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RELATIONSHIP BETWEEN VEGETABLE METAL AND SOIL-EXTRACTABLE METAL CONTENTS BY THE BCR SEQUENTIAL EXTRACTION PROCEDURE: CHEMOMETRICAL INTERPRETATION OF THE DATA

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The contents of heavy metals in soil and vegetable samples collected from an urban garden in Kayseri, Turkey, were investigated. Both wet- and dry-ashing methods were used for dissolving vegetable samples. A sequential extraction procedure proposed by the Commission of the European Communities, Community Bureau of Reference (now superseded by the Standards, Measurement and Testing Programme, SM&T) was applied to the soil samples to extract the metals which are present in exchangeable and acid soluble (i.e. bound to carbonates), reducible (bound to Fe/Mn oxides), and oxidisable forms (bound to organic matter and sulphides) in the soil samples. Trace metals in the soil and vegetable samples were determined using flame atomic absorption spectrometry (FAAS). The total metal contents acquired by summing of metal levels in all the sequential extraction steps were compared with pseudo-total metal levels obtained with aqua regia for all the soil samples. The recovery values obtained by proportioning the results obtained by the BCR procedure to those of the pseudo-total digestion were found to be satisfactory. The limits of detection for the elements investigated were in the range of 0.04 to 0.59 $\mu\text{g mL}^{-1}$ for all the extraction stages of the BCR procedure. Similarities among the variables were identified by correlation analysis, principal component analysis and hierarchical cluster analysis. The relationship between the vegetable metal and soil-extractable metal concentrations was examined in order to evaluate the bioavailability of metals.

Keywords: BCR-operational speciation; Vegetable; Bioavailability; Correlation analysis; Principal component analysis; Cluster analysis

INTRODUCTION

The accumulation of heavy metals in environmental samples (plants, sediments, soils, sewage sludges, solid residues, etc.) causes a potential risk to human health due to the transfer of these elements in aquatic media, their uptake by plants and their subsequent introduction into the food chain [1]. Soils are considered as sinks for

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trace elements, and therefore they play an important role in the environmental cycling of elements. Many potential contaminants are necessary for agricultural production but become hazardous when they occur in excess in the soil. For example, small amounts of copper, molybdenum and selenium are needed for proper plant and animal growth, but large amounts can be toxic [2].

The contamination of plants by toxic heavy metals has a major impact on both the environmental cycling of nutrients and the quality of foodstuffs. Plants can accumulate trace elements, especially heavy metals, from soils, waters or air. There is a wide variability in the bioaccumulation of trace elements among different plant species. For example, some elements such as B, Cd, Rb and Cs are readily taken up, whereas Fe and Se are only slightly available to plants [3]. Absorption processes are very complex and the main pathway of trace elements into plants is via their roots. The degree of contaminating element uptake by plant roots is dependent on many factors, including the magnitude and chemical form of the trace elements present; soil pH, moisture, aeration, temperature, organic matter and phosphate content; the presence or absence of competing ions; the plant species, rooting depth, age; and seasonal growth effects. The uptake of metals from soil by plants has already been extensively studied [4–8].

High levels of metals are usually found in soil and vegetation in areas affected by mining activities, metal factories and traffic emissions. Accurate measurements of the total metal contents in polluted soils are required to assess the potential risk of these areas. But the total metal content in polluted environmental samples is a poor indicator of its bio-availability, mobility or toxicity, because these properties depend basically on the chemical association of different components of the sample. Therefore, the leaching of soils by means of chemical extractants is a method widely used to assess the lability of heavy metals in soils. Single and sequential extraction procedures have been applied using different extractants [9–12], and chemical fractionation using sequential extraction methods has been applied to identify plant-available (mobile) forms of trace metals in soils [4,5,7,13]. Many of the sequential extraction schemes employed depend on the five-stage procedure of Tessier *et al.* [14]. Although these methods are time consuming, and suffer from non-selectivity of extractants and trace element redistribution among phases during extraction, they furnish detailed information about the origin, biological and physicochemical availability, mobilization and transport of trace metals. In particular, the BCR method, proposed by a European working group coordinated and supported by the Community Bureau of Reference, is faster and simpler (consisting of three stages only) than other methods derived from the sequential extraction procedure of Tessier *et al.* and its modifications. The BCR method has been widely applied to fractionation studies on soils and sediments [9,15–17], but, 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 metals in soils [4]. In this study, therefore, the BCR method was performed to take metals into solution from specific soil phases, in order to obtain information about the bioavailability, mobility and toxicity of metals, and to evaluate the metals' availability from soil to vegetable samples.

Statistical approaches, such as multivariate component analysis, provide good solutions to the problem of evaluating results including a large number of data. For this purpose, one of the most widely used techniques is principal components analysis (PCA) which is a tool for dimension reduction, modeling and displaying results. It is a good way to analyze very large sets of experimental data and to separate the variables

into subgroups in a data matrix. In this work, the varimax rotation mode of PCA has been used, which allows the weight of higher factor loadings to be increased and the weights of the lower values to be reduced. This leads to better understanding of the data structure [18]. Cluster analysis (CA) is a type of unsupervised pattern recognition which makes it possible to detect similarities or dissimilarities within a large group of objects characterized by a certain number of variables. In the first step of the classification the input data matrix (n objects \times m variables) is normalized to dimensionless values to avoid classification problems with objects described by variables of completely different size (e.g. concentrations varying from parts per million levels to percentage values). The Euclidean distance for similarities between objects and/or variables and an appropriate linkage algorithm (single, average, centroid linkage, Ward's linkage, etc.) is usually used to link a group (cluster) of objects and/or variables with similar distances [18].

One of the most important applications of chemical fractionation of metals in soils is to estimate bioavailability to plants. For this purpose, correlation analysis is performed in order to evaluate the relationships between extractable fractions of the metals from soils and the total metal concentrations in plants. If the accumulation of an element by a plant correlates significantly with the extractable fraction in soils, it can be assumed that the extractable fraction is readily available to plants [19].

Indeed, these statistical analysis techniques have been widely used to explore the pattern of variables and to individualize the sources of pollutants [20–22]. In this study, correlation analysis, PCA and CA were made between the acetic acid extractable fractions of the metals in soil and total metal contents in the vegetable samples. Throughout the study, the software package SPSS 9.05 was used for calculation purposes.

EXPERIMENTAL

Apparatus and Reagents

A Perkin-Elmer Model 3110 flame atomic absorption spectrometer 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 Nel 900 pH meter, an electrical heater, and an Elektromag model muffle furnace to ash vegetable samples 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 ($1000 \mu\text{g mL}^{-1}$) 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 distilled water. In the digestion and extraction procedures, concentrated nitric acid (65%), hydrochloric acid (37%), acetic acid (99.7%), hydrogen peroxide (35%), hydroxylamine hydrochloride and ammonium acetate were used.

Sampling and Sample Preparation

Ten types of vegetable and corresponding soil samples were collected from an urban vegetable garden in Kayseri, Turkey. Soil samples were taken at a depth of 10–15 cm from the soil surface and the vegetable samples were collected at the same sampling sites from which the soil samples were taken. The vegetable samples were parsley, leek, onion, radish, lettuce, spinach, dill, sheep sorrel, cabbage and mint. This order for the vegetable samples corresponds to the sample number of the corresponding soil samples in which they were collected, i.e. from soil 1 (S1-parsley) to soil 10 (S10-mint). The vegetable samples were washed successively with tap water and distilled water, then cut into small pieces and dried at 85°C for 24 h. The samples were then ground to pass through a 200-mesh sieve and stored in polyethylene containers.

Once in the laboratory the soil samples were dried at 105°C [1,14] in an oven, then crushed to obtain a fine powder and finally passed through a 200-mesh sieve (< 74 µm). They were stored in clean polyethylene bags in a desiccator until needed for analysis.

Procedures for Dissolving Vegetable Samples

The metals in vegetable samples were determined using FAAS. All sample and blank analyses were repeated in triplicate.

Wet Ashing

A 1.00 g portion of vegetable sample was transferred into a 100-mL glass beaker and 4 mL of concentrated nitric acid and 2 mL of concentrated hydrogen peroxide were added. The mixture was evaporated to near dryness. After evaporation, 2 mL of conc. nitric acid and 1 mL of conc. hydrogen peroxide were added to the residue and evaporated to dryness again. The residue was taken up in hot 1 M HNO₃ and filtered through a blue ribbon filter paper. The filtrate was diluted to 6 mL with 1 M HNO₃.

Dry Ashing

A 1.00 g subsample of vegetable was placed in a porcelain crucible and ashed at 475°C in a muffle furnace for 7 h. The ash was digested by adding 2 mL conc. HNO₃ and 1 mL conc. H₂O₂ and evaporated. The residue was taken up in hot 1 M HNO₃ and filtered through a blue ribbon filter paper. The filtrate was evaporated almost to dryness and diluted to 6 mL with 1 M HNO₃.

The BCR Three-stage Sequential Extraction Procedure for Soil Samples

Exchangeable and Acid Soluble Metals (Step 1)

40 mL of acetic acid (0.11 M) was added to 1.00 g of dry soil sample in a 50-mL polypropylene tube. The mixture was shaken for 16 h at ambient temperature (approx. 20°C) at a speed of 400 rpm. The extract was separated from the solid phase by centrifugation at 3800 rpm for 20 min. The supernatant liquid was decanted into a 100-mL beaker and then covered with a watch-glass. The residue was washed

by adding 20 mL of distilled water, shaking for 15 min and centrifuging. The second supernatant liquid was discarded without any loss of the residue.

Metals Bound to Iron and Manganese Oxides (Step 2)

Metals bound to iron and manganese oxides were extracted by adding 40 mL of hydroxylammonium chloride (0.1 M, adjusted to pH 2 with 2 M nitric acid) to the residue from Step 1. The mixture was shaken for 16 h at ambient temperature, centrifuged for 15 min and then decanted into a beaker. The residue was rinsed with 20 mL of distilled water, centrifuged, and the supernatant was discarded.

Metals Bound to Organic Matter and Sulphides (Step 3)

10 mL of hydrogen peroxide (8.8 M) was carefully added in small aliquots to the residue from Step 2 in the centrifuge tube. The tube contents were digested at room temperature for 1 h with occasional manual shaking. The procedure was continued for 1 h at 85°C and the volume reduced to a few millilitres by further heating in a water bath. A second aliquot of 10 mL of hydrogen peroxide was added to the residue and the digestion procedure repeated. The solution was heated to near dryness, and 50 mL of ammonium acetate solution (1.0 M, adjusted to pH 2 with nitric acid) was added to the moist residue. The sample solution was shaken, centrifuged, and the extract was separated as described for Step 2.

For analyses of soil samples, a concentrated acidic H₂O₂ solution is often preferred for oxidizing the organic substances, even though the oxidation of all the organic matter may not be complete. The ammonium acetate solution was used to prevent re-adsorption of extracted metals onto the oxidized soil sample.

Treatment of Residue and Extracts

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 a 100-mL beaker containing the residue from Step 3. After each addition of aqua regia solution, the residue was evaporated to near dryness on a heater. Then it was treated with successive small amounts of 1 M HNO₃, which were filtered through a filter paper. The beaker was carefully washed with the same acid solution and the filtrates were combined in a beaker.

The extracts acquired after each extraction stage were evaporated almost to dryness. Each extract was made up to 5 mL with 1 M HNO₃ except for the one from Step 3, for which the ammonium acetate extract was made up to 6 mL. The determination of Cr, Cd, Fe, Ni, Co, Cu, Zn, Pb, and Mn elements in the extracts was performed using FAAS.

RESULTS AND DISCUSSION

Recovery Percentages from Soil Samples

To determine the accuracy of the method used for the determination of the pseudo-total metal levels in the soil samples, known amounts of the target elements were added to

extracts of the samples. For this purpose, the dissolution procedure was performed by adding aqua regia solution in two 10-mL portions to a fresh soil sample. After filtration of the remaining residue with a blue ribbon filter paper, known amounts of the elements were added to the soil extract. These additions were within the range of the calibration curve standards. The analyses were repeated in triplicate using 1.0 g subsamples. The recovery values ($R\%$) were calculated by comparison of the concentrations of the metals with or without spike. The obtained percentage recoveries were 100.7 ± 0.6 for Pb, 91.3 ± 6.1 for Cr, 89.0 ± 8.2 for Co, and 89.0 ± 10.0 for Cd. Owing to their high concentrations in the investigated soil sample, Mn, Fe, Zn, Ni and Cu were not added to the extracts.

The pseudo-total metal contents obtained by dissolving the ten soil samples using the dissolution procedure with aqua regia described above were compared with the sum of the metal contents of each extraction step of the three-stage sequential extraction procedure, including the contents of the residual phase. The analyses for each soil sample were repeated in triplicate using 1.0 g of specimen. The pseudo-total metal contents, the sum of the metal contents obtained from the BCR three-stage sequential extraction procedure including the residual phase, the mean recovery values of the target elements for the ten soil samples, and typical metal contents of the soils are depicted in Table I. Recovery values of the elements were calculated as the ratio of the BCR results to those of the aqua regia digestion. Comparison of the obtained recoveries showed good agreement for Cu, Pb, Mn, Fe and Zn, but not for Ni, Co, Cd and Cr. The high recoveries for Co, Cd and Cr (136 ± 12 , 151 ± 21 and $135 \pm 14\%$, respectively) may be related to their low concentrations in the soil samples, because a low concentration of any element may generally cause high relative errors. In the case of Ni ($126 \pm 18\%$) there is a contamination risk in the BCR procedure.

Results of Soil Analysis

The mean total metal concentrations found to be the sum of Steps 1–3 of the BCR procedure and the residual phase for the ten soil samples, their standard deviation values and the detection limits (DL) of the methods for all the extraction steps are listed in Table II. In calculating the DL, the equation $3s/b$ was used; where s is the standard deviation of the blank solution and b is the slope of the calibration line. As can be seen in Table II, the DL values changed from 0.04 to $0.59 \mu\text{g mL}^{-1}$. The average total concentrations of Co, Cr, Mn, Fe, Zn and Ni obtained from the sequential extraction procedure were in the range of their typical soil metal contents (see Table I). However, average concentrations of Cu, Pb and Cd based on the sums of the three extraction stages of the sequential extraction procedure and the residual contents were higher than the upper limits of their typical soil contents ($148 > 80$; $71.5 > 20$ and $1.62 > 0.3 \mu\text{g}^{-1}$, respectively). This result is an indicator of metal pollution for by these three elements in the vegetable garden soils in an urban area. Lead, in particular, is a ubiquitous environmental pollutant, and its presence in soil is largely attributed to emissions from motor vehicles burning leaded gasoline. The emitted lead may be deposited on soil and vegetation in the vicinity of the road [2]. Copper pollution in the soils is interesting and may be attributed to the use of copper compounds, especially copper(II) sulphate, to combat pathogens, microorganisms and bacteria in agricultural areas [2,19]. The occurrence of Cd in motor oils, car tyres and Zn compounds explains

TABLE I The pseudo-total contents of metals, the sum of metal contents obtained from the BCR three-stage sequential extraction procedure and residue analysis, mean recovery values of elements in the soil samples and typical metal contents of the soils

Soil sample ^s ^a	Metals, $\mu\text{g g}^{-1}$									
	Cu	Pb	Ni	Co	Cd	Cr	Mn	Fe	Zn	
S1	$\Sigma 1234^b$ 145.1 P-total ^c 139.7 $R\%^d$ 103.9	81.8 80.5 101.6	58.0 54.4 106.5	15.0 9.90 151.4	1.53 0.93 164.4	33.4 21.8 153.1	399.0 388.1 102.8	11971 12213 98.0	148.9 167.0 89.2	
S2	$\Sigma 1234$ 143.1 P-total 148.1 $R\%$ 96.6	73.6 72.5 101.5	52.5 57.1 91.9	16.1 10.9 147.7	1.96 1.30 151.0	31.1 26.4 118.0	446.0 434.5 102.6	11883 12580 94.5	171.3 173.8 98.6	
S3	$\Sigma 1234$ 246.7 P-total 240.5 $R\%$ 102.6	85.1 84.5 100.7	68.6 58.1 70.9	15.5 10.9 142.6	1.89 1.21 156.5	37.5 26.4 142.4	399.7 421.2 94.9	13684 12764 107.2	180.2 186.2 96.7	
S4	$\Sigma 1234$ 140.7 P-total 141.1 $R\%$ 99.8	76.1 95.2 79.9	58.0 122.1 70.7	14.0 10.0 139.3	1.61 1.22 132.2	30.7 22.5 136.2	392.0 414.7 94.5	11687 12380 94.4	137.9 139.5 98.8	
S5	$\Sigma 1234$ 144.8 P-total 118.9 $R\%$ 121.7	64.7 69.9 92.6	70.7 49.1 143.8	11.4 7.95 142.7	1.68 1.02 164.5	30.7 19.8 154.9	409.3 361.6 113.2	11604 11478 101.1	135.1 118.2 114.3	
S6	$\Sigma 1234$ 126.6 P-total 128.8 $R\%$ 98.3	66.6 76.5 87.0	80.6 52.6 153.2	12.8 9.25 138.0	1.78 1.09 163.5	29.0 22.4 129.3	470 295.3 159.1	11137 9456 117.8	162.8 123.0 132.4	
S7	$\Sigma 1234$ 118.0 P-total 165.8 $R\%$ 71.2	58.2 88.5 65.7	69.8 58.4 119.7	11.8 9.85 119.8	1.41 1.22 115.6	28.4 20.2 140.3	403 430.2 93.7	12151 15080 80.6	152.4 138.9 109.7	
S8	$\Sigma 1234$ 203.8 P-total 202.8 $R\%$ 100.5	60.6 83.2 72.8	73.2 55.1 132.7	12.6 10.2 123.2	1.50 0.98 153.6	29.8 23.1 129.2	431.8 374.9 115.2	11354 12029 94.4	133.8 138.1 96.9	
S9	$\Sigma 1234$ 130.8 P-total 174.1 $R\%$ 75.1	78.9 125.2 63.0	61.2 46.2 132.5	13.3 11.4 117.0	1.46 1.16 125.7	28.5 26.4 108.3	474.8 533.9 88.9	12857 13499 95.3	154.4 161.5 95.6	
S10	$\Sigma 1234$ 80.2 P-total 89.7 $R\%$ 89.4	69.6 84.5 82.4	51.8 37.2 139.3	11.5 8.44 136.8	1.42 0.77 185.8	24.2 17.9 135.3	354.4 288.7 122.8	12068 10191 118.4	126.3 96.8 130.4	
Mean ($R\%$) \pm SD	95.9 \pm 14.5	84.7 \pm 14.5	126 \pm 18	136 \pm 12	152 \pm 21	135 \pm 14	109 \pm 21	100 \pm 12	106 \pm 15	
Typical soil contents ($\mu\text{g g}^{-1}$)	5–80	2–20	5–500	1–50	0.03–0.3	10–200	200–2000	0.5–2% (w/w)	20–300	

^aSoil samples and corresponding vegetable samples are: S1—parsley, S2—leek, S3—onion, S4—radish, S5—lettuce, S6—spinach, S7—dill, S8—sheep sorrel, S9—cabbage and S10—mint.

^bSum of the results from Steps 1–3 and aqua regia treatment of the residue.

^cP-total means pseudo-total.

^d $R\% = [(\text{Step1} + \text{Step2} + \text{Step3} + \text{Residual}) / \text{pseudo-total}] \times 100$.

TABLE II The total mean metal concentrations obtained from each stage of the sequential extraction procedure and residue analysis for the soil samples, their standard deviation values and the detection limits of metals for all the extraction steps

<i>Metal</i>	<i>Fraction^a</i>	<i>DL, µg g⁻¹</i>	<i>Average, µg g⁻¹</i>	<i>Standard deviation</i>
Cu	1	0.08	3.69	1.06
	2	0.06	4.52	3.52
	3	0.05	51.0	27.8
	4	0.08	88.7	18.9
	Σ ^b		148	46.2
Co	1	0.09	3.10	0.97
	2	0.17	1.34	0.50
	3	0.11	1.14	0.60
	4	0.04	7.81	1.41
	Σ		13.4	1.69
Mn	1	0.07	71.6	23.1
	2	0.10	140	33.9
	3	0.05	59.4	10.6
	4	0.03	147	14.5
	Σ		418	37.4
Pb	1	0.42	2.90	0.79
	2	0.19	7.31	3.86
	3	0.35	19.2	1.92
	4	0.42	42.2	5.52
	Σ		71.5	9.08
Cd	1	0.03	0.57	0.25
	2	0.06	0.20	0.13
	3	0.03	0.21	0.14
	4	0.03	0.65	0.12
	Σ		1.62	0.20
Fe	1	0.13	6.43	1.04
	2	0.37	139	71.3
	3	0.25	145	28.9
	4	0.59	11749	718
	Σ		12039	746
Ni	1	0.20	4.38	0.54
	2	0.16	3.90	1.94
	3	0.32	6.15	3.07
	4	0.13	51.3	11.4
	Σ		65.7	9.44
Cr	1	0.07	1.65	0.80
	2	0.08	< DL	0.10
	3	0.16	2.52	1.05
	4	0.10	26.1	2.81
	Σ		30.3	3.47
Zn	1	0.10	14.2	4.59
	2	0.06	31.4	11.9
	3	0.10	26.7	6.09
	4	0.11	78.0	13.8
	Σ		150	17.5

^a1, acid soluble; 2, reducible; 3, oxidizable; 4 residual.

^bΣ represents the sum of the three fractions of the sequential extraction procedure and residue analysis.

its accumulation in soils. In addition, it is also an impurity in many phosphate fertilizers because it can occur naturally in phosphate rocks [2].

The average percentages of total metals belonging to each fraction are represented in a bar diagram in Fig. 1. The extractability (%) order of the heavy metals based

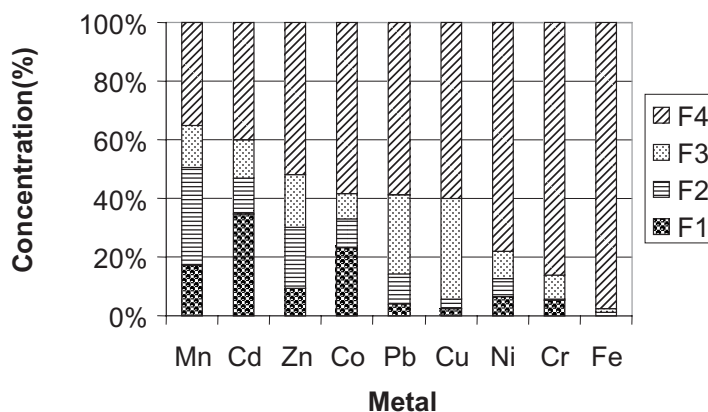


FIGURE 1 Operationally defined partitioning of the metals between the soil phases.

on the sum of the first three fractions, not including the residual phase, is $Mn > Cd > Zn > Co \approx Pb \approx Cu > Ni > Cr > Fe$. It seems that the most mobile elements are Mn ($\approx 65\%$), Cd ($\approx 60\%$) and Zn ($\approx 48\%$) followed by Co ($\approx 42\%$), Pb ($\approx 41\%$), Cu ($\approx 40\%$), Ni ($\approx 22\%$), Cr ($\approx 14\%$), Fe ($\approx 2.4\%$) while the least-mobile elements are Cr and Fe. High fractions were found in the residual phase for Zn ($\approx 52\%$), Co ($\approx 58\%$), Pb ($\approx 59\%$), Cu ($\approx 60\%$), Ni ($\approx 78\%$), Cr ($\approx 86\%$), and Fe ($\approx 97.6\%$). The high contents in the residual fraction especially for Cr and Fe showed that these metals are strongly bound to minerals and resistant components.

The highest extractable fractions (except for the residual phase) for Cu and Pb were found to be 34.5 and 26.8%, respectively, at the third extraction stage. This result indicates that they probably occurred in the forms of metal sulphides and bound to organic substances. The highest proportions for Cd and Co were found at the first extraction stage (exchangeable and acid soluble), about 35% for Co and 23% for Cd. The second stage, which involves the reducible forms of the target phase (bound to Fe/Mn oxides), has the highest percentage extractability for Mn and Zn: 33.6 and 20.9%, respectively. In addition, Mn in the soil samples was partitioned almost equally between the reducible phase (34%) and the residual phase (35%).

Cd seemed to be the most mobile element among the investigated metals. About 35% of its total concentration was measured at the first extraction stage, and about 12% and 13% were found at the second and third extraction stages, respectively. The high exchangeable content of Cd observed in fraction 1 gives more information about its mobility. Moreover, previous studies have indicated that the uptake of Cd from soil by plants is relatively easy [6,16].

Results of Vegetable Analysis

Recovery studies were performed to determine the accuracy of the wet and dry ashing procedures used for analyzing the vegetable samples. For this purpose, the extracts of the radish sample obtained by applying the procedures described above were spiked with known amounts of the analytes at three different concentrations. The analyses were made in duplicate, using 1.00 g of specimen. The recovery values (%) were calculated by comparing the concentrations of the analytes with and without spiked

sample solutions. The results are shown in Table III. The recovery values varied between about 68 and 107% and 89 and 106% for the wet- and dry-ashing procedures, respectively. As can be seen, the percentage recoveries obtained by the dry-ashing procedure were quantitative for all the elements except for Cd, which had a somewhat low recovery (89%). Dry ashing seems to be a somewhat more effective destruction procedure for the sample matrix than wet ashing.

The average element concentrations ($\mu\text{g g}^{-1}$) obtained from the wet- and dry-ashing analyses of ten vegetable samples are illustrated in Fig. 2. The concentrations of Cu, Ni, Co, Cr, Fe, Mn and Zn obtained using the two dissolution procedures were in good agreement. However, the concentration of Cd found using the wet-ashing procedure was higher than that from the dry-ashing procedure for all the vegetable samples.

TABLE III The recoveries obtained by using wet- and dry-ashing methods for the radish sample ($n = 6$, in duplicate at three different concentrations)

Element	Recovery, % \pm SD ^a	
	Wet ashing	Dry ashing
Cu	100.9 \pm 2.1	104.3 \pm 9.9
Pb	98.1 \pm 3.2	106.1 \pm 21.1
Ni	85.9 \pm 2.5	93.3 \pm 18.5
Cr	106.7 \pm 20.2	100.0 \pm 10.0
Co	80.8 \pm 4.3	100.8 \pm 15.5
Mn	74.2 \pm 3.3	n.d. ^b
Cd	67.9 \pm 0.3	88.9 \pm 7.3

^aStandard deviation.

^bNot determined.

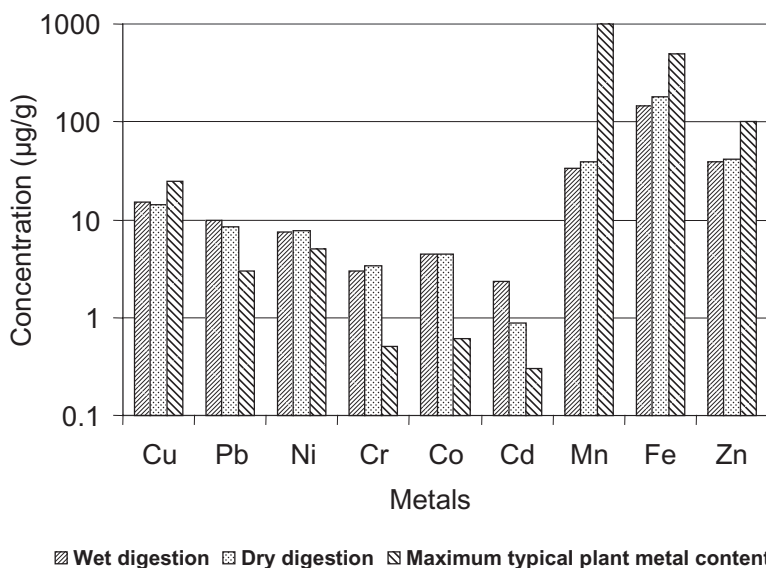


FIGURE 2 The average element contents ($\mu\text{g g}^{-1}$) of vegetable samples from wet- and dry-ashing analyses (in triplicate analyses for each sample), and upper typical plant contents.

This may be attributed to the loss of Cd during dry ashing at 475°C [23]. The mean metal contents ($\mu\text{g g}^{-1}$) of vegetable samples for wet and dry ashing and the typical plant metal contents (as upper limits) ($\mu\text{g g}^{-1}$) compiled by Grimshaw *et al.* [24] are given in Fig. 2. When the typical plant metal contents were taken into consideration, it was seen that the mean concentrations of Pb, Ni, Cr, Co and Cd elements in the vegetables were higher than the upper limit of their typical plant metal contents. The metal accumulation in the vegetable samples may well originate from traffic and industrial activities. The results obtained from the analysis of vegetable samples show that different vegetables growing under identical conditions take up different amounts of metals. The relative metal uptake order by the vegetable samples examined and their metal contents are shown in Table IV. This is also an indicator for accumulation of a metal in any vegetable sample by different modes of metal uptake. The variations of the metal contents observed in the vegetables depend on the physical and chemical nature of the soil and the absorption capacity for each metal of the plant, which is dependent on innumerable environmental and human factors and the nature of the plant [3,25]. For example, although the Cu contents in the soil samples were at pollution level, the contents for vegetable samples were within the typical plant metal content. This situation can be explained by the fact that the chelate complexes of Cu are well known to be strong [26,27].

CHEMOMETRIC DATA TREATMENT

Correlation Analysis

The correlation coefficient (r) is generally used to correlate the relationship between two variables. The ordinary correlation coefficient for variables j and k , r_{jk} , is related to the covariance by the expression

$$r_{jk} = \frac{c_{jk}}{s_j \times s_k}$$

where, c_{jk} is the sample covariance between variables j and k , and s_j and s_k are their sample standard deviations, respectively [28]. The correlation coefficients between the various metals determined in the vegetables (using wet ashing) and soil extracts (fraction 1) are presented in Table V. For all the binary correlations, significant r values ($> |\pm 0.632|$, $n = 10$) at the 95% confidence level are shown in boldface [29]. Only the nickel contents found in the vegetables and in the first fractions of the soil samples show a positive relation ($r = 0.682$) that is significant at the 95% confidence level, although some positive correlations have been observed between the metal contents of pairs of samples, like soil–soil, soil–vegetable and vegetable–vegetable, being meaningful at the same confidence level, as shown in Table V (here, the subscripts 1 and v denote the metals found in fraction 1 of the BCR procedure and in vegetable samples, respectively). Moderately positive correlations were observed between the metal concentrations obtained from soil extracts (from fraction 1) and vegetable samples for zinc and manganese, e.g. 0.521 and 0.422, respectively, at the 95% confidence level. The lack of correlation between the vegetable and soil metal contents may be explained by inter-plant differences in metal uptake, because different vegetable

TABLE V Correlation matrix between the metal concentrations in the vegetable (for wet ashing) and soil samples (found in fraction 1)

	Cu_v	Pb_v	Ni_v	Co_v	Cr_v	Cd_v	Mn_v	Fe_v	Zn_v	Cu_t	Pb_t	Ni_t	Co_t	Cr_t	Cd_t	Mn_t	Fe_t
Pb_v	0.558																
Ni_v	-0.583	-0.007															
Co_v	0.201	0.767	0.288														
Cr_v	0.386	0.788	0.392	0.642													
Cd_v	0.152	0.221	-0.402	0.193	-0.102												
Mn_v	0.491	0.247	-0.424	0.310	-0.143	0.214											
Fe_v	0.584	0.383	-0.245	0.496	0.400	-0.075	0.320										
Zn_v	0.223	0.613	0.036	0.881	0.322	0.231	0.396	0.523									
Cu_t	-0.141	0.212	0.301	0.344	0.282	0.022	0.105	-0.117	0.175								
Pb_t	-0.255	-0.292	0.218	-0.522	-0.232	-0.616	-0.300	-0.602	-0.447	-0.201							
Ni_t	-0.175	0.188	0.682	0.629	0.442	-0.068	0.180	0.275	0.359	0.300	-0.408						
Co_t	-0.782	-0.459	0.432	-0.367	-0.356	-0.499	-0.325	-0.555	-0.378	0.170	0.559	-0.065					
Cr_t	-0.749	-0.647	0.423	-0.634	-0.470	-0.404	-0.431	-0.803	-0.640	-0.043	0.736	-0.159	0.857				
Cd_t	-0.720	-0.646	0.378	-0.599	-0.453	-0.558	-0.558	-0.600	-0.489	-0.084	0.754	-0.282	0.854	0.908			
Mn_t	-0.142	0.056	-0.229	0.204	-0.345	0.685	0.422	-0.359	0.302	0.457	-0.284	-0.037	-0.077	-0.093	-0.246		
Fe_t	-0.350	-0.230	-0.104	-0.405	-0.396	-0.098	-0.002	-0.567	-0.344	0.452	0.380	-0.445	0.699	0.549	0.512	0.346	
Zn_t	-0.092	0.358	0.181	0.483	0.014	0.147	0.380	-0.312	0.521	0.248	0.197	0.168	0.003	-0.006	-0.110	0.603	0.063

Correlation coefficients greater than |0.632| (bold type) are meaningful at the 95% confidence level for 10 soil and vegetable samples.

samples were collected from each soil sampling point and the uptake of the same metal by various vegetables grown on the same soil may change with the different plant species. Positive correlations were observed in the following cases. $\text{Co}_v\text{-Zn}_v$ (0.881), $\text{Pb}_v\text{-Cr}_v$ (0.788), $\text{Pb}_v\text{-Co}_v$ (0.767), $\text{Co}_v\text{-Cr}_v$ (0.642), $\text{Pb}_v\text{-Zn}_v$ (0.613) for vegetable samples and $\text{Cr}_1\text{-Cd}_1$ (0.908), $\text{Co}_1\text{-Cr}_1$ (0.857), $\text{Co}_1\text{-Cd}_1$ (0.854), $\text{Pb}_1\text{-Cd}_1$ (0.754), $\text{Pb}_1\text{-Cr}_1$ (0.736), $\text{Co}_1\text{-Fe}_1$ (0.699) and $\text{Mn}_1\text{-Zn}_1$ (0.603) for soil samples. High negative correlations were found for: $\text{Fe}_v\text{-Cr}_1$ (-0.803), $\text{Cu}_v\text{-Co}_1$ (-0.782), $\text{Cu}_v\text{-Cr}_1$ (-0.749), $\text{Cu}_v\text{-Cd}_1$ (-0.720), $\text{Pb}_v\text{-Cr}_1$ (-0.647), $\text{Pb}_v\text{-Cd}_1$ (-0.646), $\text{Co}_v\text{-Cr}_1$ (-0.634) and $\text{Co}_v\text{-Cd}_1$ (-0.599) for soil (soluble in acetic acid) and vegetable samples. The high negative correlations for Cu_v with Co_1 , Cr_1 and Cd_1 ; Co_v and Pb_v with Cr_1 and Cd_1 might be explained by the assumption that these metals come from different origins.

In addition, correlation coefficients for the metal contents in the soil fractions and their corresponding values in the vegetables are given in Table VI. A significant negative relationship was obtained between vegetable content and oxidizable fraction of Cu and Mn in soils ($r = -0.632$ and -0.726 , respectively, at the 95% confidence level). The same negative relation was observed between the total soil level of zinc and its vegetable level ($r = -0.649$). Only the total Cr content in soils had a moderately positive correlation with vegetable Cr content ($r = 0.551$). In conclusion, no significant correlations were found between the uptake by the vegetable and the concentrations of heavy metals extracted from the soils with other extractants, except for the acetic acid. Therefore, these extractants are poor indicators from the point of view of bioavailability of metals to vegetables [4,5].

PCA Results

PCA is a powerful technique for displaying the relationships between objects and variables, so it was carried out on the correlation matrix, using the PCA subroutine of the SPSS V9.05 package program for all the computations, after transforming the raw data using logarithmic double centring [30]. For applying PCA, a 10×18 data matrix for metal levels in vegetable and soil samples (soluble in acid) was used. Afterwards, rotation of the principal components was carried out by the varimax method. As can

TABLE VI Correlation matrix (r) for the metal contents determined in the soil fractions and their corresponding values in the vegetables

Element	Fractions of the BCR procedure				Σ 1234 Total concentration
	F1 HOAc	F2 HONH ₂ ·HCl	F3 H ₂ O ₂ +NH ₄ OAc	F4 Residual	
Cu _v ^a	-0.141	-0.527	-0.632^b	-0.221	-0.514
Pb _v	-0.292	-0.501	-0.427	0.484	0.395
Ni _v	0.682^b	-0.006	-0.269	-0.005	0.417
Co _v	-0.367	0.226	0.299	-0.420	-0.190
Cr _v	-0.470	0.374	-0.019	0.067	0.551^c
Cd _v	-0.558^c	0.504	0.398	0.098	-0.073
Mn _v	0.422	-0.489	-0.726^b	0.286	-0.435
Fe _v	-0.567^c	-0.573^c	-0.444	-0.314	-0.421
Zn _v	0.521	-0.541	-0.533	0.301	-0.649^b

^aThe subscript v denotes vegetable. ^bAt the 95% confidence level. ^cAt the 90% confidence level.

TABLE VII Varimax rotated component matrix including loadings, eigenvalues, percentage of variance and cumulative variance for each principal component

<i>Metals^a</i>	<i>Component</i>			<i>Communalities</i>
	<i>1</i>	<i>2</i>	<i>3</i>	
Cr ₁ ^a	-0.964	0.155	0.168	0.877
Co _v ^b	0.934	0.286	0.114	0.663
Cd ₁	-0.914	0.230		0.978
Co ₁	-0.838	0.331	0.146	0.968
Pb ₁	-0.821		-0.325	0.802
Fe ₁	-0.802	-0.330	0.110	0.282
Pb _v	0.710	-0.169	-0.360	0.638
Zn _v	0.707	-0.129		0.815
Fe _v	0.673	-0.209	-0.564	0.526
Cr _v	0.543	0.536	-0.469	0.381
Ni _v	-0.170	0.965	0.132	0.782
Ni ₁	0.154	0.645		0.443
Mn _v	0.470	-0.615	0.198	0.834
Cd _v	0.164	-0.456	0.216	0.981
Mn ₁		-0.537	0.820	0.888
Cu _v	0.399	-0.573	-0.624	0.960
Zn ₁		-0.139	0.613	0.764
Cu ₁	0.212	0.151	0.559	0.402
Eigenvalue	6.291	3.349	2.712	
% Variance	38.450	18.607	15.065	
% Cumulative	38.450	57.057	72.122	

^{a,b}The subscripts 1 and v denote the metals found in fraction 1 of the sequential extraction procedure for soil and vegetable samples, respectively.

be seen from Table VII, only the eigenvalues contributing more than 10% to the total variance were taken into consideration and accounted for 72.1% of the total data variance. As a result of this, the number of PCs was reduced to three. The variables with higher loadings (positive or negative) are those which contribute most to explain the meaning of each principal component.

The first three principal components have the largest percentage of variance, explaining 38.4, 18.6 and 15.1% of the total, respectively. The others follow the first three, with variances decreasing from 8.6 to 0.3%. The first principal component (PC1) has high loadings for the pairs Co₁-Co_v, Pb₁-Pb_v, Fe₁-Fe_v and Cr₁-Cr_v. At the same time, PC1 is related to the toxic heavy metals, and especially to Pb and Cd, which originate mainly from traffic, because they have high loading values in this PC. In the second PC, only the Ni₁-Ni_v pair had high loading value. Consequently, the first two PCs may be attributed to the bioavailabilities of these metals to the vegetables investigated. The third PC is mainly related to acetic acid-soluble metals (Mn, Zn and Cu) in the soil samples. The Cu₁-Cu_v pair has the largest loading in this component. This factor may be attributed to agricultural processes in which compounds containing copper (especially copper(II) sulphate) are used to combat pathogens, microorganisms and bacteria in agricultural areas. Moreover, the total contents of Pb, Cd and Cu in the soils studied were higher than those of typical soils (see Table I). The latent structure of the three PCs seems to describe adequately the origin and different characteristics of the samples belonging to an urban area, because the area is mainly influenced by heavy urban traffic.

Consequently, no very clear associations between plant and soil metal (soluble in acetic acid) contents could be found except for Ni, owing to the low number the samples. Similar results have been reported elsewhere [4,5,7].

Cluster Analysis

Cluster analysis is an unsupervised method which uses the information acquired from the variables measured to reveal the natural clusters existing between the worked samples or variables. Hierarchical Cluster Analysis (HCA) was run applying Ward's method of agglomeration and Euclidean distance as a criterion of similarity. All variables were standardized by transforming the variables in the data set using the logarithmic double centring method [30]. The dendrogram obtained is depicted in Fig. 3. Two main clusters can be drawn: one is for the soil metals (Cr, Cd, Fe, Co and Pb) extractable by acetic acid. The other is formed by two groups, one for the metals (Cr, Fe and Mn) found in vegetable samples, and the other one for the metals found in soil (Mn, Zn, Ni and Cu) and vegetable (Pb, Co, Zn, Cu, Cd and Ni) samples. Here, Ni₁ and Ni_v have the highest similarity and to some extent Cu₁-Cu_v, Mn₁-Mn_v and Zn₁-Zn_v have the lowest similarities in this cluster.

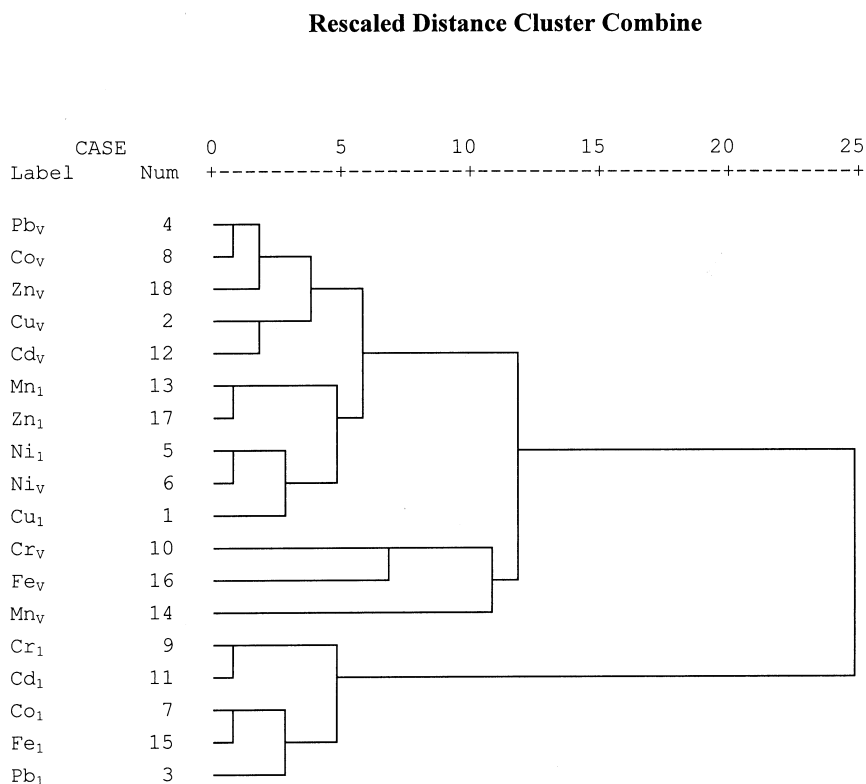


FIGURE 3 Dendrogram resulting from Ward's method of hierarchical cluster analysis for the variables (using the results of wet ashing of the vegetables and of fraction 1 of the BCR sequential extraction procedure for soils). Similarities have been calculated from the Euclidean distance based upon the transformation of the raw data on the logarithmically double centred normalized values. The subscripts 1 and v denote the metals found in fraction 1 of the BCR procedure and in vegetable samples, respectively.

CONCLUSION

In this study, extractants and extraction conditions have been tested on polluted soil and vegetable samples with three aims: (a) to determine the extractability of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in soils from an urban vegetable garden using the BCR sequential extraction scheme; (b) to determine the extractability of the metals after digestion, using wet- and dry-ashing methods, of the vegetable samples grown in corresponding soils of the urban vegetable garden; (c) to determine the chemometric relationships between the elements extracted from soils in fraction 1 of the BCR procedure and the vegetables investigated.

The results obtained from the soil and vegetable samples taken from an urban vegetable garden indicate that there is pollution of soil and vegetable samples, in which the accumulation of Cu, Cd and Pb in the soils and Pb, Ni, Cr, Co and Cd in the vegetables is obvious. The distribution of metals in the different soil fractions obtained by the BCR sequential extraction procedure and plant availability were examined using correlation analysis, PCA and HCA. The best correlations between soil and vegetable metals from the point of view of availability were obtained using acetic as the acid extractant for the BCR sequential extraction procedure. The extracted metal species from soils using acetic acid are exchangeable and bound to carbonates, and are generally considered to be the most mobile and immediately bioavailable forms from soils. Moreover, acetic acid is found among the natural organic acids secreted by plant roots, so the metals extracted by acetic acid may be very similar to the fraction of metals that is available to the plant [4]. As a result of correlation analysis, the Ni, Zn and Mn contents found in vegetables and in the first fractions of the soil samples show a positive relationship ($r = 0.682, 0.521$ and 0.422). A similar relationship was also obtained from the other statistical methods used.

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