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## **Bioavailability and speciation of potentially toxic elements in agricultural soils neighboring an industrial complex in northwest Isfahan, Iran**

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### **Abstract**

The mobility and bioavailability of trace elements in the agricultural soils are extremely important in assessing the risk of toxicity to the growing plants. A five step sequential extraction procedure (SEP) has been employed to study the speciation of As, Sb, Cr, Cu, Cd, Pb, Zn, Ni, Fe and Mn in 18 soil samples neighboring an industrial complex in Isfahan, central Iran. Enrichment factor (EF) calculation shows that in the soils studied, Cd and Fe are the most and the least enriched elements, respectively. Sequential extraction results indicate that although the total concentration of Ni, Sb, As, Cr and Cu is high, their concentration in the exchangeable plus carbonate fractions is rather low. This suggests that these elements are not easily transferred from soil to the plants. The high proportions of Cr, Cu and Fe in both organic matter and residual fractions, reduces the phytoavailability and the toxicity of these metals. On the other hand, Zn, Pb and Cd are strongly associated with exchangeable plus carbonate fractions, much more bioavailable than other studied elements. Good correlation between total contents and concentration of elements in each fraction indicates that the studied elements, excluding Fe, are mainly derived from the anthropogenic sources. Remediation measures and soil amendment practices are therefore recommended for the study site.

**Keywords:** Bioavailability; speciation; agricultural soils; Isfahan; Iran

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### **1. Introduction**

Soil is one of the most important biogeochemical sinks for metals and other pollutants. In recent years, the problem of contaminated soil is of increasing concern due to food safety issues and potential health risks via consumption of contaminated crops (Yan et al., 2007). Thus, protection of soil resources and their sustainability is of prime importance. Unlike organic pollutants, natural decomposition processes do not remove heavy metals from the contaminated soils; on the contrary they may even be enriched by organisms and can be potentially converted to organic complexes, which may be more toxic (Jain, 2004). Heavy metals remain in the pedosphere for many years even after removal of the pollution sources, and the remediation of metal pollution is often problematic due to their persistence and undegradability in the environment (Wang et al., 2010).

Total metal content of the soil often does not provide enough reliable information on the processes and dynamics of the availability, mobility

and toxicity of the metals, i.e. their potential to transfer from soil into plants and crops (Li et al., 2007; Ma et al., 2011). The capacity of plant to adsorb metals and the rate of plant metal uptake is closely related to metals mobility and bioavailability, which in turn directly depend on the chemical speciation of the metals in question (Alloway et al., 1998). Chemical speciation can be defined as the process of identification and quantification of different species, forms or phases of the elements present in a material (Naji et al., 2010). In soils, metals can be present in a number of chemical forms including exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter and in the form of residual. These forms exhibit different behaviors with respect to bioavailability; the exchangeable and carbonate-bound fractions are more bioavailable (Ma and Rao, 1997). In order to study the speciation and possible associations between metals and the soil components, sequential extraction procedures (SEPs) are often used (Ma et al., 2011), whereby several reagents are sequentially employed to extract operationally defined phases from the soil.

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In the present study, an agricultural field which is affected by industrial activities in Isfahan province has been investigated. A large industrial complex which includes oil refinery, power plants, petrochemical-textile factories and battery manufacturing units, is located to the northwest of Isfahan. Agricultural soils surrounding this center are susceptible to contamination by metals in addition to fertilizers and other agricultural practices. Previous studies in this area have reported cadmium, zinc and lead contamination (Amini et al., 2005). However, there is a lack of sufficient information on the total concentration and the speciation of different elements in the local soils. In this study, the total concentration of 10 potentially hazardous elements including As, Sb, Cr, Cu, Cd, Pb, Zn, Ni, Fe and Mn in the soil samples were determined and the speciation of target metals has been studied using Tessier et al. (1979) SEP. Based on the above information, the aims of this study are to:

- Detect the total element content in the studied soils, and to assess the degree and extent of soil contamination by various elements using enrichment factor calculations
- Study the bioavailability of elements in soils via implementing SEP
- Evaluate the relationships between total and fractional concentration of elements in the studied soils.

## 2. Materials and methods

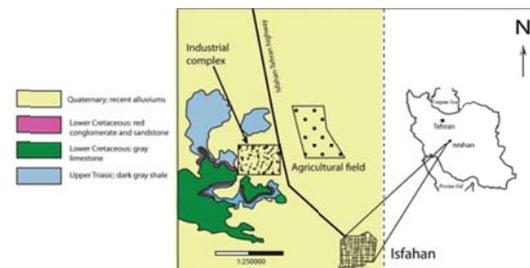
### 2.1. Site description

The experimental site is part of an agricultural field neighboring an industrial complex, 15 km to northwest Isfahan, central Iran (Fig. 1). Wheat, barley, corn, vegetables, fodder and summer crops are the main agricultural products in the region. The climate of the area is semi-arid with mean annual rainfall of approximately 140 mm and the mean temperature of 14.5°C (Amini et al., 2005). Local geology consists mainly of Quaternary alluvial deposits. The lower Cretaceous grey limestone and the upper Triassic dark grey shale units are also exposed in the western part of the area (Fig. 1).

### 2.2. Soil sampling

Surface soils (within a depth of 0-10 cm) were collected from 18 locations (Fig. 1) using a plastic spade to avoid any metal contamination. Each soil sample comprised approximately 1 kg in the form of four sub-samples taken within a 1 m×1 m square area. The collected samples were stored in plastic

bags. In the laboratory, the samples were air-dried at room temperature, disaggregated, sieved using a 2 mm sieve to remove coarse debris and finally ground to about 0.074 mm using agate mortar and pestle.



**Fig. 1.** Geological map of the study area and location of the soil samples

### 2.3. Physico-chemical analysis

General characteristics of the samples including pH, texture, organic matter (OM) and cation exchange capacity (CEC) were determined following standard methods of soil analysis. Soil pH was measured in distilled water by making a 1:2 solid/solution ratio after equilibration for 15 minutes (US EPA, 1998). Hydrometry technique was used to assess the samples textures. CEC was also determined using 1M sodium acetate and ammonium acetate solutions (US EPA, 1998). To measure organic matter content of the samples, loss on ignition (L.O.I) was implemented. For each sample, 0.3-3 g soil was dried at 105°C in an oven overnight, followed by ignition at 550°C for 12 hours in a muffle furnace.

#### 2.3.1. Total concentration and speciation of the elements

The total element contents of the soil samples were extracted using an acid digestion mixture ( $\text{HF} + \text{HClO}_4 + \text{HNO}_3$ ) in an open system. The properties of the soil samples, i.e. neutral to alkaline pH and low organic matter content, have led the authors to employ the SEP method introduced by Tessier et al. (1979). After successive extractions, separation was performed by centrifuging at 4000 rpm for 30 minutes. Supernatants were separated using pipette. The residue was then washed in 8 ml deionized water and centrifuged for a further 30 minutes. After that, the wash water was decanted. The supernatants were finally filtered through 0.45  $\mu\text{m}$  membrane filter.

#### 2.3.2. Elemental analysis and validation of sequential extraction method

Solutions resulting from the above steps were analyzed using ICP-OES. Standard addition

technique was used to prepare the blanks. The accuracy of the measurements was checked using SPEX Certi Prep standards and Merck Inc. made standard solutions. Standard solutions were freshly diluted before use and the calibration curves were prepared using linear regression approach. In this process, the concentration of metals in all blanks of each extraction step was below the detection limit. To estimate the reliability of SEP, the recovery percentage (% R) for each metal was calculated. For this, the sum of the element concentrations in the five different phases of the sequential extraction was divided by the total concentration after digestion by strong acids.

### 2.3.3. Statistical analysis

Using SPSS, version 11.5, statistical analysis was performed to calculate mean, maximum, minimum and standard deviation. These parameters were calculated for metal concentrations, pH, OM content and CEC of soil samples. Spearman regression analysis has been also carried out to determine the relationship between different fractions and soil physical properties.

## 3. Discussion

### 3.1. Soil properties

Soil sample's properties are presented in Table 1. Size wise, samples contain 33.9 % clay, 36.9 % silt and 29.2 % sand. The pH values are in the range of 6.4 and 8.5 with a mean value of 7.4; on average

samples' pH falls within the alkaline range. Organic matter content varies from 0.4% to 1.5 % with a mean value of 1 %. Maximum, minimum and mean value for CEC is 19.7, 11.4 and 16.6 meq/100 g, respectively.

**Table 1.** Descriptive statistics of the general characteristics of soil samples

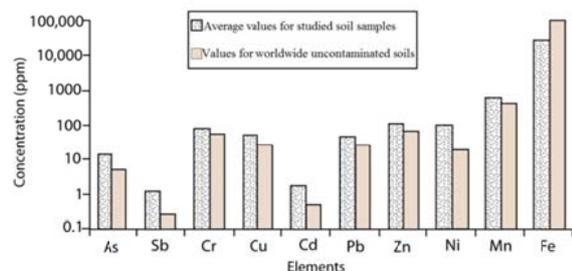
	Min.	Max.	Mean	S.D.
<b>Grain sizes</b>				
<b>Clay (%)</b>	23	52	33.9	8.4
<b>Silt (%)</b>	19	47	36.9	8.2
<b>Sand (%)</b>		35	29.2	3.8
<b>pH</b>	6.4	8.5	7.4	0.7
<b>OM content (%)</b>	0.4	1.5	1	0.3
<b>CEC (meq/100g)</b>	11.4	19.7	16.6	2.1

### 3.2. Total concentration of elements

The total concentration of elements in the samples and the mean values for the studied elements in worldwide uncontaminated soils (Kabata-Pendias and Pendias, 2001) are presented in Table 2. The mean concentration of elements (in ppm) in descending order is: Fe > Mn > Zn > Ni > Cr > Cu > Pb > As > Cd > Sb, generally higher than the worldwide uncontaminated soil values (Fig. 2). Mean concentration of Ni and Sb is four times, Cd three times and As and Cu two times greater than the worldwide uncontaminated soil values. However, Cr, Mn, Pb and Zn are only slightly higher, while Fe concentration is less than that of the uncontaminated soils.

**Table 2.** Descriptive statistics of total concentration of elements (ppm) in 18 soil samples of the studied area. The worldwide uncontaminated soils are also included.

	As	Sb	Cr	Cu	Cd	Pb	Zn	Ni	Mn	Fe
<b>Min.</b>	9	1	57	32	1.1	26	84	75	399	20901
<b>Max.</b>	16.5	1.3	105	63	2.1	68	180	120	841	31823
<b>Mean</b>	12.9	1.08	84.16	51.27	1.74	41.38	103.11	96.05	586.11	27048.16
<b>S.D.</b>	1.99	0.08	15.64	9.27	0.2	11.63	21.79	11.61	117.804	3594.13
<b>Worldwide uncontaminated soils</b> (Kabata Pendias and Pendias, 2001)	5	0.25	54	25	0.5	25	63	20	437	100000



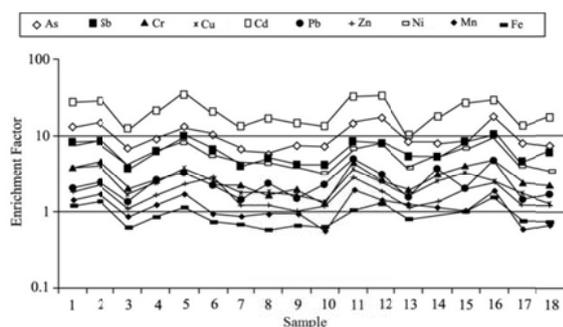
**Fig. 2.** Mean concentration of elements in the soil samples and worldwide uncontaminated values (Kabata-Pendias and Pendias 2001)

The enrichment factor (EF) is usually used to determine the degree of modification in the soil composition via man-made sources. It is based on the standardization of an metal against a reference element:

$$EF = \frac{(C_n / C_{RE})_{Soil}}{(C_n / C_{RE})_{RM}}$$

where  $C_n$  is the concentration of the element in question,  $C_{RE}$  is the concentration of a reference element and RM is the reference material. Al, Ti, Zr, Sc and Li are usually used as the reference

element in various studies. In this study, Zr has been chosen as the reference element, because it shows normal distribution in the studied samples. In addition, Zr is widely used in the geochemical investigations as a stable lithogenic element with no anthropogenic source (Wang et al., 2008). The upper continental crust composition has been selected as the reference material. The average EF values yields the following results in descending order: Cd (21.5), As (10.3), Sb (6.5), Ni (5.7), Cr (2.9), Pb (2.5), Cu (2.4), Zn (1.7), Mn (1.2) and Fe (0.9) (Fig. 3).



**Fig. 3.** Enrichment factor values for different elements in the soil samples

Sutherland (2000) has distinguished five classes of enrichment factors:  $EF < 2$  shows deficiency to low enrichment,  $2 < EF < 5$  shows moderate enrichment,  $5 < EF < 20$  shows significant enrichment,  $20 < EF < 40$  shows very high enrichment and  $EF > 40$  shows extremely high enrichment. Based on Sutherland's classification, the studied soils show either deficiency or low enrichment with respect to Fe, Mn and Zn, they are moderately enriched in Cu, Pb and Cr, significantly enriched in Ni, Sb and As and very highly enriched in Cd. Enrichment factor values lower than unity indicate crustal origin, whereas EF values greater than unity points to anthropogenic origin for the element in question (Wang et al., 2010). Thus, with the exception of Fe, all the studied elements originate partly or wholly from the anthropogenic sources. Emissions from industrial plants and agricultural activities (pesticides, fertilizers) are the two probable sources for the enriched elements in the soils of the study area.

### 3.3. Correlation between total concentrations of various elements

Inter-element relationships can provide useful information on element sources and pathways (Lu and Bai, 2010). In this study, due to the abnormality of the distribution of data set, the Spearman's correlation analysis has been carried out. Table 3 presents the correlation coefficient matrix between

the studied elements and soil properties. According to Spearman's coefficient values, significant positive correlations ( $p \leq 0.01$ ) exist between Cu and Cr (0.626), Fe and As (0.665), Ni and Cu (0.602), Cr and Al (0.777) and Zr and Fe (0.662). A high positive correlation ( $p \leq 0.05$ ) is also found between Al and Cu (0.549), Al and Fe (0.472), Al and Ni (0.495), Fe and Sb (0.545) and As and Sb (0.485).

Based on Table 3, excluding the Fe-Zr pair, no significant correlation has been observed between Zr and the rest of the elements. Zirconium is a lithogenic element without any anthropogenic sources; thus, all the studied elements, except Fe, are probably from anthropogenic sources. The positive correlation between Sb, As and Fe is likely due to the geochemical affinity of these two elements to iron oxides. Arsenic and Sb have strikingly similar geochemical behaviors (Wang et al., 2010), which explains the significant positive correlation between these two elements.

Substantial correlation between Cr, Cu and Ni is perhaps indicative of their similar pollution levels, common geochemical properties and/or the same input sources (industry and/or agriculture). Positive correlations between metals including Cu, Cr, Fe and Ni and Al suggest that the presence of these elements is partly related to the presence of Al. SEP results also confirm that the residual phases of studied soils contain various amounts of these elements (Fig. 4).

The correlation between OM and CEC is high ( $p \leq 0.05$ , 0.495). Also, high CEC values are especially associated with fine fraction of the soil, particularly clays ( $< 2 \mu\text{m}$ ) ( $p \leq 0.01$ , 0.945). With the exception of Cu and Cr, no significant correlation is found between OM and the studied elements, evidence that the organic matter may be only a minor host phase for these elements. This is likely due to the low organic matter content of the studied oxidized surface soils. Iron, Cr and Cu concentration of the studied soils are significantly correlated with soils' clay content, suggesting the presence of these metals in the clay minerals. In the following section, speciation of various groups of elements is discussed.

### 3.4. Geochemical speciation of the elements

The result of the geochemical speciation of all studied elements and the percent recoveries are illustrated in Table 4. Average recovery values range from 83.5 for Ni to 108 for Mn, indicating good agreement between total concentration and the sum of all fractions. Fig. 4 displays the mean element percentages associated with different phases in the studied samples. In this figure, each geochemical fraction is regarded as a percentage of the sum of all fractions.

**Table 3.** Spearman's rank correlation matrix of studied elements in the soil samples with texture, OM, pH, and CEC of the soils

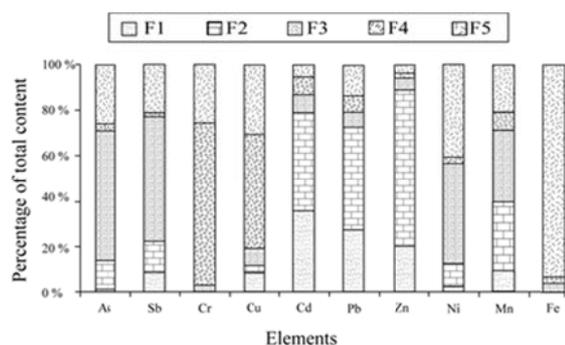
	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Sb	Zn	Al	Zr	OM	clay	silt	sand	pH	CEC
As	1.000																	
Cd	.024	1.000																
Cr	-.142	-.121	1.000															
Cu	-.142	-.060	<b>.626**</b>	1.000														
Fe	<b>.665**</b>	-.204	.321	.179	1.000													
Mn	.307	-.192	.330	.384	.455	1.000												
Ni	.147	-.169	.467	<b>.602**</b>	<b>.536*</b>	.395	1.000											
Pb	.146	.025	-.084	-.247	.038	.259	.021	1.000										
Sb	<b>.485*</b>	.024	-.033	-.057	<b>.545*</b>	-.096	-.131	-.109	1.000									
Zn	.070	.107	.375	.426	.163	.175	.167	.302	.240	1.000								
Al	.216	-.007	<b>.777**</b>	<b>.549*</b>	.472*	.307	<b>.495*</b>	-.250	.099	.355	1.000							
Zr	.332	-.085	.136	.121	<b>.662**</b>	.237	.081	.114	.400	.377	.197	1.000						
OM	-.083	.251	<b>.493*</b>	<b>.700**</b>	-.339	-.346	.240	.011	-.075	.138	-.185	-.169	1.000					
clay	.317	<b>-.536*</b>	<b>.650**</b>	<b>.650**</b>	<b>.739**</b>	-.199	-.162	-.174	.156	.084	.159	.169	.087	1.000				
silt	-.302	.078	.094	.537*	-.046	.312	.463	.045	.052	.298	.147	.087	.431	-.079	1.000			
sand	.084	-.343	.024	<b>-.495*</b>	.082	-.180	-.292	.023	-.088	-.381	-.190	-.046	-.446	-.336	<b>-.869**</b>	1.000		
pH	.116	-.332	-.024	-.288	-.232	-.149	-.100	.194	-.331	.053	-.233	-.285	.013	-.371	-.381	.361	1.000	
CEC	.300	<b>.556*</b>	-.231	.046	-.002	-.266	-.039	-.075	.213	.119	.081	.138	<b>.495*</b>	<b>.945**</b>	.068	-.446	-.359	1.000

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

**Table 4.** Metal concentrations (ppm) in various geochemical fractions of surface soils of the studied area (<d.l.: below detection limit). Recovery (%) indicates the ratio between the sum of the F1-F5 fractions and the total metal content as analyzed separately (Table 2, see text)

		Extracted concentrations (ppm)									
		As	Sb	Cr	Cu	Cd	Pb	Zn	Ni	Mn	Fe
Exchangeable	Min.	0.1	0.09	<d.l.	1	0.5	1.3	18	1.5	33.4	<d.l.
	Max.	0.5	0.1	<d.l.	4.9	0.9	16.5	43	2.3	41	<d.l.
	Mean	0.2	0.1	<d.l.	3.6	0.7	11.6	23	2.0	37	<d.l.
Carbonate bound	Min.	1.1	0.1	<d.l.	0.9	0.5	15.9	61	5.8	135	5
	Max.	1.9	0.2	<d.l.	1.2	1	28.5	109	9.8	161	18
	Mean	1.5	0.2	<d.l.	1.01	0.8	19.7	78.9	7.9	149.3	12.14
Fe-Mn oxide bound	Min.	5.2	0.6	1.8	1.6	0.1	2.1	2	23.9	175	983
	Max.	8.6	0.7	3.2	3.9	0.2	3.7	9	43.6	201	1823
	Mean	6.6	0.6	2.4	3.08	0.2	2.7	6.1	35.2	189.3	1354.6
Organic matter bound	Min.	0.1	0.01	46	12	0.1	2.1	1	1.7	68.3	325
	Max.	0.5	0.05	64	24	0.2	4.4	3	2.9	72.2	952
	Mean	0.3	0.02	56.7	19.9	0.1	2.9	1.8	2.3	70.4	569.7
Residual	Min.	2.7	0.2	20.4	2.1	0.1	4	3	27.3	129	21460
	Max.	3.6	0.3	20.8	18.1	0.1	9.1	6	38	162	29310
	Mean	3.05	0.2	20.6	12.1	0.1	5.8	4.4	32.4	145.1	26789.9
Sum of the five fractions	Min.	10.4	1.0	68.9	17.7	1.4	30.9	91	62.6	546.8	23845
	Max.	14.4	1.3	86.6	51.2	2.0	59.2	164	95.4	620	31142
	Mean	11.7	1.1	79.8	39.7	1.8	42.8	114.2	79.7	591.1	28726.3
Recovery (%)	Min.	75.9	84.6	82.1	66.2	79.7	76.1	91.1	75	79.9	99.1
	Max.	109.5	114.2	117.8	110.6	116	101.1	114.6	98.6	132.3	112
	Mean	91.5	103.2	98.9	91.1	105.3	87.6	104.1	83.5	108.0	106.5



**Fig. 4.** Percentage of metal speciation in different geochemical fractions of soil samples

#### 3.4.1. Cadmium, lead and zinc

In soil samples, these elements are mainly associated with the carbonate (F2) phase. The strong association of Cd, Pb and Zn with this fraction (on average 36 %, 26.7% and 69% of the total content, respectively) is in good agreement with findings reported by others (e.g. Li et al., 2001; Ikem et al., 2003; Li et al., 2007). The association of Cd, Pb and Zn with F2 fraction is probably because of the similar ionic radius of these elements with Ca in carbonate minerals such as calcite. Thus, calcium carbonate may act as a strong adsorbent for these metals (Korfali and Davies, 2004). It has been reported that Zn is mainly associated with Fe-Mn oxides of soils and sediments due to the high affinity of Zn for Fe-Mn oxides (Kabata-Pendias and Pendias, 2001). However, association of Zn onto the oxides has higher mobility constants than onto carbonates. Furthermore, Zn oxides are easily transformed to the other species, mainly carbonates (Sutherland, 2000). This explains the cause of higher concentration of Zn in the carbonate fraction than in the oxide fraction.

#### 3.4.2. Arsenic, Antimony, Nickel and Manganese

The speciation pattern confirms that the reducible (Fe-Mn oxide) fraction has high scavenging efficiencies for As, Sb, Ni and Mn (Fig. 4). The similar fractionation pattern of Sb and As is perhaps either due to their similar geochemical behaviors (Kreidie et al., 2011) or their similar anthropogenic source (Zhou et al., 2011). As and Sb enter soils as a result of atmospheric deposition (Machender et al., 2011) or pesticide application (Avudianayagam et al., 2003). The association of Ni with oxide fraction is mainly due to the high affinity of Ni for the ferromanganese hydrous oxides. It may also be an indication of anthropogenic source for this metal (Zhou, 2011). The high percentage of Mn in oxide

fraction is probably related to the oxidizing condition of surface studied soils and the affinity of Mn to this phase (Singh et al., 2005).

#### 3.4.3. Chromium and Copper

On average, the organic matter fraction accounted for 71.1% and 52.1% of sum of all fractions of total Cr and Cu, respectively. The affinity of Cr and Cu for organic matter is supported by other studies (e.g. Li et al., 2007; Wang et al., 2010; Zhou et al., 2011). In agricultural soils, a relatively high percentage of Cr and Cu in the organic matter phase may be due to sewage sludge soil amendment practices (Kelderman and Osman, 2007). The preferential Cu binding onto organics could be also due to the ease of complexation of Cu with materials such as particulate humic and amino acids, a well known process (Ramos et al. 1994; Du et al., 2008).

#### 3.4.4. Iron

Iron is strongly associated with the residual fraction (on average 93.1 % of total Fe). Similar situation has been reported in other studies (e.g. Tuzen, 2003). The geochemistry of Fe can also explain the low association of Fe with other fractions of the studied soils; for example, the formation of Fe-carbonates requires highly reduced conditions ( $Eh < -0.25$  mV, (Korfali and Davies, 2004). The greater the percentage of the element present in the residual phase, the less the pollution of the soil.

#### 3.5. Correlation between element fractions and total concentrations

Table 5 shows that there are significant positive correlations between element fractions and total concentrations for various elements. The correlation coefficients confirm that the distribution of elements in the various chemical phases depends on the total element content of the soil samples. The positive correlation between the proportion of elements associated with the non-residual fractions (F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>) and the total content of the elements is indicative of man-made contamination. The anthropogenically sourced elements preferentially partition to the non-residual fractions (Farkas et al., 2007). In particular, the significant positive correlation between F<sub>1</sub> fraction of Pb, Zn, Cd and Mn, and their total content, as well as significant positive correlation between F<sub>2</sub> fraction of Cd and Zn, and the total content is most likely due to the anthropogenic sources of these metals. The significant positive correlation between F<sub>5</sub> fractions

of Fe and total content proves the contribution of natural sources for this metal. Table 6 indicates that Sb and As in the F<sub>3</sub> fraction are significantly correlated with total Fe content, whereas Ni in F<sub>3</sub> fraction show significant correlation with total Mn. This suggests that Fe oxides play an important role in scavenging both As and Sb, whereas Mn oxides, in comparison to Fe oxides, scavenge Ni more efficiently.

**Table 5.** Spearman's rank correlation coefficients for the relationship between percentage of metal partitioning in the exchangeable, Carbonate, Fe-Mn oxide, organic matter, and residual fractions and the total element concentration in the studied surface soils

	<i>R</i>
Exchangeable Pb- total Pb	0.937**
Exchangeable Zn- total Zn	0.927**
Exchangeable Cd- total Cd	0.865*
Exchangeable Mn- total Mn	0.786*
Carbonate bound Zn- total Zn	0.893**
Carbonate bound Cd- total Cd	0.887**
Oxide bound As- total As	0.765*
Oxide bound Sb- total Sb	0.739*
Oxide bound Cu- total Cu	0.775*
Organic bound Pb- total Pb	0.755*
Organic bound Ni- total Ni	0.783*
Residual bound Cr- total Cr	0.757*
Residual bound Fe- total Fe	0.857*

Significant correlations: \*( $p < 0.01$ ), \*\*( $p < 0.05$ )

**Table 6.** Spearman's rank correlation coefficients between oxide bound (F<sub>3</sub>) fractions of As, Sb, Ni, and total Fe and Mn content

	<i>R</i>
Oxide bound As- total Fe	0.806*
Oxide bound As- total Mn	0.144
Oxide bound Sb- total Fe	0.732*
Oxide bound Sb- total Mn	-0.316
Oxide bound Ni- total Fe	0.821*
Oxide bound Ni- total Mn	0.500

Significant correlations: \*( $p < 0.01$ ), \*\*( $p < 0.05$ )

### 3.6. Risk assessment code (RAC)

A risk assessment code (RAC) calculates an index which shows the possible environmental risk by applying a scale to the concentration of metals present in both exchangeable and carbonate fractions (Perin et al., 1985). In general, elements in these two fractions are considered to be weakly bounded metals which may transfer easily from soil to plants (Alomary and Belhadj, 2007; Yan et al., 2010). The higher the F<sub>1</sub> and F<sub>2</sub> fractions of metal in the soil, the greater the plant uptake of that metal (Ma and Rao, 1997; Alomary and Belhadj, 2007; Yan et al., 2007; Naji et al., 2010). According to RAC, if sum of these fractions is less than 1 %, there is no risk, 1-10 % exhibits low risk, 11-30 %

shows medium risk, 31-50 % shows high risk and greater than 50% exhibits very high risk (Jain, 2004). The results of RAC calculations for studied elements (Table 7) indicate that Zn, Pb and Cd pose a very high risk, As, Sb, Cu, Ni and Mn pose a medium risk, while Cr and Fe pose no risk to the investigated soil samples.

**Table 7.** Risk Assessment Code (RAC) (%) and risk rank for studied elements in the soil samples. NR: no risk, LR: low risk, MR: medium risk, HR: high risk, VHR: very high risk (see text)

	RAC	Rank
As	14.2	MR
Sb	22.2	MR
Cr	0	NR
Cu	11.6	MR
Cd	78.7	VHR
Pb	73.2	VHR
Zn	89.2	VHR
Ni	12.4	MR
Mn	31.5	HR
Fe	0.04	NR

### 3.7. Bioavailability of elements

Elements geochemical speciation is the first step in assessing their mobility and bioavailability to plants (Cheng et al., 2011). If one assumes that bioavailability is related to solubility, then bioavailability decreases in this order: exchangeable>carbonate>oxide>organic>residual. Elements in the exchangeable and carbonate fractions are the most bioavailable fractions and are the most readily available for plant uptake (Ma and Rao, 1997; Rogan et al., 2010). Fe-Mn oxide phase is relatively stable under normal soil conditions and the release of the elements from this fraction will most likely be affected by both redox potential and pH (Hu et al., 2006). The organic fraction is not considered as mobile or available, because it is thought to be associated with stable high molecular weight humic substances that slowly release small amounts of metals (Cheng et al., 2011). In the residual fraction, generally elements are occluded in crystalline structures and have high stability (Ma et al., 2011). Therefore the elements associated with this fraction can only be mobilized as a result of weathering (Cheng et al., 2011).

Elements concentration in each chemical phase of the soil samples descends as follow:

Exchangeable: Cd > Pb > Zn > Mn > Cu > Sb > Ni > As > Cr and Fe

Carbonate: Zn > Pb > Cd > Mn > Sb > As > Ni > Cu > Fe > Cr

Oxide: As > Sb > Ni > Mn > Cd > Cu > Pb > Zn > Fe > Cr

Organic matter: Cr > Cu > Cd > Pb > Mn > Ni = As > Fe > Sb > Zn

Residual: Fe > Ni > Cu > As > Cr > Mn > Sb > Pb > Cd > Zn

The high associations of Cd, Pb and Zn with exchangeable fraction represent the very high

potential mobility of these metals. Despite the fact that total concentration of As, Cr, Cu, Ni and Sb is higher than the unpolluted worldwide values (Fig. 2), the concentration of these elements in the exchangeable fraction is low. This observation as well as the high proportion of As, Sb, Ni and Mn in both oxide and residual phases points to their low availability from soil to plant. The high proportions of Cr, Cu and Fe in the organic matter and residual fractions, restrict the phytoavailability and toxicity of these metals for plants.

#### 4. Summary and conclusions

The objective of this study was to assess the pollution status of agricultural soil samples neighboring an industrial site in NW Isfahan, central Iran. The main results of this research can be summarized as follows:

- The soil samples contain elevated concentrations of studied elements (except Fe) compared to the worldwide averages. The calculation of enrichment factor values and correlation analysis between elements indicate that As, Sb, Cr, Cu, Cd, Pb, Zn, Ni and Mn are mainly introduced into the soil system via anthropogenic inputs, e.g. emissions from industrial plants and/or pesticide and fertilizer application.
- Speciation patterns of the studied elements reveal that a high percentage of Cd, Pb and Zn are associated with carbonate phase, perhaps due to their geochemical affinity and/or their anthropogenic source. Furthermore, the presence of these metals as exchangeable phase confirms their high bioavailability and potential risk in the study area.
- Antimony, Mn, As and Ni are mainly associated with the reducible oxide fraction. Thus, they are very stable under oxidizing conditions of the surface soils. Chromium and Cu are predominantly bound to the organic matter and consequently not bioavailable. The high content of Fe in the residual fraction as well as its very low concentration in the first two fractions indicates that Fe is not possibly taken up by plants.
- Based on RAC, the mobility of elements in the studied samples decreases in the order: Zn > Cd > Pb > Sb > Mn > As > Ni > Cu > Fe > Cr.
- The speciation pattern in the studied soils warrants a further study on the concentration of elements in the local crops. As far as land management practices are concerned, it is necessary to remediate the affected soils by immobilizing biologically available elements, including Zn, Cd and Pb.

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#### References

- Alloway, B. J., Thornton, I., Smart, G. A., Sherlock, J. C., & Quinn, M. J. (1988). Metal availability. *Sci. Total Environ.* 75, 41–69.
- Alomary, A. A., & Belhadj, S. (2007). Determination of heavy metals (Cd, Cr, Cu, Fe, Ni, Pb, Zn) by ICP-OES and their speciation in Algerian Mediterranean Sea Sediments after a five-stage sequential extraction procedure. *Environ. Monit. Assess.* 135, 265–280.
- Amini, M., Khademi, H., Afyuni, M., & Abbaspour, K. C. (2005). Variability of available cadmium in relation to soil properties and landuse in an arid region in Central Iran. *Water Air Soil Pollut.* 162, 205–218.
- Avudianayagam, S., Megharaj, M., Owens, G., Kookana, R. S., Chittleborough, D., & Naidu, R. (2003). Chemistry of chromium in soils with emphasis on tannery waste sites. *Rev. Environ. Cont. Toxic.* 178, 53–91.
- Cheng, Zh., Lee, L., Dayan, S., Grinshtein, M., & Shaw, R. (2011). Speciation of heavy metals in garden soils: evidences from selective and sequential chemical leaching. *J. Soil. Sed.* 11, 628–638.
- Du, P., Xue, N., Liu, L., & Li, F. (2008). Distribution of Cd, Pb, Zn, and Cu and their chemical speciations in soils from a peri-smelter area in northeast China. *Environ. Geol.* 55, 205–213.
- Farkas, A., Erratico, C., & Vigano, L. (2007). Assessment of the environmental significance of heavy metal pollution in surficial sediments of the River Po. *Chemosphere*, 68, 761–768.
- Hu, N., Li, Z., Huang, P., & Tao, C. (2006). Distribution and mobility of metals in agricultural soils near a copper smelter in south China. *Environ. Geochem. Health.* 28, 19–26.
- Ikem, A., Egiebor, N. O., & Nyavor, K. (2003). Trace elements in water, fish and sediment from Tuskegee Lake, southeastern U.S.A. *Water Air Soil Pollut.* 149, 51–75.
- Jain, C. K. (2004). Metal fractionation study on bed sediments of River Yamuna, India. *Water Res.* 38, 569–578.
- Kabata-Pendias A., & Pendias, H. (2001). *Trace elements in soils and plants*. 3<sup>rd</sup> edn. CRC Press. LLC, Boca Raton.
- Kelderman, P., & Osman, A. A. (2007). Effect of redox potential on heavy metal binding forms in polluted canal sediments in Delft (the Netherlands). *Water Res.* 41, 4251–4261.
- Korfali, S. I., & Davies, B. E. (2004). Speciation of metals in sediment and water in a river underlain by limestone: role of carbonate species for purification capacity of rivers. *Adv. Environ. Res.*, 8, 599–612.
- Kreidie, N., Armiento, G., Cibin, G., Cinque, G., Crovato, C., Nardi, E., Pacifico, R., Cremisini, C., & Mottana, A. (2011). An integrated geochemical and mineralogical approach for the evaluation of arsenic mobility in mining soils. *J. Soil Sediment*, 11, 37–52.

- Li, X., Shen, Z., Wai, O. W. H., & Li, Y. S. (2001). Chemical forms of Pb, Zn and Cu in the sediment profiles of the Pearl River Estuary. *Mar. Pollut. Bull.*, Vol. 42, pp. 215–223.
- Li, J. X., Yang, X. E., He, Z. L., Jilani, G., Sun, C. Y., & Chen, S. M. (2007). Fractionation of lead in paddy soils and its bioavailability to rice plants. *Geoderma.*, 141, 174–180.
- Lu, S. G., & Bai, S. Q. (2010). Contamination and potential mobility assessment of heavy metals in urban soils of Hangzhou, China: relationship with different land uses. *Environ. Earth Sci.* 60, 1481–1490.
- Ma, H., Hua, L., & Ji, J. (2011). Speciation and phytoavailability of heavy metals in sediments in Nanjing section of Changjiang River. *Environ. Earth Sci.* 64, 185–192.
- Ma, L. Q., & Rao, G. N. (1997). Chemical fractionation of cadmium, copper, nickel, and zinc in contaminated soils. *J. Environ. Qual.* 26, 259–264.
- Machender, G., Dhakate, R., Prasanna, L., & Govil, P. K. (2011). Assessment of heavy metal contamination in soils around Balanagar industrial area, Hyderabad, India. *Environ. Earth Sci.* 63, 945–953.
- Naji, A., Ismail, A., & Ismail, A. R. (2010). Chemical speciation and contamination assessment of Zn and Cd by sequential extraction in surface sediment of Klang River. *Malaysia. Microchem. J.* 95, 285–292.
- Perin, G., Craboledda, L., Lucchese, M., Cirillo, R., Dotta, L., Zanetta, M. L., & Oro, A. A. (1985). Heavy metal speciation in the sediments of northern Adriatic sea. A new approach for environmental toxicity determination. In: Lakkas, T. D. (Ed.), *Heavy Metals in the Environment*, Vol. 2, CEP Consultants. *Edinburgh. P.* 454–456.
- Ramos, L., Hernandez, L. M., & Gonzalez, M. J. (1994). Sequential fractionation of copper, lead, cadmium and zinc in soils from near Donana National Park. *J. Environ. Qual.* 23, 50–57.
- Rogan, N., Dolenc, T., Serafimovski, T., Tasev, G., & Dolenc, M. (2010). Distribution and mobility of heavy metals in paddy soils of the Kocani Field in Macedonia. *Environ. Earth Sci.* 61, 899–907.
- Singh, K. P., Mohan, D., Singh, V. K., & Malik, A. (2005). Studies on distribution and fractionation of heavy metals in Gomti River sediments- a tributary of the Ganges. *India. J. Hydrol.*, 312, 14–27.
- Sutherland, R. A. (2000). Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. *Environ. Geol.* 39, 611–627.
- Tessier, A., Campbell, P. G. C., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51, 844–851.
- Tuzen, M. (2003). Determination of trace metals in the River Yesilirmak sediments in Tokat, Turkey using sequential extraction procedure. *Microchem. J.* 74, 105–110.
- US EPA (1998). *Test Methods for Evaluating Solid Waste, Method 9081A*. EPA, Washington, D.C.
- US EPA (1998). *Test Methods for Evaluating Solid Waste, Method 9081A*. EPA, Washington, D.C.
- Wang, H., Wang, C. X., & Wang, Z. J. (2008). Fractionation of heavy metals in surface sediments of Taihu Lake, East China, *Environ. Geoderma. Health*, 26, 303–309.
- Wang, X., He, M., Xie, J., Xi, J., & Lu, X. (2010). Heavy metal pollution of the world largest antimony mine-affected agricultural soils in Hunan province (China). *J. Soil. Sed.* 10, 827–837.
- Yan, C., Li, Q., Zhang, X., & Li, G. (2010). Mobility and ecological risk assessment of heavy metals in surface sediments of Xiamen Bay and its adjacent areas, China. *Environ. Earth Sci.*, 60, 1469–1479.
- Yan, S., Ling, Q. C., & Bao, Z. Y. (2007). Metals contamination in soils and vegetables in metal smelter contaminated sites in Huangshi, China. *Bull. Environ. Contam. Toxicol.* 79, 361–366.
- Zhou, Y. W., Peng, Y. Sh., Li, X. L., & Chen, G. Zh. (2011). Accumulation and partitioning of heavy metals in mangrove rhizosphere sediments. *Environ. Earth Sci.* 64, 799–807.