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Characterization of Environmental Samples in an Ophiolitic Area of Northern Italy Using ICP-OES, ICP-MS, and XRF

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INTRODUCTION

Environmental studies require accurate and precise information on trace element distribution in the ecosystems. In this respect, partition and transfer coefficients can be extremely useful to represent the distribution and flow of elements among the various environmental compartments which are often characterized by extremely complex matrices.

In environmental studies, the choice of the most suitable analytical methods and procedures by which good quality data can be obtained is of primary importance.

Generally, the use of validated methods for which precision values are well known and measurement uncertainty has already been evaluated in compliance with the latest international guidelines is recommended (1,2). Validation of these methods requires accuracy tests with certified samples (3,4) or, when not available, comparison between different independent analytical techniques on real samples (5,6).

Today, the characterization of environmental matrices is obtained using complete dissolution of the samples, followed by monoelement (FAAS or GFAAS) or multielement determination with spectroscopic

ABSTRACT

In Ponte Barberino, an ophiolitic environment in the Trebbia River Valley near the town of Piacenza, Northern Italy, rocks and soils are characterized by high concentrations of Cr and Ni. The determination of macro, micro, and rare earth elements in rock, soil, sediment, and plant samples was carried out by ICP-OES, ICP-MS, and XRF after alkaline fusion or microwave acid mineralization.

For sediment samples, microwave acid digestion (8 mL aqua regia + 2 mL HF), followed by ICP-OES, provided results comparable to those obtained by XRF.

In the case of plants, microwave acid mineralization (7 mL HNO₃ + 1 mL H₂O₂ + 0.2 mL HF) followed by ICP-OES analysis was particularly suitable for the determination of trace elements in leaves of the most common botanical species found in the investigated area. In addition, soil/plant transfer factors characterized the *Alyssum spp.* species as a hyperaccumulator of Ni.

Alkaline fusion was found to be the best dissolution procedure for rock samples; recoveries ranged between 95 and 103% for Cr and Ni, respectively.

Also, XRF and ICP-OES analysis proved to be very reliable and provided comparable results. In rock samples, ICP-MS allowed the determination of trace and rare earth elements not detectable by the other techniques.

techniques (ICP-OES or ICP-MS). Other techniques, such as X-ray fluorescence (XRF), can provide direct analysis of the sample. The current trend in analytical methods favors closed-vessel solubilization rather than the traditional open-vessel systems, since the closed-vessel method makes it possible to obtain quantitative recoveries for volatile elements of toxicological interest and improves method detection limits (MDL) by minimizing the required amount of reagents (7–10).

Multielement techniques are gradually replacing monoelement techniques because they provide a complete elemental profile of the samples (11) and ensure a significant reduction in analysis time.

In ophiolitic areas, environmental samples such as rocks, sediments, soils, and plants are characterized by high concentrations of Ni and Cr. However, the determination of these elements can pose serious problems due to the complexity of the matrices, particularly when siliceous materials are present which cannot easily be dissolved completely with common strong mineral acids (12).

Ophiolitic environments are characterized by the presence of mafic or ultramafic rocks, partly or completely serpentinized. These rocks usually show high concentrations of Ni and Cr (sometimes up to a few thousands mg/kg), which decrease progressively as the rock acidity rises.

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Since ophiolitic soils were formed as deposits (*colluvium* or *alluvium*) due to the alteration of mafic rocks, Ni and Cr are commonly found co-precipitated with Fe and Mn oxides. Unlike Fe²⁺ and Mn²⁺ ions, which precipitate *in situ*, Ni²⁺ and Cr³⁺ ions are relatively more stable in water solutions and therefore can migrate over longer distances. In these soils, Ni²⁺ and Cr³⁺ ions are often found in aluminosilicate structures or as oxides mixed with Fe³⁺; their solubility being inversely proportional to soil acidity (pH). The determination of Ni and Cr concentration in soils and plants from an ophiolitic site is crucial for environmental monitoring, since both elements can be considered natural tracers of geological origin (13).

In the present work, analyses were carried out in two different labs in order to evaluate the applicability of microwave dissolution procedures, in conjunction with multielement spectroscopic analyses (ICP-OES/ICP-MS), for the elemental analysis of rocks, sediments, and plants collected in an ophiolitic area. X-ray fluorescence was used to compare the different analytical methods, while alkaline fusion was employed as an alternative technique to closed-vessel acid digestion for the solubilization of samples with very complex matrices.

EXPERIMENTAL

Area of Study

The analyzed area (Figure 1) is located upstream of the Trebbia River Valley. This area is part of an extensive formation of serpentine outcrops, covering several hundred square kilometers, with high concentrations of Cr and Ni ranging from 500 to 1220 $\mu\text{g g}^{-1}$ and 900 to 1500 $\mu\text{g g}^{-1}$, respectively.

Samples of rocks, sediments, and some of the most common botani-

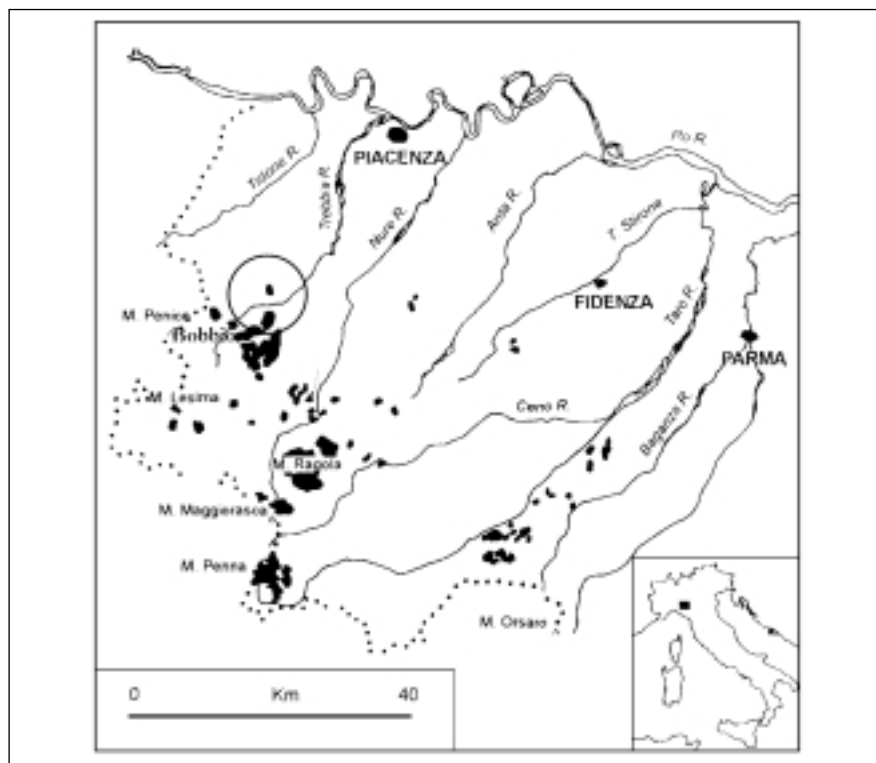


Fig. 1. Location of serpentized ophiolitic outcrops in the Apennines of the Piacenza and Parma provinces, Commune of Bobbio, Piacenza, Italy. The investigated area is marked with a circle.

cal species (i.e., *Alyssum* spp., *Helichrysum*, and *Euphorbia*) were collected in this area in September 2001.

Instrumentation

Two different microwave digesters were used in each lab: MLS 1200 Milestone (FKV, Sorisole, Bergamo, Italy) and MDS-2000 (CEM, Matthews, NC, USA). The instrumental operating conditions reported in Table I are those described in a previous paper (14).

Two inductively coupled plasma optical emission spectrometers were used: A PerkinElmer Optima™ 4300 DV simultaneous ICP-OES (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA), equipped with an AS-90 autosampler, a cross-flow nebulizer, and a Scott-type Rytan® spray chamber, and a Jobin Yvon 24 sequential ICP-

OES (Jobin Yvon, France) with a cross-flow nebulizer.

A Perkin Elmer SCIEX ELAN® 5000 inductively coupled plasma mass spectrometer was also used (PerkinElmer SCIEX Instruments, Concord, Ontario, Canada). This was equipped with a PerkinElmer GemTip™ nebulizer and a corrosion-resistant Scott-type spray chamber. The operating conditions were reported in previous papers (12,14) and are summarized in Tables II–IV.

XRF analyses were performed using a Philips PW1400 spectrometer with a Rh-target x-ray tube and Philips x-14 software package (Milan, Italy). The instrument has a four-position internal sample turret with a 72-position external sampler (PW1500/15 autosampler) which allows analysis of the ratio of the standard and several unknowns in a batch.

TABLE I
Operating Conditions for Microwave Systems

Plant Leaves ^a			
CEM 2000 / Milestone 1200			
Step	1	2	3
Power (W)	250	400	500
Hold time (min)	2	2	15

^a 250 mg of plant leaves treated with 7 mL HNO₃, 1 mL H₂O₂, and 0.2 mL HF in each of six vessels.

Soils and Sediments ^a

CEM 2000 / Milestone 1200						
Step	1	2	3	4	5 ^b	6
Power (W)	250	400	600	0	300	0
Hold time (min)	8	4	6	2	3	2

^a 250 mg of soil / sediment treated with 2 mL HF, 8 mL aqua regia, and 2 mL H₃BO₃ in each of six vessels.

^b The boric acid-saturated solution was added before step #5.

TABLE II
ICP-OES Instrumental Operating Parameters

	PerkinElmer Optima 4300 DV Simultaneous ICP	Jobin Yvon Model 24 Sequential ICP
Frequency	40 MHz free-running	40.68 MHz
Incident power	1300 W	900 W
Reflected power	<5 W	<5 W
Ar gas flow rate:		
Outer gas	15 L min ⁻¹	13 L min ⁻¹
Auxiliary gas	0.5 L min ⁻¹	<1 L min ⁻¹
Aerosol gas	0.8 L min ⁻¹	0.9 L min ⁻¹
Nebulizer		
Model	GemTip™ cross-flow	Scott-type cross-flow
Sample flow rate	1.0 mL min ⁻¹	1.5 mL min ⁻¹
Sample pump	Peristaltic pump	Gilson Minipuls™ 2
Computer	Dell® Optiplex GX150	Siemens Nixdorf PCD-4RsxA
Printer	HP LaserJet® 2200D	Fujitsu DL1150
Autosampler	Model AS90	-

TABLE III
**Wavelengths Used for the PerkinElmer
Optima 4300 DV ICP-OES**

Element	Wavelengths (nm)
Cd	214.438 – 228.802^a
Co	230.786 – 228.616^a
Cr	205.552 – 206.149 – 267.716^a
Cu	324.754 – 327.396 – 234.754^a
Mn	257.610^a – 260.569
Ni	231.604^a – 221.647
Pb	220.353^a – 261.418
Zn	206.200 – 213.856^a

^a Wavelength used for the analysis.

TABLE IV
Instrumental Parameters for ICP-MS Analysis

RF power	1085 W
Plasma argon	14.6 L/min
Nebulizer flow	1.1 L/min
Auxiliary flow	0.83 L/min
Sample flow rate	1 mL/min
Nebulizer	Cross-flow
Data	Peak-hop transient
Resolution	Normal
Reading time	150 ms
Dwell time	50 ms
Sweeps / replicates	3
Number of replicates	5
Sample time	1'06"
Sample read delay	50 s
Autosampler wash delay	60 s
Calibration mode	External calibration
Calibration standard	10, 20, 30, 50, 100 µg/L
Curve fit	Linear through zero

Isotopes	Mass	Isotopes	Mass
As	75	Mn	55
Ba	138	Nd	142
Be	9	Ni	60
Cd	114	Pb	208
Ce	140	Rb	85
Co	59	Sb	121
Cr	52	Sm	152
Cu	63	Sr	88
Cs	133	Ta	181
Ga	71	U	238
Gd	158	V	51
Hf	180	Y	89
Ho	165	Yb	174
La	139	Zn	66
Mo	98	Zr	90

Interelemental corrections:

$${}^{75}\text{As} = {}^{75}\text{As} - 3.087 {}^{77}\text{Se} + 2.619 {}^{82}\text{Se}$$

$${}^{51}\text{V} = {}^{51}\text{V} - 3.09 {}^{53}\text{Cr} + 0.353 {}^{52}\text{Cr}$$

Reagents and Standard Solutions

The following Suprapur® reagents (E. Merck, Darmstadt, Germany) were used: nitric acid (65% m/v), hydrochloric acid (37% m/v), hydrofluoric acid (40% m/v), and reagent-grade boric acid. The ICP mass spectrometer and optical emission spectrometers were calibrated using certified multielement solutions diluted with water that contained the same amount of acids as the samples. The high-purity water used in this analysis was produced with a Milli-Q™ deionizing system (Millipore, Bedford, MA, USA).

Materials and Procedures

Sediments

A four-layer sediment sample (0–15 cm, 15–25 cm, 25–38 cm and 38–50 cm) was air-dried and sieved to obtain the fine earth [fractions with a diameter (Ø) of < 2 mm].

An aliquot of each layer was then ground to 0.2 mm in a planetary mill with agate balls. About 0.250 g of this fraction (Ø < 0.2 mm) was subjected to microwave acid digestion with HF/HNO₃/HCl, following the program reported in a previous paper (12). The solutions obtained from the sample mineralization were diluted to 50 mL and analyzed by ICP-OES and ICP-MS. An aliquot of the 0.2-mm fraction was also analyzed by XRF. Eighteen elements were determined in the sediment sample. The solutions derived from the HF/HNO₃/HCl procedure were analyzed by two laboratories using two different instrumental configurations. In order to evaluate the uncertainties associated with the instrumental readings and the preparation phase, the solutions prepared by LAB A were analyzed by LAB B and vice versa.

The possibility of using two different wavelengths for every analyte in order to rapidly verify the presence of spectral interferences

was evaluated using the PerkinElmer Optima 4300 DV (Table III). The concentration difference measured for all the analytes at the two wavelengths was not larger than 4–5 %; therefore, at these operating conditions, interferences were not a problem. Using the wavelengths marked with an “a” in Table III, the short-term precision for 1 mg/L of different elements (%RSD for five replicates) was always less than 1.2%.

Plants and Soils

Trace elements were determined in plant leaves of the botanical species *Alyssum spp.*, *Helichrysum*, and *Euphorbia*. The collected samples were dried in a thermostatic oven at 40°C and then ground, without washing, in a planetary mill with agate balls in order to obtain 0.2-mm particles. Approximately 0.250 g of the dried 0.2-mm fraction was submitted to microwave acid digestion (using a mixture of 7 mL HNO₃ + 1 mL H₂O₂ + 0.2 mL HF), following the program reported in a previous paper (14) and also shown in Table I. The mineralized solutions were then diluted to 50 mL and six elements in the botanical samples were determined by ICP-OES. The same

procedure was applied for sediment samples, and six trace elements were determined in the soil obtained from beneath these plants.

Rocks

A rock sample (serpentinized ophiolite) was analyzed as follows: (a) microwave acid digestion followed by ICP-OES or ICP-MS analysis; (b) alkaline fusion with lithium tetraborate followed by ICP-OES and ICP-MS analysis; (c) direct analysis by XRF on a pellet sample. Eleven elements were determined by ICP-OES, 22 samples by ICP-MS, and 17 samples by XRF.

The accuracy of these analytical methods (expressed as % Recovery) was estimated by using the following certified samples: CRM 320 River Sediment for sediments, NIST 1570a Spinach Leaves for plants, NIM Dunite and NBS-688 Basalt for rocks. These certified materials were treated according to the manufacturer's instructions. Intralaboratory (repeatability or %r) and interlaboratory (reproducibility or %R) precisions were estimated according to ISO standard 5725 (15). The statistical methods used in this study are consistent with those reported in the literature (16).

RESULTS AND DISCUSSION

Sediments

Table V shows the concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn observed in the CRM 320 River Sediment. Acceptable accuracy (recoveries between 89 and 106%) and precision values (%r <10 and %R <26) were obtained for all of the elements determined, except Cd (uncertified), which was present at a concentration of about 0.5 µg g⁻¹.

Particularly good were the recoveries for Cr and Ni, elements abundant in ophiolitic areas and difficult to solubilize with common acid mixtures, such as HNO₃, HNO₃/HCl, and HNO₃/HClO₄. The ICP-OES/ICP-MS and XRF techniques provided comparable results as shown in Table VI. This proves that XRF is particularly suitable for a general characterization of elements since it is a quick, non-destructive technique, while ICP-OES and ICP-MS provide a wider elemental profile, including for trace elements and rare earths. XRF instrument detection limits (IDL) were several µg g⁻¹, slightly higher than the values found for ICP-OES.

The major limits of the XRF technique are related to accuracy, which depends on the types (matrices and interelement ratios) of the samples used to calibrate the instrument. On the other hand, the limits for ICP-OES are mostly due to problems arising during the dissolution steps which do not always permit a complete dissolution of the samples.

The information given in Table VI shows that Al, Ba, and K concentrations increase as the depth of the soil layers increases, while the Ca, Mg, Ni, and Cr concentrations decrease. The Cr/Ni ratio remains constant in all layers (R Cr/Ni 1.45), except the 0–15 cm depth (R Cr/Ni 1.15). This confirms the common origin of both analytes from

TABLE V
CRM 320 River Sediment
ICP-OES Determination of Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn (µg g⁻¹)
After Digestion With HF / aqua regia

Element	Certified Values (µg g ⁻¹)	Found Values (µg g ⁻¹)	Recovery (%)	r (%)	R (%)
Cd	NP	0.533 ± 0.026	ND	21	45
Co	17.3 – 22.1 ^a	20.5 ± 0.80	ND	7	26
Cr	138 ± 7.0	122 ± 6.0	88	9	12
Cu	44.1 ± 0.1	42.0 ± 2.1	95	8	9
Mn	619 – 786 ^a	697 ± 44	ND	10	12
Ni	75.2 ± 1.4	72.4 ± 2.5	96	6	12
Pb	42.3 ± 1.6	40.6 ± 10.3	96	10	22
Zn	142 ± 3	138 ± 7	97	10	14

^a Indicative values.

NP = Not Present.

ND = Not Determined.

TABLE VI
Determination of Major (%) and Minor ($\mu\text{g g}^{-1}$) Elements in a Sediment Sample
Collected in Ponte Barberino

(Analysis by XRF or ICP-OES/ICP-MS After Sample Dissolution With HF/aqua regia in a Microwave Oven)

Depth Element	0–15 cm		15–25 cm		25–38 cm		38–50 cm	
	XRF	ICP-OES/ ICP-MS	XRF	ICP-OES/ ICP-MS	XRF	ICP-OES/ ICP-MS	XRF	ICP-OES/ ICP-MS
Al (%) ^a	5.59	5.70	8.29	8.20	9.33	9.55	10.1	10.5
Ca (%) ^a	9.18	9.04	2.55	2.36	1.81	1.59	1.59	1.50
Fe (%) ^a	9.27	9.40	7.70	8.02	8.26	8.49	9.21	9.55
K (%) ^a	1.65	1.39	2.17	2.01	2.35	2.44	2.51	2.75
Mg (%) ^a	2.09	1.89	1.52	1.50	1.46	1.40	1.38	1.33
Na (%) ^a	0.31	0.28	0.22	0.20	0.20	0.19	0.14	0.15
Si (%) ^a	23.8	24.20	25.7	23.88	26.5	26.87	25.9	26.59
Ti (%) ^a	0.29	0.30	0.49	0.55	0.53	0.52	0.54	0.51
Ba ($\mu\text{g g}^{-1}$) ^a	231	250	299	320	319	330	420	411
Co ($\mu\text{g g}^{-1}$) ^b	11.7	28.0	24.2	34.5	26.0	33.6	26.4	32.0
Cr ($\mu\text{g g}^{-1}$) ^b	ND	197	ND	169	ND	149	ND	125
Cu ($\mu\text{g g}^{-1}$) ^b	31.0	51.0	43.3	44.9	44.5	43.8	42.9	38.0
Mn ($\mu\text{g g}^{-1}$) ^a	797	845	936	902	995	972	1024	942
Ni ($\mu\text{g g}^{-1}$) ^b	123	171	131	116	98	103	90.9	86
Pb ($\mu\text{g g}^{-1}$) ^b	16.4	9.12	30.3	25.2	28.9	16.0	42.8	13.4
Sr ($\mu\text{g g}^{-1}$) ^a	417	437	242	224	229	220	251	181
V ($\mu\text{g g}^{-1}$) ^b	62.6	74.0	90.8	ND	97.0	ND	11.0	ND
Zn ($\mu\text{g g}^{-1}$) ^a	90.1	101	113	123	115	149	129	123

^a Determined by ICP-OES.

^b Determined by ICP-MS.

ND = Not Determined.

ophiolitic rocks upstream of the investigated area. The Al/Ti ratio is constant at various depths (average value: 18.2 ± 1.7), which means that both analytes can be used as normalization elements in order to calculate enrichment factors for trace elements in environmental biomonitoring studies (17).

Plants and Soils

ICP-OES determination of Al, Cr, Cu, Fe, Mn, Ni, and Zn in botanical and soil samples was performed after closed-vessel microwave acid dissolution, a procedure similar to the one used for the sediment samples. Analytical accuracy and precision can be enhanced by adding HF to the solubilization mixture, as shown in a previous paper (14). This ensures the complete dissolution of materials particularly resis-

tant to acids, e.g., compounds containing Cr and Ni.

The accuracy values found in both labs for plant reference material NIST 1570a Spinach Leaves were between 89 and 100% (Table VII), with good precision values for Cr and Ni.

Table VIII shows the concentration of some analytes in the botanical species collected in the area under investigation. High Ni values were observed in the *Alyssum spp.* samples (approx. 1%), while *Euphorbia* shows a significant concentration of Mn (approx. 0.16%). Additional analyses were carried out in the soil obtained from beneath these plants using the same procedures described for sediments in order to determine the concentration of Ca, Cr, Cu, Fe, Mg, Mn, Ni, and Zn.

Table IX shows all the values found, along with the soil/plant transfer factors. This parameter (TF), important and often used in environmental studies, is the ratio between the concentration of an element in a plant and its total concentration in the soil. This provides useful information on the cycling of elements between two different compartments. As a rule, the TF values found in uncontaminated agricultural and forestry soils are less than one unit. The values given in Table IX confirm this rule, except for Mn in the *Euphorbia* species and Ni in *Alyssum spp.* While a preferential accumulation of Mn is quite evident (TF 1.26), the high Ni value (TF 8.3) in the *Alyssum spp.* species suggests hyperaccumulation, as already reported in the literature (18,19). The Ca/Mg ratio observed in

TABLE VII
NIST 1570a. Spinach Leaves, ICP-OES Determination of Cr, Cu, Fe, Mn, Ni and Zn ($\mu\text{g g}^{-1}$)
After Sample Digestion With HF/HNO₃/H₂O₂ in a Microwave Oven

Element	Certified Values ($\mu\text{g g}^{-1}$)	Found Values ($\mu\text{g g}^{-1}$)	Recovery (%)	r (%)	R (%)
Cr	NP	1.63 ± 0.45	ND	35	82
Cu	12.2 ± 0.6	10.9 ± 1.27	89	19	35
Fe	NP	260 ± 30	ND	8	36
Mn	75.9 ± 1.9	70.9 ± 8.8	93	10	38
Ni	2.14 ± 0.10	2.15 ± 0.36	100	46	47
Zn	82 ± 3	79.2 ± 8.73	97	6	34

NP = Not Present.

ND = Not Determined.

TABLE VIII
Alyssum spp., Helichrysum, and Euphorbia Leaves
ICP-OES Determination of Ca, Cr, Cu, Fe, Mg, Mn, Ni and Zn ($\mu\text{g g}^{-1}$)
After Microwave Digestion With HF/HNO₃/H₂O₂
Determination of Ca/Mg Ratio

Element	<i>Alyssum spp.</i> ($\mu\text{g g}^{-1}$)	<i>Helichrysum</i> ($\mu\text{g g}^{-1}$)	<i>Euphorbia</i> ($\mu\text{g g}^{-1}$)
Cr	23 ± 2.83	1.89 ± 0.23	7.14 ± 0.13
Cu	4.0 ± 1.41	11.4 ± 0.20	4.52 ± 0.05
Fe	1221 ± 210	197 ± 4.15	344 ± 3.21
Mn	102 ± 24.7	134 ± 1.72	1626 ± 14.8
Ni	10375 ± 1711	13.3 ± 0.31	22.9 ± 0.22
Zn	37 ± 16.3	79.2 ± 8.73	36 ± 0.83
Ca/Mg	0.050	0.82	0.81

TABLE IX
Concentrations of Cr, Cu, Fe, Mn, Ni and Zn in Ophiolitic Soil Beneath Botanical Species
Soil/Plant Transfer Factors (TF) for *Alyssum spp.*, *Helichrysum*, and *Euphorbia*

Element	Soil ($\mu\text{g g}^{-1}$)	TF <i>Alyssum spp.</i>	TF <i>Helichrysum</i>	TF <i>Euphorbia</i>
Cr	1160 ± 203	0.020	0.002	0.006
Cu	46 ± 1.99	0.087	0.248	0.098
Fe	44252 ± 9575	0.028	0.004	0.008
Mn	1289 ± 268	0.079	0.104	1.261
Ni	1260 ± 306	8.324	0.010	0.018
Zn	114 ± 11	0.319	0.415	0.315

botanical species, particularly in *Alyssum spp.*, was less than one unit, which is further evidence of the ophiolitic nature of the analyzed site, and in accordance with the results obtained by Pelosi (20) and Vergnano Gambi (21).

Rocks

Elemental determination in rocks was carried out by comparing the results obtained by three different analytical methods: XRF, ICP-OES, and ICP-MS.

Table X shows the concentration values of both major and trace elements found in the NIM Dunite certified sample and in the ophiolitic rocks collected in Ponte Barberino. These samples were analyzed using alkaline fusion for dissolution, followed by ICP-OES and XRF analysis.

Table X does not show the results of some preliminary tests performed by microwave acid digestion, since the recovery of a large number of the analytes was incomplete.

Alkaline fusion at about 1000°C ensured a complete solubilization of silicates, with recoveries between 80 and 120% for NIM Dunite (Table X) and between 76 and 114% for NBS-688 Basalt (Table XI). Cr and Ni percentage recoveries ranged from 89 to 100%.

Following the comparison between the employed techniques, the recovery of most of the analytes in the NIM Dunite material (Table X) was better with ICP-OES than XRF; Ba, Sr, Pb and Cu concentrations were not detected by X-ray fluorescence. This is probably due

to the XRF calibration which is strongly affected by the availability of certified reference materials with matrices similar to those of the samples used.

In the real ophiolite sample, the agreement between ICP-OES and XRF results was on the whole satisfactory, except for Ca and Cu which were clearly underestimated by XRF. The ICP-MS technique, due to lower instrumental detection limits (IDL), allowed the determination of approximately 20 trace and rare earth elements which cannot be determined by ICP-OES at these concentration levels. In this study, good accuracy was obtained for almost all analytes using ICP-MS, except for Ho, Sb, Zr, Sm, and Yb; for the last two analytes, only indicative values are reported in the literature.

CONCLUSION

This study demonstrates that accurate analytical data can be obtained in very complex environments, such as an ophiolitic site, by using solubilization procedures based on the type and nature of the matrices. The use of multielement independent techniques with analytical sensitivities suitable for the determination of trace metal concentrations, the use of certified reference materials with matrices similar to the samples investigated and, most of all, the use of intra- and interlaboratory comparisons are essential to verify the reliability of an analytical method in terms of accuracy and precision.

For sediment samples, the use of microwave digestion in conjunction with ICP-OES and ICP-MS provided very good analytical results, comparable to those obtained by XRF for most of the elements. The use of HF in the acid mixture employed for sample dissolution provided quantitative recoveries for the determination of the total content of analytes, particularly for Cr

TABLE X
ICP-OES and XRF Determination of
Major (%) and Minor ($\mu\text{g g}^{-1}$) Elements in
NIM Dunite and in an Ophiolitic Rock Sample

Elements	NIM Dunite			Ophiolitic Rock	
	Certified Values	Found Values		Found Values	
		ICP-OES	XRF	ICP-OES	XRF
Al (%)	0.20	0.21	0.18	0.66	0.75
Ca (%)	0.20	0.22	0.28	1.26	0.67
Fe (%)	11.87	12.15	18.1	8.82	9.08
K (%)	0.01	0.01	0.01	0.01	0.01
Mg (%)	26.1	24.7	20.1	14.7	13.6
Na (%)	0.04	0.03	0.02	0.02	< DL
Si (%)	18.18	19.0	20.3	19.6	20.1
Ti (%)	0.012	0.011	0.016	0.05	0.07
Ba ($\mu\text{g g}^{-1}$)	10.0	10.8	< DL	55	<DL
Co ($\mu\text{g g}^{-1}$)	210	198	122	103	61.9
Cr ($\mu\text{g g}^{-1}$)	2900	2760	ND	1170	ND
Cu ($\mu\text{g g}^{-1}$)	10.0	12.3	< DL	90.1	28.9
Mn($\mu\text{g g}^{-1}$)	1704	1654	1032	799	839
Ni ($\mu\text{g g}^{-1}$)	2050	2110	1971	1840	1941
Pb ($\mu\text{g g}^{-1}$)	7.00	8.43	< DL	0.70	<DL
Sr ($\mu\text{g g}^{-1}$)	3.00	4.11	< DL	5.90	4.28
V ($\mu\text{g g}^{-1}$)	40.0	43.1	20.9	83.0	90.4
Zn ($\mu\text{g g}^{-1}$)	90.0	79.7	65.5	47.2	51.7

DL = Detection Limit.
ND = Not Determined.

TABLE XI
ICP-OES/ICP-MS Determination of Macroelements (%), Trace Elements and REEs ($\mu\text{g g}^{-1}$)
in NBS-688 Basalt and in an Ophiolitic Rock After Alkaline Fusion

Element	NBS-688 Basalt			Ophiolitic Rock
	Certified Values	Found Values ICP-OES/ICP-MS	Recovery (%)	Found Values ICP-OES/ICP-MS
Al (%)	9.18 ± 0.05	9.41 ± 0.02	102	0.66
Ca (%)	8.70 ^a (8.47 ± 0.36) ^b	8.88 ± 0.09	102	1.26
Fe (%)	7.23 ± 0.03	7.43 ± 0.03	103	8.82
K (%)	0.16 ± 0.07	0.12 ± 0.05	75	0.01
Mg (%)	5.10 ^a	5.41 ± 0.04	106	14.7
Na (%)	1.60 ± 0.02	0.90 ± 0.01	56	0.02
Si (%)	22.6 ± 0.05	22.30 ± 0.03	99	19.6
Ti (%)	0.70 ± 0.06	0.68 ± 0.04	97	0.05
Ba ($\mu\text{g g}^{-1}$)	200 ^a (197 ± 12) ^b	189 ± 6	94	55
As ($\mu\text{g g}^{-1}$)	2.4 ^a	ND	ND	0.59
Be ($\mu\text{g g}^{-1}$)	NA	1.58 ± 0.86	ND	0.95
Cd ($\mu\text{g g}^{-1}$)	NA	1.10 ± 0.01	ND	1.19
Ce ($\mu\text{g g}^{-1}$)	13.3 ^a (13 ± 2) ^b	10.75 ± 0.2	81	0.47
Co ($\mu\text{g g}^{-1}$)	49.7 ^a (49 ± 3) ^b	50 ± 4.6	101	72.9
Cr ($\mu\text{g g}^{-1}$)	332 ± 9	367 ± 1	110	1170
Cs ($\mu\text{g g}^{-1}$)	(0.24 ± 0.15) ^b	ND	ND	1.19
Ga ($\mu\text{g g}^{-1}$)	17.4 ^a	16.35 ± 0.07	91	3.56
Gd ($\mu\text{g g}^{-1}$)	(3.2 ± 0.4) ^b	2.14 ± 0.08	67	< 0.2
Hf ($\mu\text{g g}^{-1}$)	1.6 ^a (1.55 ± 0.08) ^b	1.22 ± 0.01	76	< 0.2
Ho ($\mu\text{g g}^{-1}$)	(0.81 ± 0.01) ^b	0.30 ± 0.09	40	< 0.2
La ($\mu\text{g g}^{-1}$)	(5.3 ± 0.4) ^b	4.27 ± 0.01	80	0.47
Mo ($\mu\text{g g}^{-1}$)	NA	2.44 ± 0.86	ND	8.07
Nd ($\mu\text{g g}^{-1}$)	(9.6 ± 1.1) ^b	7.02 ± 0.28	79	0.47
Ni ($\mu\text{g g}^{-1}$)	(158 ± 30) ^b	162 ± 3	102	1840
Rb ($\mu\text{g g}^{-1}$)	1.91 ± 0.01	1.58 ± 0.86	83	0.95
Sb ($\mu\text{g g}^{-1}$)	(0.30 ± 0.20) ^b	1.16 ± 0.78	45	0.36
Sm ($\mu\text{g g}^{-1}$)	2.79 ^a (2.5 ± 0.2) ^b	1.89 ± 0.09	68	< 0.2
Ta ($\mu\text{g g}^{-1}$)	(0.31 ± 0.07) ^b	0.24 ± 0.01	77	< 0.2
U ($\mu\text{g g}^{-1}$)	0.37 ^a (0.31 ± 0.02) ^b	0.42 ± 0.26	114	< 0.2
V ($\mu\text{g g}^{-1}$)	250 ^a (242 ± 8) ^b	236 ± 4.9	94	154
Y ($\mu\text{g g}^{-1}$)	(19 ± 8) ^b	16.0 ± 0.35	84	2.14
Yb ($\mu\text{g g}^{-1}$)	2.09 ^a (2.05 ± 0.20) ^b	1.58 ± 0.01	76	< 0.2
Zn ($\mu\text{g g}^{-1}$)	58 ^a (84 ± 10) ^b	62.0 ± 1.41	107	48
Zr ($\mu\text{g g}^{-1}$)	(60.0 ± 1.9) ^b	46.0 ± 1.27	77	1.90

NA = Not Available.

ND = Not Determined.

^a Indicative value.

^b Consensus value reported by Gladney (22).

which is often difficult to dissolve completely.

For rock samples, alkaline fusion seems to be the most suitable procedure to obtain complete recoveries for elements such as Cr and Ni.

XRF and ICP-OES provided comparable results for a large number of elements, while ICP-MS made it possible to obtain a wider range of chemical information, including for some trace and rare earth elements. This is particularly important from a geological and environmental point of view.

Microwave acid digestion followed by ICP-OES analysis was particularly suitable for the determination of Ca, Cr, Cu, Fe, Mg, Mn, Ni, and Zn in botanical samples. The analysis of *Alyssum spp.* leaves and the calculation of the soil/plant transfer factor showed that this species is a hyperaccumulator for Ni.

Generally, the tests performed for this study suggest that for a first screening of samples, XRF is more suitable than a multielement spectroscopic technique by virtue of its reduced analytical times. On the other hand, ICP-OES and ICP-MS can perform better characterization of a wider profile of elemental concentrations, which includes both trace elements and rare earths.

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Cloud Point Extraction Preconcentration and ICP-OES Determination of Trace Chromium and Copper in Water Samples

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INTRODUCTION

Several analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) are available for the determination of trace metals with sufficient sensitivity for most applications. However, the determination of trace metal ions in natural waters is difficult due to various factors, particularly their low concentrations and matrix effects. Preconcentration and separation can solve these problems and lead to a higher confidence level and easy determination of the trace elements. There are many methods of preconcentration and separation such as liquid-liquid extraction (LLE) (1), ion-exchange techniques (2), co-precipitation (3), sorption on the various adsorbents such as chelate resin, silica gel, and activated carbon, etc. (4–7).

Separation and preconcentration based on cloud point extraction (CPE) is becoming an important and practical application in the use of surfactants in analytical chemistry (8,9). The technique is based on the property of most non-ionic surfactants in aqueous solutions to form micelles and become turbid when heated to a temperature known as the cloud point temperature. Above the cloud point, the micellar solution separates into a surfactant-rich phase of a small volume and a diluted aqueous phase, in which the surfactant concentra-

ABSTRACT

A new method is proposed for the determination of trace chromium and copper in water samples by inductively coupled plasma optical emission spectrometry after cloud point extraction (CPE). The method is based on the complexation of metal ions with 8-hydroxyquinoline in the presence of non-ionic micelles of Triton X-100. The effect of experimental conditions such as pH, concentration of chelating agent and surfactant, equilibration temperature and time on cloud point extraction was studied. Under the optimum conditions, the detection limits were 1.29 and 1.31 ng mL⁻¹ for chromium and copper, respectively, with relative standard deviations of 4.3% and 4.8%, respectively (n=11). The proposed method was applied to the determination of trace chromium and copper in water samples with satisfactory results.

tion is close to the critical micellar concentration (CMC). Any analyte solubilized in the hydrophobic core of the micelles will separate and become concentrated in the small volume of the surfactant-rich phase.

The use of preconcentration steps based on phase separation by cloud point extraction (10,11) offers a convenient alternative to more conventional extraction systems. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, economical, highly efficient, speedy, and of lower toxicity to the environment than those extractions that use organic solvents. CPE also provides results

comparable to those obtained with other separation techniques. Accordingly, any species that interacts with the micellar system, either directly (generally hydrophobic organic compounds) or after a prerequisite derivatization reaction (e.g., metal ions after reaction with a suitable hydrophobic ligand) may be extracted from the initial solution and may also be preconcentrated.

Cloud point methodology has been used to separate and preconcentrate organic compounds as a step prior to their determination in hydrodynamic analytical systems such as liquid chromatography (12,13) and capillary electrophoresis (14). The phase separation phenomenon has also been used for the extraction and preconcentration of metal ions after the formation of sparingly water-soluble complexes (15–18). U (19), Er (20), and Gd (21) were determined by spectrophotometry, Pd (22) by room temperature phosphorescence, Cu (23), Cd (24), Ni and Zn (25), Cr(III) and Cr(VI) (26) by FAAS after CPE using complexing agents.

In the present work, we report the results obtained in a study of cloud point preconcentration of chromium and copper after the formation of a complex with 8-hydroxyquinoline (8-Ox), and later analysis by inductively coupled plasma optical emission spectrometry using Triton® X-100 as the surfactant. The proposed method was applied to the determination of chromium and copper in water samples.

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EXPERIMENTAL

Instrumentation

An Optima™ 2000 DV inductively coupled plasma optical emission spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) was used. The operating conditions and the analytical wavelengths are summarized in Table I. The pH values were controlled with a Model 320-S pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, P.R. China) supplied with a combined electrode. A Model 80-2 centrifuge (Changzhou Guohua Electric Appliance Co. Ltd., Changzhou, Jiangsu, P.R. China) was used to accelerate the phase separation.

Reagents and Standard Solutions

The non-ionic surfactant Triton X-100 was obtained from Amresco and was used without further purification. Stock standard solutions of chromium and copper at a concentration of 1000 $\mu\text{g mL}^{-1}$ were obtained from the National Institute of Standards (Beijing, P. R. China). Working standard solutions were obtained by appropriate dilution of the stock standard solutions. A $1.2 \times 10^{-2} \text{ mol L}^{-1}$ solution of 8-Ox was prepared by dissolution in ethanol from the commercially available product. A stock buffer solution (0.1 mol L^{-1}) was prepared by dissolving appropriate amounts of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in doubly distilled water. All other reagents were of analytical reagent grade and all solutions were prepared in doubly distilled water. The pipettes and vessels used for trace analysis were kept in 10% nitric acid for at least 24 h and subsequently washed four times with doubly distilled water.

Procedures

For the CPE, aliquots of 10 mL of a solution containing the analytes, Triton X-100 and 8-Ox buffered at a suitable pH were kept in the ther-

TABLE I
ICP-OES Instrumental Operating Conditions and Wavelengths of Emission Lines Examined

Parameters	
RF power	1.3 kW
Plasma gas (Ar) flow rate	15 L min ⁻¹
Auxiliary gas (Ar) flow rate	1.0 L min ⁻¹
Nebulizer gas (Ar) flow rate	0.5 L min ⁻¹
Pump flow rate	0.8 mL min ⁻¹
Observation height	15 mm
Wavelength	Cr 283.6 nm; Cu 324.8 nm

mostatic bath maintained at 100°C for 25 min. Since the surfactant density is 1.37 g mL⁻¹, the surfactant-rich phase can settle through the aqueous phase. The phase separation was accelerated by centrifuging at 3000 rpm for 5 min. After cooling in an ice-bath, the surfactant-rich phase became viscous and was retained at the bottom of the tube. The aqueous phases can readily be discarded simply by inverting the tubes. In order to decrease the viscosity and facilitate sample handling prior to the ICP-OES assay, 1.0 mL 0.1 mol L⁻¹ HNO₃ was added to the surfactant-rich phase. The final solution was introduced into the nebulizer of the spectrometer by conventional aspiration.

Analysis of Water Samples

Prior to the above preconcentration procedure, all water samples were filtered through a 0.45- μm pore size membrane filter to remove suspended particulate matter and were then stored at 4°C in the dark. To a 10-mL water sample, 1.0 mL of a solution containing 3.0 g L⁻¹ Triton X-100 and $1.2 \times 10^{-2} \text{ mol L}^{-1}$ 8-Ox plus 1.0 mL of 0.1 mol L⁻¹ $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ buffer solution (pH 9.0) was added. After phase separation, 1 mL 0.1 mol L⁻¹ HNO₃ was added to the surfactant-rich phase. The samples were assayed as described in the previous section.

RESULTS AND DISCUSSION

Effect of pH

Cloud point extraction of chromium and copper was performed in different pH buffer solutions. The separation of metal ions by the CPE method involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of surfactant-rich phase, thus obtaining the desired preconcentration. The extraction yield depends on the pH at which complex formation occurs.

Figure 1 shows the effect of pH on the extraction of chromium and copper complexes. It was found that extraction was quantitative for chromium and copper in the pH range of 7.0–10.0. Hence, a middle range of pH at 9.0 was chosen for these analytes.

Effect of Buffer Concentration

The influence of buffer amount was carried out in which the other experimental variables remained constant. The results showed that above 1.0 mL of buffer solution, added to 10 mL of solution, no variation took place in the extraction yield. Thus, 1.0 mL buffer solution was added in all subsequent experiments.

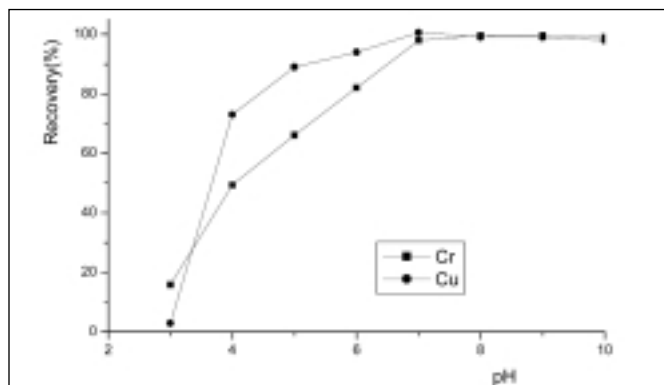


Fig. 1. Effect of pH on the extraction recovery: 50 ng mL^{-1} Cr and Cu, $1 \times 10^{-3} \text{ mol L}^{-1}$ 8-Ox, 3.0 g L^{-1} Triton X-100.

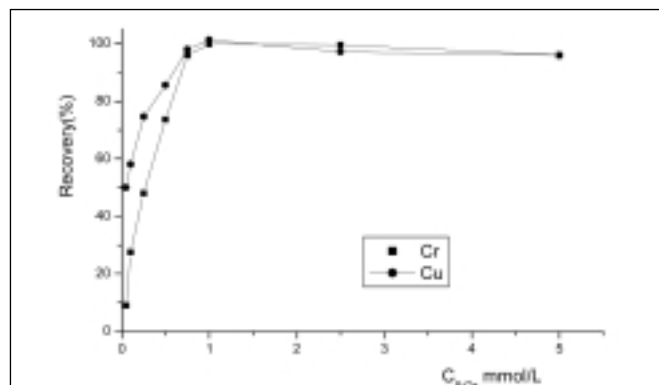


Fig. 2. Effect of 8-Ox concentration on the extraction recovery: 50 ng mL^{-1} Cr and Cu, 3.0 g L^{-1} Triton X-100, pH 9.0.

Effect of 8-Ox Concentration

For the two cations studied, 10 mL of a solution containing $0.5 \mu\text{g}$ of Cr and Cu in 3.0 g L^{-1} Triton X-100 and at a medium buffer pH of 9.0, containing various amounts of 8-Ox, were subjected to the cloud point preconcentration process. The extraction yield as a function of the concentration of the complexing agent is shown in Figure 2. The yield increases up to an 8-Ox concentration of $8 \times 10^{-4} \text{ mol L}^{-1}$ for Cr and Cu and reaches near quantitative extraction efficiency. A concentration of $1 \times 10^{-3} \text{ mol L}^{-1}$ was chosen to account for other extractable species.

Effect of Triton X-100 Concentration

A successful CPE would be that which maximizes the extraction efficiency through minimizing the phase volume ratio; thus, maximizing its concentrating factor. The variation in extraction efficiency of Cr and Cu within the Triton X-100 range of $0.1\text{--}6.0 \text{ g L}^{-1}$ was examined and the results are shown in Figure 3. Quantitative extraction was observed when the Triton X-100 concentration was above 3.0 g L^{-1} . Thus, a concentration of 3.0 g L^{-1} was chosen as the optimum surfactant concentration in order to achieve the highest possible extraction efficiency.

Effects of Equilibration Temperature and Time

It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of phases. The dependence of extraction efficiency upon equilibration temperature and time was studied with a range of $60\text{--}120^\circ\text{C}$ and $5\text{--}30 \text{ min}$, respectively. The results showed that an equilibration temperature of 100°C and an equilibration time of 25 min was adequate to achieve quantitative extraction.

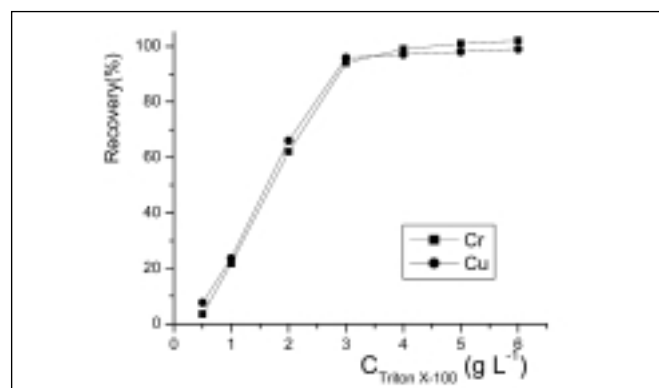


Fig. 3. Effect of Triton X-100 concentration on the extraction recovery: 50 ng mL^{-1} Cr and Cu, $1 \times 10^{-3} \text{ mol L}^{-1}$ 8-Ox, pH 9.0.

Interferences

In view of the high selectivity provided by ICP-OES, the only interferences studied were those related to the preconcentration step. Cations that may react with 8-Ox and extracted to the micelle phase were studied. The results are shown in Table II. It can be seen that the presence of major cations and anions has no obvious influence on CPE under the selected conditions.

TABLE II
Tolerance Limits of Coexisting Ions for the Studied Element Determinations

Coexisting Ions	Tolerance Limit of Ions
$\text{K}^+, \text{Na}^+, \text{Ca}^{2+}, \text{Ba}^{2+}$	1000
$\text{Mg}^{2+}, \text{Cd}^{2+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Pb}^{2+}$	100
$\text{Mn}^{2+}, \text{Zn}^{2+}$	10

Detection Limits and Precision

According to the definition of IUPAC, the detection limits (3σ) of this method were 1.29 and 1.31 ng mL⁻¹ for chromium and copper, respectively, with relative standard deviations (RSD) of 4.3% and 4.8%, respectively (n=11).

Determination of Chromium and Copper in Water Samples

The proposed method was applied to the determination of Cr and Cu in tap water and lake water. For this purpose, 10 mL of each of the samples was preconcentrated with 3.0 g L⁻¹ Triton X-100 and 1x10⁻³ mol L⁻¹ 8-Ox following the proposed method. The results are shown in Table III. For calibration purposes, the working standard solutions were subjected to the same preconcentration procedure as used for the analyte solutions.

In addition, recovery experiments for different amounts of Cr and Cu were carried out. The results given in Table III indicate that the proposed method can be reliably used for the determination of these metal ions in various waters.

CONCLUSION

Cloud point extraction offers a simple, rapid, inexpensive, and non-pollution methodology for preconcentration and separation of trace metals in aqueous solutions. Triton X-100 was chosen for the formation of the surfactant-rich phase due to its excellent physico-chemical characteristics: low CP temperature; high density of the surfactant-rich phase, which facilitates phase separation easily by centrifugation; commercial availability and relatively low price; and low toxicity. 8-Ox is a very stable and fairly selective complexing reagent. The surfactant-rich phase can be introduced into the nebulizer of an inductively coupled plasma optical emission spectrom-

TABLE III
Determination of Cr and Cu in Natural Water Samples (n = 3)

Samples	Added (ng mL ⁻¹)		Measured (ng mL ⁻¹)		Recovery (%)	
	Cr	Cu	Cr	Cu	Cr	Cu
Tap water	0	0	6.39	10.3	-	-
	10	10	16.6	20.1	102	98
	20	20	26.5	30.2	101	99.5
Lake water	0	0	8.69	3.71	-	-
	10	10	18.5	13.6	98.1	98.9
	20	20	28.8	23.8	101	100

ter by conventional aspiration after dissolving with 0.1 mol L⁻¹ HNO₃. The proposed method gives very low LODs and good RSDs, and can be applied to the determination of trace metals in various water samples.

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A Rapid Method for the Determination of Selenium in Blood and Serum by ETAAS With Zeeman Background Correction

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INTRODUCTION

Selenium is an element that belongs to group VIA of the Periodic Table of the Elements. With an atomic mass of 78.96 Daltons, it shows intermediate properties between metals and non-metals. It can be found in four states of oxidation: 0, 2⁻, 4⁺ and 6⁺. The valence of 6⁺ is the most common in inorganic selenium salts, while Se²⁻ is the most frequent for organic selenium compounds.

Selenium was discovered by Berzelius in 1817. It is not a very common element in nature; its concentration in the earth's crust scarcely reaches 0.09 mg/kg, even though in some areas it can exist in concentrations high enough to produce livestock poisoning. The main sources of exposure are industrial emission, principally from electrical, metal treatment, glass, paint and varnish production, and the chemical industries (1–3).

Selenium is an essential element to human life. A deficiency in selenium causes an endemic cardiomyopathy, the so-called Keshan syndrome, affecting mainly children and women during their child-bearing years, with symptoms such as muscular pain, striated musculature degeneracy, congestive cardiomyopathy and, finally, multifocal myocardium necrosis. Once the illness has been diagnosed, the ingestion of selenium is of no therapeutic value. There are four different types of this illness, classified by their seriousness: acute, subacute, chronic,

ABSTRACT

Selenium is an essential element to human life. Deficiency in selenium causes an endemic cardiomyopathy, also called Keshan syndrome and, in children, the Keshin-Beck disease. Therefore, selenium determination in blood, serum, and biological samples is of clinical importance.

A rapid method of analysis, based on Electrothermal Atomic Absorption Spectroscopy with Zeeman Background Correction (ETAAS), has been developed. During the method development process, the modifier concentration (palladium nitrate) has been optimized, as well as the mineralization and atomization temperatures.

A sensitive method has been obtained, with a detection limit in the samples of 0.4 µmol/L, a characteristic mass of 0.49 µmol, and a linear range of 5.07 µmol/L. Accuracy and precision determinations for the blood samples were carried out using Seronorm Trace Elements Whole Blood reference samples. An average precision and a recovery of 4.5% and 102.3%, respectively, were obtained. For serum samples, the standard addition technique was used to evaluate precision, resulting in a maximum value of 1.7%. Accuracy was calculated by participating in an interlaboratory proficiency study, conducted by Surrey University in Guilford, Surrey, United Kingdom, External Quality Assessment Scheme.

and latent. In children from five to thirteen years of age, selenium deficiency causes Keshin-Beck disease, whose main symptoms are articulation deformation and hypertrophy, and hyaline cartilage degen-

eration. Other problems related to selenium deficiency have been reported such as neutrophil bactericide capacity depletion, and reduction of 5-diiodine-ase enzyme activity (responsible for triiodothyronine production from thyroxine) (4,5).

Selenium deficiency can produce malfunction in different organs such as the brain, cardiovascular system, liver, and muscles. Epidemically, chronic deficiency has been associated to certain types of cancer, while carcinogenesis inhibitory action of the upper selenium levels has been found in some experimental models (6). Also, selenium depletion in blood has been related to some neurological degenerative illnesses similar to Alzheimer's (7).

In blood, selenium is distributed in the following way: between 40 to 45% in plasma and 55 to 60% in red blood cells, with serum and plasma concentrations approximately the same. Erythrocyte selenium regeneration is a relatively slow process that lasts more or less 120 days. In plasma it is much faster, which can be a key contributing factor for the control of selenium ingestion. In erythrocytes, most of the selenium is bound to hemoglobin and glutathione peroxidase. The activity of this enzyme represents a major part of total enzymatic blood activity, but only 3% of plasma enzymatic activity (8–10).

Selenium determination in biological samples could be of clinical importance in cases of excessive or inappropriate amounts of ingestion of this element. Most published

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studies use erythrocytes, whole blood or plasma as short-term indicators of selenium ingesta. Mainly plasma or serum samples are used in today's clinical monitoring. Acute poisoning produces nervousness, dizziness and convulsions, while chronic poisoning causes anemia, mucosae irritation, paleness, gastrointestinal problems, hepatosplenomegaly, blindness, weakness of limbs, and respiratory problems, also causing a characteristic 'garlic' odor in breath, perspiration, and urine (11).

Currently, the method of choice for selenium determination is Electrothermal Atomic Absorption Spectrometry with Zeeman Background Correction (12-14). The high volatility of selenium and its compounds in complex matrices makes the addition of a matrix modifier indispensable to fix selenium and to reach pyrolysis temperatures as high as possible, enabling the removal of the matrix without selenium loss (15).

In the past, most methods used for the determination of selenium used nickel as the matrix modifier (11). However, current methods use Pd (13,16), Pd/Mg, ascorbic acid, or hydroxylamine hydrochloride (J. Fischer, 1998) (15,17-19).

EXPERIMENTAL

Instrumentation

PerkinElmer AAnalyst™ 600 Electrothermal Atomic Absorption

TABLE I
Instrumental Operating Conditions

Wavelength	196 nm
Slit width	0.7 nm
EDL lamp current intensity	290 mA
Reading	Peak area
Integration time	3 s
Sample volume	20 µL

Spectrometer, with Longitudinal Zeeman Background Corrector and AS-800 Autosampler (PerkinElmer Life and Analytical Sciences, Shelton, CT USA).

Pyrolytically coated graphite tubes with transversely heated integrated platforms and end caps.

PerkinElmer AA WinLab™ software, Version 4.2.

PerkinElmer selenium lamp, EDL type.

Reagents

Ultrapure water (resistivity 18 MΩ x cm) purified with a Milli-Q™ system (Millipore, Bedford, MA, USA), for solution preparation.

Triton® X-100 (Merck, Darmstadt, Germany).

Nitric acid, Suprapur® grade (Merck, Darmstadt, Germany).

Selenium aqueous standard solution, 12.6 µmol/L (1 g/L) in 2% HNO₃, for atomic absorption spectroscopy (PerkinElmer).

Palladium modifier for graphite furnace, 10g/L of Pd(NO₃)₂ in 15% HNO₃ (Merck, Darmstadt, Germany).

Internal quality control samples: Nycomed (Seronorm Trace Elements Whole Blood) for blood and Nycomed (Seronorm Trace Elements Serum) for serum. In both samples, the theoretical values and acceptability ranges were indicated.

To assure measurement traceability and to assess method accuracy, the interlaboratory proficiency study of Surrey University, "External Quality Assessment Scheme," was employed.

Analytical Procedure

Experimental Conditions

Table I shows the instrumental settings used for the selenium determination in blood and serum samples.

Sample Pretreatment

Serum samples should be centrifuged for 10 minutes at 2500 rpm, while blood samples require an homogenization time of at least 20 minutes. Serum samples, blood samples, and standard solutions were diluted 1/10 (v/v) with the matrix modifier solution, and then homogenized, avoiding bubble generation during this process.

Graphite Furnace Temperature Program

Table II shows the temperature program used for selenium in graphite furnace analysis. This program has been studied in order to obtain a quick method that ensures the best accuracy and precision. Each one of the temperature ramps was evaluated with the purpose of developing a sensitive and linear method for clinical ranges and applications (20).

In ramp temperature optimization studies, elimination of the matrix effect caused by the presence of iron was taken into

TABLE II
Graphite Furnace Temperature Program

	Temp. (°C)	Ramp (s)	Time (s)	Flow (mL/min)	Gas	Reading
1	110	1	20	250	Argon	No
2	130	1	30	250	Argon	No
3	1250	5	30	250	Argon	No
4	2000	0	3	0	Argon	Yes
5	2500	1	3	250	Argon	No

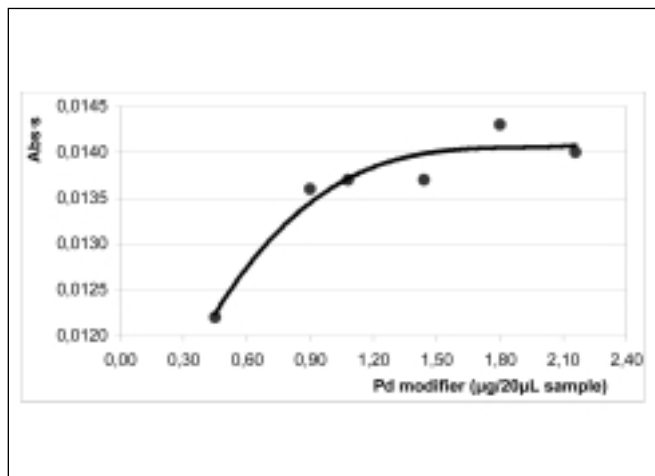


Fig. 1. Dependence of absorbance vs. modifier concentration. An aqueous standard with a final concentration of 0.125 µmol/L was used.

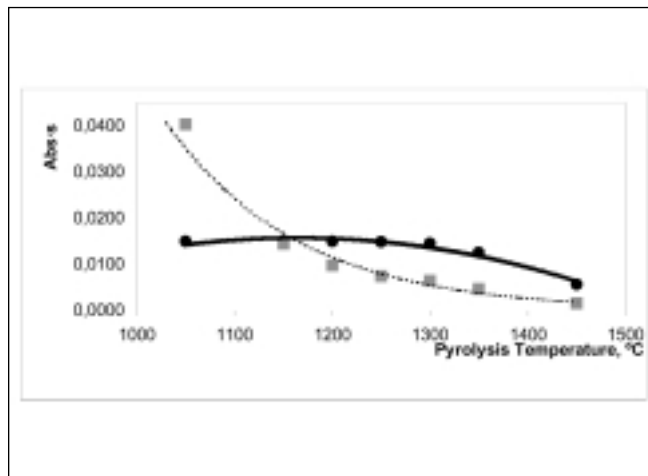


Fig. 2. Dependence of analyte absorbance (continuous line) and background absorbance (dotted line) vs. pyrolysis temperature with a fixed temperature of atomization at 2000°C. A Seronorm Trace Elements Whole Blood "level 1" sample was used.

account. This element causes lamp energy absorption at a wavelength close to that of selenium, producing a high matrix interference that even Zeeman correction cannot totally compensate for. Optimization of the analytical conditions, selection of the optimum concentration of the matrix modifier, and the temperatures of the mineralization and atomization stages are described below.

RESULTS AND DISCUSSION

Optimization of the Matrix Modifier Solution

The matrix modifier solution was prepared by adding the following amounts to a 100-mL volumetric flask: 1 mL of palladium modifier for graphite furnace analysis [10 g/L Pd(NO₃)₂ in 15% HNO₃ solution] plus 0.1 mL of Triton® X-100, then bringing the solution to volume with ultrapure water.

Figure 1 shows the dependence of absorbance with the concentration of palladium in the matrix modifier solution. The greater the palladium concentration, the higher the absorbance until reaching a concentration of 72 mg/L corresponding to 1.44 µg of palladium in each injection of sample (20 µL), where a stabilization can be observed. As a conservative approach, an optimum value of 90 mg/L (1.8 µg / 20 µL sample) was selected.

Optimization of Mineralization Stage

The pyrolysis temperature selected was based on the one that permitted the best organic matter destruction without loss of analyte, and at the same time obtained minimum matrix effect and higher signal-to-noise ratio (see Figure 2).

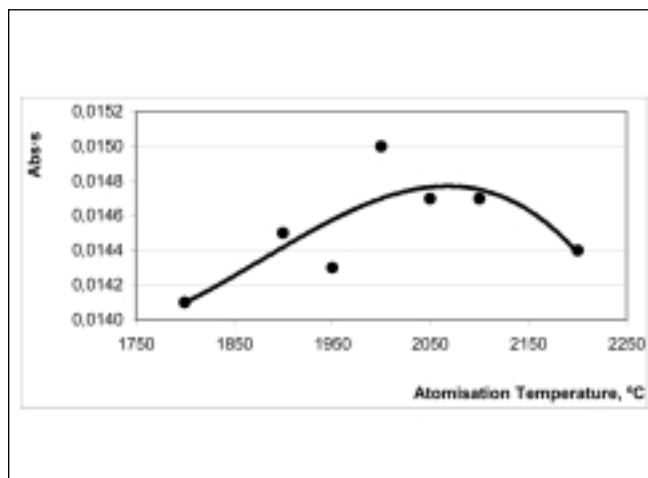


Fig. 3. Dependence of analyte absorbance vs. atomization temperature with a fixed temperature of pyrolysis at 1250°C in a Seronorm Trace Elements Whole Blood "level 1" sample.

Optimization of Atomization Stage

The temperature allowing both a higher response and a greater relation between analyte and background signals (see Figure 3) was selected.

Linearity

Calibration standards were prepared from a concentrated standard solution of 12.6 µmol/L (1g/L), initially diluted with 1% HNO₃ (v/v) in a volumetric flask until a concentration of 0.13 µmol/L (10 mg/L) was achieved. This solution is stable for at least three months in refrigerated conditions. Working standards of 0.63, 1.27, 2.53, 5.07, and 7.60 µmol/L (50, 100, 200, 400 and 600 µg/L,

respectively) were prepared by diluting this solution with ultrapure water. These working standards were diluted 1/10 (v/v) with the matrix modifier solution in order to handle the standards in the same way as the samples. As reagent blank solution, ultrapure water diluted 1/10 (v/v) with the matrix modifier solution was used.

The linearity of the calibration curve was evaluated analyzing the former working standard solutions diluted as samples in order to obtain a final range within 0 and 0.76 $\mu\text{mol/L}$ (0 to 60 $\mu\text{g/L}$). A linear response was observed up to a concentration of 0.507 $\mu\text{mol/L}$ (see Figure 4). As a working curve, the concentration range within 0 to 0.507 $\mu\text{mol/L}$ (0 to 40 $\mu\text{g/L}$) was adopted. At each point, this curve showed a maximum relative standard deviation of 3.7%.

A slope of $1.51 \cdot 10^{-4} \pm 0.01 \cdot 10^{-4}$ ($p=0.05$) was obtained. Slope variance, expressed in terms of percentage of the mean value, was 0.8%, for a probability of 95%. The correlation coefficient was 0.9992. To carry out a proportionality test, confidence limits of Y-axis intercept of the line obtained with standard solutions were calculated (95% probability). The value obtained was $7 \cdot 10^{-4} \pm 6.7 \cdot 10^{-4}$ ($n=15$, $p=0.05$), including zero.

The Matrix Effect

To prove that the measured absorbance is free of interferences from the matrix, a blood sample, spiked with increasing amounts of selenium, was analyzed. The slope of the regression line obtained after representing the measured absorbances against the added amounts was calculated.

The slope of the regression line obtained in the measurement of aqueous selenium standards ($1.51 \cdot 10^{-4} \pm 0.01 \cdot 10^{-4}$, $p=0.05$) falls inside the slope confidence interval

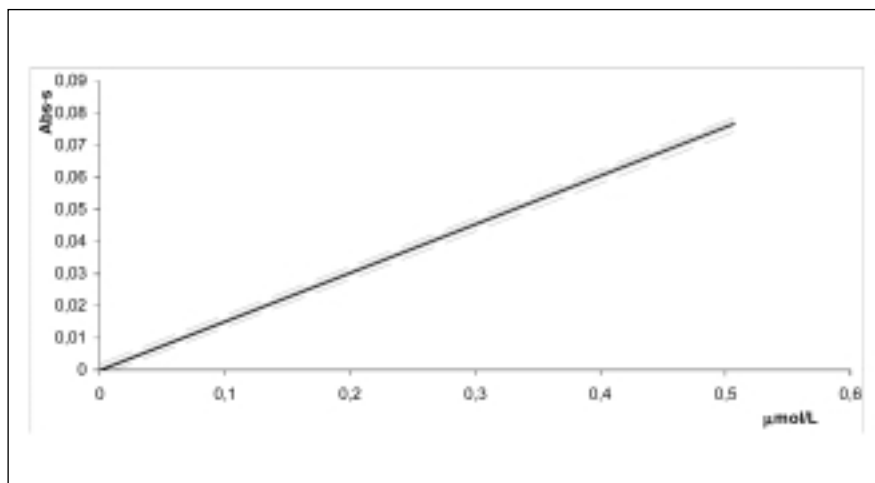


Fig. 4. Confidence and prediction intervals of the calibration curve obtained with aqueous standards for a probability of 95%.

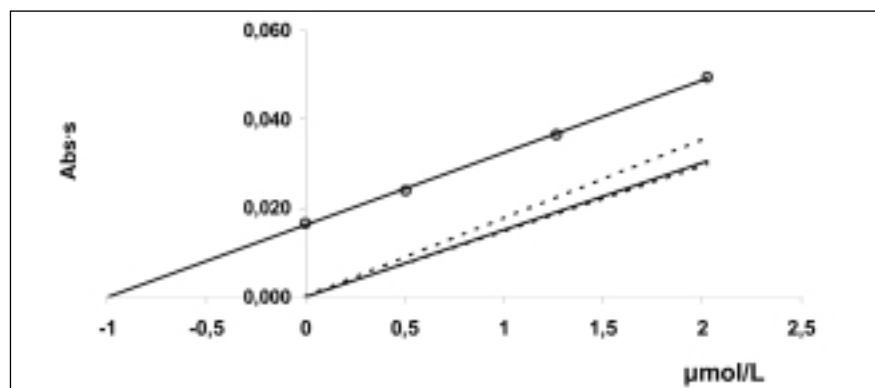


Fig. 5. Comparison between the slope of the regression line (obtained with the standard addition method, spiking selenium amounts to a blood sample) and the calibration line. The dotted lines represent the confidence interval of the calibration line (95% probability). The parallel line to that obtained with the standard addition method falls inside this interval.

of the line obtained using the standard addition method and spiked blood samples ($1.63 \cdot 10^{-4} \pm 0.14 \cdot 10^{-4}$, $p=0.05$) (see Figure 5). Thus, the sensitivity of the method does not seem to be affected by the presence of the remaining blood constituents.

In the addition tests, the recoveries obtained were 95.2, 103.2, and 106% for concentrations of 0.48, 0.76, and 1.26 $\mu\text{mol/L}$ (40, 60, and 100 $\mu\text{g/L}$), respectively.

Quantification and Detection Limits

The instrumental detection limit was calculated from the analysis of 30 reagent blank samples. A value of 0.04 $\mu\text{mol/L}$ (0.34 $\mu\text{g/L}$) was obtained for standard solutions corresponding to 0.4 $\mu\text{mol/L}$ (3.4 $\mu\text{g/L}$) for blood and serum samples when the dilution proposed in this procedure was used. The experimental characteristic mass was 495 μmol (39.1 pg), which is lower than the characteristic mass granted in the instrument manufacturer's (PerkinElmer) specifications, i.e., 570 μmol or 45 pg (21).