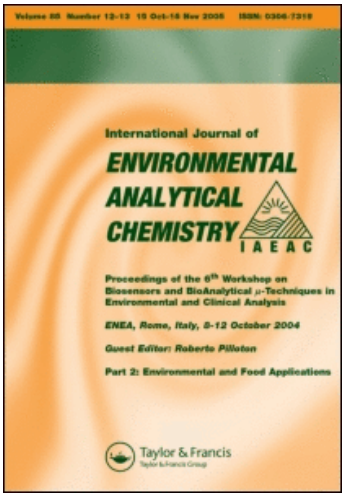

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Comparison of Different Washing, Ashing, and Digestion Methods for the Analysis of Trace Elements in Vegetation

J. Azcue[a]; A. Mudroch[a]
[a] Lakes Research Branch, National Water Research Institute, Burlington, Canada

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COMPARISON OF DIFFERENT WASHING, ASHING, AND DIGESTION METHODS FOR THE ANALYSIS OF TRACE ELEMENTS IN VEGETATION

J. AZCUE and A. MUDROCH

Lakes Research Branch, National Water Research Institute, P.O. Box 5050, Burlington, ON L7R 4A6—Canada

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Quantitative determination of the concentration of trace elements in vegetation is important in studies dealing with environmental impact, nutrition effects and geochemical exploration. However, the great diversity of sample preparation and analytical methods involved in the determination of trace elements in plant material makes the inter-comparison of the results reported in scientific literature difficult. The objective of this investigation was to compare the effects of different sample preparation, particularly cleaning, drying and digestion, used prior to the determination of the concentrations of ten trace elements (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in plant material. The results of the investigation suggested that the best cleaning method in studies of multi-element concentrations in vegetation is washing collected plant samples thoroughly with distilled water. Ashing at 550°C followed by digestion with a mixture of HNO_3:H_2SO_4 (2:1) appeared to be the best method for drying and digestion of the plant material. However, modifications of this technique can considerably improve the recoveries for some elements, such as arsenic and chromium.

KEY WORDS: Trace elements, vegetation, cleaning, drying, digestion.

INTRODUCTION

The interest in determining the concentrations of trace elements in vegetation goes back to the beginning of the nineteenth century[1]. Up to the 1920's quantitative determination of concentrations of trace elements was carried out by colorimetric and volumetric methods, with detection limits in the upper $mg.kg^{-1}$ level. Fast improvements in analytical techniques during the last decades enabled scientists to determine trace elements in plant samples at the $\mu g.kg^{-1}$ and $n \cdot kg^{-1}$ levels. However, less effort has been devoted to the optimization of the sample preparation.

A large diversity of methods for preparation of vegetation samples reported in recent literature (Table 1) makes comparison of results from different studies difficult. The diversity of the sample preparation begins with the selection of the plant organs for analysis.

Table 1 Selected methods for preparation and analysis of vegetation samples reported in the literature

Plants	*Parts analyzed*	*Cleaning procedures*	*Drying and ashing (C)*	*Chemical digestion*	*Trace elements*	*Analytical Method*	*Reference*
Mangrove	rqot-leaves	deionized	70/200	HNO3: HClO4	5 metals	AAS	2
Grasses	stem-leaves	deionized	875	HF: HNO3: HCl	Au, Pd, Pt	ICP-MS/NAA	3
Lemna minor	Whole	deionized	not mentioned	HNO3: H2SO4	Cd	AAS	4
Carrots	root-stems	deionized	90/550	HNO3	Cd, Pb	GFAAS	5
Alfalfa	root-leaves	deionized + HCl	70	HClO4: H2SO4	8 metals	AAS	6
Alfalfa-red clover	fruits-stems	deionized	40	HNO3: HClO4	Se	ICP	7
Bean	root-leave-stem	deionized	90/550	HNO3	Pb, Cd	GFAAS	8
Food spices	whole	deionized	60		14 elements	NAA	9
Spartina alteriflora	stem-leaves	deionized	60	HNO3	6 metals	AAS	10
3 plant species	leaves	deionized	freeze dried	HNO3: HClO4	Pb, Cd	AAS	11
Vegetables	edible	brush-deionized	80/450	HCl: HNO3: HF	9 metals	AAS	12
Marrow plants	root-leave-stem	distilled	90/550	HNO3	Cd, Pb	GFAAS	13
Vegetables	shoot-roots	washed	oven dried	not mentioned	Pb	AAS	14
Salt marsh plants	root-stems	distilled-dcb	80	HNO3: HClO4	Zn. Cd. Cu	AAS	15
Red maple	leaves	distilled	70	HCl	Cu, Pb	AAS	16
Food plants	leaves	distilled	not mentioned	HNO3: HClO4	Pb	ICP	17
Grasses-dwarf birch	stem-leaves	shaking-tap distilled	470/870	HNO3: HCl: HF	Au, Pt, Pd, Rh	ICP-MS	18
Forest plants	leaves-nuts-rhizo	distilled	35	HNO3	18 elements	GFAAS	19
Egg plant	root-leave-stem	distilled	90/500	HCl (6M)	Ni	AAS	20
Grasses-legumes	whole	ultrasound-distilled	air dried	HNO3: HClO4	As, Se	ICP-AES	21
Holm oak	leaves-acorns	shake-distilled	not mentioned	HNO3: HClO4	6 metals	AAS	22
Vegetables	edible	brush-water	ashed	HNO3:H2SO4:H2O2	As, Cr	GFAAS	23
Vegetables	edible	brush-water	72	HNO3:H2SO4:H2O2	Pb	AAS	24
Vegetables	leaves-roots	detergent + acid	70	HNO3	Pb, Cd, Zn	ICP	25
7 vegetables	edible	washed or peeled	not mentioned	HNO3: VO5	Cd	GFAAS	26
Lettuce-spinach	leaves-roots	Ca(NO3)2	70/450	HNO3: HCl	Cd, Zn	GFAAS	27
Reference material	—	—	—	HNO3: HCl HNO3: HF: H2O2 HNO3: HClO4	9 metals	AAS DCP-AES	28
Herbage	whole	unwashed	not mentioned	HCl: HNO3	Pb	GFAAS	29
Hay	whole	unwashed	air dried	HCl: HNO3	Pb	GFAAS	30
Trees (5 species)	leaves	unwashed	110	HNO3: HCl04	Pb	AAS	31
Grasses (3)	stem-leaves	unwashed	oven	—	Cd, Cu, Pb, Zn	AAS	32
Wild species (15)	leaves	unwashed	105	HNO3	Cd, Pb, Zn	AAS	33
Rice	root-stems	not mentioned	not mentioned	HNO3:HClO4:H2SO4	Cu	AAS	34
Foliage	leaves	not mentioned	air dried\450	H2O2: HCl	Cd, Pb	ICP-AES	35
Corn	root-leave-stem	not mentioned	40	HNO3: HClO4	Se	AF	36
Forbs, grasses	leaves-roots	not mentioned	40/50	HNO3: HCl04	Se	ICP/AF	37
Vegetables	edible	not mentioned	105	HNO3	6 metals	ICP-AES	38
Maize, barley	whole	not mentioned	not mentioned	HNO3: HClO4	8 elements	AAS	39
Grasses	whole	not mentioned	110	HNO3: H2SO4	Cd	AAS	40
Radish	stems	not mentioned	100/550	HCl (1N)	Zn	AAS	41
Rice	straw	not mentioned	—	HNO3: HClO4 (2:1)	Zn	AAS	42
Red cedar	tree core	not mentioned	—	HNO3: HClO4	Pb, Cd	ICP-AES	43
Trees	leaves	not mentioned	105/400	HCl	Cu, Pb	AAS	44
Trees (10 species)	bark	not mentioned	100/500	HCl	Zn, Cu	AAS	45
Alfalfa	stems	not mentioned	65	HNO3: HClO4	As, Se, Mo	ICP	46
Elgrass	leaves	not mentioned	80	HNO3: HCl: HF	Fe, Mn, Zn, Cu	GFAAS	47
Legum.-Grass.	stem-leaves	not mentioned	60	HNO3	Cu, Mo	ICP-AES	48
Trees (5 species)	leaves-needles	not mentioned	air dried	H2SO4: HNO3	Hg	CV-AAS	49
Azalea japonica	leaves	not mentioned	105	H2SO4: HNO3	Hg	AAS	50
Olives	leaves	not mentioned	not mentioned	tricloracetic acid	Mn	SPS	51
Eichornia crassipis	whole	not mentioned	105	HNO3: H2S04: HCl04	9 metals	AAS	52
Vegetables	leaves	not mentioned	freeze dried	HNO3: H2O2	Zn, Cd	AAS	53
Aquatic plants	whole	not mentioned	800	HNO3	Pb, Zn	AAS	54
Corn	root-stems	not mentioned	air dried	NH4OAc: NaNO3 HN03	Cd, Zn	GFAAS	55
Staghorn sumac	leaves	not mentioned	air dried	H2O2:HCl	Ni, V	ICP	56

Abbreviations:
dcb = dithionite-citrate-bicarbonate
GFAAS = graphite furnace AAS
AF = atomic fluorescnce
ICP = inductively coupled plasma
CV-AAS = cold vapor AAS
SPS = solid phase spectrophotometr
AAS = atomic absorption spectrometry
NAA = neutron activation
DCP-AES = direct current plasma atomic emission

Frequently, the trace element concentrations in vegetation are expressed without specifying analyzed plant organs (Table 1). However, it is well known that different plant organs have different capacity to accumulate trace elements[57].

The most common cleaning procedures described in the literature are washing collected plants with: a) water (tap, deionized, or distilled), b) detergent, and c) diluted acids. In studies of ingestion of trace elements by animals, vegetation samples are not washed prior to the analysis to simulate the consumption by the animals. However, in other studies the method used for cleaning the plants prior to the analysis is usually not described in reports and scientific journals. The cleaning procedure is extremely important, particularly when analyzing roots. Generally, the concentrations of trace elements in soils are up to three orders of magnitude greater than those in the plants. Considering the possibility of contamination of collected plant material, mainly with soil and dust, the omission of the description of the cleaning procedure prevents comparison among different studies of accumulation of trace elements by plants.

Common methods for sample preparation reported in the literature were tested to determine the most efficient, safe, cost effective, and least time consuming technique. The test focused on three main aspects of sample preparation: (i) sample washing to minimize external contamination from soil and dust; (ii) sample drying, which usually depends on the availability of equipment and/or cost, for example oven, furnace or freeze dryer; and (iii) sample digestion, which can be carried out by simple but time consuming methods, such as digestion by acids on a hot plate, in comparison with procedures requiring costly equipment, such as microwave oven. The efficiency of the digestion method will depend on its ability to create a balance between the most complete decomposition of the sample while minimizing reactivity/adsorption of the trace elements of interest which will prevent their quantitative determination.

EXPERIMENTAL

In this study, to maximize the continuity between samples, only one plant species, *Equisetum variegatum*, was used throughout the experiment. Large quantities of whole plants were collected within an area of 5×5 m^2 at a waste disposal site at an abandoned mine and smelter at Deloro, Ontario, in October, 1992. *Equisetum variegatum* is a vascular plant, up to 40 cm tall, that reproduces by spores. The stems and branches of the evergreen *Equisetum variegatum* are photosynthetic. For this study, the plant roots were discarded due to the difficulty of complete removal of adhering soil material similar to that reported by Freitas *et al.*[58]. The objective for collecting the samples at a mine waste site was to assure elevated levels of different trace elements in the plants. It was expected that any further decrease in the trace element concentrations caused by the different sample preparation techniques used in the experiment would remain above the detection limits and could be analytically quantified. Collected plants were placed in plastic bags and transported immediately to the laboratory. To obtain maximum homogenization, plants were cut into small pieces which were thoroughly mixed. The mixture was divided into three portions of similar weight. Each portion was used in different sample preparation and analytical procedures shown in Figure 1.

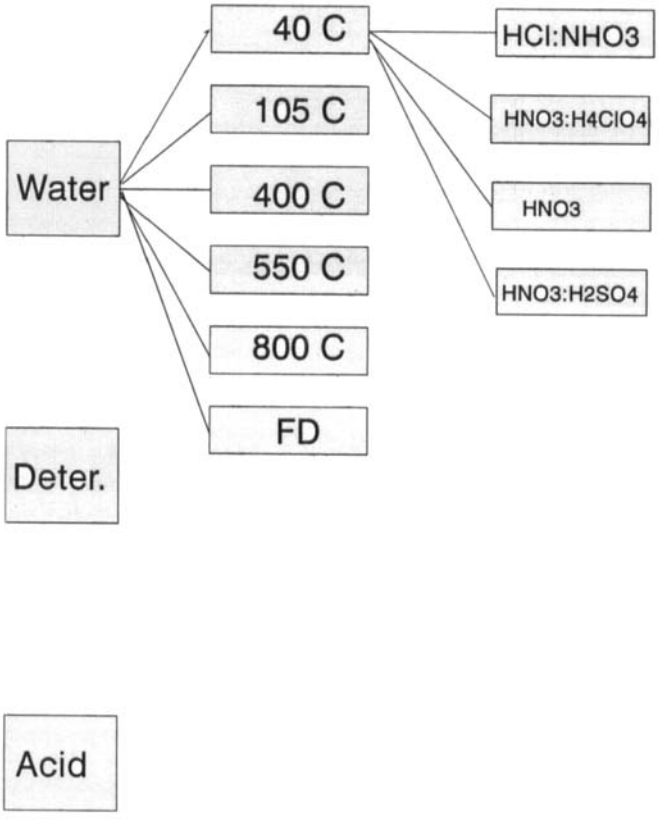

Figure 1 Schematic representation of the different analytical procedures followed in this report

Each of the three plant materials was washed by one of the three washing solutions: i) water (W); ii) detergent (Alconox 1%) (D); and iii) diluted acid solution (1% HCl) (A). Samples were soaked in washing media for approximately 3 hours, followed by ten repeated rinses with doubly distilled water. Care was taken not to rub the plant material during any of the washing steps. Blanks were collected from the final rinse and analyzed simultaneously with the samples to detect any possible remaining contamination or leaching of soluble elements during the washing. Titanium and Zr, although present in the soils, are known not to be assimilated by plants to any great extent[59]. Consequently, the presence of these elements was used as a control of the cleaning procedure.

After washing, each sample was subdivided into six subsamples to test the effects of six different drying methods on the determination of trace elements. The drying methods were: i) low temperature in an oven at 40°C, (LO), ii) high temperature in an oven at 105°C, (HO), iii) low temperature ashing in a furnace at 400°C, (LA), iv) medium temperature ashing at 550°C, (MA), v) high temperature ashing at 800°C, (HA), and vi) freeze drying at – 60°C (FD) (Figure 1). The LO and HO subsamples were dried in the oven to a constant weight.

The ashing of the subsamples LA, MA, and HA was carried out for 12 hours. The dried samples were homogenized and pulverized to approximately 177 μm in a Wiley mill equipped with stainless steel blades. The ashed samples were pulverized by hand in a porcelain mortar with a porcelain pestle. This pulverization method was found most suitable for the consistency and electrostatic properties of the ashed material. Each sample was thoroughly mixed and then further divided into four subsamples. The digestion of the four subsamples was carried out by the following methods: four different methods: 1) acid digestion with concentrated HNO_3 [N]; 2) digestion with *aqua regia* ($HCl:HNO_3$ 1:3) [H]; 3) evaporation with acid mixtures ($HNO_3:H_2SO_4$ 2:1) [S]; and 4) ($HNO_3:H_4ClO_4$ 3:2) [P]. When using perchloric acid, great care was taken to ensure the solution was not heated to complete dryness because of the explosive nature of perchloric acid as metal perchlorates.

The digestion acids were added to Teflon beakers containing 0·2–0·5g samples with subsequent mixing. All samples were allowed to degas at room temperature overnight to prevent a vigorous reaction during heating. The Teflon beakers were covered with Teflon lids to protect the sample from contamination while allowing gas to escape. The samples in $HNO_3:H_2SO_4$ mixture [S] and $HNO_3:HClO_4$mixture [P] were digested on hot plates at a maximum temperature of 200°C. The digestion with the mixture containing $HClO_4$ was carried out in a fume hood designed for $HClO_4$ digestions. The digestion was completed with the appearance of white fumes and the sample volume was reduced to approximately 0.5 mL which was usually within 6 hr. Water was added to those samples whose volume was reduced on hotplates to a final volume of approximately 10 mL. The samples in HNO_3 [N] and *aqua regia* [H] were digested in a microwave oven (Floyd, Inc. Model RMS 150). The microwave digestions followed a four stage scheme: a) 3 minutes at 30 psi, b) 5 minutes at 50 psi, c) 5 minutes at 100 psi, and d) 5 minutes at 130 psi.

Determination of the ten trace elements (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) was carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using an Jobin Yvon Model 74. The standard solutions consisted of high purity concentrations of 0.5 and 5mg.L-1 of the ten trace elements in a solution of 2% HNO_3 (Delta Scientific Laboratory Products, Canada). Instrumental conditions and information regarding the trace elements analyzed are listed in Table 2. To avoid clogging problems with the ICP-AES, all samples were filtered using 0.4 μm Nuclepore Polycarbonate filters. The detection limits, defined as that concentration equivalent to 3x standard deviation (n=19) obtained from all the blank samples are summarized in Table 2. Certified reference material of the National Institute of Standards and Technology [apple leaves—SRM 1515] was used in the quality control. Subsamples of the certified reference material were digested with the same mixtures used for the samples. Statistical calculations were performed using the Statistical Analysis System[60].

RESULTS AND DISCUSSION

Statistical significance ($p < 0.005$) for the concentration of each trace element in the plant samples was assessed with analysis of variance using the washing media, the temperature of drying/ashing and the type of digestion as class variables. Once the interaction effects

Table 2 Analytical conditions and detection limits of the ten elements analyzed using a Jobin Yvon ICP-AES.

Element	*Wavelength*	*Potential Interferences*	*Boiling Point (°C)*	*Detection Limits ($\mu g.L^{-1}$)*
As	193.759	Al, Fe, V	613	10.1
Zn	213.856	Al, Cu, Fe, Ni, Ti, V	907	2.0
Pb	220.353	Al, Cr, Fe	1740	15.5
Cd	226.502	Fe, Ni	765	7.2
Co	228.616	Cr, Fe, Ni, Ti	2870	6.1
Ni	231.604	Fe	2732	12.4
Mn	257.610	Al, Cr, Fe, V	1962	1.8
Fe	259.940	Mn, Ti	2750	4.9
Cr	267.716	Fe, Mn, V	2672	5.1
Cu	324.754	Ca, Cr, Fe, Ti	2567	3.6

were accounted for and an independent significance was obtained, the results were analyzed within each class with one way ANOVA and Duncan Test.

Washing of the plants

Table 3 summarized the concentrations of the trace elements in plant material washed by the three different solutions. The washing of the plants, with the three media (water, detergent and acid) tested in this study, has proved to be an indispensable step in the determination of the trace elements. The lowest concentrations for all the elements with the exception of Co, Mn, and Pb were obtained when the plants were washed with either detergent or water. The negligible values of the blanks from the final rinse solutions and the unlikeness of chemical leaching of the trace elements by washing with detergent or water led us to assume that the lowest concentrations represented a better cleaning method. The results for Co, Mn, and Pb were an exception with concentrations considerably lower when the plants were washed with acid solution, suggesting leaching of these elements by this washing method. This observation is in agreement with the recommendations by Richards[61] to minimize the exposure of plant parts to the cleaning solutions to prevent possible losses of water-soluble Mn. Small individual variations were observed between the results obtained after washing with detergent and plain water. However, the variations were not significantly different. Although washing with water and detergent yield similar results in our experiments (except for Fe), we recommend use of water to minimize any potential risk of contamination by using a detergent.

Drying/ashing of the plants

The effects of temperature in drying the plants prior to the acid digestion are summarized in Table 4. With the exception of Ni the choice of drying temperature affected significantly

Table 3 Concentration of trace elements (median and range) in plant material washed by three different solutions. All results expressed as $\mu g.g^{-1}$. Medias with same letter are not statistically different ($p < 0.005$).

	Water	*Detergent*	*Acid*
As	2.297 (a-b) (0.993–3.35)	1.462 (b) (0.159–3.74)	2.39 (a) (0.651–6.65)
Cd	0.061 (a) (0.013–0.211)	0.055 (a) (0.003–0.116)	0.061 (a) (0.003–0.257)
Co	0.296 (a) (0.01–0.903)	0.298 (a) (0.009–0.134)	0.134 (b) (0.007–0.354)
Cr	0.301 (a) (0.108–1.77)	0.34 (a) (0.011–0.924)	0.84 (a) (0.087–2.81)
Cu	0.246 (a) (0.094–0.894)	0.166 (a) (0.077–0.399)	0.342 (b) (0.048–1.39)
Fe	9.96 (a-b) (3.93–24.6)	4.94 (b) (0.354–13.7)	14.1 (a) (1.19–30.8)
Mn	2.15 (a) (0.563–5.47)	1.5 ± 0.95 (a) (0.134–4.24)	0.553 ± 0.35 (b) (0.147–1.4)
Ni	0.238 (a) (0.023–0.631)	0.258 (a) (0.048–0.529)	0.38 (a) (0.008–1.19)
Pb	0.314 (a-b) (0.01–0.756)	0.398 (a) (0.084–0.812)	0.281 (b) (0.001–0.672)
Zn	0.828 (a) (0.19–2.07)	0.527 (a) (0.117–1.43)	0.658 (a) (0.113–2.77)

the determination of the trace elements. Ashing has shown to be the most adequate method for all the elements with the exception of Cr. This indicates that in *Equisetum variegatum* the trace elements are present mainly as inorganic compounds with negligible volatilization at the ashing temperatures used in this study. Volatilization or adsorption on the walls of the container may be a problem in dry ashing. When the plants were ashed at a high temperature (800°C) considerable loss of As, Cd, Cu and Fe was observed. Volatilization of As was observed even when ashed at 550°C. The results indicated that ashing even at relatively low temperatures removes the organic matter and, consequently, facilitates the digestion procedure. Ashing (500–550°C) with addition of ultrapure H_2SO_4 or K_2SO_4 has been previously recommended for Pb determination in foods[62]. Hall *et al.*[3] found no losses of Au during the ashing of vegetation samples at temperature as high as 875°C. The same authors concluded that ashing concentrates the elements to levels well above the detection limits and hence reduces analytical noise and allows for a much greater original sample weight to be analyzed, therefore greatly improving the representativeness of the results.

A decrease in the concentrations of the trace elements was generally observed when the samples were freeze-dried. As observed by Fourie and Peisach[63] volatile trace element compounds, including those with relatively low vapour pressure, will be removed during freeze-drying. The best results for the determination of Cr were obtained when the samples were freeze-dried or dried at lower temperatures, such as up to 105°C. Chromium was the most sensitive element to volatilization with considerable losses at low temperature ashing

Table 4 Concentration of trace elements (median and range) in plant material dried at six different temperatures. All results expressed as $\mu g.g^{-1}$. Medias with same letter are not statistically different ($p < 0.005$).

	- 60°C	*40°C*	*105°C*	*400°C*	*550°C*	*800°C*
As	2.08 (b) 0.742–4.0	1.62 (b) 0.159–3.35	1.77 (b) 0.714–4.46	3.46 (a) 1.17–6.16	2.77 (a-b) 1.16–6.65	2.31 (b) 1.89–4.19
Cd	0.049 (b-c) 0.011–0.185	0.049 (c) 0.037–0.074	0.041 (c) 0.003–0.088	0.063 (a-b-c) 0.026–0.257	0.093 (a) 0.006–0.211	0.069 (a-b) 0.004–0.116
Co	0.175 (b) 0.007–0.255	0.193 (b) 0.01–0.345	0.180 (b) 0.012–0.317	0.414 (a) 0.166–0.903	0.456 (a) 0.019–1.34	0.427 (a) 0.009–0.647
Cr	1.27 (a) 0.111–2.13	0.702 (b) 0.166–1.97	0.865 (b) 0.188–2.81	0.310 (c) 0.082–2.21	0.213 (c) 0.011–0.571	0.106 c 0.073–0.111
Cu	0.299 (c-d) 0.055–0.385	0.211 (d) 0.117–0.381	0.163 (d) 0.099–0.277	0.338 (a-b) 0.107–1.39	0.426 (a) 0.130–0.894	0.307 (b-c) 0.048–0.235
Fe	12.1 (b-c) 5.9–17.0	7.4 (c) 0.354–19.8	12.7 (b-c) 2.57–16.6	17.6 (a-b) 2.43–22.4	18.6 (a) 1.77–24.6	13.2 (a-b-c) 1.55–30.8
Mn	1.21 (b) 0.134–2.39	1.129 (b) 0.236–2.4	1.09 (b) 0.173–2.40	1.42 (a) 0.236–5.47	2.29 (a) 0.147–4.01	1.53 (a) 0.215–3.67
Ni	0.344 (a) 0.023–0.705	0.432 (a) 0.009–1.19	0.35 (a) 0.104–0.984	0.22 (a) 0.058–0.410	0.199 (a) 0.008–0.518	0.159 (a) 0.025–0.323
Pb	0.388 (a-b) 0.064–0.609	0.329 (b) 0.241–0.478	0.265 (b) 0.038–0.50	0.356 (a-b) 0.027–0.812	0.375 (a-b) 0.01–0.679	0.396 (a) 0.122–0.52
Zn	0.535 (d) 0.113–12.2	0.635 (b-c-d) 0.123–1.12	0.556 (c-d) 0.151–2.77	0.734 (a-b-c) 0.603–1.38	0.765 (a-b) 0.267–1.9	0.822 (a) 0.191–1.83

(400°C). The results suggest that Cr in the *Equisetum variegatum* was mainly associated with organic compounds, which became volatilized under the drying conditions.

Digestion of plant material

The assumption that any mixture of concentrated acids, such as HNO_3, HCl, H_2SO_4, $HClO_4$ or HF, provides a total solubilization of trace elements in the vegetation was not proved by our experiments. Factors, such as the percentage of organic matter or the formation of compounds which are not completely soluble in the acid used in the extraction may affect the degree of trace elements recovery[64]. Reactions of the acids with some of the trace elements can form volatile compounds or complexes, giving artificially low concentrations of the elements of interest[61]. The accuracy of the digestion of plant material by the different acid mixtures was assessed by the analysis of the certified reference material (Table 5). We were not able to calculate recoveries of As, Cd, and Pb in the reference material because their certified levels were to close to the detection limit of the analytical instrument employed in this study. As shown in Table 5, the use of concentrated HNO_3 resulted in a poor recovery of Fe. On the other hand, using the HNO_3:$HClO_4$ mixture recoveries were > 110% for all the elements. Possible interferences of the $HClO_4$ during the measurement step may explain the high recoveries of the reference material and samples. The concentrations of all trace elements determined after digestion by either *aqua regia* or HNO_3:H_2SO_4 mixture were in

Table 5 Mean recovery of trace elements in cetrified reference material of the National Institute of Standards and Technology after digestion with four different acid mixtures. Average of six replicates of apple leaves (SRM-1515) in $\mu g.g^{-1}$.

	Al	Cu	Fe	Mn	Zn
SRM-1515	286 ± 9	5.64 ± 0.24	83 ± 5	54 ± 3	12.5 ± 0.3
Nitric					
mean ± s.d.	225 ± 20	6 ± 0.8	57 ± 8.6	50 ± 3.7	13 ± 1.1
recovery (%)	79	115	69	92	104
Aqua regia					
mean ± s.d.	277 ± 15	7 ± 0.6	69 ± 8.5	56 ± 6.4	13 ± 1.5
recovery (%)	97	124	83	104	106
HNO_3:H_2SO_4					
mean ± s.d.	280 ± 6	6.16 ± 0.5	77 ± 2.1	56 ± 1.9	13 ± 0.9
recovery (%)	98	109	93	104	107
HNO_3: $HClO_4$					
mean ± s.d.	329 ± 30	6.62 ± 0.7	97 ± 11.2	60 ± 5.3	14 ± 1.7
recovery (%)	115	117	117	111	113

good agreement with the values of the certified reference material. Table 6 summarizes the effects of the four acid mixtures in the digestion of the vegetation material.

In a comparison of determination of concentrations of four metals in food samples Cabanis *et al.*[65] showed that the reagents used to digest the matrix played an even more

Table 6 Concentration of trace elements (median and range) in plant material digested by four different methods. All results expressed as $\mu g.g^{-1}$. Medias withe same letter are not statistically different ($p < 0.005$).

	HNO_3:H_2SO_4	HNO_3: $HClO_4$	HNO_3	HCl: HNO_3
As	2.83 a (1.14–5.95)	2.799 a (1.17–6.65)	1.67 a (0.159–3.11)	1.96 a (1.04–4.46)
Cd	0.066 a-b (0.033–0.11)	0.087 a (0.033–0.257)	0.042 b (0.003–0.127)	0.033 b (0.006–0.073)
Co	0.399 a (0.128–1.17)	0.402 a (0.152–1.34)	0.122 b (0.007–0.393)	0.146 b (0.01–0.458)
Cr	0.82 a (0.073–2.21)	0.72 a (0.111–2.81)	0.56 a (0.082–1.97)	0.55 a (0.01–1.55)
Cu	0.224 a (0.077–0.613)	0.278 a (0.120–0.894)	0.219 a (0.055–0.663)	0.284 a (0.048–1.39)
Fe	12.9 a (1.19–30.8)	9.05 a (0.354–12.9)	5.76 a (2.4–16.6)	11.O a (1.77–24.6)
Mn	1.62 a (0.424–3.36)	1.86 a (0.215–5.47)	0.98 a (0.134–3.61)	1.28 a (0.236–4.01)
Ni	0.368 b (0.094–0.984)	0.413 a (0.164–1.19)	0.136 c (0.009–0.323)	0.193 c (0.008–0.553)
Pb	0.373 a-b (0.124–0.610)	0.516 a (0.274–0.812)	0.105 b (0.001–0.253)	0.258 b (0.03–0.609)
Zn	0.647 b (0.39–1.99)	1.37 a (0.52–2.7)	0.371 b (0.113–0.986)	0.455 b (0.257–0.90)

important role than the nature of the food material. The results obtained in this study indicated that the choice of the digestion mixture did not lead to significantly different results for As, Cr, Cu, Fe and Mn (Table 6). The amount of Ni and Zn solubilized from the vegetation was significantly greater when the [P] mixture (HNO_3:$HClO_4$ 3:2) was employed. For all other analyzed elements similar recoveries were obtained using both digestion mixtures (HNO_3:$HClO_4$ or HNO_3:H_2SO_4). Consequently, the HNO3:H_2SO_4 mixture is preferred for the digestion, mainly due to the technical difficulties in using perchloric acid, such as the need of a specifically designed fume hood and risk of explosion.

Some of the variations observed in this study may be explained by analytical problems. For instance, the presence of sulphuric acid in the matrix has been proved to markedly suppress the absorbance signal for lead[66]. However, in a comparison of digestion methods for trace metal determination in fish, Kakulu *et al.*[67] concluded that the analytical differences between AAS and ICP-AES was < 10% for the metals studied. It should be stressed that the conclusions on the ashing step are integrated with the measurement technique.

CONCLUSIONS

Several variables, such as the preferential uptake of trace elements by some plant species and by their organs were not taken into account in the present study. Some of these variables may affect the efficiency of the method used for preparation and digestion of the plant material in this study. Kovalevskii and Kovalevska[68] studied the uptake of Au in organs of 194 plant species and classified them in four significantly different groups according to their usefulness in biogeochemical prospecting. There is a lack of similar information for other trace elements. Consequently, the feasibility of any method should also be studied regarding the plant organs and plant species analyzed. Based on the results of this study, similar experiences with different chemical mixtures, such as HF and H_2O_2, and other trace elements, such as Au, Hg, and Se, are being carried out in our laboratory.

There is no universal dissolution method, but from the results of our experiment washing the plant material thoroughly with water with subsequent ashing at 550°C and digesting with a mixture of HNO_3:H_2SO_4 (2:1) appeared to be a suitable method for quantitative determination of trace elements in vegetation. However, some trace elements require slight modifications of the proposed methodology. For example, As and Cr require lower ashing temperatures to avoid losses by volatilization. Special care has to be devoted to the cleaning procedure prior to the analysis of plant roots. Alternative quality controls, such as the quantification of Zr and Ti in the plant samples, should be implemented to detect potential contamination of the plant by soil or sediment particles. The results obtained in this study allowed us to conclude that the description of the following: a) plant species and plant organ analyzed, b) cleaning method, c) drying or ashing temperature, d) digestion, and e) analytical technique, should be required in all the scientific publications dealing with the quantitative determination of trace elements in vegetation. Further, an effort should be made to unify the methods employed in the preparation and digestion of the plant material prior to analyses. This will enable comparison of results of different studies of uptake of trace elements by identical plants collected from different environments.

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