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Comparison of Different Methods for the Digestion and  
Analysis of Trace Elements in Vegetables

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

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ABSTRACT

Quantitative determination of the concentrations 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 used in the determination of trace elements in different plant material makes the inter-comparison of 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  $\text{HNO}_3:\text{H}_2\text{SO}_4$  (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 recovery of some elements, such as arsenic and chromium.

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

## INTRODUCTION

The interest in determining the concentration of trace elements in vegetation goes back to the beginning of the nineteenth century<sup>1</sup>. 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  $\text{mg.kg}^{-1}$  level. Fast improvements in analytical techniques during the last decades enabled scientists to determine trace elements in plant samples at the  $\mu\text{g.kg}^{-1}$  and  $\text{ng.kg}^{-1}$  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 scientific 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. 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<sup>57</sup>.

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 soil 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 different 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 x 5 m<sup>2</sup> 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, reproducing 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. Freitas et al.<sup>58</sup> reported similar difficulties with removal of soil particles. 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 the concentration of trace elements in the samples would remain above the detection limit and could be 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.

Each of the three plant material portions 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 the 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 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<sup>59</sup>. 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  $\mu\text{m}$  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 sample was carried out by the following methods: 1) acid digestion with concentrated  $\text{HNO}_3$  [N]; 2) digestion with aqua regia ( $\text{HCl}:\text{HNO}_3$  1:3) [H]; 3) evaporation with acid mixtures ( $\text{HNO}_3:\text{H}_2\text{SO}_4$  2:1) [S]; and 4) ( $\text{HNO}_3:\text{HClO}_4$  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 0.2-0.5 g 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  $\text{HNO}_3:\text{H}_2\text{SO}_4$  mixture [S] and  $\text{HNO}_3:\text{HClO}_4$  mixture [P] were digested on hot plates at a maximum temperature of  $200^\circ\text{C}$ . The digestion with the mixture containing  $\text{HClO}_4$  was carried out in a fume hood devoted exclusively to  $\text{HClO}_4$  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 hot plates to a final volume of 10 mL. The samples in  $\text{HNO}_3$  [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. To avoid clogging problems with the ICP-AES, all samples were filtered using 0.4  $\mu\text{m}$  Nuclepore Polycarbonate filters. Certified reference materials 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<sup>60</sup>.

## 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 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

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



and Mn 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 unlikeliness 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 and Mn 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 recommendation by Richards<sup>61</sup> to minimize the exposure of plant parts to the cleansing solution 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, 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 2. With the exception of Ni the choice of drying temperature significantly affected 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 varigatum* 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 during ashing at 500°C (Figure 2). 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<sub>2</sub>SO<sub>4</sub> or K<sub>2</sub>SO<sub>4</sub> has been previously recommended for Pb determination in foods<sup>62</sup>. Hall et al.<sup>3</sup> 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.

With the exception of Cr, a significant decrease in the concentrations of the trace elements was observed when the samples were freeze-dried. As observed by Fourie and Peisach<sup>63</sup> 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 100°C, in the oven. Chromium was the most sensitive element to volatilization with considerable losses at low temperature ashing at 400°C. The results suggests 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  $\text{HNO}_3$ ,  $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{HClO}_4$  or  $\text{HF}$ , provides a total solubilization of trace elements in the vegetation was not proved by our experiments. Factors, such as the percentage of organic matter in digested samples 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<sup>64</sup>. 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<sup>61</sup>. The accuracy of the digestion of plant material by the different acid mixtures was assessed by the analysis of the certified reference material (Table 3). We were not able to calculate recoveries of As, Cd, and Pb in the reference material because their certified values were close to the detection limit of the analytical instrument employed in this study. As shown in Table 3, the use of concentrated  $\text{HNO}_3$  resulted in a poor recovery of Fe. On the other hand, using the  $\text{HNO}_3:\text{HClO}_4$  mixture recoveries were >110% for all determined elements. The concentrations of all trace elements determined after digestion by either aqua regia or  $\text{HNO}_3:\text{H}_2\text{SO}_4$  mixture were in a good agreement with the values of the certified reference material. Table 4 summarizes the recommended acid mixtures for digestion of the vegetation material.

In a comparison of determination of concentrations of four metals in food samples Cabanis et al.<sup>65</sup> showed that the reagents used to digest the food matrix played a more important role than the nature of the food material. This study indicated that the choice of the digestion mixture lead to significantly different results for all analyzed trace elements with the exception of As, Cu and Mn. The amount of Ni and Zn solubilized from the vegetation was significantly greater when the [P] mixture ( $\text{HNO}_3:\text{HClO}_4$  3:2) was employed. For all other analyzed elements similar recoveries were obtained using both digestion mixtures ( $\text{HNO}_3:\text{HClO}_4$  or  $\text{HNO}_3:\text{H}_2\text{SO}_4$ ). Consequently, the  $\text{HNO}_3:\text{H}_2\text{SO}_4$  mixture is preferred for the digestion, mainly due to the technical difficulties in using perchloric acid, such as a need of a specially designed fume hood and risk of explosion.

Some of the variations observed in this study may be explained by analytical problems. For example, the presence of sulphuric acid in the sample matrix has been proved to markedly suppress the absorbance signal for lead<sup>66</sup>. However, in a comparison of digestion methods for trace metal determination in fish, Kakulu et al.<sup>67</sup> concluded that the analytical differences between AAS and ICP-AES was <10% for the metals studied.

## CONCLUSIONS

Several variables, such as the preferential uptake of trace elements by some plant species and by their organs that 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 Kovalevskaya<sup>68</sup> studied the uptake of Au in organs of 194 plant species and classified them into 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 species analyzed. Based on the results of this study, similar experiments with different acid and reagent mixtures, such as HF and H<sub>2</sub>O<sub>2</sub>, and other trace elements, such as Au, Hg, and Se, are presently being carried out in our laboratory.

From results of our experiment on the sample preparation and digestion of plant, washing the plant material thoroughly with water with subsequent ashing at 550°C and digesting with a mixture of HNO<sub>3</sub>:H<sub>2</sub>SO<sub>4</sub> (2:1) appeared to be the suitable methods 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 control, 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 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 the 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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**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	root-leaves	deionized	70 / 200	HNO <sub>3</sub> : HClO <sub>4</sub>	5 metals	AAS	2
Grasses	stem-leaves	deionized	875	HF : HNO <sub>3</sub> : HCl	Au, Pd, Pt	ICP-MS/NAA	3
<i>Lemna minor</i>	Whole	deionized	not mentioned	HNO <sub>3</sub> : H <sub>2</sub> SO <sub>4</sub>	Cd	AAS	4
Carrots	root-stems	deionized	90/550	HNO <sub>3</sub>	Cd, Pb	GFAAS	5
Alfalfa	root-leaves	deionized+HCl	70	HClO <sub>4</sub> : H <sub>2</sub> SO <sub>4</sub>	8 metals	AAS	6
Alfalfa-red clover	fruits-stems	deionized	40	HNO <sub>3</sub> : HClO <sub>4</sub>	Se	ICP	7
Bean	root-leave-stem	deionized	90/550	HNO <sub>3</sub>	Pb, Cd	GFAAS	8
Food spices	whole	deionized	60	—	14 elements	NAA	9
<i>Spartina alterniflora</i>	stem-leaves	deionized	60	HNO <sub>3</sub>	6 metals	AAS	10
3 plant species	leaves	deionized	freeze dried	HNO <sub>3</sub> : HClO <sub>4</sub>	Pb, Cd	AAS	11
Vegetables	edible	brush-deionized	80 / 450	HCl : HNO <sub>3</sub> : HF	9 metals	AAS	12
Marrow plants	root-leave-stem	distilled	90 / 550	HNO <sub>3</sub>	Cd, Pb	GFAAS	13
Vegetables	shoot-roots	washed	oven dried	not mentioned	Pb	AAS	14
Salt marsh plants	root-stems	distilled-dcb	80	HNO <sub>3</sub> : HClO <sub>4</sub>	Zn, Cd, Cu	AAS	15
Red maple	leaves	distilled	70	HCl	Cu, Pb	AAS	16
Food plants	leaves	distilled	not mentioned	HNO <sub>3</sub> : HClO <sub>4</sub>	Pb	ICP	17
Grasses-dwarf birch	stem-leaves	shaking-tap distilled	470 / 870	HNO <sub>3</sub> : HCl : HF	Au, Pt, Pd, Rh	ICP-MS	18
Forest plants	leaves-nuts-rhizo	distilled	35	HNO <sub>3</sub>	18 elements	GFAAS	19
Egg plant	root-leave-stem	distilled	90 / 500	HCl (6M)	Ni	AAS	20
Grasses-legumes	whole	ultrasound-distilled	air dried	HNO <sub>3</sub> : HClO <sub>4</sub>	As, Se	ICP-AES	21
Holm oak	leaves-acorns	shake-distilled	not mentioned	HNO <sub>3</sub> : HClO <sub>4</sub>	6 metals	AAS	22
Vegetables	edible	brush-water	ashed	HNO <sub>3</sub> :H <sub>2</sub> SO <sub>4</sub> :H <sub>2</sub> O <sub>2</sub>	As, Cr	GFAAS	23
Vegetables	edible	brush-water	72	HNO <sub>3</sub> :H <sub>2</sub> SO <sub>4</sub> :H <sub>2</sub> O <sub>2</sub>	Pb	AAS	24
Vegetables	leaves-roots	detergent+acid	70	HNO <sub>3</sub>	Pb, Cd, Zn	ICP	25
7 vegetables	edible	washed or peeled	not mentioned	HNO <sub>3</sub> : VO <sub>5</sub>	Cd	GFAAS	26
Lettuce-spinach	leaves-roots	Ca(NO <sub>3</sub> ) <sub>2</sub>	70 / 450	HNO <sub>3</sub> : HCl	Cd, Zn	GFAAS	27
Reference material	---	---	---	HNO <sub>3</sub> : HCl HNO <sub>3</sub> : HF : H <sub>2</sub> O <sub>2</sub> HNO <sub>3</sub> : HClO <sub>4</sub>	9 metals	AAS DCP-AES	28

Table 1 (Continuation)

Plant	Parts analyzed	Cleaning procedures	Drying and ashing (C)	Chemical digestion	Trace elements	Analytical Method	Reference
Herbage	whole	unwashed	not mentioned	HCl : HNO <sub>3</sub>	Pb	GFAAS	29
Hay	whole	unwashed	air dried	HCl : HNO <sub>3</sub>	Pb	GFAAS	30
Trees (5 species)	leaves	unwashed	110	HNO <sub>3</sub> : HClO <sub>4</sub>	Pb	AAS	31
Grasses (3)	stem-leaves	unwashed	oven	—	Cd, Cu, Pb, Zn	AAS	32
Wild species (15)	leaves	unwashed	105	HNO <sub>3</sub>	Cd,Pb,Zn	AAS	33
Rice	root-stems	not mentioned	not mentioned	HNO <sub>3</sub> :HClO <sub>4</sub> :H <sub>2</sub> SO <sub>4</sub>	Cu	AAS	34
Foliage	leaves	not mentioned	air dried \ 450	H <sub>2</sub> O <sub>2</sub> : HCl	Cd, Pb	ICP-AES	35
Corn	root-leave-stem	not mentioned	40	HNO <sub>3</sub> : HClO <sub>4</sub>	Se	AF	36
Forbs, grasses	leaves-roots	not mentioned	40 / 50	HNO <sub>3</sub> : HClO <sub>4</sub>	Se	ICP/AF	37
Vegetables	edible	not mentioned	105	HNO <sub>3</sub>	6 metals	ICP-AES	38
Maize, barley	whole	not mentioned	not mentioned	HNO <sub>3</sub> : HClO <sub>4</sub>	8 elements	AAS	39
Grasses	whole	not mentioned	110	HNO <sub>3</sub> : H <sub>2</sub> SO <sub>4</sub>	Cd	AAS	40
Radish	stems	not mentioned	100 / 550	HCl (1N)	Zn	AAS	41
Rice	straw	not mentioned	—	HNO <sub>3</sub> : HClO <sub>4</sub> (2:1)	Zn	AAS	42
Red cedar	tree core	not mentioned	—	HNO <sub>3</sub> : HClO <sub>4</sub>	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	HNO <sub>3</sub> : HClO <sub>4</sub>	As, Se, Mo	ICP	46
Elgrass	leaves	not mentioned	80	HNO <sub>3</sub> : HCl : HF	Fe, Mn, Zn, Cu	GFAAS	47
Legum.- Grass.	stem-leaves	not mentioned	60	HNO <sub>3</sub>	Cu, Mo	ICP-AES	48
Trees (5 species)	leaves-needles	not mentioned	air dried	H <sub>2</sub> SO <sub>4</sub> : HNO <sub>3</sub>	Hg	CV-AAS	49
<i>Azalea japonica</i>	leaves	not mentioned	105	H <sub>2</sub> SO <sub>4</sub> : HNO <sub>3</sub>	Hg	AAS	50
Olives	leaves	not mentioned	not mentioned	trichloroacetic acid	Mn	SPS	51
<i>Eichornia crassipis</i>	whole	not mentioned	105	HNO <sub>3</sub> :H <sub>2</sub> SO <sub>4</sub> :HClO <sub>4</sub>	9 metals	AAS	52
Vegetables	leaves	not mentioned	freeze dried	HNO <sub>3</sub> : H <sub>2</sub> O <sub>2</sub>	Zn, Cd	AAS	53
Aquatic plants	whole	not mentioned	800	HNO <sub>3</sub>	Pb, Zn	AAS	54
Corn	root-stems	not mentioned	air dried	NH <sub>4</sub> OAc:NaNO <sub>3</sub> HNO <sub>3</sub>	Cd, Zn	GFAAS	55
Staghorn sumac	leaves	not mentioned	air dried	H <sub>2</sub> O <sub>2</sub> :HCl	Ni,V	ICP	56

## Abbreviations:

dcb= dithionite-citrate-bicarbonate  
GFAAS= graphite furnace AAS  
AF= atomic fluorescence

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

<b>Table 2. Comparison of the different drying and ashing temperatures.</b>		
	<b>Recommended</b>	<b>Observations</b>
As	LA	-MA and HA volatilization -LO, HO and FD considerably lower
Cd	MA	-HA volatilization -LO, HO and FD considerably lower
Co	LA - MA - HA	-LO, HO and FD considerably lower
Cr	FD	-avoid any ashing
Cu	MA	-HA volatilization -LO, HO and FD considerably lower
Fe	MA	-HA volatilization -LO, HO and FD considerably lower
Mn	LA - MA - HA	-LO, HO and FD considerably lower
Ni	LA, MA, HA, LO, HO, FD	no significant differences
Pb	LA - MA - HA	-LO, HO considerably lower
Zn	LA - MA - HA	-LO, HO and FD considerably lower

(LA= ashing at 400°C; MA= ashing at 550°C; HA= ashing at 800°C; LO= 40°C; HO= 105°C and FD= freeze drying at -60°C)

**Table 3.** Mean recovery of trace elements in centrifuged 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-1571) in  $\mu\text{g}\cdot\text{g}^{-1}$ .

	Al	Cu	Fe	Mn	Zn
<b>SRM-1571</b>	286±9	5.64±0.24	83±5	54±3	12.5±0.3
<b>Nitric</b>					
mean ± s.d.	225±20	6±0.8	57±8.6	50±3.7	13±1.1
recovery (%)	79	115	69	92	104
<b>Agua regia</b>					
mean ± s.d.	277±15	7±0.6	69±8.5	56±6.4	13±1.5
recovery (%)	97	124	83	104	106
<b>HNO<sub>3</sub>:H<sub>2</sub>SO<sub>4</sub></b>					
mean ± s.d.	280±6	6.16±0.5	77±2.1	56±1.9	13±0.9
recovery (%)	98	109	93	104	107
<b>HNO<sub>3</sub>:HClO<sub>4</sub></b>					
mean ± s.d.	329±30	6.62±0.7	97±11.2	60±5.3	14±1.7
recovery (%)	115	117	117	111	113

**Table 4. Suitable acid mixtures for digestion of vegetation material. [P] = (HNO<sub>3</sub>:HClO<sub>4</sub>=3:2); [N] =HNO<sub>3</sub>; [H] = (HCl:HNO<sub>3</sub>=1:3); and [S] = (HNO<sub>3</sub>:H<sub>2</sub>SO<sub>4</sub> =2:1).**

	<b>Recommended</b>	<b>Not Suitable</b>
<b>As</b>	P - S - H - N	-
<b>Cd</b>	S - P	N - H
<b>Co</b>	S - P	N - H
<b>Cr</b>	S - P	--
<b>Cu</b>	P - S - H - N	--
<b>Fe</b>	S	--
<b>Mn</b>	P - S - H - N	
<b>Ni</b>	P	N - H
<b>Pb</b>	P - S	N - H
<b>Zn</b>	P	S - N - H

## Figure Captions

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

Figure 2. Effects of drying and ashing in the determination of Cu, Mn, Fe, As, and Cr. For comparison purposes the concentrations of the trace elements (average of 12 samples) has been normalized to percentage. Bars with the same letter are not statistically different. (FD = freeze drying)

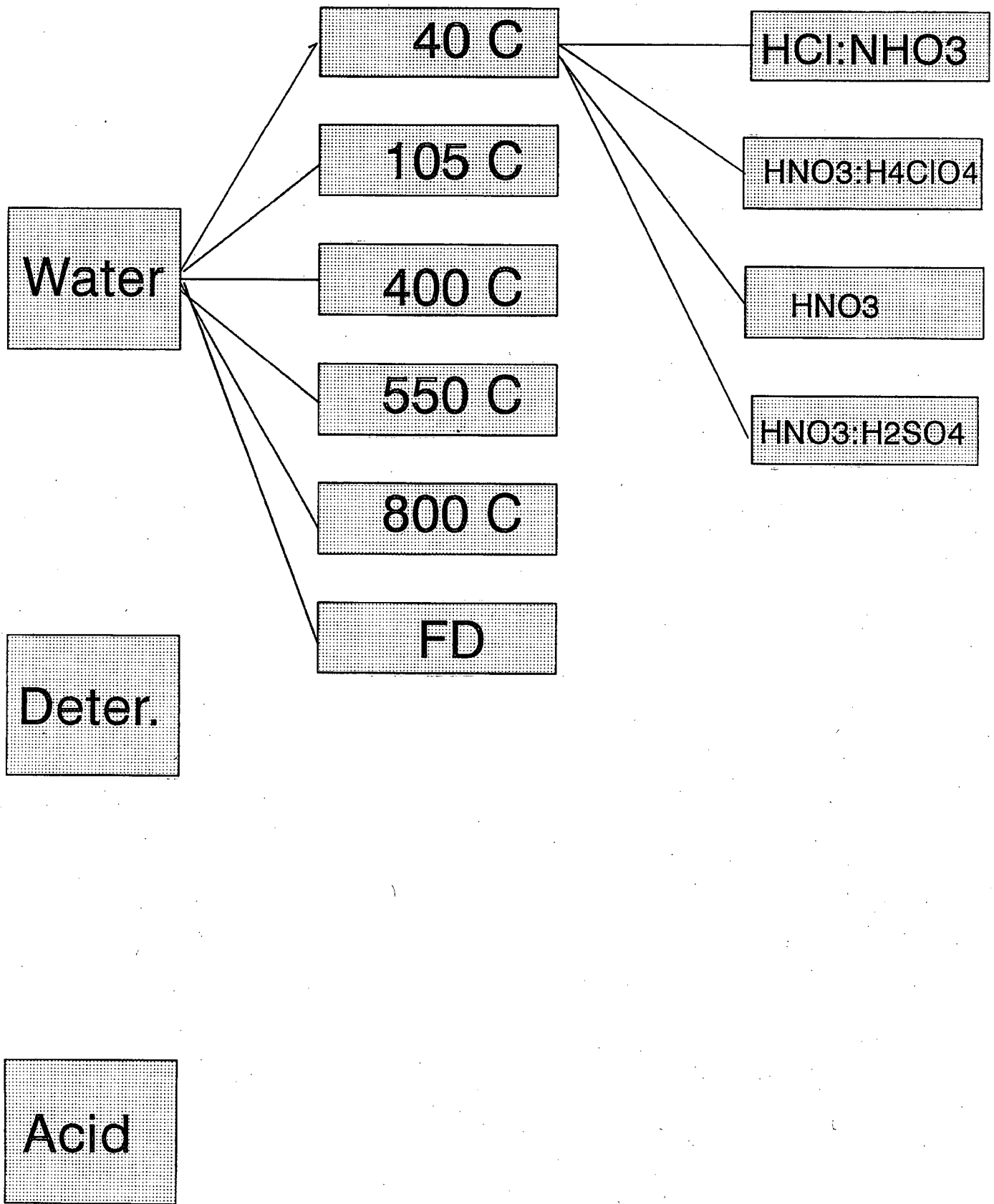
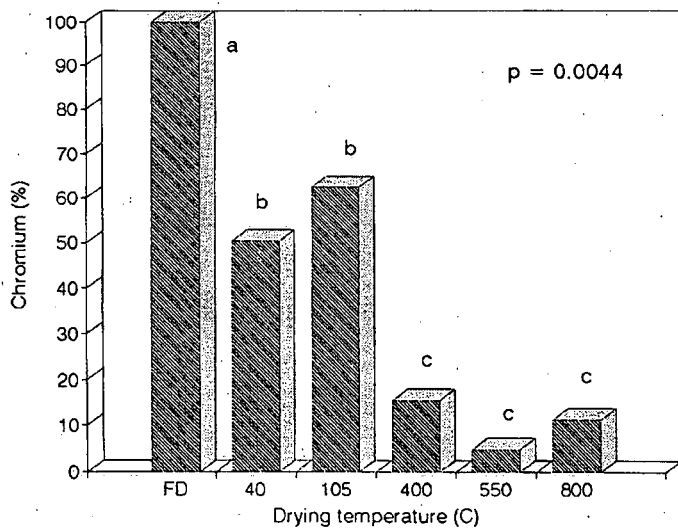
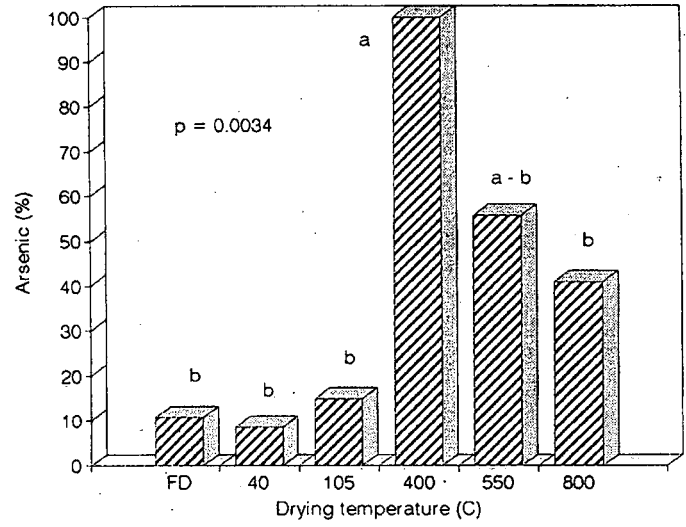
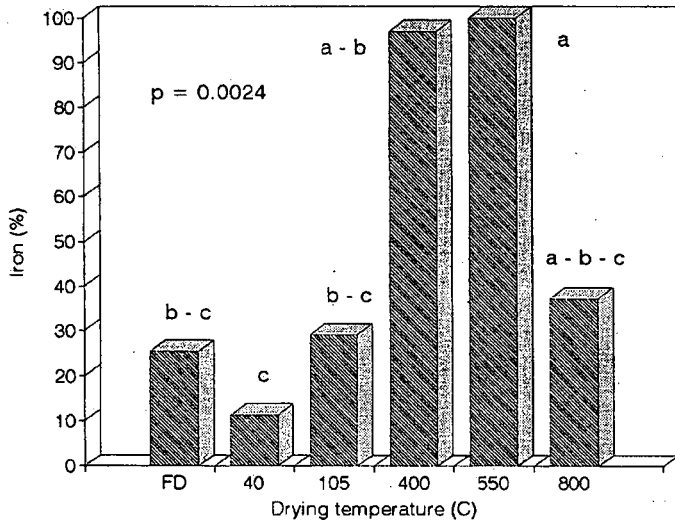
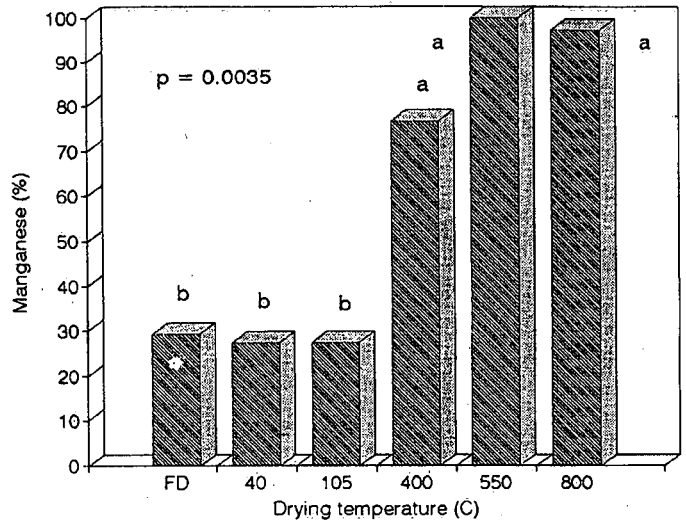
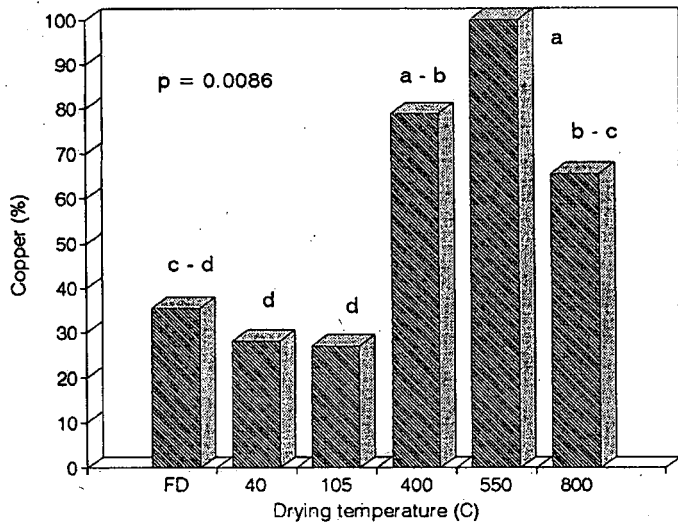


Fig 1





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