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Investigation of trace elements in agricultural soils by BCR sequential extraction method and its transfer to wheat plants

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Abstract In this study, soil samples were collected from Edirne, Turkey in both summer and winter seasons and subjected to the modified Community Bureau of Reference (BCR) sequential extraction procedure in order to investigate the chemical partitioning of metals in soils and to predict heavy metals uptake by wheat grains which grown at the same soils. The samples were subjected to a three stage extraction procedure proposed by the BCR. The three phases that were separated out in the following order: (1) carbonate, exchangeable, (2) Fe–Mn oxides, and (3) organic matter. Metal concentrations of soil fractions and grain samples were determined by inductively coupled plasma atomic emission spectroscopy. The wheat samples were prepared to analysis using microwave acid digestion procedure. The pseudo-total concentrations of metals were determined after aqua regia digestion. The analytical accuracy of the method was evaluated by using the Standard Reference Materials (BCR 142R Light Sandy Soil, NIST 2711 Montana Soil, and NIST 2704 Buffalo River Sediment). The sum of the metal contents obtained from the modified BCR sequential extraction procedure and pseudo-total metal contents for soil samples were used to calculate recovery values. In order to evaluate the bioavailability of metals, the relationships between the wheat-metal and soil-extractable metal concentrations were compared.

Keywords Modified BCR sequential extraction · Soil · Wheat grain · Bioavailability · Inductively coupled plasma atomic emission spectroscopy

Introduction

Trace elements enter an ecosystem through both natural and anthropogenic activities. The anthropogenic activities have caused a remarkable release of trace elements into agricultural soils. These activities include inputs of trace elements through use of fertilizers, liming minerals, organic manures, sewage sludge, and other wastes, irrigation waters, and atmospheric deposition from industrial, and road emissions (Adriano 1986; Senesi et al. 1999). Although some trace elements are required for plant nutrition, an excessive amount may cause soil contamination and toxicity to plants. In addition, toxic trace elements may contaminate the food chain and water resources. Therefore, they have potentially harmful effects to human and animal health (Senesi et al. 1999; Marin et al. 2001).

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Trace elements in soils can be classified as water-soluble, exchangeable, oxide-bound, carbonate-bound, organic matter-bound, and residual that is bound to resistant minerals (silicates) and non-extractable in order to determine their mobility and bioavailability (Shuman 1991). Water-soluble and exchangeable fractions are considered to be bio-available; oxide-, carbonate-, and organic-matter-bound fractions may be potentially bio-available, but the residual fraction is mainly not available to plants and microorganisms. Several soil properties, such as pH, clay, silt, organic matter content, redox potential, cation exchange capacity, as well as amounts and forms of oxides and carbonates in these fractions, affect the bio-availability and mobility of trace elements between the soils and agro ecosystem (He et al. 2005). Although the separation of various chemical fractions of trace elements is very difficult, the use of selective chemical extraction methods is a common approach for understanding associations of trace elements with soil components (Ure and Davidson 2002). The aim of such extractions is to determine operationally defined metal fractions, as well as to bio-available, potentially mobile or toxic phases of a contaminant. Sequential extraction procedures provide an important approach in these subjects, because the total concentration of trace elements in soils does not give usable information about the bio-availability of potentially toxic elements.

There is wide variety of sequential extraction procedures available in soil analysis based on a different sequence of extractants and/or different operating conditions (Lopez-Sanchez et al. 1993; Filgueiras et al. 2002; Parat et al. 2003). The most recent sequential extraction method has been developed by the Community Bureau of Reference, BCR (Quevauviller et al. 1993) (now the Measurements and Testing Program), as enterprise to standardize different sequential extractions schemes for the determination of trace elements in soils. BCR proposed a standard three-step extraction procedure was widely accepted and used to metal extraction in sediment (Petit and Rucandio 1999; Mester et al. 1998) and soil samples (Rauret et al. 1999; Pueyo et al. 2003). This method started with inter-laboratory exercises to develop a three-step procedure using; acetic acid (step 1); hydroxylamine (step 2); and hydrogen peroxide (step 3). Detailed information of the conditions of use at each step as well as preparation of extracting solutions is given to avoid any difference that could impair the reproducibility of the procedure. This method was then applied to the certification of a sediment reference material (CRM 601) (Quevauviller et al. 1997; Lopez-Sanchez et al. 1998). The developed version was proposed by Rauret et al. (1999). They used an aqua regia digestion of both the sample and residue after extraction according to the ISO 11466 method, for internal control. The modified BCR extraction procedure is now frequently used in recent studies.

The main purpose of this work was to determine the total and available concentrations of trace elements in various agricultural soils by using modified BCR sequential method and to investigate correlation between wheat metal concentration and metal fractions extracted from corresponding soils. Another purpose of this work was to determine the influence of seasonal changes on trace element concentrations in soil samples.

Experimental

Instruments and apparatus

A horizontal flasker, electric shaker (Nüve ST 402) was used for shaking samples; 38 R type Hettich Zentrifugen Rotina centrifuge; speed range 0–10,000 rpm, timer 0–60 min used for centrifugation. An Orion 720 pH meter was used for pH adjustments of the reagents. A microwave oven equipped with PTFE vessels model Marsh extraction (MarshXpress) was used for sample digestion. The determination of metals in extracts and digests was carried out by means of an inductively coupled plasma atomic emission spectroscopy (ICP-OES) spectrometer (Varian Vista-MPX CCD simultaneous). Operating RF power was 1.05 kw. Argon gas flow rate (in L/min): plasma 15; auxiliary 1.5; nebulizer 0.9. Integration time was 5 s.

Reagents

All chemicals and reagents were of analytical reagent grade. All glass and plastic bottles was acid-cleaned (soaked in 20% nitric acid for at least 24 h) and rinsed thoroughly with distilled water prior to use. All solutions were stored in high-density polyethylene bottles. Multi-element stock solutions containing 100 mg/L of each element were obtained from Merck (Darmstadt, Germany). Analytical calibration standards were prepared daily by diluting suitable volumes of stock solutions with 0.5 M HNO_3. The Indium internal standard solution (10 μg/L) was prepared from stock solution (1,000 mg/L).

In order to verify the accuracy and precision of the proposed method, three standard reference materials were supplied by the Community Bureau of Reference Sample (BCR), and the National Institute of Standards and Technology (NIST): BCR 142 “Light Sandy Soil,” NIST 2711 “Montano Soil,” SRM 2704 “Buffalo River Sediment.” Samples were handled according to the supplier’s specifications.

Procedures

Sampling and preparation of soil and wheat samples

Soil samples were collected in seasons as follows: winter (January 2007), summer (end of the June 2007–mid-July). However, wheat grain samples were taken randomly across the field during summer (end of the June 2007–mid-July) season. Soil samples were collected from seven locations of the agricultural area in Edirne. All of the locations are close to road in the vicinity of a city center. The sampling was carried out so that each soil was a composite sample consisting of five subsamples randomly taken in the surface (0–20 cm depth) and in an area of 25 × 25 m. Sample preparation was performed according to Ure (1996). A part of each sample was air-dried, ground, and passed through a 2-mm stainless steel screen and transferred to polyethylene bottles until analysis.

About 0.25 g dried and ground wheat grain sample was transferred into pre-cleaned PTFE vessel for total digestion, and then 4 mL of 65% $HNO_3 + 2$ mL of 30% H_2O_2 were added; then the vessels were placed inside a rotor of microwave digestion system, finally submitted to a microwave digestion program (Table 1). After cooling, the digest was filtered through a whatman type 41 filter papers (Nardi et al. 2009) and then indium was added as an internal standard. Finally the volume was diluted to 50 mL with distilled water. The digests were stored at 4°C until analysis.

Table 1 Microwave oven heating program for the digestion of wheat samples

Step	Temperature (°C)	Power (W)	Time (min)
1	160	1,000	4.5
2	160	0	0.5
3	230	1,000	5.0
4	230	1,000	15.0
5	0	0	20.0

Digestion procedure for soils

For pseudo-total metal content determination, one gram of sample was digested directly into each pre-cleaned vessel and 15 mL of %37 HCl and then 5 mL of %65 HNO_3 were added; then the vessels were placed inside a rotor of the microwave digestion system and finally submitted to a microwave digestion program (Table 2). After cooling, the digest was filtered, to remove the silicate content, through a whatman type 41 filter paper and then 10 μg/L of indium was added, and finally diluted to 50 mL with distilled water. The sample solutions were stored in plastic containers and finally measured for their metal contents by ICP-OES. Each sample was prepared five times. The residues, after step 3 (modified BCR sequential extraction), were also performed as the same microwave digestion procedure below.

Table 2 Microwave oven heating program for the decomposition of soil samples

Step	Power (W)	Time (min)
1	250	6.0
2	400	6.0
3	600	6.0
4	250	6.0
5	0	20.0

Table 3 Modified BCR three-stage sequential extraction procedure

Step	Solid phase	Extraction procedures
F1	Water and weak-acid soluble and exchangeable	1 g of soil sample, 40 mL of 0.11 mol L^{-1} CH_3COOH, shake 16 h, room temperature
F2	Reducible	40 mL of 0.5 mol L^{-1} $HONH_2.HCl$ (pH 1.5), shake 16 h, room temperature
F3	Oxidiseable	10 mL of 8.8 mol L^{-1} H_2O_2 (pH 2), shake 1 h, room temperature and 1 h at 85°C Then 10 mL of 8.8 mol L^{-1} H_2O_2 (pH 2), shake 1 h at 85°C, cool, add 50 mL of 1 mol L^{-1} NH_4OAc (pH 2), shake 16 h at room temperature
R^a	Residual	15 mL of 12 mol L^{-1} HCl+5 mL of 15.8 mol L^{-1} HNO3 under microwave digestion procedure (Table 2)

[a] Digestion of the residual phase is not a step of the BCR procedure

Modified BCR three-step sequential extraction procedure for soils

A simple, three-step modified sequential procedure was performed for extraction of trace elements in soil samples. The procedure was performed to extract trace elements from three supposed phases of soil, F1: water and acid soluble, and exchangeable; F2: reducible (bound to Fe/Mn oxides); F3: oxidiseable (bound to organic matter and sulphides); R: residual (metals bound to the primary and secondary minerals which strongly bound to soil). Five independent replications were performed for each sample. The extractants used, the extraction conditions, and the defined target phases of trace elements to be extracted are shown in Table 3 (Rauret et al. 1999, 2000).

Results and discussion

Pseudo-total metal contents in soils

Pseudo-total metal contents in soil were determined by digestion with aqua regia. The results obtained from aqua regia digestion for soil samples, whose corresponding wheat samples were compared with the sum of results obtained by the modified BCR sequential extraction procedure. The results are showed in Tables 4 and 5.

The five-fold samples were good in agreement. An internal check was performed on the results of the modified BCR sequential extraction steps plus residual by comparing the total content of elements extracted from the pseudo-total digestion (aqua regia). In all cases, metal recovery was calculated as follows:

$$\text{Recovery}(\%) = \Big[(\text{step 1 (F1)} + \text{step 2 (F2)} + \text{step 3 (F3)} + \text{Residual(R)})/\text{Pseudo-total}\Big] \times 100$$

The average recovery values were in the range of 90–111%.

The mean concentration of the metals increased in the order Mn>Zn>Cu>Pb>Cr≈Ni>Co>Cd. Concentrations of Cd, Cu, and Ni were higher than typical soil ranges reported (He et al. 2005; Kabata-Pendias and Pendias 2001). The mean concentrations of all metals were lower than the maximum permitted values by European Soil Bureau Research Report (European Soil Bureau 1998).

Analytical accuracy and precision

The accuracy of the pseudo-total digestion method was evaluated by analyzing three certified reference materials (BCR 142, NIST 2711, and SRM 2704). The obtained results are shown in Table 6. No significant statistical differences at 95% confidence level were observed between certified values and found values for certified reference materials. The precision for most of the metals analyzed were lower than 12%. The detection limits based on three times the standard deviation of the blank ($n = 10$) were found in the

Table 4 Concentrations (μg g^{-1}) of Cd, Co, Cr, and Cu obtained by sequential extraction and pseudo-total digestion

Element	Soil[a] sample	F1[b] (μg g^{-1})	F2[b] (μg g^{-1})	F3[b] (μg g^{-1})	R^b (μg g^{-1})	BCR+residual[b,c] (μg g^{-1})	Pseudo-total[b] (μg g^{-1})	R (%)[d]
Cd	A	0.61 ± 0.11	0.32 ± 0.13	0.43 ± 0.16	0.49 ± 0.10	1.84 ± 0.08	1.77 ± 0.09	104
	B	0.78 ± 0.11	0.32 ± 0.11	0.71 ± 0.11	0.95 ± 0.21	2.75 ± 0.21	2.60 ± 0.15	105.9
	C	0.58 ± 0.11	0.31 ± 0.11	0.39 ± 0.18	0.48 ± 0.12	1.17 ± 0.14	2.75 ± 0.08	92.4
	D	0.66 ± 0.11	0.36 ± 0.09	0.40 ± 0.13	0.53 ± 0.07	1.96 ± 0.16	1.91 ± 0.11	102.4
	E	0.77 ± 0.09	0.30 ± 0.12	0.46 ± 0.10	0.33 ± 0.07	1.09 ± 0.11	1.06 ± 0.12	103.5
	F	0.72 ± 0.09	0.30 ± 0.13	0.41 ± 0.13	0.23 ± 0.07	1.66 ± 0.10	1.53 ± 0.07	108.2
	G	0.67 ± 0.13	0.35 ± 0.13	0.47 ± 0.07	0.33 ± 0.07	1.81 ± 0.10	1.72 ± 0.08	105.5
Co	A	1.29 ± 0.11	3.75 ± 0.05	1.10 ± 0.20	22.8 ± 0.8	28.9 ± 1.8	25.9 ± 2.6	111.4
	B	1.40 ± 0.09	5.41 ± 0.10	2.28 ± 0.13	29.7 ± 1.1	33.1 ± 0.9	37.9 ± 2.4	102.3
	C	1.48 ± 0.08	2.85 ± 0.06	1.40 ± 0.11	25.3 ± 0.6	31.8 ± 3.6	28.4 ± 1.5	109.1
	D	1.77 ± 0.06	2.17 ± 0.14	1.20 ± 0.12	25.6 ± 0.8	29.6 ± 1.3	28.3 ± 1.4	108.7
	E	1.35 ± 0.09	2.51 ± 0.07	1.61 ± 0.08	24.1 ± 1.7	26.8 ± 1.4	27.5 ± 1.3	107.5
	F	1.30 ± 0.10	3.03 ± 0.23	1.16 ± 0.10	21.3 ± 0.7	28.7 ± 1.0	31.1 ± 1.0	90.1
	G	1.36 ± 0.60	2.68 ± 0.08	1.20 ± 0.09	23.4 ± 3.4	33.4 ± 1.5	26.7 ± 1.5	107.3
Cr	A	3.37 ± 0.51	1.21 ± 0.20	9.97 ± 0.21	26.4 ± 0.60	40.9 ± 1.8	41.0 ± 1.2	99.8
	B	3.09 ± 0.08	1.43 ± 0.08	14.4 ± 0.51	38.0 ± 1.2	56.9 ± 1.7	63.4 ± 1.5	89.7
	C	3.08 ± 0.11	1.19 ± 0.10	9.46 ± 0.48	22.9 ± 0.33	36.7 ± 1.5	37.1 ± 1.3	98.8
	D	3.12 ± 0.16	1.24 ± 0.13	8.04 ± 0.78	17.6 ± 0.13	30.0 ± 1.2	31.7 ± 1.5	94.8
	E	3.15 ± 0.13	1.15 ± 0.15	11.5 ± 0.28	38.8 ± 1.9	54.6 ± 1.3	57.1 ± 1.2	95.7
	F	3.02 ± 0.14	1.06 ± 0.18	8.86 ± 0.10	30.7 ± 1.5	43.6 ± 1.3	42.9 ± 2.1	101.6
	G	3.21 ± 0.19	1.13 ± 0.18	9.17 ± 0.18	30.4 ± 1.7	43.8 ± 1.5	39.5 ± 1.6	110.9
Cu	A	2.56 ± 0.08	18.3 ± 0.53	10.4 ± 0.53	10.6 ± 0.81	41.9 ± 1.8	41.9 ± 2.9	99.9
	B	3.85 ± 0.14	18.5 ± 0.64	18.5 ± 0.63	33.2 ± 1.1	74.1 ± 2.9	72.4 ± 3.3	102.4
	C	3.12 ± 0.20	26.3 ± 0.77	15.3 ± 0.70	17.2 ± 1.4	62.0 ± 1.3	67.2 ± 3.9	92.3
	D	2.31 ± 0.11	16.8 ± 0.36	11.6 ± 0.69	15.8 ± 0.81	46.6 ± 3.2	47.8 ± 2.3	97.4
	E	3.75 ± 0.17	20.6 ± 5.0	16.9 ± 0.46	12.8 ± 0.48	54.0 ± 1.6	58.9 ± 1.7	91.8
	F	3.95 ± 0.27	29.5 ± 1.1	19.1 ± 0.78	28.6 ± 1.7	81.2 ± 2.9	81.1 ± 2.7	100.1
	G	3.76 ± 0.11	25.6 ± 0.83	17.4 ± 0.46	25.9 ± 1.5	72.7 ± 3.5	78.2 ± 3.8	93.0

[a] Soil samples taken from agricultural lands at A (Karaağaç), B (Kirişhane), C (Kıyık), D (Üniversite), E (Ticaret borsası), F (Göçmenevleri), G (Yeniimaret) corresponding to wheat samples (summer season), $n = 5$

[b] mean ± SD

[c] sum of the fractions F1 + F2 + F3 + residual (Table 3)

[d] Recovery (R%) = [(F1 + F2 + F3 + residual)/pseudo-total] × 100

Table 5 Concentrations (μg g^{-1}) of Mn, Ni, Pb, and Zn obtained by sequential extraction and pseudo-total digestion, $n = 35$

Element	Soil[a] sample	F1[b] (μg g^{-1})	F2[b] (μg g^{-1})	F3[b] (μg g^{-1})	R^b (μg g^{-1})	BCR+residual[b,c] (μg g^{-1})	Pseudo-total[b] (μg g^{-1})	R (%)[d]
Mn	A	53.5 ± 1.2	228 ± 16	119 ± 8	257 ± 22	658 ± 51	657 ± 37	100.2
	B	23.7 ± 1.3	470 ± 51	180 ± 5	542 ± 47	1,216 ± 83	1,226 ± 98	99.2
	C	23.6 ± 2.7	229 ± 30	144 ± 5	216 ± 18	613 ± 42	707 ± 29	100.9
	D	44.6 ± 4.8	190 ± 13	117 ± 7	267 ± 25	619 ± 39	685 ± 25	90.3
	E	34.3 ± 2.6	257 ± 13	154 ± 6	190 ± 13	635 ± 40	666 ± 41	95.3
	F	29.5 ± 1.5	306 ± 32	142 ± 5	192 ± 10	669 ± 35	710 ± 45	94.2
	G	51.3 ± 1.4	317 ± 27	183 ± 8	178 ± 16	780 ± 42	782 ± 62	99.7
Ni	A	1.94 ± 0.15	3.28 ± 0.25	4.19 ± 0.17	24.5 ± 2.4	33.9 ± 2.2	31.5 ± 2.4	107.6
	B	1.61 ± 0.12	5.44 ± 0.26	11.9 ± 1.1	37.5 ± 2.6	56.5 ± 2.5	53.1 ± 4.1	106.5
	C	1.24 ± 1.0	3.20 ± 0.20	6.81 ± 0.65	26.2 ± 2.5	37.4 ± 2.5	36.9 ± 2.6	101.3
	D	1.65 ± 0.20	2.59 ± 0.23	2.86 ± 0.32	24.5 ± 1.7	31.6 ± 2.8	32.6 ± 3.2	96.9
	E	2.01 ± 0.20	4.47 ± 0.28	21.1 ± 2.0	52.3 ± 2.8	79.9 ± 5.1	78.9 ± 5.7	101.3
	F	1.33 ± 0.13	3.33 ± 0.27	5.84 ± 0.13	32.3 ± 1.6	33.8 ± 2.6	32.6 ± 2.9	103.6
	G	1.43 ± 0.15	3.56 ± 0.17	7.55 ± 0.48	38.5 ± 2.5	44.8 ± 3.8	43.1 ± 4.2	104.0
Pb	A	10.5 ± 0.5	12.6 ± 1.7	11.0 ± 1.0	23.6 ± 1.2	57.8 ± 4.4	52.6 ± 3.9	109.9
	B	11.3 ± 0.8	15.3 ± 1.3	12.7 ± 0.8	41.9 ± 0.7	69.9 ± 5.9	71.8 ± 7.1	97.4
	C	10.3 ± 1.2	13.7 ± 2.1	10.5 ± 1.1	25.3 ± 2.1	59.8 ± 4.3	55.8 ± 5.3	107.3
	D	10.3 ± 0.8	11.9 ± 1.1	10.6 ± 0.9	21.8 ± 2.2	54.6 ± 4.2	57.4 ± 6.0	95.0
	E	10.2 ± 0.9	12.4 ± 0.7	11.2 ± 1.2	26.5 ± 1.7	60.4 ± 5.1	64.0 ± 5.2	106.0
	F	11.6 ± 0.6	17.8 ± 0.9	10.6 ± 1.2	25.2 ± 1.3	53.6 ± 3.9	50.6 ± 3.8	95.6
	G	11.5 ± 0.7	22.0 ± 1.2	13.5 ± 1.0	46.6 ± 3.4	82.1 ± 6.9	85.9 ± 6.2	94.5
Zn	A	16.9 ± 1.6	20.8 ± 1.7	39.6 ± 2.5	28.9 ± 2.0	106 ± 9.5	112 ± 10	94.6
	B	21.2 ± 2.3	26.1 ± 1.6	53.3 ± 3.8	65.5 ± 4.8	165 ± 11	173 ± 15	95.2
	C	17.4 ± 1.5	23.9 ± 1.5	35.1 ± 2.7	38.7 ± 2.6	115 ± 10	116 ± 11	99.6
	D	15.7 ± 1.2	20.1 ± 1.6	30.7 ± 2.9	29.1 ± 1.8	96 ± 9	96 ± 9	100.0
	E	17.5 ± 1.1	20.9 ± 1.7	31.7 ± 3.0	39.3 ± 3.2	109 ± 8.9	104 ± 8.6	105.2
	F	19.4 ± 2.2	23.3 ± 2.0	32.8 ± 2.9	49.5 ± 3.9	125 ± 12	128 ± 12	97.9
	G	18.9 ± 0.9	25.9 ± 2.1	33.5 ± 2.5	48.0 ± 4.0	126 ± 12	132 ± 10	95.7

[a] Soil samples taken from agricultural lands at A (Karaağaç), B (Kirişhane), C (Kıyık), D (Üniversite), E (Ticaret borsası), F (Göçmenevleri), G (Yeniimaret) corresponding to wheat samples (summer season), $n = 5$

[b] mean ± SD

[c] sum of the fractions F1 + F2 + F3 + residual (Table 3)

[d] Recovery (R%) = [(F1 + F2 + F3 + residual)/pseudo-total] × 100

Table 6 Determination of heavy metals in the soil reference materials (BCR 142, NIST 2711 and SRM 2704) after analysis with the pseudo-total digestion method

Element	BCR 142		NIST 2711		SRM 2704	
	Light sandy soil		Montano soil		Buffalo river sediment	
	Certified value ($\mu g\ g^{-1}$)	Found value ($\mu g\ g^{-1}$)	Certified value ($\mu g\ g^{-1}$)	Found value ($\mu g\ g^{-1}$)	Certified value ($\mu g\ g^{-1}$)	Found value ($\mu g\ g^{-1}$)
Cd	0.22 ± 0.10	<0.20	41.7 ± 0.25	40.4 ± 3.6	3.45 ± 0.22	3.27 ± 0.12
Co	*nr*	*nd*	10[a]	9.69 ± 0.87	14.0 ± 0.6	14.3 ± 0.5
Cr	44.4 ± 5.4	46.6 ± 4.8	47[a]	48.5 ± 3.7	135 ± 5	129 ± 7
Cu	25.3 ± 2.0	26.2 ± 1.9	114 ± 2	115 ± 14	98.6 ± 5	97.3 ± 4.6
Mn	527 ± 35	529 ± 17	*nr*	*nd*	*nr*	*nd*
Ni	28.9 ± 8.0	29.7 ± 1.5	20.6 ± 1.1	17.2 ± 1.3	44.1 ± 3	46.2 ± 2.9
Pb	30.9 ± 6.7	29.6 ± 1.4	1162 ± 31	1158 ± 54	161 ± 17	165 ± 10
Zn	79.6 ± 11.7	87.4 ± 8.6	350.4 ± 4.8	345 ± 13	438 ± 12	426 ± 20

Results are given mean ± 95% confidence interval of five separate analyses
nr not reported, *nd* not determined
[a] NIST indicative value

range of 0.1–1.0 μg/L for all extraction steps when the ICP-OES was used.

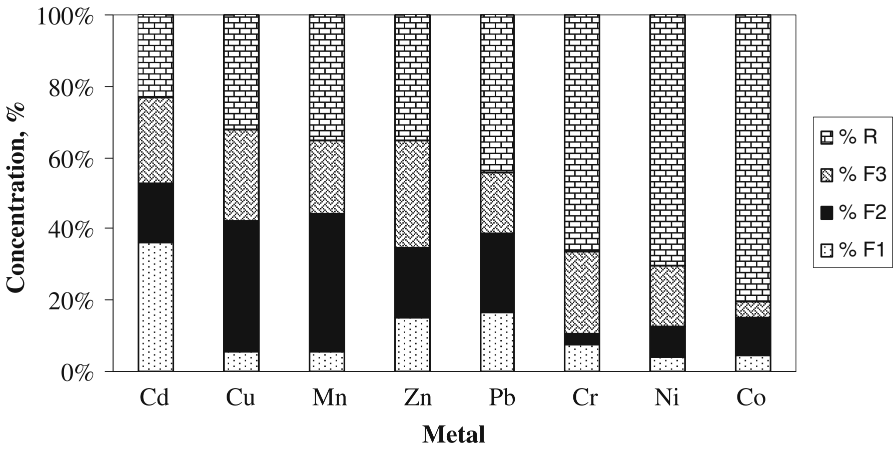
Heavy metals in soil fractions

The distribution of the metals studied into the three fractions of BCR sequential extraction (F1 + F2 + F3) as well as residual fraction (R) for the summer and winter seasons are shown in Figs. 1 and 2. The graphs, as well as Tables 4 and 5 indicated that substantial differences exist between soil samples for given fractions. At the summer season, the studied metal concentrations were lower than that of winter season. Many studies have shown a decrease of metal concentrations in the growing season compared with winter. These authors proposed that this was caused by a dilution effect due to slower uptake than growth during favorable periods and to a large decrease in plant metabolic rates in autumn-winter seasons (Brekken and Steinnes 2004; Kim and Fergusson 1994).

Exchangeable and acid-soluble fraction (F1)

Metal extracted in this fraction would include weakly sorbed metal species, particularly those caught on the soil surface by relatively weak electrostatic interactions and those that can be released by ion-exchange processes. This fraction indicates the amount of metal ions that would be released into the environment if conditions

Fig. 1 Fraction of heavy metals in soil samples (in percent of content) using BCR sequential extraction procedure at summer season ($n = 35$)



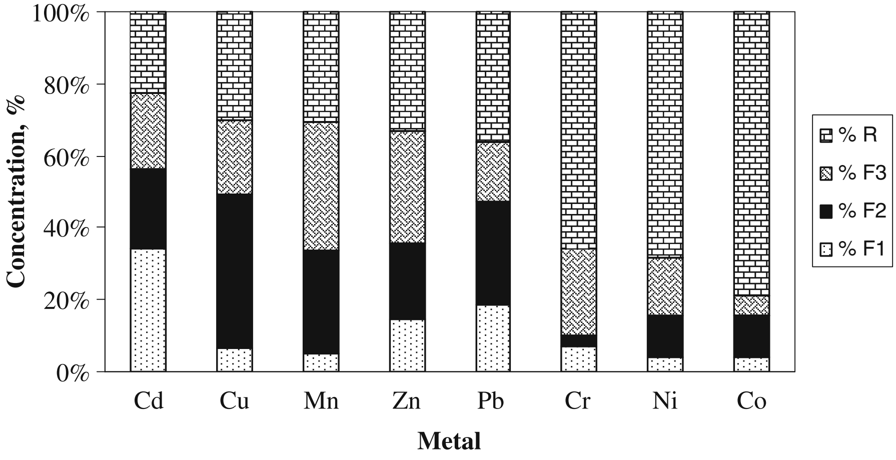


Fig. 2 Fraction of heavy metals in soil samples (in percent of content) using BCR sequential extraction procedure at winter season ($n = 35$)

became more acidic and those gives dangerous effect to the environment (Nemati et al. 2009). The extractable amount of Cd in this fraction, which was found to be the most extractable analyte, is varied from 0.58–0.78 μg/g which corresponding to 27.32–70.32% (Fig. 1).

Reducible fraction (F2)

Metals released in this fraction in reducible conditions such as those bound to hydrous oxides of Mn and Fe. The fraction obtained when metals bound to iron and manganese oxides, a reducing solution is used as the extractant (Gleyzes et al. 2002). In this fraction, 3.20 ± 0.1 μg/g Co, 22.26 ± 1.3 μg/g Cu, 285 ± 26 μg/g Mn, and 15.10 ± 1.3 μg/g Pb were released (Tables 4, 5, and 6). The reducible percentages of Co, Cu, Mn, and Pb were 8.0%, 28.6%, 29.8%, and 18.8%, respectively (Fig. 1).

The oxidizing fraction (F3)

Metals may be released through complexation or bioaccumulation process with various forms of organic matter such as living organism, organic coatings on inorganic particles and detritus. Metals associated with this fraction are assumed to remain in the soil for longer time but may be mobilized by decomposition processes (Kennedy et al. 1997). Treatment of organic matter with oxidizing agents can release of soluble metals bound to this fraction. In addition, amount of metals bound to sulfides might be extracted during this fraction (Marin et al. 1997). This fraction is not considered very mobile or available. Because it is associated with high molecular weight humic matters which release small amounts of metals in slow manner (Filgueiras et al. 2002). Zinc, nickel, and chromium concentrations were found to be at the highest levels in this fraction of the soil samples.

Soil organic matter is known to be capable of bonding Zn in stable forms; therefore, the Zn accumulation in organic soil horizons and in some peats is observed. However, stability constants of Zn-organic matter in soils are relatively low, but a high proportion of Zn is bound to organic matter in mineral soils (Kabata-Pendias and Pendias 2001). The Cr content of surface soil increases due to pollution from various sources, such as several industrial wastes and municipal sewage sludge. Ni has recently become a serious pollutant that is released in the emissions from metal processing operations and from the increasing combustion of coal and oil. The application of sludge and certain phosphate fertilizers may also be important sources Ni. Nickel distribution in soil fraction is released to either to organic matter or to amorphous oxides and clay fractions. The contents of heavy metals in this fraction, associated with the organic matter strongly, were Zn (23.7%), Cr (18.3%), and Ni (13.5%).

The distribution of heavy metals in the soil samples allows us to investigate their mobility and bioavailability (Tokalioglu et al. 2003). The mobility orders of the sum of F1, F2, and F3 fractions, except residual, at summer and winter seasons are given in Figs. 1 and 2. According to Fig. 1, the mobility order at summer season was Cd>Cu>Mn>Zn>Pb>Cr>Ni>Co. As shown in Fig. 2, the mobility order for winter season is similar to that for summer season. In general, at two seasons, Cd (76.7%) seems to be easily mobilized while Co (19.6%) is the minimum mobilizable metal. For summer season, the mobility order in the first fraction was Cd>Pb>Zn>Cr>Cu≥Mn>Co>Ni. In addition, mobility orders of the heavy metals for the second and third fractions were Mn>Cu>Pb>Zn≥Cd>Co>Ni>Cr and Zn>Cd>Cu>Cr>Mn>Pb>Ni>Co, respectively. Since the mobility order of heavy metals at winter season is similar to that of summer season, individual values of fractions were not given for the sake of simplicity whereas only the fraction patterns of winter were given in Fig. 2.

Residual fraction (R)

The residual fraction is mainly not available to either plants or microorganism. This fraction of heavy metals is strongly bound to crystalline structures of minerals. Cr and Ni are often associated with residual mineral in soils and sediments (Davidson et al. 1998). As shown in Figs. 1 and 2, the residual fraction of soil samples showed about ∼80% Co, ∼70% Ni, and ∼66% Cr was released to this fraction of soils.

Heavy metals in wheat grains

Concentrations of Cd, Co, Cr, Mn, Ni, Pb, and Zn in wheat grain grown at the same soils sampled together with maximum permission limit and Bioconcentration Factors (BCF) are shown in Table 7.

BCF is used in the environmental contamination study (Huang et al. 2008). BCF ratio is calculated as fallows:

$$\mathrm{BCF} = \mathrm{C}_{\mathrm{plat}}/\mathrm{C}_{\mathrm{soil}}$$

C_p the concentration of heavy metals in wheat grains;
C_s the concentration of heavy metals in soils.

The order of BCF of heavy metals in wheat samples were as follows: Zn>Cu>Cr>Co>Mn>Cd>Ni>Pb. Zn and Cu were the most abundant metals in wheat grains, because those are the essential micronutrients for plants (Ramesh et al. 2004; Marschnar 1995). The concentrations of Zn and Cu in wheat grains were 5.95 and 1.40 μg/g, respectively. Mean concentrations of Zn and Cu wheat grains were not higher than the maximum allowable concentration of these metals in wheat grains (Table 7). Wang et al. have suggested that the high concentration of heavy metals in wheat grain is related to that in soil. The high value of BCF means that the metal transferred from soil to wheat grains (Wang et al. 2005).

The Mn and Cr levels in wheat grains are not in agreement with those in soil. The most likely explanation is that those heavy metal concentrations in grain may be affected by some anthropogenic activities, automotive emission, and use of metal-enriched materials, including chemical fertilizers, sewage sludge's and waste water irrigation.

The main sources of heavy metals in plants are their growth media, such as nutrient solutions or soils. In addition to plant-specific ability, the uptake of trace elements by plants is affected by

Table 7 Concentrations of heavy metals (μg g^{-1}) in wheat grains

Element	Range[a]	Mean[a]	Critical concentrations of heavy metals in wheat grains[b]	Bioconcentration Factor[a]
Cd	*nd*	0.016	0.21	0.009
Co	0.10–0.67	0.39	*nd*	0.013
Cr	2.03–0.26	0.68	0.02	0.016
Cu	0.76–3.21	1.40	3.0	0.023
Mn	4.82–16.4	8.80	2.0	0.012
Ni	0.023–0.52	0.25	1.63	0.0058
Pb	0.14–0.26	0.12	0.43	0.002
Zn	3.97–11.9	5.95	27.4	0.051

nd not determined
[a] $n = 5$
[b] FAO/WHO (1984)

soil factors namely pH, redox potential, Eh, water regime, clay content, organic matter content, cation exchange capacity, nutrient balance and the concentration of other trace elements. Also, climatic conditions are shown to influence the rate of trace metal uptake which may be partly an indirect impact due to the water flow phenomenon (Kabata-Pendias and Pendias 2001).

Correlation between heavy metal concentrations in different fractions of soils and wheat grain samples

In this study, a single correlation analysis was used to investigate the relationships between heavy metal concentrations in different fractions ofsoils and metal contents in wheat grain samples. Significant *r* values ($>|\pm0.325|, n = 35$) at the 95% confidence level (Yamane 1973) are represented in bold in Table 8. Significant positive correlations were found Cr, Mn and Ni concentrations determined in wheat grain samples and in different fractions of the modified BCR sequential extraction procedure for the soils. The significant correlation shows bioavailability between the metal concentrations in wheat grains and in different fractions of soils. The correlation coefficient between wheat grains and soil samples were found the highest in the first, second and third extraction steps of the BCR sequential extraction procedure. A water soluble and exchangeable fractions from the soils at the first step are considered to be most mobile and immediately bioavailable forms to plants, oxide-, carbonate-, and organic matter-bound fractions may be potentially bioavailable to plants, but the residual fractions is mainly not available to either plants and microorganism (He et al. 2005). In this work, the correlation analyses indicated that residual and sum of the metal concentrations of heavy metals extracted with the sequential extraction procedure were inadequate indicators for heavy metal uptake by plants. There is higher negative correlations were found between Cd in the first and second fractions and Cu in the third fraction because of antagonistic effect in plants (Kabata-Pendias and Pendias 2001).

Conclusions

In this work, the results of heavy metal fraction in soil samples obtained by modified BCR extraction procedure in agricultural soil samples and metal uptake of corresponding wheat grain samples were compared using a correlation analyses. The proportions of each fraction vary between soils and largely determine the availability and mobility of heavy metals in the soil. Many soil factors such as pH, organic matter content, amounts and forms of oxides and carbonates, charge characteristics, as well as mineral composition influence metal contents of these fractions, the bioavailability and transport of metal from soil to plant. The highest correlation coefficients between wheat grain and soil samples were found from first, second and third extraction steps of the modified BCR sequential extraction procedure. The mobility orders of the sum of first, second and third steps of the BCR sequential extraction procedure for the soils studied at

Table 8 Correlation coefficients (*r*) between heavy metal fractions (i.e., F1, F2, F3, Residual (R), BCR+R (F1+F2+F3+R), and their corresponding values in wheat grains ($n = 35$))

Element	Soil fractions				
	F1	F2	F3	R	BCR+R
Cd	**−0.414**	**−0.404**	−0.058	−0.216	0.091
Co	**0.540**	0.194	**0.343**	0.244	**0.375**
Cr	**0.356**[a]	−0.232	0.242	0.226	0.278
Cu	−0.132	**0.336**	**−0.480**	−0.272	**−0.352**
Mn	**0.386**[a]	−0.164	0.003	−0.002	−0.011
Ni	**0.525**[a]	0.176	**0.326**	**0.380**	**0.406**
Pb	0.046	**−0.319**	−0.089	**−0.435**	−0.218
Zn	**0.621**	0.286	**0.715**	**0.506**	**0.575**

[a] Correlation is significant at 95% confidence level ($r_{critical} = 0.325, n = 35$)

summer and winter seasons was found to be highest mobility. The mobility order for the soils studied at summer season was ranged as follows: Cd>Cu>Mn>Zn>Pb>Cr>Ni>Co. The highest mobility was Cd while the lowest was Co for two seasons. Wheat grain samples from those agricultural land had the highest Mn and Cr concentrations, reflecting natural/or anthropogenic contamination by both elements. In addition, bioconcentration factors were obtained between the concentration of heavy metals in soils and the respective concentration in wheat grains. The highest BCF of Zn and Cu, showing that wheat plants from this region are more efficient uptakers of these elements.

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