

Fractionation and mobility risks of heavy metals and metalloids in wastewater-irrigated agricultural soils from greenhouses and fields in Gansu, China

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ABSTRACT

Wastewater irrigation reduces the pressure on freshwater usage but leads to the accumulation of heavy metal (loid)s in soils. This study investigated heavy metal(loid)s (As, Cr, Cu, Ni, Pb, and Zn) in the acid-soluble (F1), reducible (F2), oxidizable (F3), and residual (F4) fractions of soils from greenhouses and fields in Baiyin City, Gansu, China, which had been irrigated with treated industrial and municipal wastewater. Risk assessment (RAC) and modified risk assessment (mRAC) codes were used to estimate the environmental risks based on metal mobility. Results showed that more than half of each studied heavy metal(loid) (72.6%–97.4%) was present in the residual fraction (F4), which is non-bioavailable. Both the concentrations and percentages of metals in the bio-accessible fractions (F1, F2, and F3) showed no significant differences ($p > 0.05$) between rhizosphere and bulk soils. The greenhouse soils had higher concentrations and percentages of metals (except Pb) in bio-accessible fractions compared with field soils. Similarly, compared with irrigation using treated municipal wastewater, irrigation with treated industrial wastewater resulted in higher concentrations and percentages of all the studied metals in the bio-accessible fractions of soils. The average RAC value of each heavy metal(loid) in soils suggested medium risks from Zn, low risks from As, Cu, Ni, and Pb, and no risks from Cr. According to the mRAC values for 120 soil samples, there were only 13.3% of soils with no potential adverse effect, but 83.3% of soils with a low potential adverse effect and 3.3% of soils with a medium potential adverse effect.

1. Introduction

Water scarcity has gradually emerged as one of the most important issues in many regions and countries (Saffari and Saffari, 2013; Pontoni et al., 2016). Agriculture is one of the largest freshwater consumers, and its sustainability is threatened by the increasing pressures from freshwater shortages (Aydin et al., 2015). Large amounts of wastewater effluents are annually discharged from industrial and municipal wastewater treatment plants to the surrounding water bodies (i.e., rivers and lakes) (Ullah and Khan, 2015; Balkhair and Ashraf, 2016). Using treated wastewater to replace freshwater in agricultural irrigations can reduce the pressures of water scarcity (Ayoub et al., 2016). Treated wastewater containing certain amounts of nutrients (i.e., nitrogen, potassium, and

phosphorus) can improve soil fertility (Samia et al., 2013). These benefits have promoted the wide replacements of freshwater with treated wastewater in agricultural irrigation, particularly in arid and semi-arid regions (i.e., the Mediterranean and the Middle East Region) (Aydin et al., 2015; Cary et al., 2015; Qureshi et al., 2016).

However, treated wastewater still contains small amounts of contaminants (i.e., heavy metal(loid)s and toxic organic compounds), whereas untreated wastewater may contain considerably higher amounts of such contaminants. Using treated or untreated wastewater for irrigation would therefore introduce these contaminants into agricultural soils. Among these contaminants, heavy metal(loid)s are considered the most dangerous group of pollutants due to their toxic, persistent, and bio-accumulative properties (Liu et al., 2013; Aydin

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et al., 2015). The effects of irrigation with treated (Ayoub et al., 2016; Lu et al., 2016; Qureshi et al., 2016; Turner et al., 2016) and untreated (Aydin et al., 2015; Khan and Bano, 2016; Meng et al., 2016) wastewater on the accumulation of heavy metal(loid)s in soils have frequently been reported and are well summarized by Elgallal et al. (2016). The elevated concentrations of heavy metal(loid)s in soils from wastewater irrigation have been consistently observed in these studies, and heavy metal(loid) pollution is expected to be more severe in soils irrigated with untreated wastewater (Liang et al., 2015; Meng et al., 2016). In addition, wastewater irrigation in greenhouses, which are widely adopted for increasing agricultural production, may lead to substantial accumulations of heavy metal(loid)s in soils due to the associated intensive anthropogenic activities (e.g., extensive use of fertilizers) (Tian et al., 2016). Furthermore, food crops (e.g., vegetables) growing in contaminated soils can contain high concentrations of heavy metal(loid)s in their edible parts, and potential adverse health risks to humans through dietary exposure can be expected (Balkhair and Ashraf, 2016; Bednarova et al., 2016; Cao et al., 2016).

Currently, studies on soils contaminated by heavy metal(loid)s through wastewater irrigation are mainly focusing on contaminant concentrations (Liu et al., 2013). However, the adverse health risks are related not only to the total concentrations of heavy metal(loid)s but also to their mobility and availability in soils (Cui et al., 2016). Heavy metal(loid)s exist in various forms in soils, including dissolved in the soil solution, adsorbed on exchangeable sites, bound to carbonates and Fe/Mn oxides, complexed with soil organic matter and sulfides, and embedded in mineral matrices (Hu et al., 2016; Ma et al., 2016). Their toxicity, mobility, and bioavailability depend strongly on their forms or binding states and soil physicochemical properties [i.e., pH, cation exchange capacity (CEC), and organic matter] (Ghrefat et al., 2012; Gasparatos et al., 2015; Cui et al., 2016). Moreover, root activities greatly affect the physicochemical properties of rhizospheric soils and thus the speciation distributions of heavy metal(loid)s (Tao et al., 2003). Additionally, immobilization of heavy metal(loid)s in soils by the addition of soil amendments is considered one of the beneficial methods for reducing their associated environmental risks (Khan et al., 2015), including health risks through dietary exposure. Therefore, a good understanding of the chemical fractions of heavy metal(loid)s in contaminated soils would be beneficial for assessing their potential environmental risks (Liu et al., 2013; Yang et al., 2013).

The objectives of this study were as follows: (1) to investigate the fraction concentrations of heavy metal(loid)s (As, Cr, Cu, Ni, Pb, and Zn) in agricultural greenhouse and field soils irrigated with treated industrial and municipal wastewater and sown with four vegetable species (carrot, rape, Chinese lettuce, and leafy lettuce); (2) to evaluate the effects of irrigation type (treated industrial and municipal wastewater), cultivation mode (greenhouse and field), and soil compartment (rhizosphere and bulk soil) on the mobility and bioavailability of heavy metal(loid)s in soils; and (3) to estimate the environmental risks of heavy metal(loid)s based on their potential mobility and bioavailability in soils.

2. Materials and methods

2.1. Study area

In 2015, the Chinese Ministry of Finance and Chinese Ministry of Environmental Protection provided funding to 30 priority areas, with metal(loid)-contaminated soil, for remediation (http://jjs.mof.gov.cn/zhengwuxinxi/tongzhigonggao/201506/t20150602_1248397.html). The first listed area was Baiyin City, Gansu Province, in northwestern China, also named “Tong Cheng” (which means “Copper City” in Chinese) (Cao et al., 2016). The mean annual air temperature, precipitation, and evapotranspiration in this area are 7.5 °C, 315 mm, and 2101 mm, respectively (Cao et al., 2016; Cao et al., 2018). Since the 1950s, this city has been an important non-ferrous metal mining and

smelting base in China (Nan and Zhao, 2000; Li et al., 2006). On the basis of its watersheds, the city is divided into the Xidagou Basin (or “West Big Ditch,” 428 km²) and the Dongdagou Basin (or “East Big Ditch,” 368 km²) (Nan and Zhao, 2000). As a consequence of water shortages, industrial and municipal (or domestic) wastewater has been used for irrigation since the 1960s (Nan et al., 1999; Nan and Li, 2000). Specifically, farmers in the Xidagou and Dongdagou basins have primarily used treated municipal wastewater and industrial wastewater as irrigation water, respectively (Nan and Zhao, 2000). Other than in field, greenhouse cultivation is also common in both these basins (Cao et al., 2016). As and Cd concentrations in the irrigation wastewater in the two basins are higher than the limits prescribed by the Chinese standards for irrigation water quality (State Standard of China, 2005), as previously reported by Cao et al. (2016).

The two basins in Baiyin City have important farmland that provides vegetables for the local communities (Cao et al., 2016). These vegetables include mainly carrot (*Daucus carota* L.), Chinese lettuce (*Lactuca sativa* L.), rape (*Brassica chinensis* L.), leafy lettuce (*Lactuca sativa* var. *longifolia* L.), Chinese cabbage (*Brassica pekinensis* L.), tomato (*Lycopersicon esculentum* L.), zucchini (*Cucurbita pepo* L.), and eggplant (*Solanum melongena* L.). In the present study, we selected two representative greenhouses and fields in the Xidagou and Dongdagou basins as experimental sites (Fig. 1). Four types of vegetables, carrot, Chinese lettuce, rape, and leafy lettuce, were planted in the greenhouses (average temperature ~25 °C) on February 1, 2014 and harvested on April 10, 2014 (a cycle of ~70 days), and were watered once before sowing and once on day 45 of growth. In the fields (average temperature ~7.1 °C), the same four vegetables were planted on February 20, 2014 and harvested on May 10, 2014 (a cycle of ~80 days), and were watered once before sowing and once on day 50 of growth.

2.2. Soil sampling and analysis

The local soil is calcareous (Sierozem), with a cultivation layer of 0–19 cm, plow pan of 19–30 cm, subsoil of 30–87 cm, and loess parent material below 87 cm. The soil is classified as Aridosol according to National standards of China (2009) and Calcisol/Gypsisol according to World Reference Base (2014). The farmlands have a slope of < 15°. At the end of the growing seasons, both rhizosphere and bulk soils were collected from a depth of 0–5 cm. The rhizosphere soil was collected by carefully brushing off the soil attached to the plant roots, and the bulk soils were collected at a distance of approximately 1–8 cm from the plant roots (Wang et al., 2014; Chen et al., 2015). Each soil sample was a composite of four subsamples, with each subsample collected from around at least 10 individual plants of the same type of vegetable species. In total, 60 composite rhizosphere soils and 60 bulk soils (3–4 composite rhizosphere and 3–4 bulk soil samples for each of the 4 vegetable types and each of the 4 sites) were collected. Specifically, among these 120 soils, there was a quarter of samples collected from each of the 4 sites, i.e., the Xidagou greenhouses, Xidagou fields, Dongdagou greenhouses, and Dongdagou fields. Any fine root fragments in soil samples were carefully excluded to reduce potential errors caused by possibly large differences between root and soil metal(loid) concentrations (Wang et al., 2012; Guo et al., 2013; Fahey et al., 2017).

All soil samples were air-dried at room temperature and passed through a 100-mesh sieve. For digestion, soil samples (0.500 g) were mixed with 10 ml concentrated HCl, 10 ml concentrated HNO₃, 10 ml concentrated HF, and 2 ml concentrated HClO₄ in a 300 ml Teflon beaker. After digesting for 3 h at 240 °C, the solution was transferred to a volumetric flask, which was then filled to 100 ml with Milli-Q water. Inductively coupled plasma atomic emission spectroscopy (ICP-AES: IRIS Intrepid XSP; Thermo Fisher Scientific, MA, US) was used to detect the concentrations of Cu, Ni, and Zn in the digested soil samples. Concentrations of As, Cr, and Pb in the digested soil samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS:

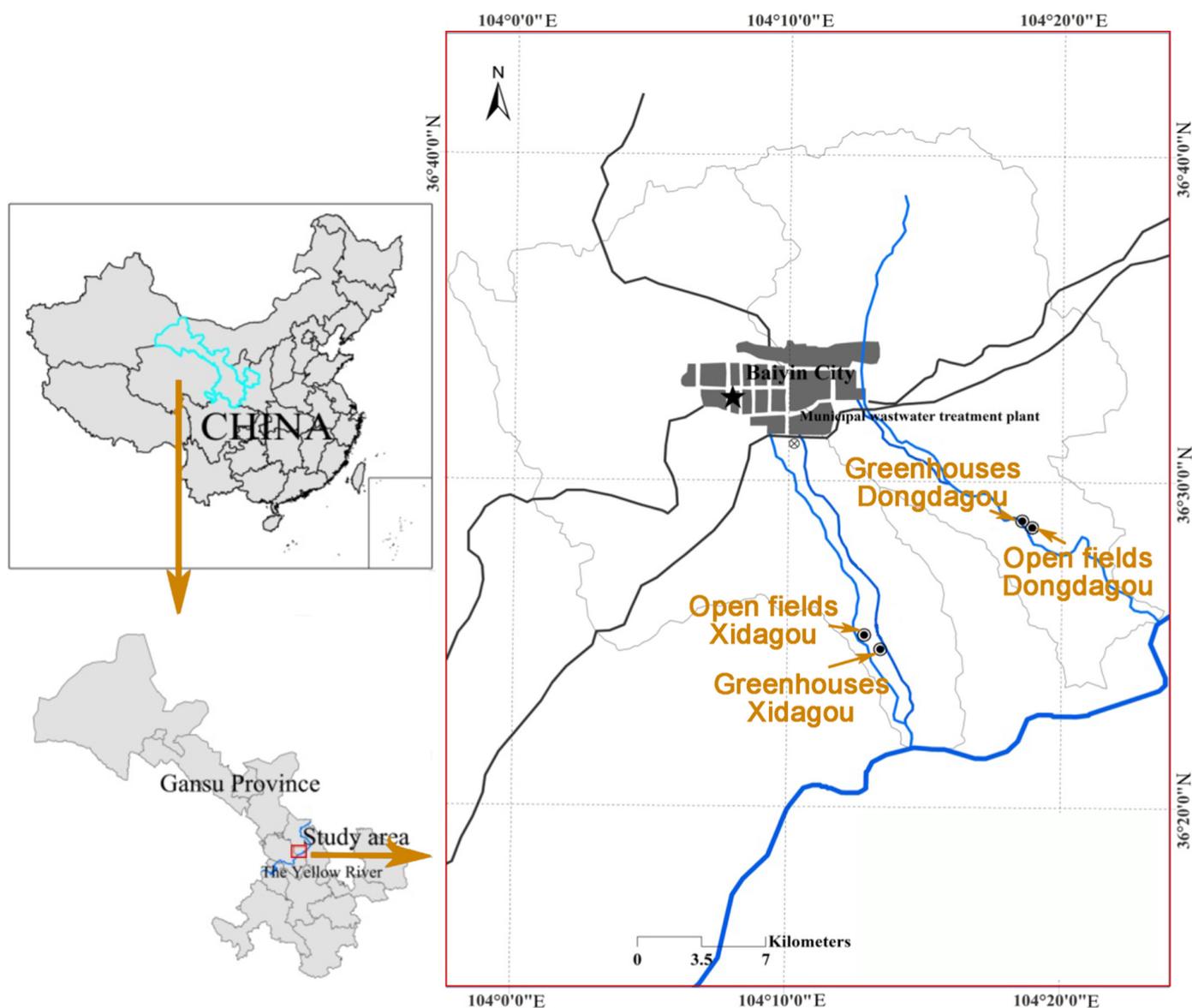


Fig. 1. Location of the sampling sites.

Source: Cao et al. (2016).

X7; Thermo Fisher Scientific, MA, US). Recoveries for all the studied metal(loid)s ranged between 86.8% and 116.9%, and the repeated tests on standards and procedure blanks showed acceptable relative standard deviation ($< 20\%$).

Ten gram of sieved soil sample was mixed with 25-ml deionized water (1:2.5, w/v) for 3 min and the pH value was measured after equilibrating for 30 min using PHS-3C pH meter (Rex Instrument Factory, Shanghai, China). Soil total carbon and total nitrogen contents (including organic and inorganic contents) in the dried and sieved soil samples were analyzed using an elemental analyzer (Vario EL III; Elementar, Germany). Soil organic matter was quantified using the traditional chromic acid oxidation and titration method (Walkley and Black, 1934). Specifically, a 0.200-g dried and sieved (160-mesh) soil was mixed with 0.100 g Ag_2SO_4 and 10 ml $\text{K}_2\text{Cr}_2\text{O}_7$ -concentrated- H_2SO_4 solution (0.068 mol l^{-1} of $\text{K}_2\text{Cr}_2\text{O}_7$) and boiled for 5 min at $170\text{--}180^\circ\text{C}$ in oil bath. After cooling down to the room temperature, the residue dichromate was titrated using 0.2 mol l^{-1} FeSO_4 . The oxidizable organic C was then calculated based on the amount of FeSO_4 consumed, and the soil organic matter was defined as 1.724 times of soil organic C. Soil CEC was determined by the ammonium acetate

(NH_4OAc) method (Institute of Soil Science at Chinese Academy of Sciences, 1978; Sumner and Miller, 1996). Briefly, a 1.000-g dried and sieved (60-mesh) soil was shaken with an $\text{EDTA-NH}_4\text{OAc}$ solution (5 mol l^{-1} EDTA and 1 mol l^{-1} NH_4OAc), stood for 16 h, and centrifuged at $1000g$ for 5 min. The supernatant was removed, and the soil was washed with NH_4OAc for four more times and with 95% methanol for eight times to remove excess cations. The ammonium remaining in soil was exhaustively exchanged with 1 mol l^{-1} KCl for eight times and was then quantified by a Kjeldahl auto analyzer (K9840, NADE Scientific Instrument Co., Ltd., China). The CEC value was calculated as the exchangeable number of moles of the ammonium in the soil.

2.3. Sequential extraction

A three-step sequential extraction technique was developed by the Commission of the European Communities Bureau of Reference (BCR) (Ure et al., 1993), which can be used to separate heavy metal(loid)s into four forms: an acid-soluble fraction (F1) of soluble and exchangeable metal(loid)s, a reducible fraction (F2) of metal(loid)s bound to Fe/Mn oxides, an oxidizable fraction (F3) of metal(loid)s bound to organic

matter, and a residual fraction (F4) of residual metal(loid)s such as silicates. A modified version of the BCR procedure for fractionating heavy metal(loid)s in soil samples (Yang et al., 2013; Ma et al., 2016) is described below:

Step 1: One gram of sieved dry soil sample was weighed and placed in a 100 ml tube. After adding 40 ml of 0.1 mol l⁻¹ acetic acid (HOAc) solution, the tube was shaken gently for 16 h at 25 °C and then centrifuged at 9689 × g for 10 min. The supernatant in the tube was transferred to a 50 ml volumetric flask and diluted with 2% nitric acid (HNO₃) solution to a volume of 50 ml. The 50 ml diluted supernatant was considered the acid-soluble fraction and stored at 4 °C. The remaining pellet was then rinsed twice with 0.1 mol l⁻¹ HOAc solution prior to the next extraction.

Step 2: After adding 40 ml of 0.5 mol l⁻¹ hydroxylamine hydrochloride (NH₂OH·HCl) solution, the tube was shaken for 16 h at 25 °C, and then centrifuged at 9689 × g for 10 min. The supernatant in the tube was transferred to a 50 ml volumetric flask and diluted with 2% HNO₃ solution to a volume of 50 ml. The 50 ml diluted supernatant was considered the reducible fraction and stored at 4 °C. The remaining pellet was then rinsed twice with 0.5 mol l⁻¹ NH₂OH·HCl solution prior to the next extraction.

Step 3: After slowly adding 10 ml of 30% hydrogen peroxide (H₂O₂) solution (pH = 2–3), the tube was evenly stirred at room temperature for 1 h. The tube was placed in a water bath at 85 ± 2 °C until the sample volume was reduced to 3 ml. After adding 10 ml H₂O₂, the tube was again placed in a water bath at 85 ± 2 °C until the sample volume was condensed to 1 ml. After adding 50 ml of 1 mol l⁻¹ ammonium acetate (NH₄OAc) solution, the tube was shaken gently for 16 h at 25 °C, and then centrifuged at 9689 × g for 10 min. The supernatant in the tube was transferred to a 50 ml volumetric flask and diluted with 2% HNO₃ to 50 ml. The 50 ml diluted supernatant was considered the oxidizable fraction and stored at 4 °C.

Residual Fraction: The sample left in the tube was considered the residual fraction. Concentrations of heavy metal(loid)s in F4 were obtained by subtracting the concentrations in fractions F1, F2, and F3 from the total concentrations (Table S1 in the supplementary information).

2.4. Mobility risk assessments

The speciation distribution of heavy metal(loid)s is important in assessing the potential mobility and bioavailability of heavy metal(loid)s in soils (Moore et al., 2015). The risk assessment code (RAC) is one of the prevalent indices developed by Perin et al. (1985) for evaluating the mobility of heavy metal(loid)s. It is the proportion of metal(loid) concentration in the exchangeable and carbonate fractions to the total concentration [Eq. (1)]. On the basis of the RAC, Saeedi and Jamshidi-Zanjani (2015) developed a new index [named the modified risk assessment code (mRAC)] by taking into consideration metal(loid) toxicity [Eq. (2)]. The classifications of the environmental risks based on the RAC and mRAC values are shown in Table 1.

$$RAC_i = \frac{C_{ex,i} + C_{carb,i}}{C_{t,i}} \times 100\% \quad (1)$$

$$mRAC = \frac{\sum_{i=1}^n T_{ri} RAC_i}{\sum_{i=1}^n T_{ri}} \quad (2)$$

where $C_{ex,i}$ is the concentration of metal(loid) i in the exchangeable fraction; $C_{carb,i}$ is the concentration of metal(loid) i in the carbonate fraction; $C_{t,i}$ is the total concentration of metal(loid) i ; RAC_i is the RAC value of metal(loid) i ; T_{ri} is the toxic factor of metal(loid) i ; and n is the total number of metal(loid)s. The suggested toxic factors (T_r) for As, Cr, Cu, Pb, Ni, and Zn are 10, 2, 5, 5, 2, and 1, respectively (Hakanson, 1980; Saeedi and Jamshidi-Zanjani, 2015). The metal(loid) concentration in the exchangeable and carbonate fractions in Eq. (1) is equal to

Table 1

Criteria for risk assessment code (RAC) and modified risk assessment code (mRAC).

Source: Perin et al. (1985) and Saeedi and Jamshidi-Zanjani (2015).

Index values	Indications
RAC (%)	
RAC < 1	No risk
1 ≤ RAC < 10	Low risk
10 ≤ RAC < 30	Medium risk
30 ≤ RAC < 50	High risk
RAC ≥ 50	Very high risk
mRAC	
mRAC < 1	No potential adverse effect
1 ≤ mRAC < 10	Low potential adverse effect
10 ≤ mRAC < 30	Medium potential adverse effect
30 ≤ mRAC < 50	High potential adverse effect
mRAC ≥ 50	Very high potential adverse effect

the concentrations in the acid-soluble fraction (F1) in our study.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate whether the effect of operations (*i.e.*, different types of vegetable planting, rhizosphere *versus* bulk soils, treated industrial *versus* municipal wastewater irrigation, and greenhouses *versus* fields) on metal(loid) speciation was statistically significant. RStudio Desktop version 1.0.44 (Boston, MA, USA) was employed in our study for data and statistical analyses.

3. Results

Table 2 shows the average fraction concentrations of heavy metal(loid)s in the 120 analyzed soil samples, along with their percentages of the total concentrations shown in Table 3. The mean concentrations of As in F1, F2, F3, and F4 were 2.35, 1.26, 0.80, and 96.87 mg kg⁻¹, corresponding to 3.65%, 1.91%, 1.31%, and 93.14% of the total concentrations, respectively (Tables 2 and 3). For both concentrations and percentages, the descending order was F4 ≫ F1 > F2 > F3. This indicates that As predominated mainly in the residual fraction. Heavy metal(loid)s in the residual fraction (F4) are relatively stable and unlikely to be released because they are strongly bound to the crystalline structures of minerals in soil matrices (Nemati et al., 2011; Matong et al., 2016). The mean concentrations of Cr in F1, F2, F3, and F4 were 0.08, 0.13, 1.04, and 48.27 mg kg⁻¹, which accounted for 0.17%, 0.29%, 2.20%, and 97.35% of the total concentrations, respectively (Tables 2 and 3). The descending order of concentrations and percentages for Cr was F4 ≫ F3 > F2 > F1. Among all the metal(loid)s, Cr had the lowest concentrations in both the acid-soluble and reducible fractions, but the highest percentage in the residual fraction. In this study, we found that almost all of the Cr was present in the residual fraction. Compared with the greenhouse and field soils without wastewater irrigation examined by Sungur et al. (2016), the Cr in our study had higher values in F4 but lower values in F1, F2, and F3, in terms of both concentrations and percentages, indicating greater stability and less bioavailability.

Cu had the mean concentrations of 1.24, 1.83, 10.09, and 46.48 mg kg⁻¹ in F1, F2, F3, and F4, corresponding to the percentages of 2.09%, 2.75%, 16.13%, and 79.03%, respectively (Tables 2 and 3). The descending order for Cu distribution was F4 ≫ F3 > F2 > F1. Among all the metal(loid)s, Cu had the highest percentage in the oxidizable fraction. Heavy metal(loid)s in the oxidizable fraction are bound to organic matter and sulfides and will be released into the environments under oxidative conditions (Nemati et al., 2011; Matong et al., 2016). The mean concentration of Cu was higher in F1 and F4 but lower in F2 in our study compared with soils in Nannoni and Protano

Table 2Average mass concentrations (mg kg⁻¹) of heavy metal(loid)s in soils. Numbers in parentheses are the standard deviations (n = 120).

Metal(loid)s	This study	Uncontaminated soils in Nannoni and Protano (2016)	Contaminated soils in Nannoni and Protano (2016)	Field soil in Sungur et al. (2016)	Greenhouse soils in Sungur et al. (2016)
Acid-soluble fraction (F1)					
As	2.35 (4.13)	–	–	–	–
Cr	0.08 (0.08)	0.13 (0.03)	–	3.35 (0.91)	7.36 (1.58)
Cu	1.24 (1.15)	0.16 (0.02)	0.43 (0.26)	0.27 (0.18)	0.40 (0.13)
Ni	0.87 (1.16)	2.30 (0.55)	–	1.31 (0.44)	2.00 (0.67)
Pb	0.69 (0.54)	0.15 (0.09)	1.0 (0.7)	0.42 (0.15)	1.65 (0.71)
Zn	29.20 (25.83)	1.5 (0.5)	6.1 (3.2)	3.57 (0.64)	11.23 (1.85)
Reducible fraction (F2)					
As	1.26 (2.33)	–	–	–	–
Cr	0.13 (0.13)	2.2 (0.66)	–	10.73 (2.39)	18.05 (1.91)
Cu	1.83 (3.69)	2.2 (0.8)	6.2 (5.9)	2.12 (0.21)	6.15 (1.52)
Ni	1.09 (1.49)	4.00 (0.99)	–	9.68 (0.98)	12.40 (3.17)
Pb	2.09 (1.79)	12.1 (3.9)	61.6 (44.3)	9.13 (1.06)	11.72 (1.37)
Zn	25.37 (18.98)	7.4 (1.8)	31.6 (21.1)	4.75 (0.58)	17.99 (5.66)
Oxidizable fraction (F3)					
As	0.80 (1.24)	–	–	–	–
Cr	1.04 (0.91)	9.4 (1.6)	–	11.76 (1.34)	14.31 (1.41)
Cu	10.09 (12.10)	2.1 (0.2)	11.1 (7.5)	3.61 (1.30)	5.13 (2.97)
Ni	3.64 (2.24)	4.5 (1.3)	–	8.73 (1.37)	12.74 (3.13)
Pb	1.65 (2.74)	1.60 (0.79)	14.7 (13.9)	1.39 (0.18)	1.52 (0.23)
Zn	17.32 (28.12)	4.0 (2.1)	14.9 (8.2)	3.75 (0.93)	9.36 (0.72)
Residual fraction (F4)					
As	96.87 (61.53)	–	–	–	–
Cr	48.27 (8.38)	70.7 (13.1)	–	44.41 (4.99)	40.05 (9.19)
Cu	46.48 (22.76)	20.8 (1.2)	40.0 (11.2)	28.95 (5.84)	31.78 (10.66)
Ni	28.01 (6.97)	35.9 (6.8)	–	24.94 (5.01)	25.85 (6.65)
Pb	35.05 (28.48)	12.8 (4.5)	36.3 (15.9)	4.69 (0.96)	4.65 (0.29)
Zn	207.11 (137.12)	70.6 (15.0)	104.0 (19.3)	31.85 (6.08)	40.20 (4.03)

–: not available.

Table 3

Average mass percentages (%) of heavy metal(loid)s in soil. Numbers in parentheses are the standard deviations (n = 120).

Metal(loid)s	This study	Uncontaminated soils in Nannoni and Protano (2016) ^a	Contaminated soils in Nannoni and Protano (2016) ^a	Field soil in Sungur et al. (2016)	Greenhouse soils in Sungur et al. (2016)
Acid-soluble fraction (F1)					
As	3.65 (6.85)	–	–	–	–
Cr	0.17 (0.17)	–	–	4.8	9.2
Cu	2.09 (1.40)	0.6	–	0.8	0.9
Ni	2.76 (3.26)	–	–	2.9	3.8
Pb	1.80 (1.34)	0.5	0.9	2.7	8.4
Zn	11.69 (7.10)	2	–	8.1	14.3
Reducible fraction (F2)					
As	1.91 (3.77)	–	–	–	–
Cr	0.29 (0.32)	–	–	15.3	22.6
Cu	2.75 (6.17)	9	11	6.1	14.2
Ni	3.15 (4.18)	9	–	21.7	23.4
Pb	5.23 (3.45)	44	50	58.4	60.0
Zn	9.62 (4.33)	9	–	10.8	22.8
Oxidizable fraction (F3)					
As	1.31 (2.30)	–	–	–	–
Cr	2.20 (1.99)	11	–	16.8	17.9
Cu	16.13 (8.49)	8	18	10.3	11.8
Ni	11.07 (6.15)	10	–	19.5	24.0
Pb	3.48 (2.89)	5	11	8.9	7.8
Zn	6.11 (4.44)	–	–	8.5	11.9
Residual fraction (F4)					
As	93.14 (12.56)	–	–	–	–
Cr	97.35 (2.02)	–	–	63.2	50.2
Cu	79.03 (12.09)	82	70	82.8	73.1
Ni	83.07 (10.02)	76	–	55.9	48.8
Pb	89.46 (6.87)	49	39	30.0	23.8
Zn	72.62 (13.89)	84	–	72.5	49.0

–: not available.

^a Median value.

(2016) (uncontaminated and contaminated soils) and Sungur et al. (2016) (field and greenhouse soils). The mean concentrations of Ni in F1, F2, F3, and F4 were 0.87, 1.09, 3.64, and 28.01 mg kg⁻¹, which accounted for 2.76%, 3.15%, 11.07%, and 83.07% of the total concentrations, respectively (Tables 2 and 3). The descending order for Ni was F4 ≫ F3 > F2 > F1. Pb had the concentrations of 0.69, 2.09, 1.65, and 35.05 mg kg⁻¹, corresponding to the percentages of 1.80%, 5.23%, 3.48%, and 89.46%, respectively (Tables 2 and 3). The descending order for Pb was F4 ≫ F2 > F3 > F1. Compared with the previous studies (Nannoni and Protano, 2016; Sungur et al., 2016), the Ni and Pb in our study had the higher percentages in F4, indicating greater stability. The mean concentrations of Zn in F1, F2, F3, and F4 were 29.20, 25.37, 17.3, and 207.11 mg kg⁻¹, corresponding to the percentages of 11.69%, 9.62%, 6.11%, and 72.62%, respectively (Tables 2 and 3). The descending order for Zn was F4 ≫ F1 > F2 > F3. Zn was mostly present in the residual fraction and was accordingly considered to be non-bioavailable. Notably, among all the studied heavy metal(loid)s, Zn had the highest concentrations in F1, F2, F3, and F4.

4. Discussion

4.1. Heavy metal(loid) fractionations in soils

Heavy metal(loid)s in the reducible fraction are bound to Fe/Mn oxides and will be released into the environment along with the decomposition of the oxides or hydroxides (Matong et al., 2016). In contrast, heavy metal(loid)s in the acid-soluble fraction are loosely bound on soil matrices, and thus considered labile, bioavailable, and highly toxic (Moore et al., 2015). On the basis of the BCR fractions, heavy metal(loid)s can be separated into bioavailable (F1), potentially bioavailable (F2 + F3), bioaccessible (F1 + F2 + F3), and non-bioavailable (F4) heavy metal(loid)s (Devi and Saroha, 2014; Nannoni and Protano, 2016). The descending order for percentages in the residual fraction was Cr (97.35%) > As (93.14%) > Pb (89.46%) > Ni (83.07%) > Cu (79.03%) > Zn (72.62%). More than half of each of the heavy metal(loid)s examined in our study was detected in the residual fraction and accordingly considered non-bioavailable. This therefore suggests that the environmental risks of these heavy metal(loid)s in soils may be low in our study area. High percentages of metal(loid) in the residual fraction have also been reported in previous studies. For example, > 60% of As, Cr, Mo, V, and Zn were shown to be present in the residual fraction of waste clay sediments discharged from a phosphate beneficiation process (Al-Hwaiti et al., 2015). Similarly, Zn and Cu percentages of 81.8% and 69.6% were detected in the residual fraction of soils irrigated with freshwater, and percentages of 77.6% and 66.2% were detected in soils irrigated with treated municipal wastewater (Saffari and Saffari, 2013).

4.2. Factors affecting bioaccessible metal(loid)s in soils

Table 4 shows the concentrations and percentages of metal(loid)s in F1 + F2 + F3 under different operational parameters. These parameters include: (1) soil compartment (rhizosphere and bulk soils); (2) cultivation mode (greenhouse and field soils); and (3) irrigation type (soils irrigated with treated industrial and municipal wastewater). Analysis of variance (ANOVA) was conducted to evaluate whether differences in these operational parameters had caused the significant differences in metal(loid) concentrations and percentages in F1 + F2 + F3, and the results are shown in Table 5.

4.2.1. Rhizosphere versus bulk soils

With no specific amendments in rhizosphere soils in our study, both the concentrations ($p > 0.05$) and percentages ($p > 0.05$) of metal(loid)s in F1 + F2 + F3 showed no significant differences across the four types of rhizosphere soils (planted with four different vegetables)

or between rhizosphere and bulk soils. This finding differs from previously reported shifts in the metal(loid) speciation of rhizosphere soil with plant growth based on laboratory-based rhizobox studies (Tao et al., 2003; Tao et al., 2004). This discrepancy can probably be attributed to that a single growth cycle of selected vegetables is not sufficient to cause any significant change in soil metal(loid) speciation. This is evident from soil chemical properties, such as soil pH, CEC, and organic matter content, which did not differ significantly between rhizosphere and bulk soils (Table 6). Moreover, the more complex conditions of the field environment compared with the strictly controlled laboratory conditions may also have introduced interferences (e.g., watering with metal(loid)-rich wastewater) to narrow the differences in metal(loid) speciation between rhizosphere and bulk soils. There were, however, slightly higher percentages of As, Cr, Ni, Pb, and Zn in the F1 + F2 + F3 of rhizosphere soils and for Cu in bulk soils.

Significant differences in metal(loid) concentrations in F1, F2, and F3 between rhizosphere and bulk soils have, nevertheless, been observed by Li et al. (2016) in soils amended with sulfur and phosphorus. Zhao et al. (2016) observed higher percentages of potentially bioavailable heavy metal(loid)s in rhizosphere soils, along with higher percentages in the residual and bioavailable fractions of bulk soils. Cheng et al. (2015) showed lower percentages of exchangeable and carbonate Pb and higher percentages of Pb bound to Fe/Mn oxides in rhizosphere soils. Available Pb in rhizosphere soils of *Athyrium wardii* was found to be 17 times higher than that in bulk soils (Zou et al., 2012); in contrast, available Cu in rhizosphere soils has been shown to be significantly lower than that in bulk soils (Motaghian and Hosseinpour, 2015). Variations in the metal(loid) fractions between rhizosphere and bulk soils could be attributed to many factors (i.e., soil physicochemical properties, soil microorganisms, and root activities), which are conversely employed to immobilize the metal(loid)s and make them less bioavailable in rhizosphere soils (Azimzadeh et al., 2014; Tang et al., 2016).

4.2.2. Greenhouse versus field soils

Because larger amounts of irrigation water and fertilizers are used in greenhouses for higher productions in shorter times and out of season (Kong et al., 2014; Sungur et al., 2016), reported metal(loid) concentrations in greenhouse soils tend to be higher than those in field soils (Yang et al., 2015; Tian et al., 2016). The average total concentrations (mg kg⁻¹) in field and greenhouse soils were 132.18 and 63.68 for As, 58.58 and 71.22 for Cu, and 31.18 and 49.69 for Pb, respectively (Table S1). There were, however, no significant differences in the concentrations of the other studied metal(loid)s. The significantly higher soil As concentrations in fields compared with greenhouses are presumed to be due to the long-term difference in atmospheric As deposition (Cao et al., 2016), because it could not be explained by the similar As concentrations in irrigation water (Cao et al., 2016) or higher agrochemical application in greenhouses.

Importantly, our study showed significant differences in the percentages of heavy metal(loid)s ($p < 0.05$) in F1 + F2 + F3 between the greenhouse and field soils. Compared with field soils, greenhouse soils had higher concentrations and percentages of heavy metal(loid)s in F1 (except Pb and Ni), F2 (except Cr, Cu, and Pb), F3, and F1 + F2 + F3 (except Pb) (Tables S1–S2). These results are consistent with the findings of previous studies (Massas et al., 2009; Ramos-Miras et al., 2011; Sungur et al., 2016). Ramos-Miras et al. (2011) have also shown that both the mean and median concentrations of EDTA-extractable metal(loid)s (Cd, Pb, Ni, Zn, Cu, and Co) were higher in greenhouse soils than in control soils. Sungur et al. (2016) attributed the higher mobile fractions (F1 + F2 + F3) of Cd, Pb, Ni, Cr, Zn, and Cu in greenhouse soils to the intensive inputs, decreased pH, and increased organic matter. Indeed, the greenhouse soils examined in the present study had slightly lower average pH and higher organic matter content compared with field soils (Table 6).

Table 4

Mass concentrations and percentages of the bioaccessible metal(loid)s in soils under various parameters. Numbers in parentheses are the standard deviations ($n = 60$).

Metal(loid)s	Soil compartment		Cultivation mode		Irrigation type	
	Rhizosphere	Bulk	Field	Greenhouse	Industrial wastewater	Municipal irrigation
F1 + F2 + F3 (mg kg ⁻¹)						
As	4.56 (7.80)	4.25 (7.37)	2.08 (1.12)	7.21 (10.54)	7.66 (9.61)	1.10 (0.32)
Cr	1.30 (0.92)	1.19 (0.89)	0.68 (0.43)	1.93 (0.86)	1.35 (1.19)	1.15 (0.50)
Cu	12.14 (15.59)	14.43 (12.81)	10.76 (6.72)	18.39 (23.29)	16.70 (18.29)	9.56 (7.77)
Ni	5.72 (4.24)	5.48 (3.36)	4.50 (1.77)	7.22 (5.20)	7.24 (4.46)	4.35 (2.61)
Pb	4.83 (5.27)	4.01 (3.67)	4.69 (2.76)	4.11 (6.01)	7.39 (4.79)	1.50 (1.15)
Zn	71.67 (60.08)	71.87 (66.48)	62.61 (27.04)	85.30 (92.77)	115.29 (76.21)	38.58 (8.90)
F1 + F2 + F3 (%)						
As	8.10 (15.19)	5.63 (9.27)	2.07 (1.82)	12.64 (16.91)	11.50 (16.11)	2.13 (3.46)
Cr	2.70 (1.96)	2.60 (2.10)	1.51 (1.16)	4.03 (1.99)	2.85 (2.60)	2.47 (1.25)
Cu	19.35 (9.88)	23.09 (14.35)	19.68 (11.33)	23.82 (13.40)	22.94 (10.61)	19.00 (13.24)
Ni	17.13 (10.41)	16.74 (9.72)	14.82 (6.80)	20.05 (12.90)	22.05 (9.73)	13.03 (8.40)
Pb	10.79 (6.60)	10.29 (7.19)	14.54 (6.15)	5.89 (4.27)	13.60 (6.67)	7.53 (5.67)
Zn	27.45 (13.58)	27.31 (14.31)	26.54 (11.92)	28.62 (16.46)	35.64 (12.83)	21.08 (11.16)

Acid-soluble fraction (F1); Reducible fraction (F2); Oxidizable fraction (F3).

Table 5

Evaluating the effects of controlled factors on metal(loid) concentrations and percentages in F1 + F2 + F3 by fitting an analysis of variance (ANOVA) model.

Parameters	df	Concentrations		Percentages	
		F value	p value	F value	p value
Soil compartment	1	0.000	0.993	0.146	0.703
Cultivation mode	1	2.966	0.087	4.423	0.037
Irrigation type	1	11.815	< 0.001	24.919	< 0.001
Vegetable species	3	0.534	0.659	1.148	0.331

4.2.3. Treated industrial versus municipal wastewater irrigation

Accumulations of heavy metal(loid)s in soils due to irrigation with treated industrial and municipal wastewater have been widely reported (Sinha et al., 2006; Qureshi et al., 2016). In the present study, we observed significant differences, in both the concentrations ($p < 0.05$) and percentages of heavy metal(loid)s ($p < 0.05$) in F1 + F2 + F3, between the irrigations using treated industrial and municipal wastewater. Compared with irrigation using treated municipal wastewater, irrigation with treated industrial wastewater resulted in higher concentrations and percentages of all the studied heavy metal(loid)s in F1 + F2 + F3 in soils. This is because concentrations of heavy metal(loid)s were higher in the treated industrial wastewater than in the treated municipal wastewater. Irrigation with wastewater affects the physicochemical properties of soils, total metal(loid) concentrations, and the distribution of heavy metal(loid) speciation (Lucho-Constantino et al., 2005; Saffari and Saffari, 2013; Ma et al., 2015). After soil had been irrigated with treated municipal wastewater, the mobility factor (percentage in exchangeable, sorbed, and carbonate fractions) was

Table 6

The physicochemical properties of rhizosphere soils, bulk soils, greenhouse soils, field soils, and soils irrigated with treated industrial or municipal wastewater in our study. Numbers in parentheses are the standard deviations ($n = 30$).

Parameters	pH	CEC (cmol kg ⁻¹)	Total carbon (mg kg ⁻¹)	Total nitrogen (mg kg ⁻¹)	Organic matter (mg kg ⁻¹)
Soil compartment					
Rhizosphere	8.12 (0.32)	17.40 (1.91)	26.12 (5.90)	1.29 (0.58)	26.14 (10.97)
Bulk	8.06 (0.42)	17.06 (1.79)	26.56 (6.51)	1.47 (0.96)	26.91 (10.86)
Cultivation mode					
Field	8.27 (0.35)	17.41 (2.05)	21.28 (2.80)	0.79 (0.14)	17.17 (2.48)
Greenhouse	7.89 (0.28)	17.03 (1.59)	32.12 (3.01)	2.06 (0.68)	37.22 (5.18)
Irrigation type					
Industrial wastewater	7.95 (0.28)	17.42 (1.49)	27.34 (5.03)	1.55 (0.93)	26.41 (10.60)
Municipal wastewater	8.23 (0.40)	17.05 (2.15)	25.34 (7.07)	1.22 (0.60)	26.64 (11.24)

CEC: cation exchange capacity.

reduced from 12.31% to 12% for Zn and increased from 20.82% to 24.95% for Cu (Saffari and Saffari, 2013).

4.3. Risk assessment code (RAC) and modified risk assessment code (mRAC)

Table 7 shows the RAC and mRAC of heavy metal(loid)s in the 120 soil samples and their associated environmental risks. RAC values for As, Cr, Cu, Ni, Pb, and Zn were 0%–46.55%, 0%–0.78%, 0%–30.60%, 0%–16.94%, 0%–5.13%, and 0%–31.43%, respectively. The maximum RAC value for Cr (0.78%) is lower than 1% and indicates that Cr presented no risks in all the soils. According to the classifications of environmental risks by RAC values, there were 37.50%, 10.83%, and 0.83% of soils with low, medium, and high As risks; 61.67%, 9.17%, and 0.83% of soils with low, medium, and high Cu risks; 57.50% and 4.17% of soils with low and medium Ni risks; 66.67% of soils with low Pb risks; and 40.00%, 44.17%, and 2.50% of soils with low, medium, and high Zn risks, respectively. The descending average RAC values were in the order of Zn (10.2) > Cu (3.9) > As (3.6) > Ni (2.4) > Pb (1.8) > Cr (0.2). This suggests there are medium risks from Zn, low risks from Cu, As, Ni, and Pb, and no risks from Cr. mRAC values for the 120 soil samples ranged from 0.57 to 18.90, with a mean value of 3.2. On the basis of the classifications of environmental risks according to mRAC values, there were only 13.33% soils with no potential adverse effect, but 83.33% of soils with low potential adverse effect and 3.33% of soils with medium potential adverse effect. Although more than half of each of the heavy metal(loid)s present in the residual fraction was considered non-bioavailable, the environmental risks posed by the percentages of metal(loid)s in the bioavailable

Table 7

Risk assessment code (RAC) and modified risk assessment code (mRAC) values of soils along with associated environmental risk. Numbers in parentheses are the standard deviations.

Parameters	RAC						mRAC
	As	Cr	Cu	Ni	Pb	Zn	
Soil compartment							
Rhizosphere (n = 60)	4.2 (8.4)	0.2 (0.2)	1.6 (1.2)	2.3 (3.0)	1.9 (1.3)	9.7 (7.1)	3.0 (3.4)
Bulk (n = 60)	2.9 (4.7)	0.1 (0.1)	6.1 (8.5)	2.6 (3.3)	1.7 (1.4)	10.8 (8.2)	3.4 (2.3)
Cultivation mode							
Field (n = 60)	1.0 (1.0)	0.1 (0.1)	2.0 (1.4)	2.7 (2.2)	2.7 (1.1)	11.1 (6.1)	2.0 (0.8)
Greenhouse (n = 60)	6.5 (9.1)	0.2 (0.2)	6.0 (8.9)	2.0 (4.0)	0.8 (0.6)	9.2 (9.2)	4.5 (3.8)
Irrigation type							
Industrial wastewater (n = 60)	6.4 (8.7)	0.2 (0.2)	1.4 (1.3)	2.7 (3.6)	2.3 (1.2)	11.1 (8.7)	4.0 (3.5)
Municipal wastewater (n = 60)	0.8 (1.1)	0.2 (0.1)	6.3 (8.4)	2.1 (2.7)	1.3 (1.3)	9.4 (6.4)	2.4 (1.9)
Data range							
Mean (n = 120)	3.6 (6.8)	0.2 (0.2)	3.9 (6.4)	2.4 (3.2)	1.8 (1.3)	10.2 (7.7)	3.2 (2.9)
Max	46.55	0.78	30.60	16.94	5.13	31.43	18.90
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.57
Environmental risk (n = 120)							
% of no risk	50.83	100.00	28.33	38.33	33.33	13.33	13.33
% of low risk	37.50	0.00	61.67	57.50	66.67	40.00	83.33
% of medium risk	10.83	0.00	9.17	4.17	0.00	44.17	3.33
% of high risk	0.83	0.00	0.83	0.00	0.00	2.50	0.00
% of very high risk	0.00	0.00	0.00	0.00	0.00	0.00	0.00

fraction and their toxicity cannot be ignored in agricultural soils irrigated with treated industrial and municipal wastewater.

5. Conclusions

This study investigated the speciation distributions of six heavy metal(loid)s (As, Cr, Cu, Ni, Pb, and Zn) in soils irrigated with treated industrial and municipal wastewater. A three-step BCR sequential extraction technique was adopted to separate the heavy metal(loid)s in soils into four chemical forms: an acid-soluble fraction (F1), a reducible fraction (F2), an oxidizable fraction (F3), and a residual fraction (F4). Our study showed that the mean percentages of As, Cr, Cu, Ni, Pb, and Zn in the residual fraction were 93.14%, 97.35%, 79.03%, 83.07%, 89.46%, and 72.62%, respectively. More than half of each of the studied heavy metal(loid)s was detected in the residual fraction and considered non-bioavailable. According to the ANOVA analysis, cultivation mode and irrigation type had significant effects on the metal percentages in F1 + F2 + F3. Greenhouse soils had higher concentrations and percentages of heavy metal(loid)s (except Pb) in F1 + F2 + F3 compared with field soils. Soils irrigated with treated industrial wastewater had higher concentrations and percentages of all the studied heavy metal(loid)s in F1 + F2 + F3 than soils irrigated with treated municipal wastewater. Although the percentages of each studied heavy metal (loid) in the residual fraction were higher than 50%, the environmental risks posed by the proportions of metal(loid)s in the bioavailable fraction and their toxicity cannot be ignored. In order to reduce these environmental risks, remediation technologies for the immobilization of heavy metal(loid)s in the soils [e.g., *in-situ* chemical immobilization by soil additives (Kumpiene et al., 2008) and phytoremediation through the accumulation of metal(loid)s from soils to plants (Padmavathamma and Li, 2007)] and the reduction of their concentrations in wastewater should be considered.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://>

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