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Statistical Evaluation of the Bioavailability of Heavy Metals from Contaminated Soils to Vegetables

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Plants are important components of ecosystems. The main sources of trace elements to plants are their growth media, e.g., nutrient solutions or soils. One of the most important factors that determines the biological availability of a trace element is its binding to soil constituents. In generally, plants readily take up the species of trace elements that are dissolved in the soil solutions in either ionic or chelated and complexed forms. The bioavailability of elements to plants is controlled by many factors associated with soil and climatic conditions, plant genotype and agronomic management, including: active/passive transfer processes, speciation, redox states, the type of plant root system and the response of plants to elements in relation to seasonal cycles. The ability of different plants to absorb trace elements varies greatly. Some elements such as Cd, B, Br, Cs and Rb are extremely easily taken up, while Ba, Ti, Zr, Sc, Bi, Ga and, to an extent Fe and Se, are but slightly available to plants (Kabata-Pendias and Pendias 1992; Chojnacka et al. 2004).

The quantification of different chemical fractions of heavy metals in soil is necessary for information on metal mobility, as well as on their bioavailability or phytotoxicity. Therefore, information on total concentrations of metals alone is not sufficient to assess the environmental impact of polluted soils because heavy metals are present in different chemical forms in soils, which determine their mobilization capacity and bioavailability (Morillo et al. 2002). Vegetation can be used as a biomonitor of site pollution, by identifying the mobilisation of heavy metals and by providing an understanding of their bioavailability. Several studies have shown that the available fraction of heavy metals mainly decided the mobility, bioavailability or phytotoxicity of heavy metals in soils (Morillo et al. 2002; Pueyo et al. 2004). Jin et al. (2005) used $CaCl_2$ solution to assess Pb bioavailability in tea garden soil. Tokalıoğlu et al. (2004) used the single extractants to evaluate the bioavailability of metals in soils to grape samples. Chojnacka et al. (2005) tested several extracting reagents and examined correlation coefficients between the citrate-soluble and total fraction of soil and content in plant for elements. Also, sequential extraction procedures have been used to partition heavy metals in soils into fractions and to correlate metals in these fractions with plant concentrations or uptake (Maiz et al. 2000; Ma et al. 2003; Tokalıoğlu and Kartal 2003). Kment et al. (2005) were examined chemical composition of wines and soil extracts using statistical multivariate analyses.

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In this study, it was investigated the uptake of Cu, Pb, Cd, Mn, Cr, Co, Ni, Fe and Zn elements by lettuce (*Lactuca sativa L.*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*) and onion (*Allium cepa L.*) samples growing in the three vegetable gardens by using statistical methods such as correlation analysis, principal component analysis (except for onion), and cluster analysis. The modified three-step sequential extraction procedure proposed by the European Community Bureau of References (BCR), now the European Comission Standards, Measurements and Testing Programme (Rauret et al. 1999), was used in order to evaluate trace elements mobility in soil samples, and heavy metals uptake by vegetables. The relationships between acetic acid-extractable fractions of these elements in soils and their total concentrations in the vegetable samples were evaluated.

MATERIALS AND METHODS

A Perkin Elmer 3110 model flame atomic absorption spectrometer equipped with 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 model end-over-end shaker, 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). Deionized and distilled water was used for preparing solutions and making dilutions. Standard solutions prepared (1000 μg/mL) for all the studied elements were stored in polyethylene containers. In the digestion and extraction procedures, concentrated nitric acid, hydrochloric acid, acetic acid, hydrogen peroxide, hydroxylammonium chloride and ammonium acetate were used.

Three urban vegetable gardens (Sahabiye, Çevreyol, and Altınoluk gardens) from Kayseri, Turkey, were selected for this investigation. Three samples of each vegetable, lettuce (n=9), parsley (n=9), dill (n=9), and onion (except for Altınoluk garden, n=6), and corresponding surface (0-10 cm) soil samples were collected from each garden. Air-dried, ground and powdered soil samples were passed through a sieve with 200 mesh sizes (<74 μm). The vegetable samples were washed, dried, ground and sieved (<74 μm).

4 mL of concentrated nitric acid and 2 mL of concentrated hydrogen peroxide were added twice to the vegetable samples of 1.0 g. This mixture was heated until a clear solution was obtained and made up to 5 mL with 1 M HNO_3. The chemical partitioning of metals in soils was determined by means of the modified BCR three-step sequential extraction procedure (Rauret et al. 1999). This scheme consists of three successive extractions that make it possible to determine the association of the metals in three phases: acid soluble, reducible and oxidizable (Table 1). In this study, the results obtained from the first step of the BCR procedure were used to evaluate trace elements mobility in soil samples, and heavy metals uptake by the

vegetables. More detailed information in relation to the results of analysis of the soil and corresponding vegetable samples was given in our previous study (Tokalıoğlu et al. in press).

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

Step	Target phases	Extractant	Shaking time and temperature
1	Water and acid soluble, and exchangeable	40 mL of 0.11 M CH_3COOH	16 h at room temperature
2	Reducible	40 mL of 0.5 M $HONH_2$ HCl (pH 1.5)	16 h at room temperature
3	Oxidisable	10 mL of 8.8 M H_2O_2 (pH 2) then 10 mL of 8.8 M H_2O_2 (pH 2) cool, add 50 mL of 1 M NH_4OAc (pH 2)	1 h at room temperature and 1 h at 85 °C 1 h at 85 °C 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

According to the results obtained, 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, Mn > Cd > Cu > Pb > Zn > Cr > Ni > Co > Fe for Çevreyol garden, and Mn > Cd > Pb > Cu > Zn > Cr > Ni > Co > Fe for Altınoluk garden. The pH values measured for the three garden soils were found in the range of 6.9-8.1 for Sahabiye garden, 7.1-7.7 for Çevreyol garden and 7.0-7.6 for Altınoluk garden.

The data were treated statistically by using the software package SPSS 10.0. The correlations between the contents of the elements in the soils and vegetables were expressed by Pearson correlation coefficients. For this purpose, a data matrix with dimensions of 9×18 was formed the basis for the correlation matrix, principal component analysis (PCA), and cluster analysis. The matrix for onion samples was 6×18.

PCA is an important chemometric tool that seeks to establish combinations of variables capable of describing the principal data tendencies observed. In mathematical terms, PCA relies upon an eigenvector decomposition of the covariance or correlation matrix (Morales et al. 1999).

The cluster analysis technique comprises an unsupervised classification procedure that involves measuring either the distance or the similarity between objects to be clustered. Objects are grouped in clusters in terms of their similarity. The initial assumption is that the nearness of objects in the space defined by the variables reflects the similarity of their properties (Frías et al. 2003; Mendiguchía et al. 2004).

RESULTS AND DISCUSSION

The highest correlations between the various metals determined in the vegetables and soil extract (fraction 1) are illustrated in Figure 1 (a-h). Fraction 1 contains the most mobile metals found in soil and immediately bioavailable forms to plants (Morillo et al. 2002). In this study, therefore, the correlations between metals extracted by acetic acid from soil and in each vegetable samples was taken into consideration. As can be seen in Figure 1 a-h, the highest correlations for the each vegetable sample and the corresponding soil sample were found for Zn in onion, r^2= 0.89, at 95% confidence level. The decreased absolute correlations between the other elements in the vegetables and soil samples are as follows: 0.87 for Pb and 0.84 for Mn in onion, 0.77 for Cu and 0.75 for Ni in lettuce, 0.66 for Cr in lettuce, 0.65 for Zn in dill, 0.64 for Co and 0.64 for Fe in onion, 0.46 for Fe in lettuce, and 0.43 for Fe in parsley. The high correlations obtained for the onion and lettuce samples show that bioavailability for these samples are higher than those of the parsley and dill samples. The highest correlation for Cd was observed for onion samples (r^2=0.35), and the relationships between Cd concentrations in onion and soil samples were adverse to each other.

The principal component analysis technique allows a considerable reduction in the number of variables and the detection of structure in the relationships between metals, that would give information about the relation between soil and plant systems (Maiz et al. 2000). By extracting the eigenvalues from the correlation matrix, the number of significant factors, the percent of variance explained by each of them was calculated by using the software package SPSS 10.0. For exploring the correlations between vegetable-soil heavy metal concentrations, the variables were simplified to only two principal component (≥ ca. 79% of the total variance). A varimax rotation was carried out to mimimize the number of variables influencing each factor and to facilitate the interpretation of the results. The obtained PCA results for each of the lettuce, parsley and dill samples are given in Table 2. Loading factors > 0.5 are shown in boldface.

The first component for the lettuce sample that explains ca. 41% of total variance and is highly loaded by the pairs of $\mathrm{Cr_v}$-$\mathrm{Cr_l}$, $\mathrm{Co_v}$-$\mathrm{Co_l}$ and $\mathrm{Ni_v}$-$\mathrm{Ni_l}$. PC1 is related to the bioavailabilities of Cr, Co and Ni metals to the lettuce samples. In addition, this factor implies that of Pb and Zn in the soils may originate from similar pollution sources, such as the deposition of aerosol particles emitted by vehicular traffic. The second PC has high loadings for $\mathrm{Cu_v}$-$\mathrm{Cu_l}$ and $\mathrm{Mn_v}$-$\mathrm{Mn_l}$. This factor may be attributed to agricultural processes, because the compounds containing copper and manganese are used to combat with microorganisms. For parsley samples, PCA gave two significant components (PC1 and PC2) explaining ca. 44% and 40% of the total variance, respectively. While PC1 included high loading for the pairs of $\mathrm{Cu_l}$-$\mathrm{Cu_v}$ and $\mathrm{Ni_l}$-$\mathrm{Ni_v}$, PC2 is associated with the pair of $\mathrm{Pb_v}$-$\mathrm{Pb_l}$. As regards dill samples, PCA has a total variance of 78.8%, to be 51.2 and 27.6%, respectively. PC1 is related to $\mathrm{Ni_l}$-$\mathrm{Ni_v}$, $\mathrm{Fe_l}$-$\mathrm{Fe_v}$ and also antagonistly with $\mathrm{Z_v}$-$\mathrm{Zn_l}$, respectively. PC2 showed high values only for $\mathrm{Fe_v}$-$\mathrm{Fe_l}$ pair. The negative loadings for metals in each factor suggest an antagonistic effect in respect to the positive loadings and also different sources of the elements (Filguerias et al. 2004).

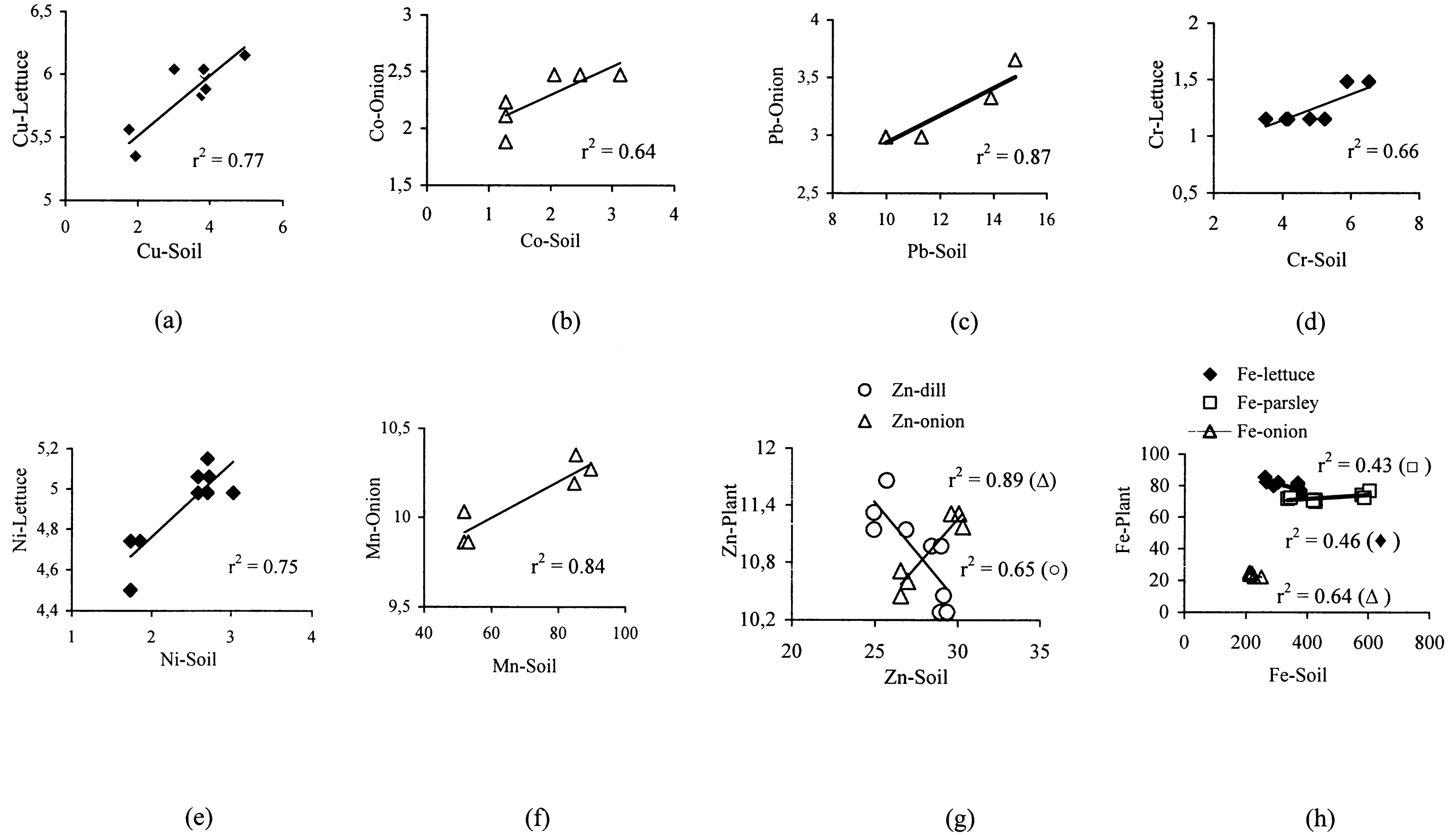

Figure 1(a-h). Correlations between the lettuce, parsley, dill and onion and corresponding soil samples (conc., µg/g).

Table 2. Varimax rotated component matrix including loadings, eigenvalues, percent of variance and cumulative variance for each principal component (PC) for vegetable- and soil-metals concentrations.

Metals	Lettuce		Parsley		Dill	
	PC1	PC2	PC1	PC2	PC1	PC2
Cu_1[a]	0.496	**-0.809**	**-0.755**	**0.609**	-0.221	-0.128
Pb_1	**0.773**	0.414	-0.266	**0.904**	-0.333	**-0.869**
Ni_1	**-0.940**		**-0.823**	0.373	**0.718**	**-0.685**
Co_1	**0.937**	-0.327	0.308	**0.892**	0.333	**-0.886**
Cd_1	-0.320	0.458	**0.910**	0.288	0.471	0.302
Cr_1	**0.897**	-0.230	-0.243	**0.878**		0.145
Mn_1	**-0.682**	**0.687**	**0.651**	**-0.730**	-0.388	**0.878**
Fe_1	0.211	**0.931**	**0.909**	-0.400	**0.805**	**0.560**
Zn_1	**0.962**	-0.198	**0.918**	-0.127	**-0.904**	0.210
Cu_v[b]	0.321	**-0.883**	**-0.620**	0.242	**0.986**	-0.113
Pb_v		**-0.941**	0.162	**0.837**	**0.870**	0.294
Ni_v	**-0.877**		**-0.910**		**0.976**	0.110
Cr_v	**0.789**		**0.796**			**0.987**
Co_v	**0.534**	0.470	**0.947**		**0.968**	-0.194
Mn_v		**0.689**	**-0.785**	-0,524	**0.810**	-0.151
Fe_v	0.432	**-0.866**	0.438	**-0.746**	**0.750**	**0.639**
Zn_v	0.303	**0.886**		**-0.983**	**0.957**	0.165
Cd_v	**0.547**	**0.786**	0.132	**-0.976**	**0.921**	-0.343
Eigenvalue	8.191	6.220	9.369	5.823	9.462	4.728
Variance %	40.6	39.4	44.4	40.0	51.2	27.6
Cumulative variance %	40.6	80.1	44.4	84.4	51.2	78.8

[a,b] The subscripts 1 and v denote the metal contents from step 1 of the three-step BCR sequential extraction procedure and the metal contents of the vegetables.

The aim of cluster analysis is to identify relatively similar, that is, homogeneous, groups of objects in the space of measured features. The agglomerative hierarchical cluster analysis according to Ward's method was applied to detect the grouping of sampling locations (i.e., cases) on the basis of their similarities in chemical composition (the metal concentrations in the soil and vegetable samples).

The dendrograms resulting from the cluster analysis according to the Ward's method are represented in Figures 2, 3, 4 and 5. Two well differentiated clusters can be seen, one formed by two subgroups including the samples # 1, 2 and 3 from Altınoluk garden and the ones # 7, 8 and 9 from Sahabiye garden, and the second cluster contains the samples # 4, 5 and 6 from Çevreyol garden.

Hierarchical cluster analysis was used to group sampling stations based on the similarity of the soil and vegetable chemical compositions. The Ward's method was applied to normalised data using squared Euclidean distance to quantify similarity between the sampling stations. Figures 2-5 show the dendrograms obtained by the Ward's method. Both vegetable and soil samples are very well

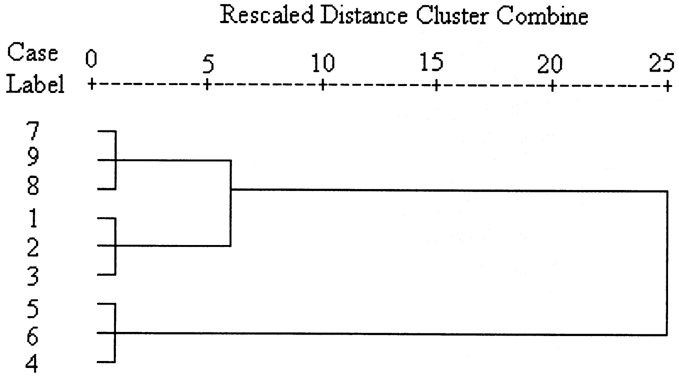

Figure 2. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of the 9 parsleys and soil samples (for 18 variables).

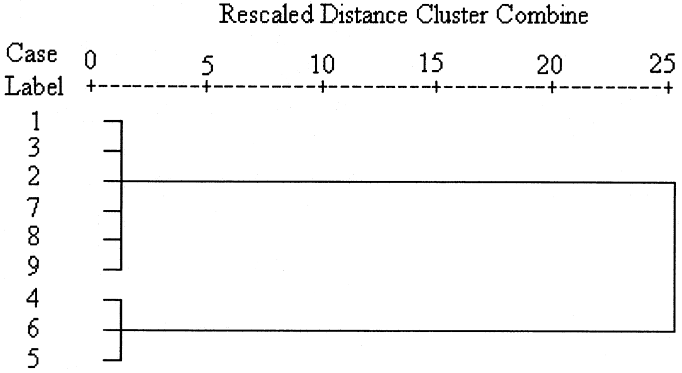

Figure 3. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of the 9 dills and soil samples (for 18 variables).

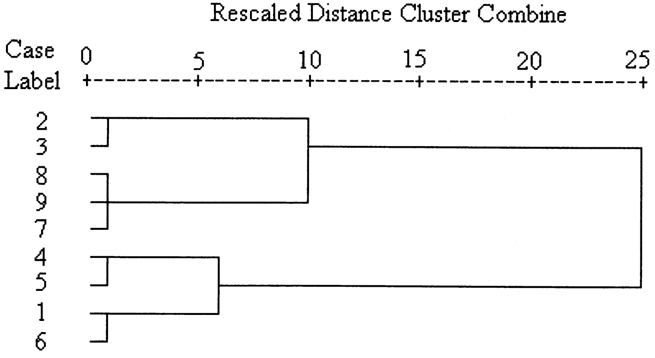

Figure 4. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of the 9 lettuces and soil samples (for 18 variables).

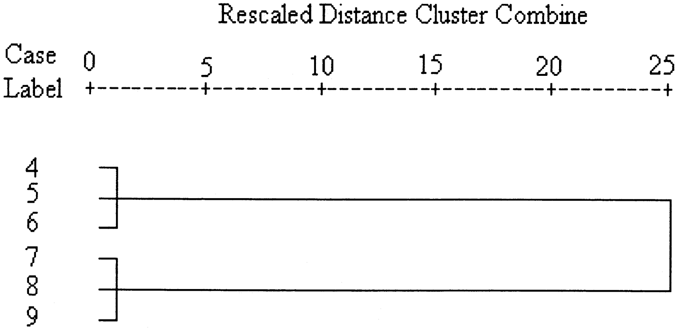

Figure 5. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of the 6 onions and soil samples (for 18 variables).

clustered according to their locality. For Figure 2, two very well differentiated clusters were observed. The one placed sampling stations # 1, 2 and 3, which show the parsley and corresponding soil samples from the Altınoluk garden, and the second subcluster contents (# 7, 8 and 9) corresponds to the samples from the Çevreyol garden. The second cluster includes samples from Sahabiye garden (the sampling locations 4, 5 and 6). The similar results were also observed for Figure 3, which gives two very well differentiated clusters for the dill and corresponding soil samples. For lettuce samples (Figure 4), mainly two cluster were obtained.The first main cluster contains the lettuce-soil samples from Altınoluk (2 and 3) and Sahabiye gardens (7, 8, and 9). The second cluster corresponds to Çevreyol garden samples (4, 5 and 6), and also #1 from the Altınoluk garden, as unexpected. Figure 5 includes two cluster. The first group contains the samples # 4-6 (Çevreyol onion and soil samples) and the second contains the samples # 7-9 from Sahabiye garden. Consequently, the samples taken from Çevreyol are differentiated from the other two garden's samples in respect to their chemical compositions, metal uptaking by the vegetables and soil characteristics.

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