type of irrigation water was considered the only effect. The statistical significance of the differences in the means was determined using Tukey's honest significance difference *post hoc* test at the 5% probability level. Bivariate statistical methods were applied to verify the data for correlations among the different heavy metal contents for irrigation water, soil, and marketable yield. The ANOVA and bivariate statistical methods were performed using the JMP software package, version 8.1 (SAS Institute Inc., Cary, NC, USA), and figures were constructed using Sigma Plot Software (Systat Software, Chicago, IL, USA).

3. Results and Discussion

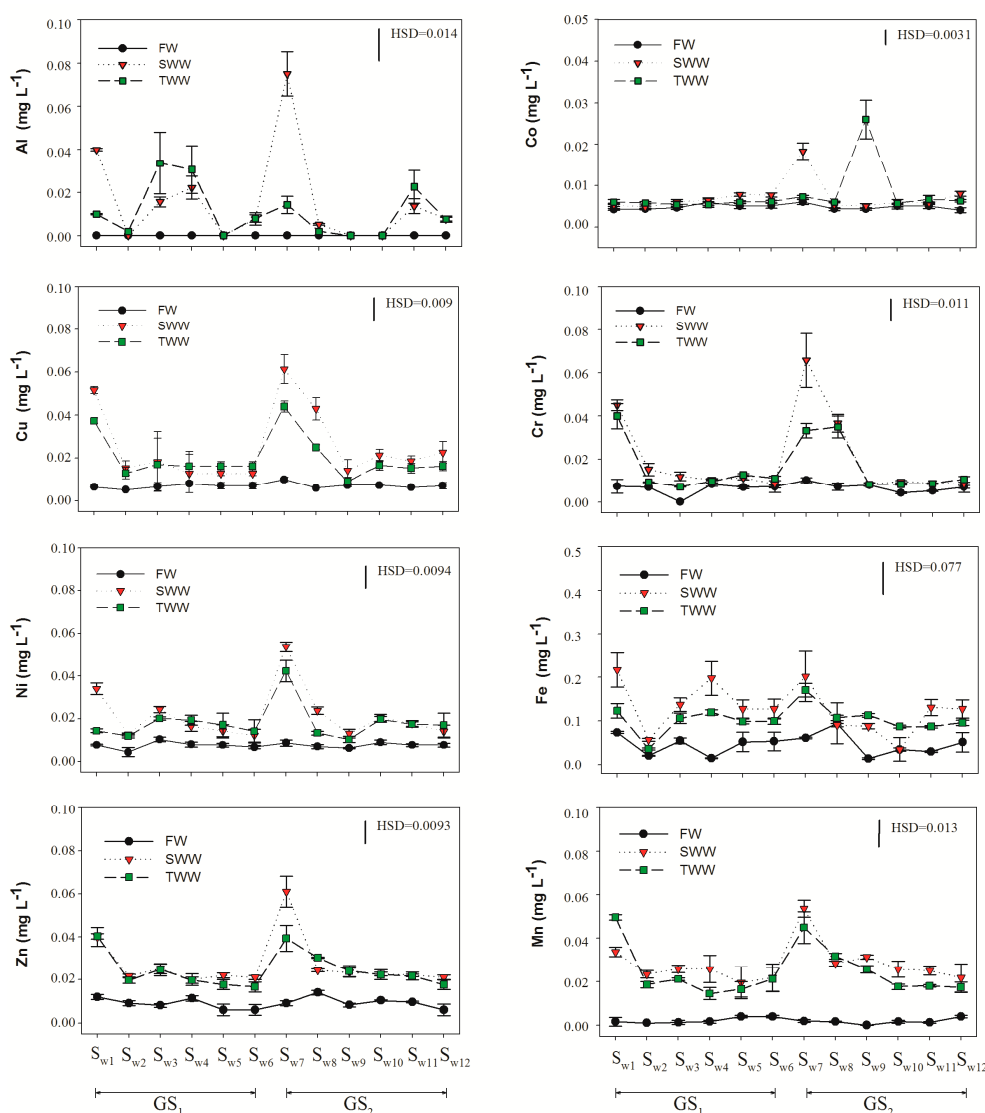*3.1. Heavy Metal Content of Irrigation Water*

The ANOVA of the data for the three irrigation waters showed significant differences in heavy metal content for both, irrigation water type (IW) and water sampling date (S_W) as well as for their interaction. Figure 1 shows the levels of individual heavy metals in FW, SWW and TWW measured on the 12 sampling dates (GS_1, S_{W1}–S_{W6}; GS_2, S_{W7}–S_{W12}). The Cd and Pb contents were not considered being below the detection limits (0.001 and 0.008 mg L^{-1}, respectively) probably because recycled municipal wastewaters do not usually contain these industrial waste contaminants [15,34]. As expected, the concentrations of all heavy metals in FW were lower than the concentrations in SWW and TWW (Figure 1). The heavy metal content of SWW and TWW showed high variability as a function of water sampling date. In particular, the Cr, Cu, Zn, and Mn contents were significantly higher ($p < 0.05$) at S_{W1} and S_{W7} (July 2012 and July 2013, respectively) than at the other sampling dates (Figure 1).

This variability might be due to seasonal variability in the quality of the water that formed the input to the wastewater treatment plant. Indeed, the input water for the wastewater treatment plant came from Trinitapoli, which is a small town of 15,000 inhabitants [28], which increase during the summer holidays (July–August); this might result in variation in the chemical characteristics of the wastewater.

The Co content (about 0.007 mg L^{-1}) did not show significant differences as a function of water sampling date, with the exception of S_{W7} and S_{W9} for SWW and TWW, respectively (Figure 1). The Al and Fe contents of SWW and TWW were highly variable, ranging from 0.075 mg L^{-1} to 0.005 mg L^{-1} and from 0.20 mg L^{-1} to 0.05 mg L^{-1}, respectively. Comparison of the measured heavy

metal concentrations with national [35] and international (United Nations Food and Agriculture Organization (FAO), US Environmental Protection Agency) guidelines showed that the levels of heavy metals in SWW and TWW were below the recommended threshold values (Table 1).

The heavy metal concentrations in the irrigation water that were used in this study are in agreement with the report of Shiekh et al. (1987) [36] on the efficiency of municipal wastewater treatment processes for the reduction of heavy metal content. Indeed, high contents of heavy metals are normally found in industrial sewage effluent, whereas the concentrations of these metals in domestic wastewaters are generally low following the settling of solids during treatment [37]. However, heavy metal accumulation in the soil does not exclusively depend on the levels of heavy metals in treated wastewater; it also depends on the rate of irrigation and on the soil type [38]. Therefore, although the heavy metal levels in the irrigation wastewater used in this study are below the recommended thresholds, it remains important to evaluate the soil bioaccumulation of these metals over both the medium and long term.

Figure 1. Heavy metal contents of the three irrigation water types applied as function of water sampling date, showing the irrigation water × water sample date interaction (IW × S_W). The data are the means ± standard errors of three replicates. GS_1, first growing season; GS_2, second growing season. S_{W1}–S_{W12} are the water sampling dates for the first (S_{W1}–S_{W6}) and second (S_{W7}–S_{W12}) growing seasons. Honest significant differences (HSDs) calculated according to Tukey's test are also reported.

3.2. Heavy Metal Content of the Soil-Plant System

3.2.1. Heavy Metal Content of the Soil

　　Table 2 lists the mean concentrations of individual heavy metals in the soil as a function of irrigation water type (IW), soil depth (H), and soil sampling time (Ss). Only the soil depth had significant effects on the total metal contents ($p \leq 0.05$), while no significant interactions were found between these different experimental factors. The Co soil content was not determined because it was below the detection limit of 0.015 mg kg^{-1}; on the other hand, also in the irrigation water the Co content was, in general, very low (about 0.007 mg L^{-1}). Also for the soil, all the heavy metals examined were lower than the threshold values (Table 1) that were listed in the national [39] and international [40] guidelines.

Table 1. National and international guidelines for maximum limits of heavy metals in irrigation water, soil and cultivated vegetables.

Heavy Metals	Wastewater Guidelines (mg L^{-1})			Soil Guidelines (mg kg^{-1} dry weight)			Vegetable Guidelines (mg kg^{-1} fresh weight)
	[1] IG$_w$	[2] FAO	[3] US EPA	[4] IG$_s$	[5] EUs	[6] IR	[7] FAO/WHO
Al	1.0	5.0	5.0	-	-	-	-
Cd	0.005	0.01	0.005	2.0	3.0	3.0 [e]	0.1 (0.050)
Co	0.05	0.05	0.05	20	-	50 [e]	50
Cr	0.10	0.10	0.10	150	150	100 [e]	2.3
Cu	1.0	0.20	0.2	120	140	100 [e]	73
Fe	2.0	5.0	5.0	-	-	50,000 [f]	425
Ni	0.20	0.20	0.20	120	75	50 [e]	67
Pb	0.10	0.50	0.50	100	300	100 [e]	0.3 (0.1)
Zn	0.50	0.20	2.0	150	300	300 [e]	100
Mn	0.20	0.20	0.20	-	-	2000 [f]	500

Note: The maximum limits for vegetables according to European guidelines, EC 1881/2006 [41], are reported in parentheses. [1] IG$_w$, Italian guidelines for agricultural wastewater reuse: Ministerial Decree n. 185/2003 [35]. [2] Ayers, R.S.; Westcot 1985 [42]. [3] United States Environmental Protection Agency, Washington, DC, 2012 [43]. [4] IG$_s$, Italian guidelines for soil content: Ministerial Decree n. 152/2006 [39]. [5] EUs, European union standards (EU 2002) [40]. [6] IR, International references. [7] FAO/WHO, 2001. Food additives and contaminants [44]. [e] Ewers, 1991 [45]. [f] Pendias and Pendias, 1992 [46]. **-** no prescribed limits.

Table 2. Main effects of the irrigation waters, soil depths, and soil sampling dates on the total soil heavy metal contents.

Experimental Factor	Total Heavy Metal Content (mg kg^{-1})							
	Al	**Co**	**Cr**	**Cu**	**Fe**	**Ni**	**Zn**	**Mn**
Irrigation water (IW)	ns		ns	ns	ns	ns	ns	ns
FW	2827.9 ± 45.8	nd	21.4 ± 1.7	21.0 ± 1.7	18,007.6 ± 298.1	18.3 ± 1.8	51.8 ± 3.1	504.1 ± 13.9
SWW	2839.8 ± 45.2	nd	22.7 ± 1.7	21.6 ± 1.8	17,554.4 ± 193.2	19.3 ± 1.5	50.3 ± 2.7	528.0 ± 133
TWW	2915.6 ± 57.8	nd	22.3 ± 0.6	21.1 ± 0.9	18,228.1 ± 244.4	19.1 ± 0.5	51.9 ± 4.1	524.9 ± 12.1
Soil depth (H)	***		***	***	*	***	***	***
H$_1$	3007.2 ± 24.9 [a]	nd	24.3 ± 0.8 [a]	25.8 ± 2.2 [a]	17,615.1 ± 183.4 [b]	20.4 ± 0.9 [a]	56.7 ± 2.3 [a]	562.3 ± 6.5 [a]
H$_2$	2715.1 ± 33.3 [b]	nd	20.0 ± 0.9 [b]	18.3 ± 0.3 [b]	18,244.7 ± 214.7 [a]	17.5 ± 1.0 [b]	46.2 ± 1.5 [b]	475.8 ± 6.7 [b]
Soil sampling date (S$_S$)	ns		ns	ns	ns	ns	ns	ns
S$_{S1}$	2959.4 ± 54.2	nd	22.2 ± 1.6	21.2 ± 1.7	18,179.5 ± 288.8	18.2 ± 1.4	51.6 ± 2.5	517.7 ± 13.3
S$_{S2}$	2845.8 ± 53.1	nd	22.3 ± 1.9	22.3 ± 1.8	17,632.2 ± 226.3	19.4 ± 1.6	51.5 ± 4.2	516.2 ± 12.7
S$_{S3}$	2832.2 ± 42.3	nd	21.8 ± 0.7	22.1 ± 1.0	17,978.2 ± 247.8	19.2 ± 0.9	50.9 ± 3.5	523.1 ± 13.7

Note: Data are means ±standard error (*n* = 3). For each experimental factor, data followed by different superscripted letters (a and b) within each column are significantly different ($p \leq 0.05$; Tukey's test); ns, not significant. FW, fresh water; SWW, secondary treated wastewater; TWW, tertiary treated wastewater; H$_1$, top soil profile (0–40 cm); H$_2$, bottom soil profile (40–80 cm). S$_{S1}$, S$_{S2}$, S$_{S3}$, first (before transplanting), second (end of GS$_1$), and third (end of GS$_2$) soil sampling dates, respectively; nd, not detected; * $p \leq 0.05$; *** $p \leq 0.001$ (Fisher's test).

　　The concentrations of Al, Cr, Cu, Ni, Zn, and Mn were significantly higher ($p \leq 0.05$) in H$_1$ (top soil profile) than in H$_2$ (bottom soil profile). In contrast, higher concentrations of Fe were found in H$_2$ than in H$_1$ (18,244.7 vs. 17,615.1 mg kg^{-1}).

In order to determine the BAF, many studies refer to the concentrations of a heavy metal in the soil as the 'total' amount. To the best of the authors' knowledge, very few studies have considered the concentrations of the 'extractable' heavy metals using DTPA, or other methods. Because total heavy metal content is not an efficient predictor of the availability and mobility of heavy metals in soils [47], in this study, the potentially plant-available heavy metal contents, as represented by the DTPA-extractable contents, were also evaluated to determine whether they can move into the soil and uptake by the crop. The DTPA-extractable metals do not represent the total soil metals that are available for the plant, however, they are good indicators of the potential metals quantities that the crop may uptake [48].

The effects of type of irrigation water used, soil depth, and soil sampling time (IW, H, S_S experimental factors) on the mean soil concentrations of DTPA-extractable heavy metals are reported in Table 3. Also, in this case, Co content was not determined as it was below the detection limit (0.015 mg kg^{-1}). DTPA-extractable Al, Cu, Ni, and Zn levels were significantly higher ($p \leq 0.05$) in SWW and TWW than in FW. The concentrations of Cr in TWW was not significantly higher than those in FW.

Table 3. Main effects of irrigation waters, soil depths, and soil sampling dates on the exchangeable soil heavy metal contents (after DTPA extraction).

| Experimental Factor	Exchangeable Heavy Metal Content (mg kg^{-1})					
	Al	**Co**	**Cr**	**Cu**	**Ni**	**Zn**
Irrigation water (IW)	***		*	**	***	**
FW	0.64 ± 0.02 [b]	nd	0.34 ± 0.01 [b]	3.02 ± 0.15 [b]	0.29 ± 0.02 [b]	6.78 ± 0.28 [b]
SWW	0.74 ± 0.01 [a]	nd	0.41 ± 0.02 [a]	3.52 ± 0.20 [a]	0.41 ± 0.02 [a]	7.60 ± 0.24 [a]
TWW	0.70 ± 0.02 [a]	nd	0.39 ± 0.02 [ab]	3.55 ± 0.20 [a]	0.38 ± 0.01 [a]	8.39 ± 0.57 [a]
Soil depth (H)	***		***	**	***	*
H$_1$	0.73 ± 0.01 [a]	nd	0.44 ± 0.01 [a]	3.56 ± 0.18 [a]	0.40 ± 0.01 [a]	7.33 ± 0.20 [b]
H$_2$	0.66 ± 0.02 [b]	nd	0.33 ± 0.02 [b]	3.17 ± 0.13 [b]	0.32 ± 0.02 [b]	7.95 ± 0.25 [a]
Soil sampling date (Ss)	ns		ns	***	ns	ns
S$_{S1}$	0.71 ± 0.02	nd	0.38 ± 0.02	2.53 ± 0.08 [b]	0.34 ± 0.02	7.79 ± 0.17
S$_{S2}$	0.67 ± 0.03	nd	0.39 ± 0.02	3.71 ± 0.16 [a]	0.35 ± 0.02	7.39 ± 0.37
S$_{S3}$	0.70 ± 0.02	nd	0.36 ± 0.03	3.86 ± 0.17 [a]	0.37 ± 0.02	7.60 ± 0.39

Note: Data are means ± standard error (n = 3). For each experimental factor, data followed by different superscripted letters (a, b and ab) in each column are significantly different ($p \leq 0.05$; Tukey's test). FW, fresh water; SWW, secondary treated wastewater; TWW, tertiary treated wastewater; H$_1$, top soil profile (0–40 cm); H$_2$, bottom soil profile (40–80 cm); S$_{S1}$, S$_{S2}$, S$_{S3}$, first, second, and third soil sampling dates, respectively; ns, not significant.; nd, not detected; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Fisher's test).

Moreover, for all of the metals considered, with the exception of Zn, the DTPA-extractable content was higher in the top soil profile than in the bottom one. Finally, as for soil sampling date (S$_{S1}$, S$_{S2}$, S$_{S3}$), only Cu showed a different DTPA-extractable heavy metal content. The mean Cu content of the soil sampled at the end of the first (S$_{S2}$) and second (S$_{S3}$) artichoke crop cycles exceeded the background value (S$_{S1}$). This might be due to the plants uptake Cu at a rate that is lower than the rate at which it was supplied by the irrigation water.

The DPTA-extractable soil content of Fe and Mn varied significantly with type of irrigation water and soil depth (IW × H interaction; Figure 2A,B). In the top soil profile (H$_1$), SWW and TWW both significantly increased the Fe and Mn soil content (3–5 mg kg^{-1} and 10–15 mg kg^{-1}, respectively; $p \leq 0.05$ for both) compared to FW. In contrast, in the bottom soil profile (H$_2$) there were no significant effects ($p > 0.05$) of SWW or TWW on the Fe and Mn soil concentrations. These data are in agreement with the results reported by Achah et al. (2014) [15], who found a close relationship between Fe content in the irrigation water and Fe content of the soil and vegetable samples. Also Al-Lahham et al. (2007) [34], in a study on tomato crops irrigated with wastewater, reported increased concentrations of Mn, and Fe in the soil correlated with the high concentrations of these metals in the wastewater used for irrigation.

In terms of the $S_S \times H$ interaction effects (Figure 2C,D), the DTPA-extractable Fe and Mn soil content in the top soil profile (H_1) increased from S_{S1} (3.26, 10.55 mg kg^{-1}, respectively) to S_{S3} (4.43, 15.32 mg kg^{-1}, respectively), indicating that these metals accumulated gradually in the top soil; no such effect was observed for the bottom soil profile (H_2). The accumulation of Fe and Mn in the top soil profile was related to the higher content of Fe and Mn in SWW and TWW (Figure 1), and to the low mobility of these heavy metals, especially in alkaline soils (pH ~8) [38]. Moreover, combining this with the irrigation water demand of artichoke plants defined in terms of the active root depth (40–50 cm), the heavy metals that were present in the added SWW and TWW would be expected to accumulate specifically in the top soil profile.
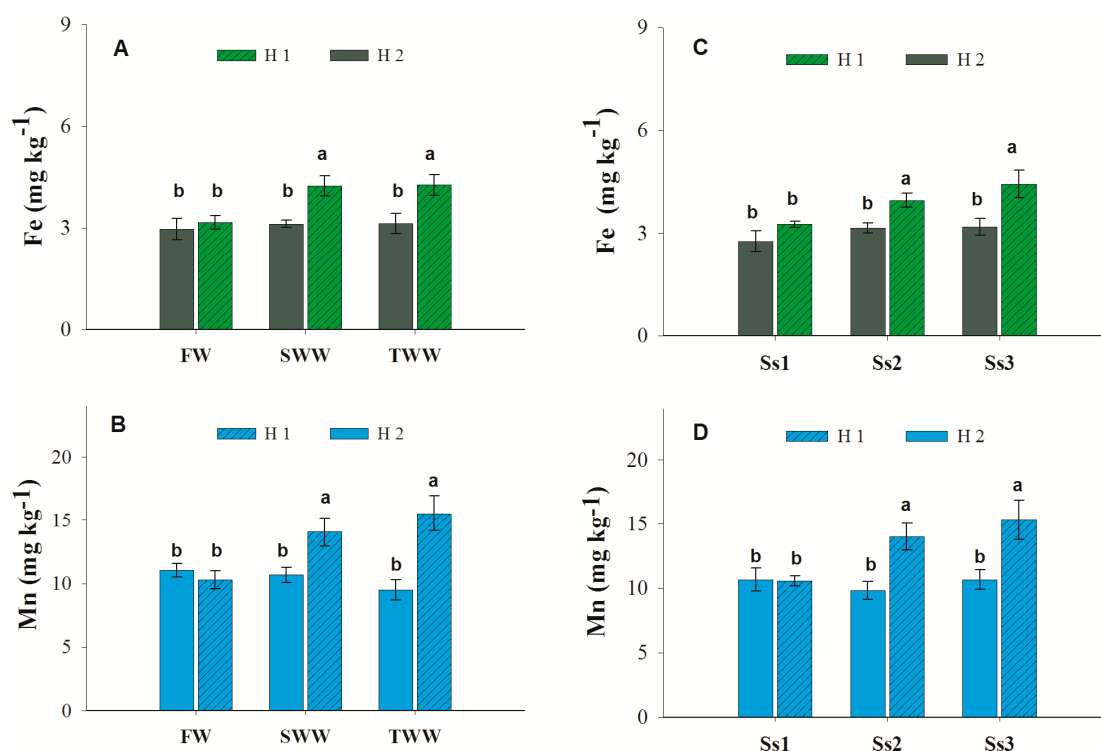
Figure 2. Diethylenetriaminepentaacetic acid (DTPA)-extractable soil content of iron (**A,C**) and manganese (**B,D**) according to the first-order interaction factors *irrigation water × soil depth* (**A,B**) and *soil sampling date × soil depth* (**C,D**). Different letters (a–b) indicate significant differences ($p \leq 0.05$; Tukey's test). The data shown are the means ± standard errors of three replicates. S_{S1}, S_{S2}, S_{S3} refer to the first, second and third soil sampling dates, respectively. FW, fresh water; SWW, secondary treated wastewater; TWW, tertiary treated wastewater; H_1, top soil profile (0–40 cm); H_2, bottom soil profile (40–80 cm).
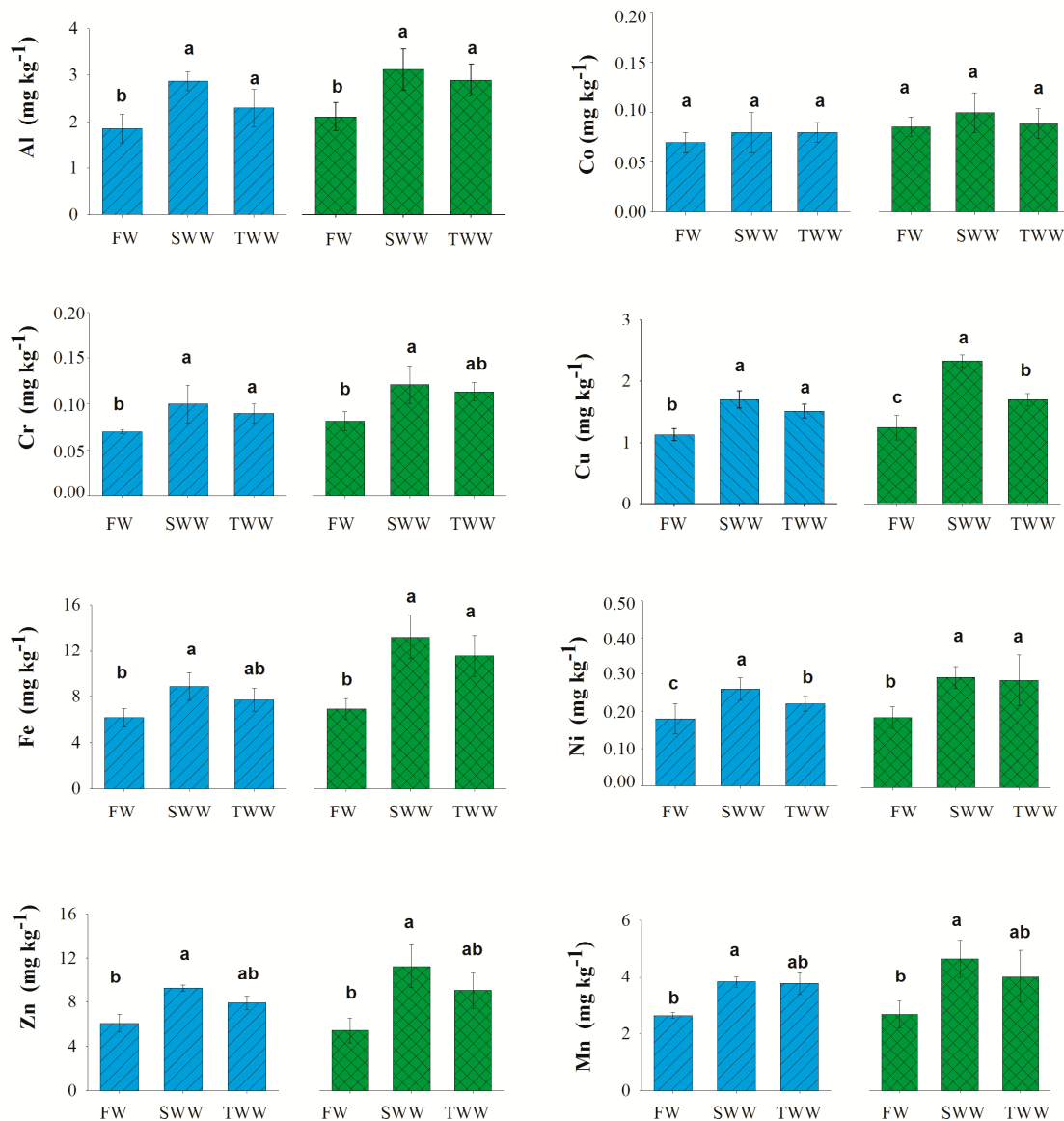
3.2.2. Heavy Metal Content of the Plants and of the Marketable Yield

Artichoke plants and heads irrigated with different types of water differed in all of the heavy metal contents that were considered, except for Co (Figure 3).

The heavy metal content of the artichoke plants resulted higher under SWW and TWW irrigation when compared to FW irrigation, although these always remained below the vegetable guidelines (Table 1). Al, Fe, and Ni showed the same behavior, with similar SWW and TWW values that were significantly higher than FW. Instead, Cr, Zn, and Mn, showed the highest values under SW, without significant differences between TW and FW. Finally, only Cu showed significant differences among the three irrigation water types, having SWW with highest value and FW the lowest.

The heavy metal concentrations in the artichoke heads were on average about 20% lower than those in the artichoke plants. Several studies have indicated that edible plant organs are not the

main site of accumulation of heavy metals [7,49]; instead, heavy metals appear to accumulate mainly in the shoots and roots of plants, and lower levels are generally found in the marketable yield [14]. In particular, Al and Cu showed the same behavior, with similar SWW and TWW value significantly higher than FW. Instead, Cr, Fe, Zn, and Mn, showed highest values under SW without significant differences between TW and FW. Finally, only Ni showed significant differences among the three irrigation water type having SWW the highest value and FW the lowest.

Figure 3. Heavy metal content of artichoke crops as plants (right, green bars) and as artichoke heads (left, blue bars), as a function of the type of irrigation water used. Different letters (a–b) indicate statistically significant differences ($p \leq 0.05$; Tukey's test). The vertical bars indicate standard errors (n = 6, 3 replicates × 2 growing seasons).

Generally, the artichoke heads contained low concentrations of heavy metals, below the limits specified in the European [41] and international [44] guidelines for vegetables used for human consumption (Table 1). Indeed, the heavy metal contents of artichoke heads under SWW and TWW irrigation were about 12-fold and 50-fold lower than the international threshold values for Zn (8 vs. 100 mg kg^{-1} fresh weight) and Fe (9 vs. 425 mg kg^{-1} fresh weight), respectively.

These data are in agreement with Christou et al. (2014) [50], who evaluated the impact of tomato crop irrigation with treated wastewaters on the soil geochemical properties and the safety of the consumption of the tomato fruit. Under all of the irrigation treatments used in that study, the heavy metal contents of Zn, Mn, Ni, Cu, and Co in the tomato fruits and leaves were below the maximum levels set for tomato fruit safety and below the critical tissue concentrations for phytotoxicity. Similar data have been reported for potato crops [51].

With the exception of Al and Co, the heavy metal contents in the irrigation waters were significantly correlated ($p \leq 0.01$, $p \leq 0.001$), with their contents in artichoke plants and heads (Table 4). In addition, Table 4 gives the correlations between the heavy metal contents of the plant biomass (both plants and heads) and the soil as total and DTPA-extractable heavy metals.

The significant correlations between the Cr, Fe, Zn, and Mn DTPA-extractable soil fractions and the artichoke heads content suggest that this fraction represents a good predictor for the heavy metal contents of the marketable yield [51,52]. However, for the other heavy metals that were examined (Al, Cu and Ni), the DTPA-extractable soil content was not related to the heavy metal content of the heads. This behavior could be due to a different Al, Cu, and Ni uptake rate by artichoke plant.

Table 4. Pearson's correlation coefficient values between the heavy metal content in artichoke fraction (plant or yield/head) and irrigation water and soil heavy metal content.

Artichoke Fraction	Heavy Metal							
	Al	Co	Cr	Cu	Fe	Ni	Zn	Mn
versus Irrigation water								
Plant	ns	ns	0.77 ***	0.78 ***	0.61 ***	0.76 ***	0.60 **	0.80 **
Head	ns	ns	0.83 ***	0.75 ***	0.55 **	0.74 ***	0.76 ***	0.73 ***
versus Total soil content								
Plant	ns	ns	ns	ns	ns	ns	ns	ns
Head	ns	ns	ns	ns	ns	ns	ns	ns
versus DTPA-extractable soil content								
Plant	ns	ns	0.71 ***	ns	0.76 ***	ns	0.51 **	0.74 ***
Head	ns	ns	0.50 **	ns	0.67 ***	ns	0.37 *	0.77 ***

Note: ns, correlation not significant (*p*-value > 0.05). np, correlation analysis not performed because the soil heavy metal content was below the detection limit. *, correlation significant at the 0.05 level (*p*-value $\leq$ 0.05). **, correlation significant at the 0.01 level (*p*-value $\leq$ 0.01). ***, correlation significant at the 0.001 level (*p*-value $\leq$ 0.001).

3.3. Bioaccumulation Factors

Soil-to-plant transfer of toxic heavy metals is one of the key processes through which humans are exposed to heavy metals through the food chain [53]. Table 5 gives the BAF for both the total (BAF_t) and the DTPA-extractable (BAF_e) soil heavy metal concentrations and shows how they are affected by the irrigation water type.

The irrigation water type significantly influenced only BAF_t for Zn ($p \leq 0.01$); the value of this parameter was higher for SWW and TWW than for FW. For Al, Cr, Fe, Ni, and Mn, BAF_t was consistently lower, ranging from 0.002 (Fe under FW) to 0.38 (Cu under SWW).

Many studies have reported that $BAF_t \leq 1$ indicates that even if the plant uptakes the heavy metal, there is not accumulation in the plant, whereas $BAF_t > 1$ suggests that the plant accumulates the heavy metal in the shoot, root or edible fractions [15,53]. Finally, according to Mollazadeh (2014) [54], $BAT_t < 0.1$ suggests that there is no uptake of the heavy metal by the plant. The author [54] indicated that when the plant-to-soil ratio for a specific chemical element is 0.1, then it can be considered that the plant to exclude the chemical element from its tissues.

In the present study, the highest BAF_t values were observed for Zn, with the highest value being recorded for artichoke heads after SWW irrigation (0.92). The high BAF_t values for Zn, although being less than one, highlight the potential for contamination of the artichoke heads by irrigation with

treated wastewater. Sponza and Karaoglu (2002) [55] reported that there is a need for environmental monitoring if BAF > 0.5, as this indicates the possibility of metal contamination of edible vegetables. Generally, under the present experimental conditions, the BAF values suggested that, apart from Zn, the heavy metals are not dangerously accumulated in the edible part of the artichoke.

Studies on the effects of heavy metals on the artichoke growth have been conducted to determine whether this crop can be used in the remediation of polluted soils [56,57]. These studies showed that artichoke plants can tolerate the presence of heavy metals, including arsenic and cadmium, in the soil without great impact on plant growth.

Several studies have emphasized that the assessment of hazardous levels of heavy metals based on the total soil content of individual metals may be inappropriate and that the bioavailability of the metals is of greater relevance to their potential environmental hazard [19,48,58]. Thus, to ensure the more relevant assessment of potential risk, the procedures for determination of metal transfer from soil to crops and the consequent ecotoxicological guidelines should be based on the bioavailable fraction of a particular metal in the soil [48]. Therefore, in the present study, the bioavailable fractions of these heavy metals in terms of their extraction using DTPA were also determined, and the BAF_e were calculated. The effects of irrigation water type on BAF_e are also reported in Table 5.

Table 5. Effect of the type of irrigation water used on bioaccumulation factors for the given heavy metals in the total (BAF_t) and DTPA-extractable (BAF_e) soil fractions. The data shown are the means±standard errors for each heavy metal as determined for 6 samples (3 replicates $\times$ 2 growing seasons).

[1] IW	Bioaccumulation Factor Values for Heavy Metals						
	Al	Cr	Cu	Fe	Ni	Zn	Mn
BAF_t	ns	ns	ns	ns	ns	**	ns
FW	0.004 ± 0.0010	0.018 ± 0.006	0.30 ± 0.05	0.002 ± 0.0004	0.05 ± 0.007	0.44 ± 0.03 [b]	0.03 ± 0.007
SWW	0.005 ± 0.0007	0.023 ± 0.003	0.38 ± 0.09	0.003 ± 0.0008	0.07 ± 0.004	0.92 ± 0.04 [a]	0.04 ± 0.008
TWW	0.005 ± 0.0006	0.022 ± 0.004	0.37 ± 0.04	0.003 ± 0.0004	0.06 ± 0.004	0.87 ± 0.04 [a]	0.04 ± 0.007
BAF_e	*	ns	**	ns	ns	*	*
FW	17.8 ± 0.45 [b]	1.14 ± 0.05	1.95 ± 0.06 [b]	5.38 ± 0.28	3.11 ± 0.09	3.99 ± 0.08 [b]	1.30 ± 0.06 [b]
SWW	21.4 ± 1.02 [a]	1.18 ± 0.02	2.24 ± 0.04 [a]	5.77 ± 0.20	3.29 ± 0.08	6.10 ± 0.44 [a]	1.43 ± 0.08 [a]
TWW	18.83 ± 1.09 [ab]	1.19 ± 0.03	2.14 ± 0.10 [ab]	5.64 ± 0.21	3.21 ± 0.08	5.63 ± 0.18 [ab]	1.42 ± 0.05 [ab]

Note: [1] IW, Irrigation water; FW, fresh water; SWW, secondary treated wastewater; TWW, tertiary treated wastewater. For each heavy metal and bioaccumulation factor, means followed by different superscripted letters (a, b and ab) within each column are significantly different ($p \leq 0.05$; Tukey's test). * $p \leq 0.05$; ** $p \leq 0.01$.

In contrast to the data for BAF_t, the type of irrigation water used influenced the BAF_e values for Al, Cu, Zn, and Mn. The use of SWW significantly increased BAF_e when compared to FW ($p \leq 0.05$), although there were no significant differences between SWW and TWW and between TWW and FW. Therefore, this BAF expressed with respect to the soil extractable metals fraction provides improved the understanding of the effects of the different irrigation water types on the metal accumulation in the plants.

Generally, a value of BAF_e > 1.0 indicates a tendency of the exchangeable fraction of a heavy metal to accumulate in the plant. Among the tested heavy metals, the highest values of BAF_e were obtained for Al, Fe, and Zn; these metals showed greater propensities for accumulation, whereas the BAF_e of Cr and Mn, in particular, were close to unity. As indicated, BAF_t is the ratio between the total plant content and the total soil content of a specific heavy metal, while BAF_e represents the ratio between the total plant content and the bioavailable soil content of the given heavy metal, which is here defined as the soil DPTA-extractable fraction. However, it should also be noted that the method used to determine this bioavailable fraction not accurately represent the fraction that can be assimilated by the plant. This will also affect the calculation and interpretation of BAF_e and should to be considered in particular for some of these heavy metals, such as Al, for which BAF_e is much higher than 1.0. Therefore, the relevance of expressing BAF according to this bioavailable soil fraction, rather than the total soil fraction, will depend primarily on the suitability of the extraction method used.

3.4. Potential Health Risk of Heavy Metal Ingestion Due to Consumption of Artichoke Heads

The HIs calculated for the heavy metals considered in this study, based on the estimated annual consumption of artichoke heads by adults and children, are given in Table 6. Both SWW and TWW significantly affected HI. However, HIs was never >1.0 for artichoke head consumption, suggesting that the health risk that is associated with exposure to these heavy metals due to consumption of artichoke heads is negligible.

The data in Table 6 are in agreement with the results of other studies that have attempted to measure the human risk that is related to the consumption of horticultural crops that have been irrigated with wastewater effluents. Ackah et al. (2014) [15] investigated the risk to human health due to the consumption of lettuce and sorrel that had been irrigated with wastewater. Based on the measured HIs, which were significantly <1.0, they concluded that people who consume these vegetables appear not to be at risk. Another study of the risk to human health from the ingestion of heavy metals (Cu, Zn, Pb, Cd, Hg, and Cr) via consumption of cabbage, fennel, and spinach food crops grown on contaminated soil showed HIs < 1 for both adults and children [16].

Table 6. Main effects of the type of irrigation water used in artichoke cultivation on hazard indices for specific heavy metals. The hazard indices were calculated to define the exposure to non-carcinogenic risk in terms of the estimated consumption of artichoke heads by adults and children. The data shown are the means±standard errors for each heavy metal and were determined on 6 samples for each water source (3 replicates × 2 growing seasons).

[1] IW	Hazard Indices for Specific Heavy Metals						
	Al ($\times 10^{-3}$)	Cr	Cu	Fe ($\times 10^{-3}$)	Ni	Zn ($\times 10^{-3}$)	Mn
Adults	**	**	**	**	**	**	**
FW	0.10 ± 0.006 [b]	0.25 ± 0.013 [b]	0.018 ± 0.001 [b]	1.07 ± 0.06 [b]	0.037 ± 0.002 [b]	2.0 ± 0.1 [b]	0.05 ± 0.003 [b]
SWW	0.16 ± 0.010 [a]	0.36 ± 0.013 [a]	0.027 ± 0.004 [a]	1.55 ± 0.15 [a]	0.054 ± 0.005 [a]	4.0 ± 0.2 [a]	0.08 ± 0.005 [a]
TWW	0.14 ± 0.008 [a]	0.30 ± 0.046 [a]	0.022 ± 0.001 [a]	1.30 ± 0.10 [a]	0.045 ± 0.004 [ab]	3.0 ± 0.4 [a]	0.07 ± 0.004 [ab]
Children	**	**	**	**	*	*	*
FW	0.12 ± 0.007 [b]	0.29 ± 0.015 [b]	0.020 ± 0.001 [b]	1.25 ± 0.06 [b]	0.043 ± 0.002 [b]	2.9 ± 0.15 [b]	0.06 ± 0.002 [b]
SWW	0.18 ± 0.011 [a]	0.42 ± 0.026 [a]	0.031 ± 0.002 [a]	1.81 ± 0.11 [a]	0.063 ± 0.004 [a]	4.2 ± 0.26 [a]	0.09 ± 0.006 [a]
TWW	0.17 ± 0.010 [a]	0.35 ± 0.022 [a]	0.026 ± 0.002 [a]	1.51 ± 0.10 [a]	0.052 ± 0.004 [ab]	3.5 ± 0.20 [a]	0.08 ± 0.005 [ab]

[1] IW, Irrigation water; FW, fresh water; SWW, secondary treated wastewater; TWW, tertiary treated wastewater. For each heavy metal, means followed by different superscripted letters (a, b and ab) in each column are significantly different ($p \leq 0.05$; Tukey's test). * $p \leq 0.05$; ** $p \leq 0.01$ (Fisher's test).

When considering that the presence of two or more heavy metals in artichoke heads might produce additive or interactive toxic effects, we also defined the addition risk hypothesis based on the AHI, the sum of individual metal HIs relative to each of the species that are considered in this study. As Figure 4 shows, the use of SWW and TWW significantly ($p \leq 0.05$) increased AHI compared to FW. This result indicate that the use of treated wastewater might represent a health risk to the consumer. However, because the AHIs for all three types of irrigation water were <1.0, the health risk from collective exposure to all of the heavy metals that are present in the artichoke heads does not appear to represent a significant concern. The estimated AHIs for the consumption by adults of the artichoke heads in this study were lower of the AHIs for children for each of the three types of irrigation water tested (Figure 4). The major contribution to risk from consumption of artichoke heads from plants irrigated with SWW and TWW, both for adults and children, was due to Cr (about 68%).
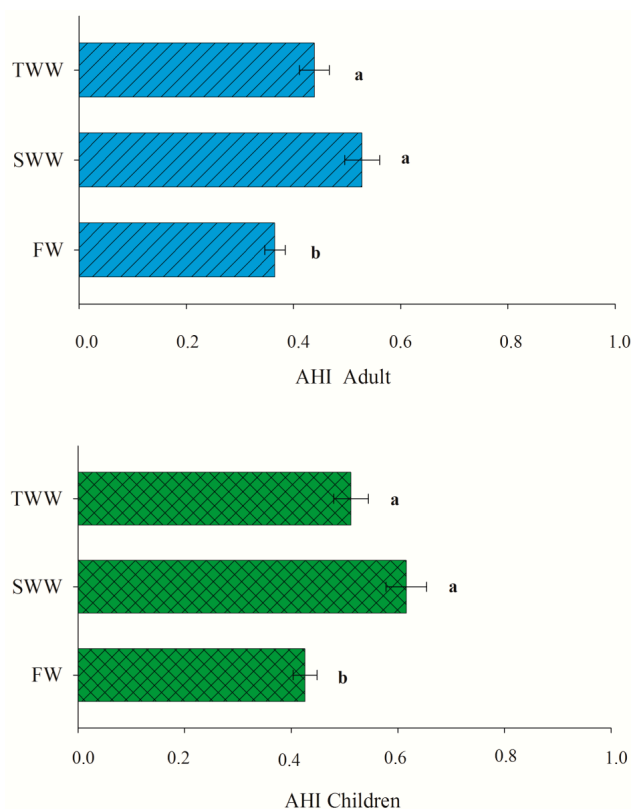
Figure 4. Additive hazard risk index (AHI) for adults and children for the combined heavy metals detected in artichoke heads. FW, fresh water; SWW, secondary treated wastewater; TWW, tertiary treated wastewater. Different letters (a–b) indicate statistically significant differences ($p \leq 0.05$; Tukey's test). The data shown are the means±standard errors of 6 samples (3 replicates × 2 growing seasons).

4. Conclusions

The aim of the study was to determine whether SWW and TWW could be used for the irrigation of artichoke plants without compromising the quality of the soil or the safety of the final product for human consumption. The data led to several interesting conclusions: (i) although Cr, Cu, Zn, Mn contents of SWW and TWW showed great variability as a function of water sampling date, they remained below the heavy metal concentration thresholds as defined by the national [35] and international [40,44] guidelines; (ii) only the exchangeable heavy metal fraction in the soil (DTPA-extractable soil content) highlighted the effects of the different irrigation waters on the heavy metal contents of the artichoke plants and heads, although the national and international thresholds were not exceeded for any of these heavy metals; (iii) the highest BAF_t values that were observed for Zn, (0.9), highlighting the potential for contamination of the artichoke heads by irrigation of the plants with treated wastewater; (iv) generally, the quality of the artichoke heads, as defined by their heavy metal content, remained good after irrigation of the plants with either of the treated municipal wastewaters; (v) the HIs (and AHIs) based on the consumption of the artichoke heads remained <1.0 for both adults and children, thus further indicating that the health risks involving the single (and collective) heavy metals are not significant. Although the data from the present study were obtained using a specific crop (artichoke), they define the key aspects for closer consideration in areas where crop irrigation is carried out with such treated municipal wastewaters.

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