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International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713640455>

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Online publication date: 17 August 2004

To cite this Article Tokalioğlu, Şerife, Kartal, Şenol and Güneş, Alfer A. (2004) 'Statistical evaluation of bioavailability of metals to grapes growing in contaminated vineyard soils using single extractants', *International Journal of Environmental Analytical Chemistry*, 84: 9, 691 – 705

To link to this Article: DOI: 10.1080/03067310410001688444

URL: <http://dx.doi.org/10.1080/03067310410001688444>

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STATISTICAL EVALUATION OF BIOAVAILABILITY OF METALS TO GRAPES GROWING IN CONTAMINATED VINEYARD SOILS USING SINGLE EXTRACTANTS

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(Received 28 August 2003; In final form 19 February 2004)

This article describes the statistical evaluation of concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Ca, and Mg metals determined in twenty-two grape and corresponding soil samples from around a zinc smelter in Kayseri, Turkey. In the analyses of soil samples, three different extractants, 0.1 M HCl in 0.025 M H₂SO₄, 1 M NH₄OAc, and aqua regia (1HNO₃ + 3HCl), were used to extract and determine the leachable metal contents which are acid soluble, exchangeable, and total, respectively. A mixture of HNO₃ and HClO₄ was used to dissolve grape samples. The determinations of metals were performed by flame atomic absorption spectrometry (FAAS). The analytical data were evaluated by using principal component analysis (PCA), hierarchical cluster analysis (HCA), correlation analysis (CA), and enrichment factors (EF). The relationship between the grape-metal and soil-extractable-metal concentrations was examined to evaluate the bioavailability of metals in soil to grape samples. As a consequence of statistical evaluations, Cd, Pb, Zn, Mn, and Fe labile levels in soil were related to total grape-metal contents indicating the method's suitability for bioavailability studies in polluted soil-plant systems.

Keywords: Bioavailability; Grape; Metal pollution; Multivariate analysis; Correlation

INTRODUCTION

Concentrations of heavy metals in soils are associated with biological cycles and influenced by anthropogenic activities such as agricultural practices, transport, industrial activities, and waste disposal. Knowledge of both the total concentration and chemical speciation is necessary to characterize the behaviour of heavy metals in soils. Although the total concentration of heavy metals in soils affords valuable information about overall pollution levels, it is insufficient to estimate the bioavailability of heavy metals for living organisms. It is well known that metals are present in soils in different chemical forms, which influence their reactivity and hence their mobility and bioavailability. Therefore, measurement of the total amount of metals should be complemented with measurements of their available fractions [1,2].

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In recent years, there has been much interest in and concern about the uptake of trace metals by plants from contaminated soils, because excess metals getting into the food chain through uptake by plants might be deleterious to human health [3–5]. The uptake of nutrient and/or contaminant elements by plant roots is dependent on many factors, including the magnitude and chemical form of trace elements present; soil pH, moisture, aeration, temperature, organic matter and phosphate content; the presence or absence of competing ions; plant species, rooting depth, age, and seasonal growth effects [6,7]. In soils, elements of interest are present in several different forms and associated with various geochemical phases. Generally, the water-soluble and exchangeable forms of metals are considered to be most available to plants, and metals bound to organic matter are also found to be available [2,8]. The uptake of metals by plants from soil has already been extensively studied [5,7,9–11]. To assess the lability of heavy metals in soils and sediments, single and sequential extraction procedures have widely been applied using different extractants [8,12,13]. A number of single extractants including ethylenediaminetetraacetic acid (EDTA), acetic acid, ammonium acetate, calcium chloride, and hydroxylamine hydrochloride have been tested to identify metal species as exchangeable, carbonate-bound, Fe- and Mn-oxide bound, or organically bound, and to estimate the plant-available trace metals [11,14,15].

One of the most important applications of chemical fractionation of metals in soils is to estimate bioavailability of metals to plants. For this purpose, correlation analysis is performed to evaluate the relationships among extractable fractions of the metals in soils and their total metal concentrations in plants. If the accumulation of an element by a plant correlates significantly with its extractable fraction in soils, it can be assumed that the extractable fraction is readily available to plants [7,16].

In this research on metal availability to grape plants (*Vitis vinifera* L.), correlation analysis and the other multivariate statistical techniques PCA and HCA were used to reveal the relationships between plant metal concentration and metal fractions from soils. Throughout the study, the software package SPSS 10.0 was used for statistical 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 determination of metals. The elements Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Ca, and Mg were determined according to the operating conditions suggested in the manufacturer's manual. A Nel 900 pH meter and an electrical heater were used throughout the experiments.

All reagents were of analytical reagent grade (Merck, Darmstadt, Germany). Throughout the analytical work, distilled water was used. Standard solutions ($1000 \mu\text{g mL}^{-1}$) prepared 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, 1 M HNO_3 , 0.1 M HCl in 0.025 M H_2SO_4 , 1 M NH_4OAc , and aqua regia solutions were used.

Site, Sampling, and Sample Preparation

In the zinc smelter about 170 000 tonnes of zinc ore per year is processed as metal carbonate and/or oxide. Particulate matter emission of the smelter is nearly 1250 tonnes per year. The zinc ore with carbonate coming from the mine quarries to the smelter consists of 21% Zn, 2–3% Pb, 0.07% Cd, 15% Fe, 0.022% F, 0.0003% Cl, 6% Al₂O₃, 8% SiO₂, 0.60% MgO, and 10–15% CaO. The annual capacity of the smelter is about 33 650 tonnes of electrolytic ingot zinc, 6000 tonnes of metallic lead, 124 tonnes of metallic cadmium, and 4.5 tonnes of silver.

Twenty-two grape and corresponding soil samples were collected along 1100 m towards the west from the smelter. Soil samples were taken from a depth of 10–15 cm below the soil surface, and the grape samples were collected from the same sampling sites as the soil samples. Detailed sample preparation procedures were mentioned in our previous paper [15].

Single Extraction Procedures for Soil Samples and Dissolution of Grape Samples

Three single-extraction procedures were applied to soil samples. Firstly, a mixture of 10 mL diluted acid (0.1 M HCl in 0.025 M H₂SO₄) was added to a 2.5-g subsample of soil to extract acid-soluble metals. After shaking for 25 min, the suspension was filtered through a blue-band filter paper using distilled water, and the filtrate was made up to a volume of 10 mL. Secondly, 15 mL of 1 M NH₄OAc (pH 7) was added to a 1.5-g subsample of soil to extract exchangeable metals. After shaking for 15 min, the mixture was passed through a blue-band filter paper, the residue was washed with distilled water, and the filtrate was diluted to 15 mL. Thirdly, 10 mL of aqua regia was added to a 10-g subsample of soil in a beaker. The mixture was evaporated nearly to dryness and then 5 mL of concentrated HClO₄ was added to the residue. The solution was heated to dryness and this procedure was repeated with 10 mL aqua regia once again. The final residue was taken up in diluted nitric acid, filtered, and the filtrate was made up to 10 mL with distilled water. The metal contents of all the extracts were determined by FAAS.

To dissolve the grape samples, 15 mL of concentrated nitric acid was added to a beaker containing 2.0 g of the grape sample. The mixture was heated until dense fumes of NO₂ were evolved. After cooling, 2 mL of concentrated HClO₄ was added, and after evaporating to near dryness, the remaining residue was made up to 5 mL with 1 M HNO₃.

Multivariate Statistical Analysis

Principal Component Analysis

Principal component analysis enables a reduction in data and description of a given multidimensional system by means of a small number of new variables. The application of varimax rotation of standardized component loadings enabled us to obtain a clear system as a result of maximizing component loadings variance and eliminating invalid components [17–20].

Cluster Analysis

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. In our study, we used Ward's method as an amalgamation rule and the squared Euclidean distance as a distance measure [21].

Correlation Analysis

In the presence of one independent and one dependent variable, the classical measure of linear relationship is given by the correlation coefficient. The correlation coefficient has often been considered as another measure of similarity between the variables [20].

Enrichment Factors

This popular technique of evaluating the relative importance of pollution sources on the determined trace metals in any environmental sample (airborne particles, soils, sediments, dusts, etc.) is used to compare the relative abundance of species in source material with that found in the Earth's crust. In the case of a terrigenous source, this comparison is made by examining the enrichment or metals in samples studied over what is found in soil or crustal material. If there are no anthropogenic sources, concentrations of metals should be explained by the sample itself. However, the composition of the sample may be modified by contributions from various external sources. The degree of modification of the sample's chemical composition may be different at each sampling point because of differing magnitudes of source contributions at each location. Crustal enrichment factors (EF_c) of elements are frequently used to determine the degree of modification in studies of atmospheric aerosols, sediments, and soils. In this study, it was applied to the soil samples from around a zinc smelter. The enrichment figures are calculated according to the following equation:

$$EF_c = (C_X/C_{Fe})_{\text{soil}} / (C_X/C_{Fe})_{\text{Earth's crust}}$$

where (C_X/C_{Fe}) is the ratio of the concentration of the element determined (C_X) to that of Fe (C_{Fe}) in the soil sample and reference earth's crust. The total C_X and C_{Fe} in the soil samples were calculated using the analyte concentration found from the aqua regia procedure. Enrichment factors were calculated for each individual sample relative to the abundance of the elements in the Earth's crust, choosing Fe as the reference element. The iron content of soils is almost constant over time owing to its high levels in the Earth's crust, provided that no large point source exists near a specific environment. An enrichment factor of unity would indicate that the relative concentration of a given metal is identical to that which is present in soil. If an enrichment factor is greater than unity, this indicates that the metal is relatively more abundant in the sample than in the Earth's crust. However, enrichment factors less than 5 may be not considered significant, although they indicate metal accumulation, because such small enrichments may arise from differences in the compositions of local sample material and the reference Earth's crust used in the EF_c calculations. If the EF_c values are greater than 5, in this case they are considered to indicate sample pollution by relevant metals [22].

RESULTS AND DISCUSSION

Results for Soil and Grape Samples

The concentrations of metals interested and their basic statistical values for 22 grape samples are given in Table I, and the metal levels found using HCl/H₂SO₄, NH₄OAc, and aqua regia extractants in 22 soil samples are presented in Tables II, III and IV, respectively.

Statistical Evaluation of the Results

Correlation Analysis

One of the most important applications of chemical speciation and/or fractionation of metals in soils is to estimate bioavailability to plants. The relationships between the extractable fractions of metals from soils and the total metal concentrations in plants can also be used to estimate this connection. If the accumulation of an element by a plant correlates significantly with its extractable fraction in soils, it can be assumed that the extractable fraction of metal is readily available to plants [10]. To establish the relationship between the speciation of heavy metals and plant availability, correlation analysis was performed between extractable fractions of the metals from the 22 soils and their corresponding grape contents. Significant relationships have been obtained, especially between the Zn, Cd, and Pb contents of soils using the HCl/H₂SO₄,

TABLE I Concentrations of metals determined in grape samples and their mean and standard deviation values ($\mu\text{g g}^{-1}$, by dry weight)

Sample No.	Variables										
	Pb _g	Cd _g	Zn _g	Cu _g	Co _g	Ni _g	Cr _g	Mn _g	Ca _g	Mg _g	Fe _g
1	8.18	0.36	40.7	4.52	1.86	3.26	1.11	7.45	4058	1283	38.3
2	8.41	0.28	39.3	5.47	1.81	2.97	1.62	7.94	3502	1275	28.4
3	6.29	0.27	35.3	5.45	1.99	2.76	1.32	6.98	3455	1175	28.1
4	7.50	0.38	37.7	5.05	1.42	2.55	2.38	7.18	3830	917	62.8
5	6.15	0.32	33.0	3.30	1.35	2.62	1.22	7.67	3370	842	37.3
6	8.30	0.34	34.0	6.06	1.79	3.26	1.97	5.70	3355	1500	26.8
7	8.95	0.39	35.3	4.02	1.85	3.34	2.36	7.12	3383	1325	29.8
8	8.73	0.32	36.7	4.52	1.80	4.32	1.57	6.38	4857	1275	33.4
9	7.92	0.34	31.3	4.12	1.48	4.78	2.04	6.61	3730	1283	40.1
10	8.07	0.32	36.0	4.35	1.71	3.58	1.59	8.69	4358	1283	20.5
11	10.7	0.33	35.3	3.48	1.59	3.56	1.57	8.11	4358	1133	28.4
12	9.76	0.33	39.3	5.06	1.72	3.42	2.01	8.43	2615	1117	29.3
13	8.65	0.26	33.0	3.96	1.60	2.84	1.71	7.48	3128	1350	29.9
14	7.55	0.29	33.7	3.18	1.77	2.87	2.01	5.38	3127	1183	29.1
15	6.45	0.20	24.3	5.22	2.15	2.99	2.28	5.37	3072	1267	20.6
16	5.73	0.09	21.0	2.66	2.11	2.92	2.03	6.76	3487	1167	38.3
17	5.68	0.12	22.0	3.74	1.68	2.54	1.99	5.42	2603	1042	23.7
18	3.93	0.08	22.3	2.96	1.41	2.46	1.62	5.35	3233	1275	23.2
19	4.52	0.07	20.3	3.85	1.48	2.88	1.80	5.55	3282	1083	23.6
20	4.71	0.10	20.5	3.41	1.39	2.05	1.56	5.08	2897	1050	25.4
21	4.66	0.09	17.5	5.03	0.97	1.92	1.02	4.50	2903	1042	20.4
22	5.08	0.05	17.7	4.00	0.89	1.81	0.99	4.19	2845	1025	15.9
Mean	7.09	0.24	30.3	4.24	1.63	2.98	1.72	6.52	3429	1177	29.7
SD	±1.87	±0.12	±7.8	±0.91	±0.32	±0.70	±0.41	±1.30	±579	±154	±9.81
SD/mean	0.26	0.49	0.26	0.22	0.20	0.24	0.24	0.20	0.17	0.13	0.33

TABLE II Concentrations of metals determined in 0.1 M HCl/0.025 M H₂SO₄ extracts of soil samples and their mean and standard deviation values ($\mu\text{g g}^{-1}$, by dry weight)

Sample No.	Variables										
	Pb ₁	Zn ₁	Cd ₁	Fe ₁	Cr ₁	Ni ₁	Cu ₁	Co ₁	Mn ₁	Ca ₁	Mg ₁
1	9.60	6.00	0.44	18.0	7.10	5.16	1.46	3.40	223	2672	46.0
2	9.00	6.20	0.44	18.1	7.14	5.18	1.56	2.86	194	2542	40.0
3	8.80	5.00	0.44	18.0	6.84	5.18	1.50	3.02	197	2760	44.0
4	13.0	8.60	0.46	15.9	7.58	5.08	1.32	3.30	232	2606	38.0
5	10.4	7.40	0.38	15.4	6.62	4.60	1.58	3.18	230	2744	40.0
6	10.0	6.20	0.34	15.4	6.22	4.08	0.90	2.82	236	2680	34.0
7	12.8	7.80	0.46	14.8	6.02	4.54	1.10	2.64	229	2662	32.0
8	8.60	4.60	0.42	13.9	6.24	5.18	1.02	2.70	198	2610	28.0
9	9.40	5.80	0.38	14.0	5.72	5.08	0.94	2.70	204	2624	30.0
10	10.0	5.80	0.46	12.8	5.72	5.10	0.98	2.60	192	2536	32.0
11	12.2	8.80	0.42	13.0	5.76	3.86	0.96	2.44	184	2382	26.0
12	12.6	4.60	0.38	5.94	5.40	3.90	0.90	2.52	182	2392	30.0
13	8.60	5.00	0.40	6.36	5.62	3.82	1.00	2.60	189	2424	24.0
14	6.60	7.40	0.12	7.86	6.36	3.48	1.02	2.78	207	2170	26.0
15	6.00	4.60	0.14	7.46	4.94	3.32	0.72	2.62	180	2154	28.0
16	6.20	4.20	0.10	6.80	3.84	3.58	0.58	2.24	184	2218	32.0
17	4.60	3.80	0.18	7.08	3.74	3.56	0.74	2.12	173	2000	26.0
18	3.80	3.00	0.18	7.26	4.00	3.06	0.44	2.08	176	1914	22.0
19	3.40	1.80	0.16	5.18	3.18	3.08	0.46	1.94	172	1866	20.0
20	3.40	1.80	0.04	5.22	3.26	2.96	0.54	1.86	170	1780	24.0
21	3.40	2.00	0.06	4.96	3.20	2.64	0.48	1.72	169	1582	22.0
22	3.20	1.60	0.04	4.84	3.30	2.68	0.46	1.58	168	1576	20.0
Mean	7.98	5.09	0.29	10.8	5.36	4.05	0.94	2.53	195	2313	30.2
SD	±3.33	±2.17	±0.16	±4.9	±1.44	±0.91	±0.37	±0.50	±22	±380	±7.6
SD/mean	0.42	0.43	0.55	0.46	0.27	0.22	0.39	0.20	0.12	0.16	0.25

NH₄OAc, and aqua regia extractants and the total metal contents in grape samples. The correlations obtained are illustrated in Figs. 1–3 for Zn, Cd, and Pb, respectively, and shown in Table V for the other elements. The metals extracted with HCl/H₂SO₄ were probably predominantly in acid-soluble forms and bound to carbonate. As can be seen in Fig. 1, significant positive correlations ($r_{\text{critical value}} = 0.406$ for $n = 22$, at 95% confidence level) existed between Zn contents of grape samples and Zn concentrations of soils obtained using the extractants HCl/H₂SO₄, NH₄OAc, and aqua regia ($r = 0.781$, 0.858 , and 0.928 , respectively). The highest correlation was observed with aqua regia.

The correlation coefficients (r) obtained for Cd among the grape values and those from each of the three extractants for soils were very close to each other (0.864 , 0.877 , and 0.845 for HCl/H₂SO₄, NH₄OAc, and aqua regia, respectively), indicating that Cd uptake from soil to grape samples was almost the same for the three extractants.

The highest correlation coefficient for Pb was obtained between the grape samples and the HCl/H₂SO₄ extracts ($r = 0.850$). This indicates that Pb probably occurs in acid-soluble forms in soils. When NH₄OAc extraction was used to extract metals from soil, r was found to be 0.703 , while this value was 0.618 for aqua regia digestion.

As can be seen in Table V, the Cr contents of the analyzed grape samples were not dependent on the Cr contents of soils obtained using HCl/H₂SO₄, NH₄OAc, and aqua regia. The contents of the other elements determined in grape samples did

TABLE III Concentrations of metals determined in 1 M CH₃COONH₄ extracts of soil samples and their mean and standard deviation values ($\mu\text{g g}^{-1}$, by dry weight)

Sample No.	Variables										
	Pb ₂	Zn ₂	Cd ₂	Fe ₂	Cr ₂	Ni ₂	Cu ₂	Co ₂	Mn ₂	Ca ₂	Mg ₂
1	16.8	7.60	0.55	3.80	9.36	3.85	1.15	7.80	140	3505	14.0
2	16.2	7.15	0.50	3.80	9.55	3.80	1.10	8.15	138	3275	14.0
3	15.4	8.10	0.55	3.25	9.50	3.45	1.05	6.45	138	3625	12.0
4	13.6	7.75	0.50	3.80	9.60	3.20	0.95	5.30	141	3635	12.0
5	13.8	6.40	0.35	3.30	9.10	3.15	0.80	5.60	136	3350	13.0
6	15.4	7.05	0.40	3.65	8.75	3.60	0.80	5.85	136	3285	14.0
7	16.2	7.80	0.50	2.95	8.35	2.85	0.85	6.55	134	3380	14.0
8	16.1	5.90	0.30	2.70	8.70	2.50	0.75	6.05	122	3235	13.0
9	9.65	4.70	0.40	2.80	7.15	2.35	0.95	5.10	136	3285	14.0
10	9.45	3.80	0.40	2.70	7.55	2.25	0.80	5.15	134	3160	12.0
11	13.2	7.90	0.45	2.80	7.50	2.05	0.75	4.45	126	3170	12.0
12	14.2	7.85	0.45	2.60	5.10	2.00	0.70	2.65	124	2900	12.0
13	14.2	8.15	0.40	2.60	6.30	1.90	0.60	2.70	122	2755	12.0
14	12.8	4.50	0.40	2.55	6.65	1.65	0.55	3.45	128	2655	13.0
15	8.80	4.75	0.20	2.50	4.55	1.80	0.45	3.70	126	2660	13.0
16	8.70	3.20	0.30	2.60	4.75	1.65	0.55	2.80	126	2260	12.0
17	8.55	3.70	0.15	2.10	4.10	1.90	0.50	2.35	122	2275	11.0
18	8.65	2.45	0.10	2.30	4.35	1.55	0.40	3.15	124	2580	11.0
19	8.85	2.75	0.10	2.25	3.80	1.50	0.40	2.75	120	2290	11.0
20	8.60	2.70	0.10	1.90	3.45	1.60	0.50	2.50	122	2170	10.0
21	8.55	2.55	0.10	1.85	3.40	1.40	0.40	2.40	120	2235	10.0
22	8.75	2.55	0.10	1.75	3.50	1.45	0.40	2.40	118	2205	10.0
Mean	12.1	5.42	0.33	2.75	6.59	2.34	0.70	4.42	129	2904	12.2
SD	±3.2	±2.20	±0.16	±0.63	±2.32	±0.82	±0.24	±1.86	±8	±508	±1.3
SD/mean	0.26	0.41	0.48	0.23	0.35	0.35	0.34	0.42	0.06	0.18	0.11

not linearly change with their soil concentrations for all the extractants used, but significant relationships were obtained at 0.1 and 0.05 probability levels.

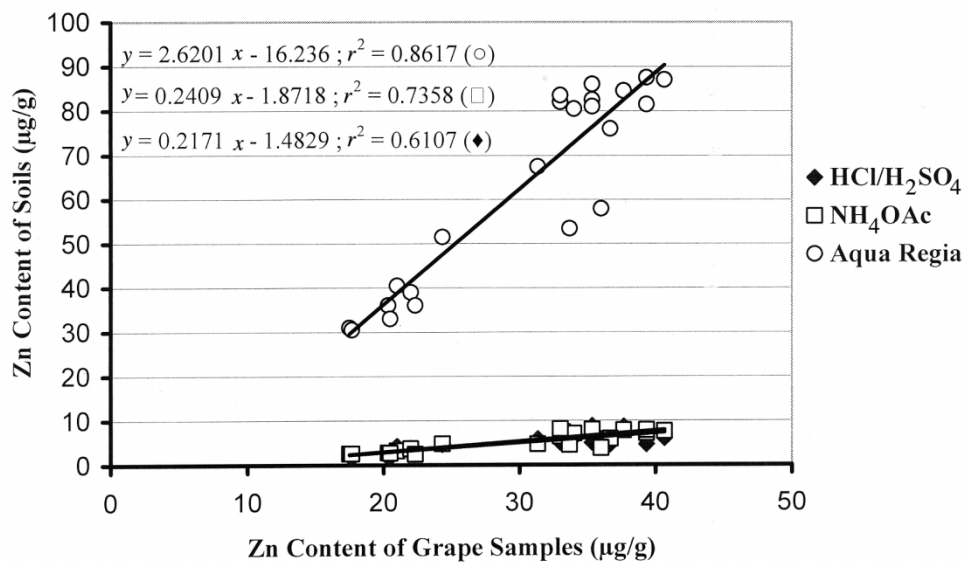
PCA Results

PCA is a powerful technique for displaying the relationships between objects and variables. PCA was carried out on the correlation matrix using the PCA subroutine of the SPSS V 10.0 package program for all the computations. For applying PCA, the data matrix 22 × 22 for metal levels in grape and soil samples (for each of the three extractants) was used. Afterwards, the rotation of the principal components was carried out by the varimax method. Table VI shows the application of PCA to the 0.1 M HCl in 0.025 M H₂SO₄ extractant. Only the eigenvalues having a contribution larger than 18% to the total variance were taken into consideration and accounted for 70.2% of the total variance. As a result of this, the number of PCs was reduced to two. The variables with higher loadings (positive or negative) are those that contribute most to explain the meaning of each principal component.

The two principal components have the largest percentage of total variance, explaining 51.9 and 18.3% of the total, respectively. The first principal component (PC1) has high loadings for the pairs Cd_s-Cd_g, Pb_s-Pb_g, Zn_s-Zn_g, Fe_s-Fe_g, and Mn_s-Mn_g. At the same time, PC1 is related to the toxic heavy metals, especially Pb, Cd, and Zn, that originate mainly from the zinc smelter. In addition PC1 has high loadings for the elements Cu_s, Co_s, Cr_s, Ni_s, Ca_s, Mg_s, and Fe_s, the major crustal and non-enriched elements.

TABLE IV Concentrations of metals determined in aqua regia extracts of soil samples and their mean and standard deviation values ($\mu\text{g g}^{-1}$, by dry weight)

Sample No.	Variables										
	Pb_3	Zn_3	Cd_3	Fe_3	Cr_3	Ni_3	Cu_3	Co_3	Mn_3	Ca_3	Mg_3
1	122	87.0	4.60	9670	38.8	368	29.6	16.8	1160	11 170	321
2	117	87.5	4.60	9550	39.1	371	29.8	17.6	1138	10900	350
3	119	86.0	4.35	9610	39.3	363	28.6	17.4	1140	11 125	390
4	109	84.5	4.30	9110	39.4	356	28.8	17.7	1158	11 030	390
5	109	82.0	4.05	9100	38.6	346	27.6	16.8	1126	10980	350
6	119	80.5	4.15	8800	37.4	344	27.7	18.3	1102	10810	370
7	120	82.5	3.85	8470	38.1	336	28.6	16.8	1098	10 890	360
8	120	76.0	3.60	8270	38.2	351	26.8	15.8	1044	10 680	350
9	106	67.5	3.55	7960	36.2	328	27.4	15.4	1102	10 750	340
10	106	58.0	3.65	7930	37.2	319	26.7	15.5	998	10 530	320
11	102	81.0	3.70	7730	37.4	323	23.6	15.8	1022	10 620	330
12	104	81.5	3.85	7160	37.2	313	22.8	14.6	978	10 420	320
13	105	83.5	3.55	7330	37.1	280	23.2	15.0	944	10 250	310
14	99.6	53.3	3.50	7070	38.3	286	23.2	14.6	902	10 140	310
15	99.4	51.5	3.25	6250	29.2	291	22.6	14.6	974	10 310	330
16	86.8	40.5	3.05	6420	36.2	278	21.8	14.1	974	9920	320
17	87.4	39.0	2.85	6290	36.4	275	19.4	13.8	934	9670	290
18	99.2	36.0	2.95	6180	29.6	264	18.8	13.8	930	9510	280
19	99.1	36.0	2.75	5900	28.8	252	19.2	12.8	904	9420	270
20	84.4	33.0	2.50	5690	26.8	251	18.8	12.6	904	9260	260
21	83.1	31.0	2.55	5110	25.3	244	18.4	12.3	890	9070	250
22	82.6	30.5	2.35	5050	25.6	240	18.2	12.2	888	9020	240
Mean	104	63.1	3.52	7484	35.0	308	24.2	15.2	1014	10294	320
SD	± 13	± 22.1	± 0.68	± 1480	± 4.8	± 44	± 4.1	± 1.8	± 98	± 694	± 42
SD/mean	0.12	0.35	0.19	0.20	0.14	0.14	0.17	0.12	0.10	0.07	0.13

FIGURE 1 Relationship between the Zn concentrations in grape and soil samples ($n=22$).

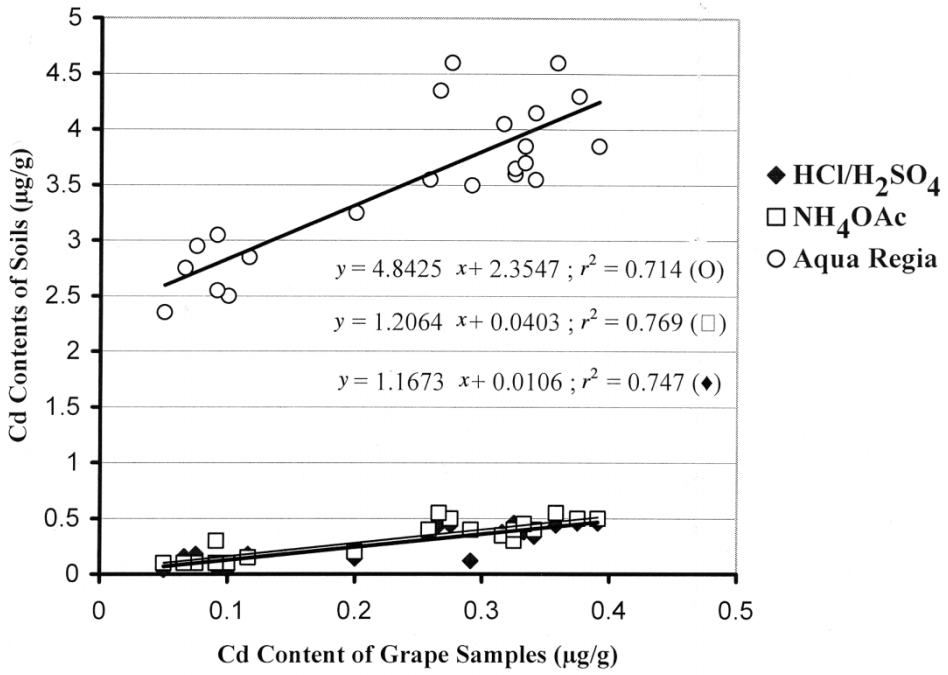


FIGURE 2 Relationship between the Cd concentrations in grape and soil samples ($n=22$).

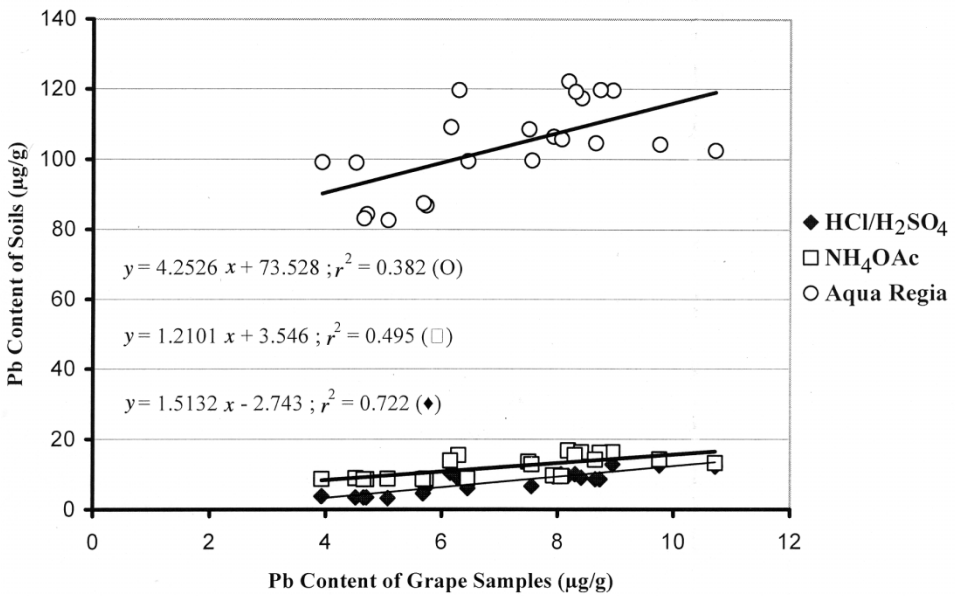


FIGURE 3 Relationship between the Pb concentrations in grape and soil samples ($n=22$).

TABLE V Correlation coefficients (*r*) between the soil-metals from three single extractions and the grape-metals

Element	Soil extractants		
	HCl/H ₂ SO ₄	NH ₄ OAc	Aqua regia
Cu	0.344	0.442^b	0.454^b
Co	0.507^b	0.397^a	0.486^b
Ni	0.631^b	0.285	0.549^b
Cr	0.158	0.051	0.299
Mn	0.396^a	0.560^b	0.592^b
Fe	0.489^b	0.625^b	0.557^b
Ca	0.559^b	0.600^b	0.554^b
Mg	0.073	0.549^b	0.253

^a*r* = 0.345 for *n* = 22, at 90% confidence level.^b*n* = 0.406 for *n* = 22, at 95% confidence level.

TABLE VI Varimax rotated component matrix including loadings, eigenvalues, percentage of variance and cumulative variance for each principal component (PC) for grape- and soil-metals obtained from single extractions

Metals ^a	HCl/H ₂ SO ₄		NH ₄ OAc		Aqua regia	
	PC1	PC2	PC1	PC2	PC1	PC2
Pb _g	0.54	0.67	0.54	0.66	0.60	0.56
Cd _g	0.82	0.47	0.79	0.49	0.84	0.38
Zn _g	0.84	0.43	0.84	0.44	0.87	0.35
Cu _g	0.34	0.16	0.47		0.41	0.14
Co _g	0.26	0.64	0.23	0.72	0.30	0.72
Ni _g	0.33	0.78	0.30	0.78	0.40	0.71
Cr _g		0.56		0.64		0.57
Mn _g	0.68	0.38	0.64	0.39	0.71	0.27
Ca _g	0.50	0.37	0.49	0.33	0.53	0.27
Mg _g		0.78		0.71		0.82
Fe _g	0.68		0.59		0.66	-0.13
Pb _s	0.80	0.43	0.82	0.27	0.83	0.39
Zn _s	0.78	0.33	0.79	0.34	0.91	0.26
Cd _s	0.83	0.41	0.85	0.40	0.94	0.22
Fe _s	0.89	0.16	0.92	0.19	0.96	0.17
Cr _s	0.93	0.26	0.95	0.22	0.82	0.35
Ni _s	0.87	0.31	0.94		0.95	0.22
Cu _s	0.95		0.94	0.19	0.93	0.25
Co _s	0.92	0.21	0.88	0.19	0.92	0.26
Mn _s	0.81	0.14	0.89		0.94	
Ca _s	0.87	0.43	0.93	0.26	0.93	0.32
Mg _s	0.88		0.64	0.61	0.85	0.32
Eigenvalue	11.42	4.03	11.31	4.12	12.56	3.60
Variance (%)	51.90	18.31	51.40	18.75	57.08	16.34
Cumulative (%)	51.9	70.2	51.4	70.2	57.1	73.4

^aThe subscripts g and s denote grape and soil, respectively.

The first PC may be attributed to the bioavailabilities of Pb, Zn, Cd, and Fe metals to the grape samples. The second PC, for Pb_g, Co_g, Ni_g, Cr_g, and Mg_g, had high loading values, except for Mg_s, and this PC probably originates from traffic. As regards 1 M NH₄OAc extraction, PCA gave two significant components explaining 51.4 and 18.7% of the total variance, respectively (totally 70.2%). The first principal component

has high loadings for the pairs Cd_s-Cd_g , Pb_s-Pb_g , Zn_s-Zn_g , Fe_s-Fe_g , and Mn_s-Mn_g . In the second PC, Pb_g , Co_g , Ni_g , Cr_g , and the pair Mg_g-Mg_s had high loading values. PCA results acquired using NH_4OAc extraction were in agreement with those of PCA obtained using HCl/H_2SO_4 extraction.

The application of PCA to the data obtained from aqua regia extraction led to two significant principal components, explaining 57.1 and 16.3 of the total variance, respectively (totally 73.4%). The obtained results were similar to those of the first two extractions used for leaching the metals from soils. In conclusion, high loading values have been observed for the pairs of Cd_s-Cd_g , Pb_s-Pb_g , Zn_s-Zn_g , Fe_s-Fe_g , and Mn_s-Mn_g with particular consideration of the uptake of metals from soils by grape samples. It is well known, from studies performed previously, that the metals Pb, Cd, and Zn are the most mobile elements in soils [23,24].

Cluster Analysis

Cluster analysis is an unsupervised method that uses the information acquired from the variables measured to reveal the natural clusters existing between the worked samples or variables. HCA was run applying Ward's method of agglomeration and squared Euclidean distance as a criterion of similarity. Figure 4 depicts the dendrogram

Dendrogram using Ward's method

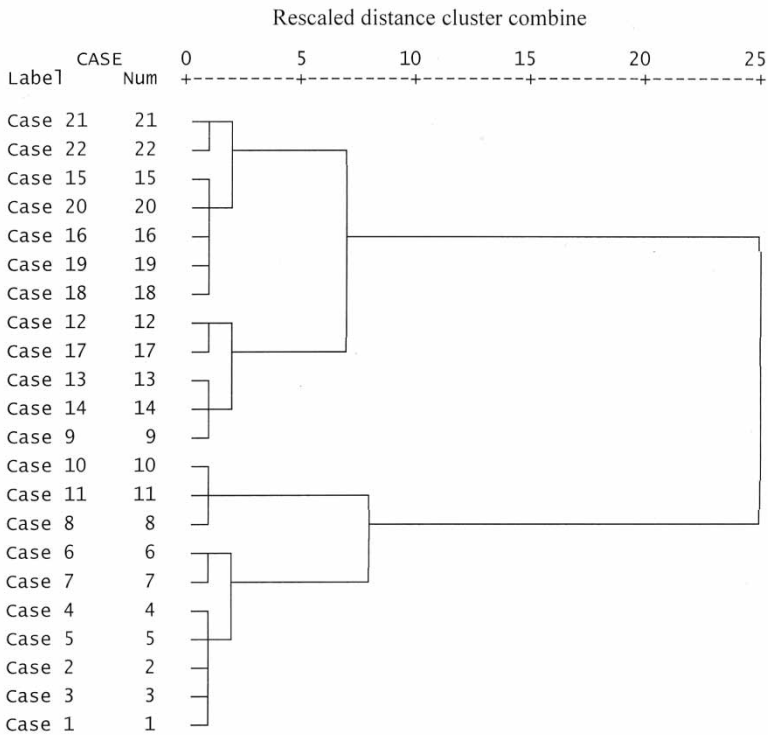


FIGURE 4 Dendrogram resulting from Ward's method of hierarchical cluster analysis of the 22 soil (aqua regia extraction) and grape samples. Similarities have been calculated from squared Euclidean distance.

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Dendrogram using Ward's method

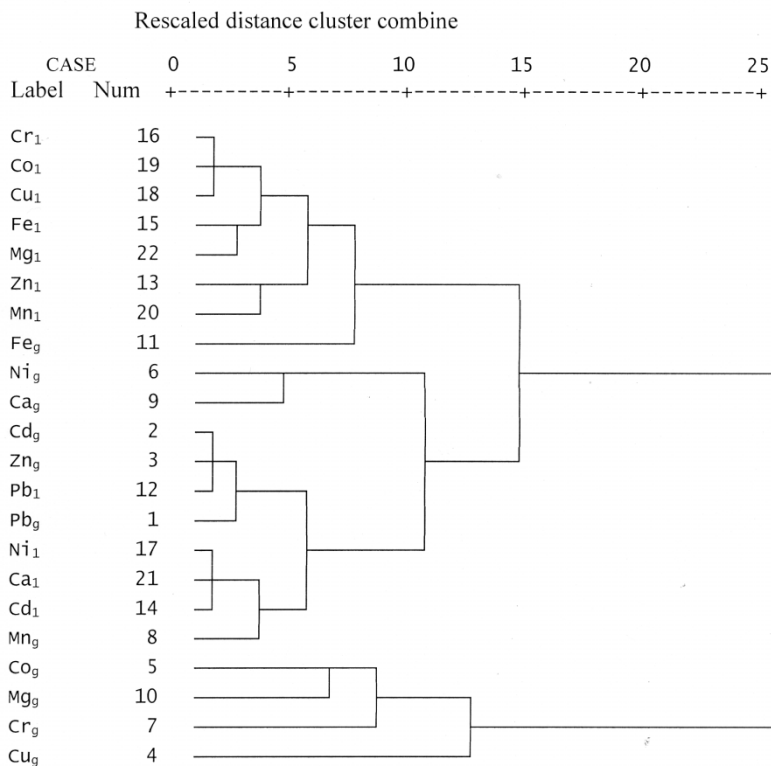


FIGURE 5 Dendrogram resulting from Ward's method of hierarchical cluster analysis for the 22 grape samples and 22 soil samples leaching with HCl/H₂SO₄.

obtained when the soil (for aqua regia extraction) and grape samples were considered together. Two main clusters can be drawn: the first cluster contains the first 10 samples (apart from sample 9) taken from the sampling sites close to the zinc smelter; the second cluster contains the samples between 11 and 22 taken from sampling sites further away from the smelter. When the dendrograms were examined taking into consideration the variables (metals) separately for three soil extractants, each of the three dendrograms had two main large subgroups. When the metal contents obtaining with the HCl/H₂SO₄ extractant were used in clustering (Fig. 5) the first subgroup of the cluster contains pairs of Cd₁-Cd_g, Pb₁-Pb_g, Zn₁-Zn_g, Fe₁-Fe_g, and Mn₁-Mn_g, and the second subgroup contains Co_g, Mg_g, Cr_g, and Cu_g variables. When the cluster was examined using the results of NH₄OAc extraction (Fig. 6), one of the two subclusters contains pairs of Cd₂-Cd_g, Pb₂-Pb_g, Zn₂-Zn_g, Cu₂-Cu_g, and Mn₂-Mn_g, and the second includes the pair Mg_g-Mg₂, and Ni_g, Co_g, and Ca_g variables. When the metal contents extracted with aqua regia were considered (Fig. 7), pairs of Cd₃-Cd_g, Pb₃-Pb_g, Zn₃-Zn_g, Cu₃-Cu_g, and Mn₃-Mn_g were in the first subcluster, and Mg_g-Mg₃, Cr_g, Ni_g, Fe_g, Co_g, Mg_g, and Ca_g were in the second. Compounds containing copper (especially copper(II) sulphate) and manganese are used to combat pathogens, microorganisms, and bacteria in agricultural areas particularly in vineyard soils [18].

Dendrogram using Ward's method

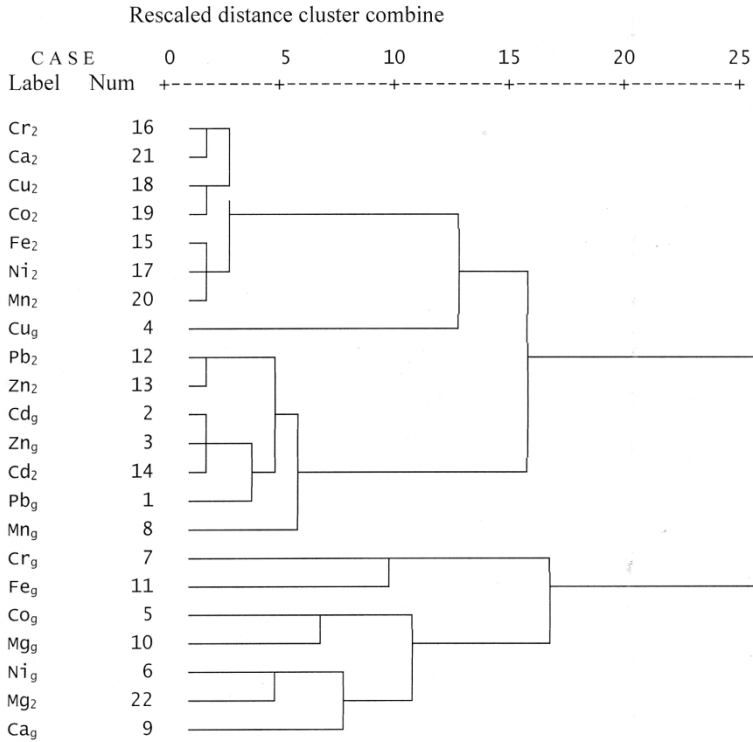


FIGURE 6 Dendrogram resulting from Ward's method of hierarchical cluster analysis for the 22 grape samples and 22 soil samples leaching with NH₄OAc.

Enrichment Factors

Figure 8 shows enrichment factors of metals considered together with their standard deviations. Pb, Cd, Zn, Ni, and Mn have EF_c values higher than 5, i.e., 63.1 ± 6.4 , 132 ± 7 , 6.57 ± 1.40 , 31.2 ± 2.2 , and 8.17 ± 1.00 respectively. These high EF_c values indicate that the source of accumulation of Pb, Cd, Zn, and Ni originates from anthropogenic contributions, mainly from the zinc smelter, although traffic can also cause significant accumulation of Pb, Cd, and Zn. Indeed, Cd, Pb, Zn, and Ni are good indicators for contamination in soils because they exist in gasoline (especially Pb in leaded gasoline), car components, oil lubricants, and industrial and incinerator emissions. The bioavailability and toxicity of heavy metals in soil samples depend not only upon total concentrations but also upon the chemical form of the metals. The presence of more soluble metal compounds makes the metals much more available to the environment. It can be concluded that the elements with the highest enrichment factors are also those with higher potential mobility and bioavailability because an increase in the total level of any metal in the sample may cause an increase in its mobile-phase fraction contents. The EF_c values found for the other metals were 3.3 ± 0.19 for Cu, 4.63 ± 0.42 for Co, and 2.66 ± 0.27 for Cr. These enrichment factors indicate that there was no significant enrichment for these elements.

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Dendrogram using Ward's method

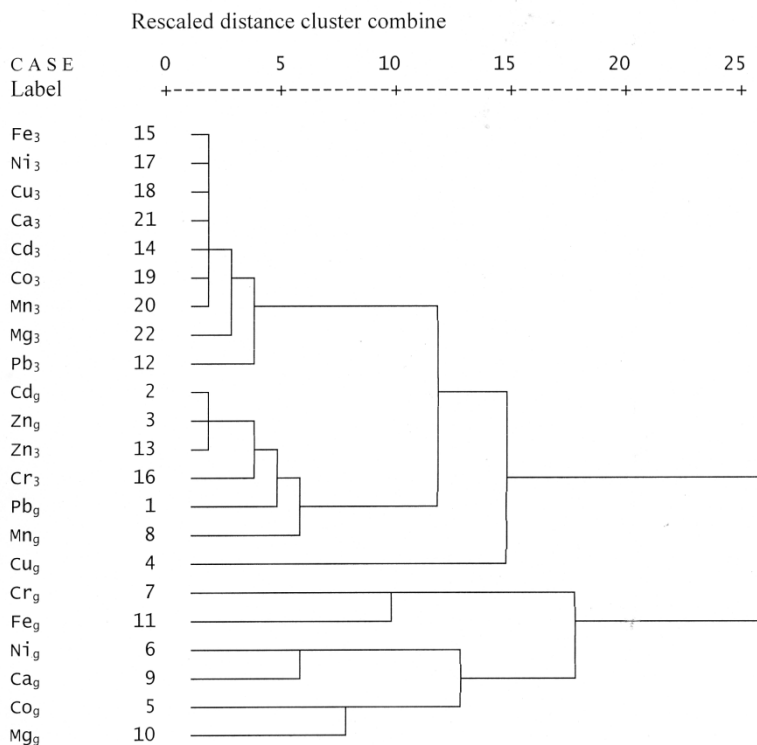


FIGURE 7 Dendrogram resulting from Ward's method of hierarchical cluster analysis for the 22 grape samples and 22 soil samples leaching with aqua regia.

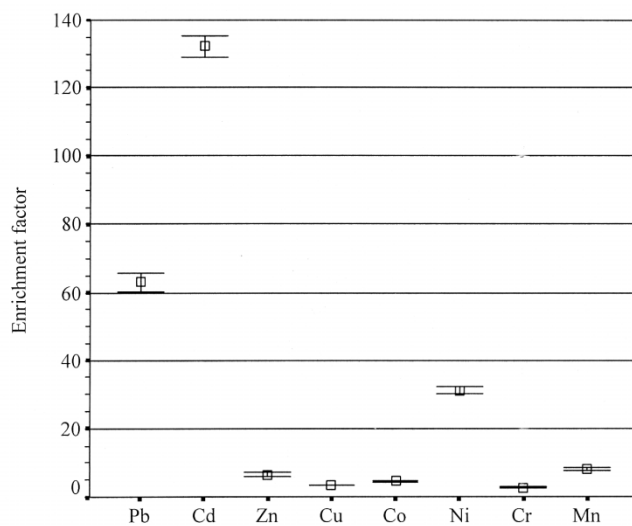


FIGURE 8 Enrichment factor values for some metals studied ($n=22$).

CONCLUSIONS

The main aims of this study were: (1) to assess the bioavailability of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Ca, and Mg metals to grape samples in contaminated vineyard soils, (2) to establish a relationship between metal concentrations in grape and soil, and (3) to evaluate the efficiency of chemical extraction methods to predict metal bioavailability.

The results from the correlation analysis show that, in particular, the zinc, cadmium, and lead contents in the grape samples are highly correlated with those of the corresponding soil samples. The highest r value (0.850) for Pb was obtained with HCl/H₂SO₄ extractant, for Cd (0.877) with NH₄OAc extraction, and for Zn (0.928) using aqua regia digestion. Significant relationships were also supported by the results of other statistical methods such as PCA and HCA.

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