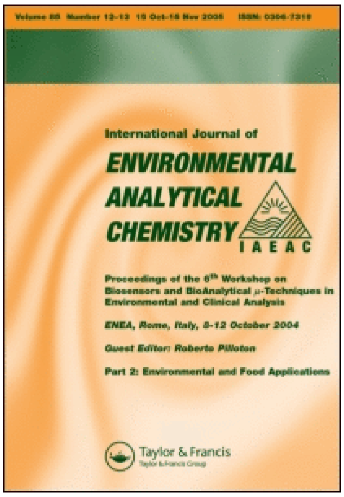

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method

Şerıfe Tokalioğlu[a]; Şenol Kartal[a]; Arıf Gültekın[a]
[a] Department of Chemistry, Faculty of Arts and Sciences, Erciyes University, Kayseri, Turkey

To cite this Article Tokalioğlu, Şerıfe , Kartal, Şenol and Gültekın, Arıf(2006) 'Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method', International Journal of Environmental Analytical Chemistry, 86: 6, 417 — 430

To link to this Article: DOI: 10.1080/03067310500352387

URL: http://dx.doi.org/10.1080/03067310500352387

Intern. J. Environ. Anal. Chem.
Vol. 86, No. 6, 15 May 2006, 417–430

Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method

ŞERİFE TOKALIOĞLU*, ŞENOL KARTAL and ARİF GÜLTEKİN

Department of Chemistry, Faculty of Arts and Sciences,
Erciyes University, TR-38039, Kayseri, Turkey

(*Received 31 March 2005; in final form 30 May 2005*)

The heavy metal (Cu, Fe, Co, Ni, Cd, Cr, Pb, Zn, and Mn) concentrations in soils and in vegetable samples, i.e. lettuce (*Lactuca sativa* L.), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), and onion (*Allium cepa* L.), taken from three urban vegetable gardens in Kayseri, Turkey, were determined by FAAS. The modified three-step sequential extraction procedure proposed by the European Bureau of References (BCR), now the Standards, Measurements and Testing Programme, was used in order to evaluate trace elements mobility in soil samples, and heavy-metal uptake by vegetables. Three operationally defined fractions were isolated using the BCR procedure: acid extractable (i.e. bound to carbonates), reducible (bound to Fe/Mn oxides), and oxidizable (bound to organic matter and sulphides). The vegetable samples were prepared to analysis using the wet-ashing procedure. To estimate the accuracy of the method used in analysis of the vegetable samples, the standard reference material (NIST SRM 1573a, Tomato leaves) was used. The results of recovery for all the elements were relatively satisfactory (87.7–108%). For the soil samples, the recovery values were calculated by proportioning the sum of the steps of the BCR procedure to those of the pseudototal digestion (i.e. aqua regia). In soils, the mobility of heavy metals followed the order $Mn > Cd > Cu > Pb > Zn > Cr > Ni > Co > Fe$. The relationship between the vegetable–metal and soil–extractable metal concentrations was examined in order to evaluate the bioavailability of metals, and the positive correlation, especially for the first (i.e. water, acid-soluble and exchangeable fraction) and for the third (i.e. oxidizable fraction) extraction steps, was obtained.

Keywords: Modified BCR sequential extraction; Lettuce; Parsley; Dill; Onion; Bioavailability

1. Introduction

Agricultural soils are contaminated by heavy metals that mainly originate from mining activities, industrial and traffic emissions, or the application of sewage sludge. Elevated heavy-metal concentrations in the soil can lead to enhanced crop uptake and also negatively affect crop growth. At higher concentrations, they interfere with metabolic processes and inhibit growth, sometimes leading to plant death. Excessive metals in human nutrition can be toxic and can cause acute and chronic diseases [1, 2].

---

*Corresponding author. Fax: +90-352-4374933. Email: serifet@erciyes.edu.tr

International Journal of Environmental Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online
DOI: 10.1080/03067310500352387

Soil is an important sink for heavy metals due to soils' high metal retention capacities. It is an environmental, biochemical reaction system with three important phases: solid (i.e. mineral particles, organic debris, plant roots), solution (i.e. groundwater, rain water, biological excreta, products of biochemical reactions), and gas (i.e. atmospheric, products of biochemical reactions) which move towards equilibrium with one another [3, 4]. Heavy metals may be in many forms in soil solution (ionic, organic complexes, inorganic complexes) on soil cation exchange sites, more tightly bound to soil adsorption sites, occluded into soil oxide materials, incorporated into soil micro-organisms and/or soil animals or in the lattice structure of primary and secondary soil minerals. Soil solution is thought to play an important role in plant nutrient and pollutant uptake from soils and soil-to-plant cycling processes [3, 5].

It is well known that the different fractions of soils vary considerably chemical reactivity and bioavailability of metals. Therefore, single extraction of metals in soil using a selective chemical extractant, such as strong chelating agents or neutral salt, and recently chemical fractionation procedures have been used to indicate the bioavailability or mobility of heavy metals and to identify the relationship between the bioavailable fraction of a metal in soil and its content in plants [6–11]. Sequential approach provides knowledge about metal affinity to the soil components and the strength with which they are bound to the matrix. Unlike the single extraction technique, sequential extraction gives information about mobile and stabile fractions of metals in soil, which evaluates the actual and potential mobility of metals [12]. The development and use of these types of extraction schemes started in the early 1980s and aimed to evaluate the metal fractions available to plants (for estimating the related phytotoxic effects and/or nutritional properties of elements) and the environmentally accessible trace metals (evaluation of the mobility of metals). Most of the sequential extraction schemes mimic the five-stage procedure initially developed for sediments by Tessier *et al.*, who applied it for the fractionation of metals into the following fractions: (i) exchangeable fraction, representing the most easily available metals, (ii) carbonate fraction, (iii) Fe, Mn, and Al oxide fraction, (iv) organic matter fraction, and (v) residual fraction, which is tightly bound to the silicate matrix of the sample. The BCR method, proposed by a European working group coordinated and supported by the Community Bureau of Reference, is faster and simpler than the methods which mimic Tessier *et al.*'s sequential extraction procedure [13], because it consists of three stages. The BCR method has been widely applied to fractionation studies on soils and sediments [14–18]. The original BCR protocol involves successive treatment of sample with 0.11 M acetic acid, 0.10 M hydroxylammonium chloride at pH 2, and hydrogen peroxide followed by 1.0 M ammonium acetate at pH 2. The only difference of the modified BCR from the original one is at the second step. At this step, it was recommended that the concentration of hydroxylammonium chloride be increased to 0.5 M and the pH of the extractant decreased to pH 1.5. This step of the modified extraction procedure provides better attack on the iron-based components of the reducible matrix for a wide range of soils and sediments [19, 20]. Mossop and Davidson compared the results of the original and the modified BCR sequential extraction procedures for fractionation of copper, iron, lead, manganese, and zinc in soils and sediments [21]. Sahuquillo *et al.* applied the modified BCR procedure to the sediments to assess potential remobilization of As [22]. Van Hullebusch *et al.* discussed in detail the results of the fractionation of key metals important for anaerobic metabolism (Co, Ni, Cu, Mn, and Fe) within two different anaerobic granular sludges [23]. Up to now, the various acid and/or acid mixtures have been used to dissolve vegetable samples. For example, Mohamed *et al.* used a mixture of concentrated $HNO_3/HClO_4/HF$ acids for wet ashing of vegetables [7]. Helgesen and Larsen wet-ashed carrot samples using nitric acid in a Teflon-lined pressure steel bomb at 160°C for 4 h [9]. Lee *et al.* used 1 mL of concentrated HNO_3 and 1 mL of 14% HNO_3 to dissolve vegetable samples on a hot plate at 80°C [24]. Although heavy-metal uptake by plants in contaminated soils has been extensively studied using various extraction methods, to our knowledge, there are very few papers in the literature on the application of the BCR sequential extraction procedure for evaluating plant availability of heavy metals in soils [25, 26].

In the present study, single correlation analysis was used to estimate the relationship between vegetable metal concentration and metal fractions extracted from corresponding soils using the modified BCR sequential extraction protocol. The main objectives of the study were (1) to take metals into the solution from the specific soil phases, (2) to find out about bioavailability, mobility and toxicity of metals, and (3) to evaluate the relationship between the uptake of metals by vegetable samples and different forms of metals in soil.

2. Experimental

2.1 *Apparatus and reagents*

A Perkin Elmer Model 3110 flame atomic absorption spectrometer (FAAS) equipped with a hollow cathode lamp was used for the metal determinations. The measurements of the elements Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were performed according to the operating conditions suggested in the manufacturer's manual. A centrifuge (Hettich Rotofix 32, Germany) for complete separation of the extracts from the residues, a Clifton shaker with end-over-end shaking, a Jenco 672 model pH meter, and an electrical heater were used throughout all the experiments.

All reagents were of analytical reagent grade (Merck, Darmstadt, Germany). Throughout the analytical work, distilled water was used. Standard solutions prepared ($1000\,\mu g\,mL^{-1}$) for all the studied elements were stored in polyethylene containers. Glassware and polyethylene bags used for sampling were treated with diluted nitric acid (1 : 1) for 24 h before use and then rinsed with tap and distilled water, respectively. In the digestion and extraction procedures, concentrated nitric acid (65%), hydrochloric acid (37%), acetic acid (99.7%), hydrogen peroxide (35%), hydroxylammonium chloride (i.e. hydroxylamine hydrochloride) and ammonium acetate were used.

2.2 *Sampling and preparation of soil and vegetable samples*

A total of 33 soil samples and corresponding vegetable samples were collected from the three urban vegetable gardens (hereafter referred to simply as Sahabiye garden (GA), Çevreyol garden (GB), and Altınoluk garden (GC)) in areas of heavy traffic in Kayseri, Turkey. All soil samples were taken from the surface layer (0–15 cm). Four different species and three samples of each vegetable, i.e. lettuce (L1, L2 and L3), parsley (P1, P2 and P3), dill (D1, D2 and D3) and onion samples (O1, O2 and O3, except for garden C), were collected from each garden (12 samples from Sahabiye garden (GA), 12 from Çevreyol garden (GB), and nine from Altınoluk garden (GC)). Air-dried, ground and powdered soil samples were passed through a sieve with 200 mesh sizes ($<74\,\mu$m). Soil pH was measured using a soil/water ratio of 1 : 5 (w/v; weight/volume) [27]. The vegetable samples were washed thoroughly with tap water followed by distilled water, dried at 110°C, and ground to pass a 200 mesh sieve. Both vegetable and soil samples were stored in clean polyethylene bags in a desiccator until analysis.

2.3 *Wet ashing of vegetables*

One gram of dried and ground vegetable sample was digested with a mixture of 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (35%). This mixture was heated almost to dryness. After evaporation, 4 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 were added to the residue and heated until a clear digest was obtained (digestion time was about 3 h at 130°C). The digest was made up to 5 mL with 1 M HNO_3.

2.4 *Modified BCR three-stage sequential extraction procedure for soils*

One gram of the air-dried soil sample was put into a 50 mL polypropylene centrifuge tube for fractionation analysis with the modified BCR sequential extraction procedure [19]. As defined in table 1, the procedure was employed to extract heavy metals from three operationally defined target phases of soil, F1: water-soluble, exchangeable and bound to carbonates; F2: bound to Fe–Mn oxides (reducible); and F3: bound to organic matter and sulphides (oxidizable). Residual fraction (R) contains mainly primary and secondary minerals which occlude the trace metals within their crystal structures. The analysis of the residue was performed using aqua regia. For this purpose, a 10 mL aliquot of aqua regia solution was added twice sequentially to the remaining residue. After each addition of aqua regia solution, the mixture was evaporated almost to dryness on a heater. The residue was taken into the solution with 1 M HNO_3 and then filtered through a filter paper using the same acid solution in small amounts.

All the extractions and blank analyses were made in triplicate. After each extraction, the mixtures were centrifuged and the supernatant solutions separated by decantation. The residues were washed with distilled water and the washings discarded. The extracts acquired after each extraction stage were evaporated almost to dryness, and the final volumes were made up to 5 mL with 1 M HNO_3.

Table 1. Modified BCR sequential extraction scheme used for metal speciation.

Step	Soil phases	Extractant	Shaking time and temperature
F1	Water- and acid-soluble, and exchangeable	40 mL of 0.11 M CH_3COOH	16 h at room temperature
F2	Reducible	40 mL of 0.5 M $HONH_2 \cdot HCl$ (pH 1.5)	16 h at room temperature
F3	Oxidizable	10 mL of 8.8 M H_2O_2 (pH 2)	1 h at room temperature and 1 h at 85°C
		Then 10 mL of 8.8 M H_2O_2 (pH 2)	1 h at 85°C
		Cool, add 50 mL of 1 M NH_4OAc (pH 2)	16 h at room temperature
R	Residual[a]	10 mL of aqua regia, twice	Heating on hot plate to dryness

[a] Digestion of the residual material is not a step of the BCR protocol.

3. Results and discussion

3.1 *Pseudototal metal concentrations in soils*

The pseudototal metal concentrations obtained with aqua regia digestion for three soil samples taken from Altınoluk garden (GC), whose corresponding vegetable samples were lettuce (L2), parsley (P2), and dill (D2), were compared with the sum of results obtained by the modified BCR procedure. In order to estimate the effect of soil/extractant ratio on the determination of pseudototal metal concentrations of the soil samples, different amounts of soil samples, i.e. 0.20, 0.50 and 1.00 g, were treated by the same procedure used in the residual fraction analysis. The results are given in table 2. When the sum of the metal concentrations obtained from the sequential extraction of 1.00 g of soil sample was compared with their pseudototal metal contents found using 0.20, 0.50 and 1.00 g specimens of the soil sample, a very good agreement was achieved for all the elements. The recoveries obtained between 91 and 114% show that the aqua regia procedure used to dissolve the residual fraction (ca. 0.2 g) can also be used to obtain pseudototal metal concentrations of soil samples with 0.50 and 1.00 g.

3.2 *Accuracy of the wet-digestion method*

Validation of the wet-digestion method for the vegetable sample analysis was checked by the determination of metals in the reference plant material NIST SRM 1573a (tomato leaves). The results are listed in table 3. A good agreement was found between the data obtained by the present method and the certified values for the reference plant material, except for Zn having a recovery of 88%. The *t*-test was used to compare if the observed difference between experimental mean and true value for all the elements is higher than that computed at 95% confidence level. Only for Fe and Zn the differences were found to be greater than their critical values.

3.3 *Detection limits*

The detection limits based on three times the standard deviation of the blank ($n = 10$) were found in the range of 0.03–0.43, 0.02–0.56, 0.02–0.40, and 0.04–0.56 $\mu g\,mL^{-1}$ for the steps F1, F2, and F3 of the modified BCR sequential extraction method, and the residual phase (R), respectively. In the same way, the detection limits of the wet digestion method for the vegetables were found between 0.03 and 0.35 $\mu g\,mL^{-1}$ for the metals investigated.

3.4 *Heavy metals in soils*

Soil pH plays a major role in the sorption of heavy metals as it directly controls the solubilities of metal hydroxides, as well as metal carbonates and phosphates, and also affects metal hydrolysis, ion-pair formation, organic matter solubility, as well as surface charge of iron, manganese, and aluminium oxides, organic matter, and clay edges [1, 4]. The pH values measured for three garden soils were found in the range of 6.9–8.1 for Sahabiye garden (GA), 7.1–7.7 for Çevreyol garden (GB) and 7.0–7.6 for Altınoluk garden (GC).

Table 2. Sum (Σ) of metal contents obtained from the modified BCR three-stage sequential exreaction procedure plus residual fraction, pseudo-total metal contents, and mean recovery (*R*, %) values of the elements (soil samples taken from GC (Altınoluk garden) corresponding to lettuce, parsley and dill samples), $n = 3$.

Element	Soil[a] sample	Amount of soil (g)	Σ ($\mu g\,g^{-1}$)	Pseudo-total ($\mu g\,g^{-1}$)	R^b (%)	Element	Σ ($\mu g\,g^{-1}$)	Pseudo-total ($\mu g\,g^{-1}$)	*R* (%)	Element	Σ ($\mu g\,g^{-1}$)	Pseudo-total ($\mu g\,g^{-1}$)	*R* (%)
Cu	L2	0.2		41.3	104	Ni		49.0	104	Co		21.9	105
		0.5		43.8	98			50.2	102			22.6	102
		1.0	42.9	42.7	101		51.1	50.2	102		23.1	21.7	106
	P2	0.2		40.7	103			41.9	105			34.6	104
		0.5		46.2	91			56.0	79			35.3	101
		1.0	42.1	41.6	101		44.0	43.3	102		35.8	34.6	104
	D2	0.2		45.4	102			39.5	106			13.1	110
		0.5		48.6	95			41.2	102			13.8	104
		1.0	46.3	45.0	103		41.8	41.4	101		14.4	13.4	108
Pb	L2	0.2		113	104	Cr		43.4	106	Mn		361	101
		0.5		119	98			40.9	113			363	101
		1.0	117	119	98		46.2	46.9	98		365	348	105
	P2	0.2		129	105			37.6	102			316	101
		0.5		134	102			33.9	114			310	103
		1.0	136	138	99		38.5	38.7	100		320	303	106
	D2	0.2		60.8	105			39.5	106			324	102
		0.5		62.3	102			37.0	113			325	102
		1.0	63.8	64.2	99		41.9	42.7	98		331	310	107
Fe (%)	L2	0.2		1.28	101	Zn		216	101	Cd		2.39	105
		0.5		1.29	100			217	101			2.46	102
		1.0	1.29	1.27	102		218	210	104		2.50	2.49	100
	P2	0.2		1.18	102			159	101			1.74	113
		0.5		1.21	100			161	100			1.82	108
		1.0	1.21	1.19	102		161	158	102		1.97	1.96	100
	D2	0.2		1.39	101			147	101			1.93	108
		0.5		1.41	100			149	100			2.04	102
		1.0	1.41	1.39	101		149	148	101		2.08	2.04	102

[a] Soil samples taken from GC (Altınoluk garden) and their corresponding vegetable samples. [b] Recovery ($R\% = [(F1 + F2 + F3 + \text{residual})/\text{pseudo-total}] \times 100$, $\Sigma = $ sum of the fractions $F1 + F2 + F3 + $ residual (see table 1).

Table 3. Determination of heavy metals in the reference material NIST SRM 1573 a (tomato leaves), $\mu g\,g^{-1}$.

Element	This method[a]	Certified value	Recovery (%)
Cu	4.92 ± 0.14	4.70 ± 0.14	105
Ni	1.54 ± 0.20	1.59 ± 0.07	96.9
Cd	1.48 ± 0.05	1.52 ± 0.04	97.4
Cr	2.15 ± 0.36	1.99 ± 0.06	108
Co	0.56 ± 0.11	0.57 ± 0.02	98.2
Fe	390 ± 6	368 ± 7	106
Mn	243 ± 3	246 ± 8	98.8
Zn	27.1 ± 0.5	30.9 ± 0.7	87.7
Pb	7.39 ± 0.75	–[b]	–

[a] Mean and standard deviation from four determinations. [b] No certified.

The fractionation results of heavy metals in the soil samples from the three vegetable gardens, obtained by the modified BCR sequential extraction procedure, are given in table 4. When the first three fractions were taken into consideration, except residual, the highest concentrations (exchangeable and bound to carbonates) at the first stage (F1) were observed for Co and Cd in three garden soils, and also for Fe in the garden A soils. According to the results in table 4, the highest metal concentrations in reducible fractions (F2) of the soil were found for Cu and Mn at Sahabiye garden (GA), for Cu, Ni, and Mn at Çevreyol garden (GB), and for Pb, Ni and Mn at Altınoluk garden (GC). Correspondingly, the Pb, Ni, Cr and Zn concentrations in soil at Sahabiye garden (GA), the Pb, Cr, Fe and Zn concentrations at Çevreyol garden (GB), and the Cu, Cr, Fe, and Zn concentrations at Altınoluk garden (GC) were found to be at the highest levels in the oxidizable fraction (F3) of the soil samples. The mean total Pb concentrations of 67.2, 61.9 and 108 $\mu g\,g^{-1}$ and the mean total Cd concentrations of 1.48, 1.76 and 2.11 $\mu g\,g^{-1}$ in soils of the same sequence at the Sahabiye garden (GA), Çevreyol garden (GB), and Altınoluk garden (GC) were higher than the upper limits of their typical soil concentrations of 20 and 0.3 $\mu g\,g^{-1}$, respectively [28]. Both the Pb and Cd have toxic effects on living organisms because it is well known that they are not required as nutrient elements [29].

The fractionation patterns of the metals based on the sum of F1, F2 and F3 fractions of the sequential extraction procedure for all the soil samples are represented in figure 1. The comparative mobility (%) order of the heavy metals based on the sum of the first three fractions, except for the residual phase, is Mn > Cd > Cu > Pb > Zn > Cr > Ni > Co > Fe. It seems that the most mobile elements are Mn (~86%), Cd (~81%), Cu (~75%), Pb (~70%) and Zn (~66%) followed by Cr (~44%), Ni (~34%) and Co (~30%), while the most non-mobile element is Fe (~6.0%). In addition, the order of comparative trace-metal affinities in each site for the first three fractions was as follows: Mn > Cd > Cu > Zn > Pb > Cr > Ni > Co > Fe for Sahabiye garden (GA), Mn > Cd > Cu > Pb > Zn > Cr > Ni > Co > Fe for Çevreyol garden (GB), and Mn > Cd > Pb > Cu > Zn > Cr > Ni > Co > Fe for Altınoluk garden (GC).

The mobility of Mn, Cd, Cu, Pb, and Zn indicates that the metals originated from anthropogenic sources remained in relatively weakly bound forms [12]. High fractions were found in the residual phase for Cr (~56%), Ni (~66%), Co (~70%), and Fe (~94%), which are possibly related to the solid residual phases of soils. The high contents in the residual fraction expecially for Fe showed that this metal is strongly bound to minerals and resistant components. When the first three fractions of the sequential

Table 4. Mean and sum concentrations (μg g^{-1}) of heavy metals in various fractions of soil samples taken from three vegetable gardens at GA (Sahabiye), GB (Çevreyol), and GC (Altınoluk).

Sampling site	Fraction	Cu	Pb	Ni	Cr	Co	Mn	Fe	Zn	Cd
GA (*n* = 12)	F1	2.55 ± 0.96	11.3 ± 3.1	3.27 ± 0.56	3.50 ± 0.71	2.78 ± 0.76	78.7 ± 18.5	382 ± 140	27.7 ± 2.5	0.70 ± 0.13
	F2	29.7 ± 22.4	17.1 ± 2.4	4.57 ± 0.59	1.46 ± 0.85	1.29 ± 0.82	160 ± 5	141 ± 14	16.1 ± 3.4	0.27 ± 0.05
	F3	17.6 ± 3.7	18.4 ± 4.7	5.35 ± 0.79	9.27 ± 1.95	1.11 ± 0.73	54.7 ± 10.2	217 ± 38	49.1 ± 28.6	0.22 ± 0.19
	R	13.4 ± 4.4	20.4 ± 3.5	26.9 ± 7.8	23.7 ± 8.6	12.2 ± 2.5	49 ± 28	1.19 ± 0.15 (%)	34 ± 39	0.30 ± 0.17
	Σ	63.3 ± 18.0	67.2 ± 4.8	40.1 ± 8.9	38 ± 9	17.4 ± 3.1	342 ± 24	1.26 ± 0.14 (%)	128 ± 48	1.48 ± 0.44
GB (*n* = 12)	F1	3.53 ± 0.74	12.1 ± 3.0	3.66 ± 0.84	3.98 ± 0.69	2.71 ± 0.99	56.9 ± 9.6	271 ± 44	25.2 ± 1.8	0.59 ± 0.20
	F2	32.6 ± 26.1	14.7 ± 2.5	5.41 ± 1.23	2.55 ± 1.06	2.35 ± 0.84	170 ± 21	160 ± 26	16.8 ± 2.0	0.52 ± 0.20
	F3	15.1 ± 4.7	17.4 ± 2.2	5.03 ± 2.10	11.0 ± 2.7	1.35 ± 0.42	44.9 ± 7.6	280 ± 84	41.5 ± 22.8	0.38 ± 0.18
	R	16.9 ± 2.4	17.6 ± 2.6	27.4 ± 9.0	18.2 ± 2.3	15.2 ± 4.4	38.4 ± 25.4	1.22 ± 0.19 (%)	50.0 ± 33.2	0.27 ± 0.23
	Σ	68.2 ± 24.6	61.9 ± 5.0	41.5 ± 10.1	35.8 ± 5.0	21.6 ± 5.1	310 ± 13	1.29 ± 0.19 (%)	133 ± 57	1.76 ± 0.44
GC (*n* = 9)	F1	3.21 ± 0.52	12.1 ± 2.4	3.03 ± 0.96	5.03 ± 0.79	3.35 ± 1.66	58.3 ± 5.7	379 ± 40	28.7 ± 3.0	0.69 ± 0.15
	F2	8.33 ± 2.06	34.7 ± 14.6	7.18 ± 1.30	4.14 ± 1.03	2.59 ± 0.35	179 ± 15	186 ± 25	20.3 ± 2.5	0.59 ± 0.15
	F3	17.6 ± 2.4	28.8 ± 9.0	5.82 ± 1.14	10.5 ± 2.1	1.14 ± 0.35	54.2 ± 5.8	415 ± 147	64.9 ± 21.2	0.41 ± 0.10
	R	14.3 ± 1.7	32.7 ± 11.9	28.5 ± 3.8	22.9 ± 2.6	17.1 ± 7.8	47.9 ± 22.2	1.20 ± 0.10 (%)	65.4 ± 7.7	0.43 ± 0.10
	Σ	43.5 ± 1.8	108 ± 35	44.5 ± 4.3	42.6 ± 5.3	24.2 ± 9.2	340 ± 26	1.30 ± 0.09 (%)	179 ± 31	2.11 ± 0.25

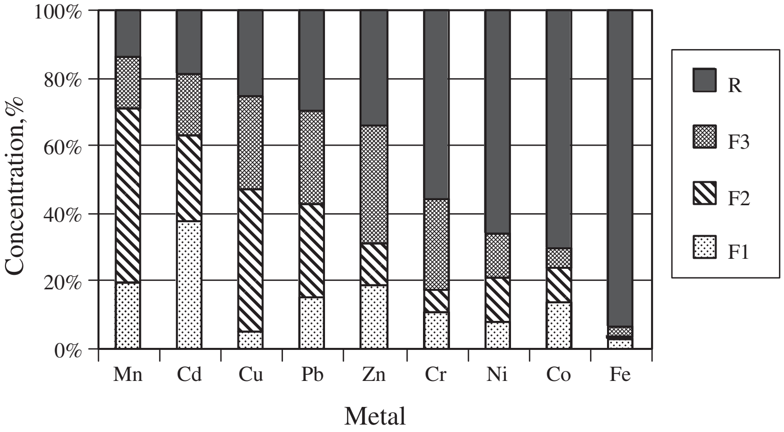

Figure 1. Fractionation patterns of the elements in the soil samples using the modified BCR sequential extraction ($n = 33$).

extraction procedure were taken into consideration, the highest proportions for Co and Cd were found in the first extraction stage (exchangeable and acid soluble), about 14 and 37.4%, respectively. It is known that the carbonates in soil contain significant trace-metal concentrations, which are sensitive to changes in pH. The Cd in this fraction is the most labile; hence, it may be available for uptake by the total biota [30]. The second stage which involves the reducible forms of the target phase (bound to Fe/Mn oxides) has the highest percent mobility for Mn, Cu, and Ni with proportions of 51.2, 41.8 and 13.4%, respectively. Pueyo *et al.* [31] reported that Mn was mainly associated with reducible fractions in the slightly contaminated soils, such as Mn oxides. The adsorption by Fe-Mn oxides controls the Cu and Ni levels in soils. The high surface area and adsorbing capacity of Fe/Mn oxides coupled with the ability of Cu to replace Fe^{2+} in some Fe oxides may be responsible for such adsorption [32]. Pb in the soil samples was partitioned almost equally in both the reducible phase (27.5%) and oxidizable phase (27.3%). The adsorption of Pb cations on the hydrous (amorphic) oxides of Fe/Mn is considered a reasonably universal fixation mechanism, and the ability of lead to form organic complexes is known [32]. The highest extractable fractions for Zn and Cr elements were found to be 35.2 and 26.6%, respectively, at the third extraction stage. This result indicates that in soils these two metals probably occurred in the forms of metal sulphides and/or bound to organic substances.

3.5 *Heavy metals in vegetables*

The results of metal analyses in the lettuce, parsley, dill and onion samples are illustrated in figure 2a, b, c and d, respectively. However, the chromium concentrations are not presented in figure 2d because they were under the detection limit in the onions. As can be seen from figure 2, when compared with the soil types, significant differences were observed on the basis of vegetable samples for Mn, Fe and Zn. For example, the mean Mn concentrations in the lettuce, parsley and dill were 94, 66 and 94 $\mu g\, g^{-1}$, respectively, while the Mn concentrations in the onion samples were only about 10 $\mu g\, g^{-1}$. The mean Fe concentrations were between approximately 70 and

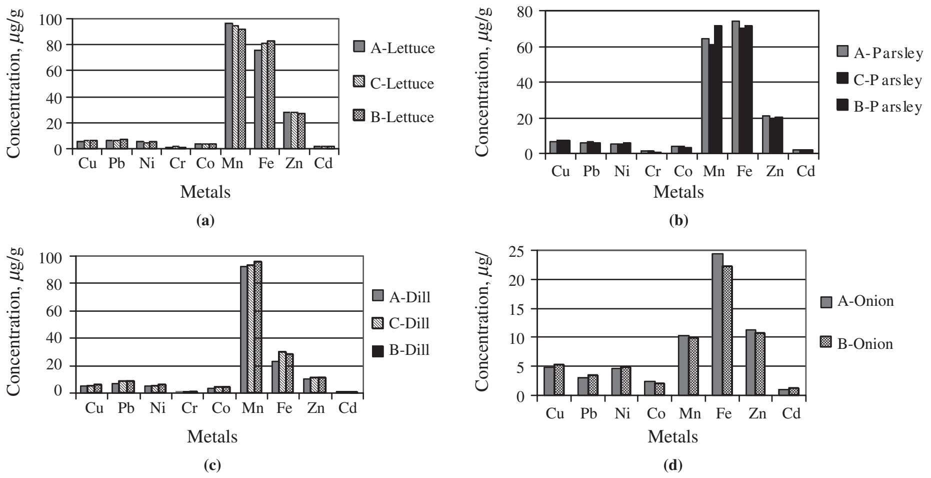

Figure 2. Results of metal analyses (a) in the lettuce (*Lactuca sativa* L.), (b) in parsley (*Petroselinum crispum*), (c) in dill (*Anethum graveolens*), and (d) in onion (*Allium cepa* L.).

80 μg g^{-1} in the lettuce and parsley but between 20 and 30 μg g^{-1} in the dill and onion samples. The mean Zn concentrations in the lettuce, parsley, dill and onion were approximately 28, 20, 11 and 11 μg g^{-1}, respectively. This indicates that different plants can take up and/or accumulate metals in various amounts. From the results obtained, no important difference was observed between the metal uptake by the same kind of vegetable sample taken from the same and/or different garden soil, although soils contained different amounts of the metals [1–3, 17].

The metal concentrations in the vegetable samples were compared with their typical plant values [28]. The results show that all the vegetable samples except onion were polluted by Cr, Pb, Cd and Co, probably originating from human activities such as traffic, atmospheric deposition, or fertilizer.

3.6 *Correlation between heavy-metal concentrations in different fractions of soils and vegetable samples*

One of the most important applications of chemical speciation and/or fractionation of metals in soils is to estimate their bioavailabilities to plants. Different forms of labile metals in soils extracted by chemical reagents were usually assessed for their availabilities to plants based on the correlation between metal concentrations in the plant tissue and different labile forms of the metal in the soils. If the accumulation of any element by a plant correlates significantly with the extractable fraction of soil, it can be assumed that the extractable fraction of the metal is readily available to plants [25, 33]. Therefore, in this work, a single correlation analysis was performed to investigate the relationships between concentrations of metals in different soil fractions and vegetables. For all the binary correlations, significant r values ($>|\pm 0.334|$, $n = 33$) at the 95% confidence level [34] are shown in bold in table 5. According to table 5, the significant correlations from the viewpoint of bioavailability between the metal concentrations determined in the vegetable samples and in different extraction steps of the modified BCR sequential extraction procedure for the soils were acquired.

Table 5. Correlation coefficients (*r*) between the metal contents in different soil fractions (i.e. F1, F2, F3, Residual (*R*), Total) and their corresponding values in the vegetables ($n = 33$).

	Fraction				
Element	F1	F2	F3	*R*	Total
Cu_v	−0.177	**0.450**	**−0.784**	0.193	**0.371**
Pb_v	0.063	0.081	0.179	0.057	0.110
Ni_v	**0.624**[a]	0.161	−0.050	**−0.408**	−0.274
Co_v[b]	**0.405**	0.254	0.293	**0.492**	**0.537**
Cd_v	**−0.442**	−0.129	0.159	−0.144	−0.209
Cr_v	**0.564**	**0.362**	**0.605**	0.222	**0.523**
Mn_v	−0.146	**0.462**	0.064	−0.086	0.147
Fe_v	**0.471**	**0.448**	**0.683**	−0.133	−0.040
Zn_v	−0.113	**0.424**	**0.841**	0.145	**0.534**

[a]Significant at 95% confidence level ($r_{critical} = 0.334$, $n = 33$). [b]Co_v: cobalt concentrations in vegetables, etc.

The highest correlation coefficients between vegetable and soil samples were obtained from first and third extraction steps of the BCR sequential extraction procedure. There were meaningful positive relationships between Ni_v–Ni_1 (0.624), Co_v–Co_1 (0.405), Cr_v–Cr_1 (0.564), Fe_v–Fe_1 (0.471), Cu_v–Cu_2 (0.450), Cr_v–Cr_2 (0.362), Mn_v–Mn_2 (0.462), Fe_v–Fe_2 (0.448), Zn_v–Zn_2 (0.424), Cr_v–Cr_3 (0.605), Fe_v–Fe_3 (0.683), Zn_v–Zn_3 (0.841), Co_v–Co_R (0.492), Cu_v–Cu_t (0.371), Co_v–Co_t (0.537), Cr_v–Cr_t (0.523) and Zn_v–Zn_t (0.534). A similar relationship for Ni was also found in our another study [25]. The high negative correlations were observed between Cd_1–Cd_v (–0.442), Cu_3–Cu_v (−0.784) and Ni_R–Ni_v (−0.408) due to the antagonistic effect in plants [2]. Where 1, 2, 3 and *R* denote the metals determined in different fractions of the BCR procedure and residual, respectively, *v* denotes vegetable content, and *t* denotes metal content based on the sum of fractions of the BCR procedure including the residual phase. The high correlation coefficients obtained from the first and third extraction steps of the modified BCR procedure are in good agreement with the literature [25, 26]. The extracted metal species from the soils, exchangeable and bound to carbonates, with acetic acid at the first step, are generally considered as the most mobile and immediately bioavailable forms to plants, and metals bound to organic matter are also found to be available to plants. Acetic acid extractant used is found in the nature of organic acids secreted by plant root, and therefore, the metal forms extracted by acetic acid may be considerably similar to those of metals that are available to the plant [25, 26]. Also, the correlation analysis results indicated that residual contents of the heavy metals in soils and the sum of metal contents extracted with the sequential extraction procedure were poor indicators of heavy-metal uptake by the plant.

The correlation coefficient between metal concentrations of each type vegetable sample and their corresponding in soil fractions are shown in table 6. For all the binary correlations, significant *r* values at the 95% confidence level are shown in boldface. The highest positive correlations were obtained between Cd_t–$Cd_{lettuce}$ (0.935), Cd_R–$Cd_{lettuce}$ (0.935), Zn_3–$Zn_{lettuce}$ (0.910), Pb_R–$Pb_{parsley}$ (0.847), Pb_3–$Pb_{parsley}$ (0.902), Pb_2–$Pb_{parsley}$ (0.899), Cd_2–Cd_{dill} (0.965), Ni_2–Ni_{dill} (0.938), Co_t–Co_{dill} (0.938), Co_R–Co_{dill} (0.938), Zn_t–Zn_{onion} (0.972), Zn_R–Zn_{onion} (0.975), Zn_3–Zn_{onion} (0.966), Zn_2–Zn_{onion} (0.940), Zn_1–Zn_{onion} (0.946), Pb_1–Pb_{onion} (0.932), Mn_1–Mn_{onion} (0.918), Fe_t–Fe_{onion} (0.914), and Fe_R–Fe_{onion} (0.914). An interesting consequence observed in

Table 6. Correlation coefficients (*r*) between the metal contents in soil fractions (i.e. F1, F2, F3, Residual (*R*), Total) and the lettuce, parsley, dill, and onion samples.

Metal	Fraction	Lettuce	Parsley	Dill	Onion	Metal	Fraction	Lettuce	Parsley	Dill	Onion	Metal	Fraction	Lettuce	Parsley	Dill	Onion
Cu	F1	**0.879**[a]	0.469	−0.164	0.406	Co	F1	0.388	0.261	0.510	**0.798**	Mn	F1	0.520	−0.152	−0.481	**0.918**
	F2	−0.030	−0.112	0.110	**−0.969**		F2	−0.006	−0.364	**0.921**	**−0.866**		F2	−0.401	−0.358	**0.736**	**0.835**
	F3	−0.115	−0.221	**−0.919**	−0.153		F3	0.479	**0.870**	**0.631**	**−0.883**		F3	**0.732**	0.173	−0.303	**0.722**
	R	**0.719**	0.472	0.558	**−0.929**		*R*	0.529	−0.110	**0.938**	0.406		*R*	0.158	**−0.787**	**−0.719**	**−0.903**
	Total	0.099	−0.050	0.069	**−0.928**		Total	0.462	−0.058	**0.938**	−0.610		Total	0.587	**−0.630**	−0.575	**0.803**
Pb	F1	−0.337	0.595	−0.548	**0.932**	Cr	F1	**0.814**	−0.066	0.088	–[b]	Fe	F1	**−0.682**	**0.653**	−0.244	**−0.803**
	F2	−0.089	**0.899**	−0.614	**−0.840**		F2	**0.654**	−0.230	−0.393	–		F2	**0.789**	**−0.790**	−0.172	−0.608
	F3	−0.065	**0.902**	**−0.849**	**0.821**		F3	0.156	0.323	−0.373	–		F3	**0.684**	**−0.700**	−0.241	**−0.936**
	R	−0.098	**0.847**	0.510	**−0.847**		*R*	−0.167	**0.731**	**0.895**	–		*R*	**−0.734**	−0.514	**0.777**	**0.914**
	Total	−0.104	**0.888**	**−0.904**	−0.388		Total	0.214	0.445	**0.895**	–		Total	**−0.732**	−0.578	**0.792**	**0.914**
Ni	F1	**0.867**	**0.763**	**0.610**	**−0.710**	Cd	F1	−0.038	−0.135	0.249	−0.588	Zn	F1	0.140	0.132	**−0.808**	**0.946**
	F2	**−0.830**	−0.085	**0.938**	−0.397		F2	0.596	**−0.767**	**0.965**	**0.925**		F2	0.122	0.287	**0.830**	**0.940**
	F3	**−0.841**	**−0.921**	**0.853**	**0.861**		F3	−0.594	**−0.973**	**−0.818**	**0.901**		F3	**0.910**	−0.571	**0.861**	**0.966**
	R	**−0.685**	**−0.835**	**−0.758**	0.654		*R*	**0.935**	**−0.637**	−0.439	**0.908**		*R*	**−0.708**	**−0.921**	**0.817**	**0.975**
	Total	**−0.801**	**−0.810**	−0.501	0.661		Total	**0.935**	**−0.924**	−0.439	**0.929**		Total	−0.474	**−0.914**	**0.833**	**0.972**

[a] Significant correlation coefficients at 95% confidence level ($r_{\text{critical}} = 0.602$, $n = 9$ for lettuce, parsley and dill; $r_{\text{critical}} = 0.707$, $n = 6$ for onion). [b] Not detected.

relation to the uptake of Zn by onion samples was the highly meaningful correlations existing between Zn contents of the vegetable and each of the soil fractions.

4. Conclusion

In this study, the distribution of heavy metals in the different soil fractions obtained by the modified BCR procedure for the three garden soils and metal uptake of corresponding vegetable samples were examined using a correlation analysis. Soil is a complicated system, and interactions between biota and soil are intricate. Therefore, more detailed information is needed to evaluate soil heavy-metal availability to plants. However, the highest correlation coefficients between vegetable and soil samples were obtained from first and third extraction steps of the BCR sequential extraction procedure, i.e. Ni_v–Ni_1 (0.624), Co_v–Co_1 (0.405), Cr_v–Cr_1 (0.564), Cr_v–Cr_3 (0.605), Fe_v–Fe_3 (0.683), and Zn_v–Zn_3 (0.841). Moreover, the metals having the highest concentrations on average in the vegetable samples were Mn (from 10 to 94 $\mu g\,g^{-1}$), Fe (from 20 to 80 $\mu g\,g^{-1}$) and Zn (from 11 to 28 $\mu g\,g^{-1}$), while the other metals investigated had concentrations lower than 10 $\mu g\,g^{-1}$. The results of vegetable analyses show that no important difference was observed between the metal uptake by the same kind of vegetable sample taken from the same and/or different garden soil, although soils contained different amounts of the metals. The heavy-metal mobility order (%) based on the sum of the first three fractions of the BCR procedure for the soils studied was found to be $Mn > Cd > Cu > Pb > Zn > Cr > Ni > Co > Fe$. The recovery values of metals for the methods used in analyses of both soil and vegetable samples were relatively satisfactory.

References

[1] U. Schmidt. *J. Environ. Qual.*, **32**, 1939 (2003).
[2] I. Maiz, I. Arambarri, R. Garcia, E. Millian. *Environ. Pollut.*, **110**, 3 (2000).
[3] V.H. Kennedy, A.L. Sanchez, D.H. Oughton, A.P. Rowland. *Analyst*, **122**, 89R (1997).
[4] C. Apel, L. Ma. *J. Environ. Qual.*, **31**, 581 (2002).
[5] M. Pueyo, J.F. López-Sánchez, G. Rauret. *Anal. Chim. Acta*, **504**, 217 (2004).
[6] M. Yaman, Y. Dilgin, Ş. Güçer. *Anal. Chim. Acta*, **410**, 119 (2000).
[7] A.E. Mohamed, M.N. Rashed, A. Mofty. *Ecotox. Environ. Safe*, **55**, 251 (2003).
[8] N. Calace, B.M. Petronio, M. Picciolo, M. Pietroletti. *Commun. Soil Sci. Plant Anal.*, **33**, 103 (2002).
[9] H. Helgesen, E.H. Larsen. *Analyst*, **123**, 791 (1998).
[10] C. Parat, J. Lévêque, S. Dousset, R. Chaussod, F. Andreux. *Anal. Bioanal. Chem.*, **376**, 243 (2003).
[11] A.P. Murphy, M. Coudert, J. Barker. *J. Environ. Monit.*, **6**, 621 (2000).
[12] C. Kabala, B.R. Singh. *J. Environ. Qual.*, **30**, 485 (2001).
[13] A. Tessier, P.G.C. Campbell, M. Bisson. *Anal. Chem.*, **51**, 844 (1979).
[14] Ph. Quevauviller. *Trends Anal. Chem.*, **17**, 289 (1998).
[15] Ş. Tokalıoğlu, Ş. Kartal, G. Birol. *J. Environ. Monit.*, **5**, 468 (2003).
[16] J. Hlavay, K. Polyák. *Microchem. J.*, **73**, 65 (2002).
[17] Ş. Tokalıoğlu, Ş. Kartal, L. Elçi. *Anal. Chim. Acta*, **413**, 33 (2000).
[18] R.A. Sutherland, F.M.G. Tack. *Sci. Total Environ.*, **256**, 103 (2000).
[19] G. Rauret, J.F. López-Sánchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, Ph. Quevauviller. *J. Environ. Monit.*, **1**, 57 (1999).
[20] M. Pueyo, G. Rauret, D. Lück. *J. Environ. Monit.*, **2**, 243 (2001).
[21] K.F. Mossop, C.M. Davidson. *Anal. Chim. Acta*, **478**, 111 (2003).
[22] A. Sahuquillo, G. Rauret, A. Rehnert, H. Muntau. *Anal. Chim. Acta*, **476**, 15 (2003).
[23] E. van Hullebusch, S. Utomo, M.H. Zandvoort, P.N.L. Lens. *Talanta*, **65**, 549 (2005).
[24] Y.Z. Lee, S. Suzuki, T. Kawada, J. Wang, H. Koyama, F. Rıvai, N. Herawati. *Bull. Environ. Contam. Toxicol.*, **63**, 711 (1999).
[25] Ş. Tokalıoğlu, Ş. Kartal. *Int. J. Environ. Anal. Chem.*, **83**, 935 (2004).

[26] Z. Wang, X.-Q. Shan, S. Zhang. *Chemosphere*, **46**, 1163 (2002).

[27] F. Bayraklı (Çeviren ve Derleyen). *Toprak ve Bitki Analizleri*, p. 77, Ondokuz Mayis Üniversitesi Yayinlari, Samsun (1986).

[28] H.M. Grimshaw, S.E. Allen, J.A. Parkinson. In *Chemical Analysis of Ecological Materials*, 2nd edn, S.E. Allen (Ed.), pp. 81–159, Blackwell Scientific, London (1989).

[29] Ş. Tokalıoğlu, Ş. Kartal, G. Birol. *Turk. J. Chem.*, **27**, 333 (2003).

[30] C. Yuan, J. Shi, B. He, J. Liu, Li Liang, G. Jiang. *Environ. Int.*, **30**, 769 (2004).

[31] M. Pueyo, J. Sastre, E. Hernández, M. Vidal, J.F. López-Sánchez, G. Rauret. *J. Environ. Qual.*, **32**, 2054 (2003).

[32] A.D.K. Banerjee. *Environ. Pollut.*, **123**, 95 (2003).

[33] S. Zhang, A. Lu, X.-Q. Shan, Z. Wang, S. Wang. *Anal. Bioanal. Chem.*, **374**, 942 (2002).

[34] T. Yamane. *Statistics: An Introductory Analysis*, 3rd edn, p. 1092, Harper & Row, New York (1973).