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# The Use of Hyphenated Spectrometric Techniques for the Environmental Forensic Assessment of Non-Traditional Pollutants and Degradates in the Greater Florida Everglades

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

THE USE OF HYPHENATED SPECTROMETRIC TECHNIQUES FOR THE  
ENVIRONMENTAL FORENSIC ASSESSMENT OF NON-TRADITIONAL  
POLLUTANTS AND DEGRADATES IN THE GREATER FLORIDA EVERGLADES

A dissertation submitted in partial fulfillment of the  
requirements for the degree of  
DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Luis E. Arroyo-Mora

2009

To: Dean Kenneth Furton  
College of Arts and Sciences

This dissertation, written by Luis E. Arroyo-Mora, and entitled The Use of Hyphenated Spectrometric Techniques for the Environmental Forensic Assessment of Non-Traditional Pollutants and Degradates in the Greater Florida Everglades, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: February 24, 2009

The dissertation of Luis E. Arroyo-Mora is approved.

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Dean Kenneth Furton  
College of Arts and Sciences

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Dean George Walker  
University Graduate School

Florida International University, 2009

## DEDICATION

To my wife Tatiana and my daughter Montserrat, my two angels on Earth ...



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ABSTRACT OF THE DISSERTATION

THE USE OF HYPHENATED SPECTROMETRIC TECHNIQUES FOR THE  
ENVIRONMENTAL FORENSIC ASSESSMENT OF NON-TRADITIONAL  
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by

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Florida International University, 2009

Miami, Florida

Professor Piero R. Gardinali, Major Professor

A comprehensive investigation of sensitive ecosystems in South Florida with the main goal of determining the identity, spatial distribution, and sources of both organic biocides and trace elements in different environmental compartments is reported. This study presents the development and validation of a fractionation and isolation method of twelve polar acidic herbicides commonly applied in the vicinity of the study areas, including e.g. 2,4-D, MCPA, dichlorprop, mecoprop, picloram in surface water. Solid phase extraction (SPE) was used to isolate the analytes from abiotic matrices containing large amounts of dissolved organic material. Atmospheric-pressure ionization (API) with electrospray ionization in negative mode (ESP<sup>-</sup>) in a Quadrupole Ion Trap mass spectrometer was used to perform the characterization of the herbicides of interest.

The application of Laser Ablation-ICP-MS methodology in the analysis of soils and sediments is reported in this study. The analytical performance of the method was evaluated on certified standards and real soil and sediment samples. Residential soils were analyzed to evaluate feasibility of using the powerful technique as a routine and

rapid method to monitor potential contaminated sites. Forty eight sediments were also collected from semi pristine areas in South Florida to conduct screening of baseline levels of bioavailable elements in support of risk evaluation. The LA-ICP-MS data were used to perform a statistical evaluation of the elemental composition as a tool for environmental forensics.

A LA-ICP-MS protocol was also developed and optimized for the elemental analysis of a wide range of elements in polymeric filters containing atmospheric dust. A quantitative strategy based on internal and external standards allowed for a rapid determination of airborne trace elements in filters containing both contemporary African dust and local dust emissions. These distributions were used to qualitative and quantitative assess differences of composition and to establish provenance and fluxes to protected regional ecosystems such as coral reefs and national parks.

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## CHAPTER 1

### 1.0. Introduction

## 1.1. Introduction

One of the main objectives of environmental research laboratories consists to develop new techniques, to reduce costs and to improve existing methods for the analysis of pollutants. Developing methods for environmental analysis is challenging because the analytes of interest are typically present in complex matrices and at low concentration levels. The incorporation of suitable sampling, extraction and clean up procedures to isolate and enrich the analytes is generally applied. Moreover, the proper characterization of organic and inorganic compounds in environmental and forensic sciences requires the use of state of the art instrumentation, specially hyphenated spectrometric techniques. This instrumentation not only provides appropriate sensitivity but also unique capabilities for identification and confirmation of the species of interest.

Under this scope this research focused on the development of novel analytical protocols as alternatives to undertake the quantification of both organic and inorganic analytes on several matrices.

Within the suite of organic contaminants, polar herbicides were considered a priority because of their extensive use in current agricultural and domestic and their possible impact on sensitive ecosystems located in South Florida. The development of a solid phase extraction (SPE) procedure to pre-concentrate twelve acidic herbicides from water samples is presented in Chapter 2 in conjunction with the chromatographic separation via High Performance Liquid Chromatography (HPLC), followed by detection by Electrospray Ionization and Ion Trap Mass Spectrometry (LC/QIT/MS).

Chapter 3 describes the optimization and validation of a Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) methodology, as an

alternative tool for the elemental analysis in soils and sediments. This micro-sampling technique represents the first attempt to apply the inherent advantages of laser ablation's sampling for the routine quantitative analysis of metals of environmental relevance. The performance of LA-ICP-MS was evaluated and compared to commonly used digestion protocols. The optimized method was then applied to real soil and sediment samples, targeting both pristine and contaminated conditions.

Chapter 4 explores the possibility of using a laser ablation approach to get qualitative and quantitative information of metals in airborne particulate matter collected in filters. Research in the area of wind-borne dust deposition is of great interest due to its role as a source of iron microbes and other elements and its implications in coral reef health. This study explores the identification of trace elements as a tool to segregate natural and anthropogenic sources of elements typically linked to African dust storms. Although much work is needed in this area, we provided a reliable methodology to assess the composition of dust by either laser ablation or digestion methods.

## CHAPTER 2

### 2.0. Environmental Monitoring of Acidic Herbicides in Surface Waters by Electrospray Ionization Quadrupole Ion Trap Mass Spectrometry (LC-QIT/MS)



## 2.1. Pesticides Residues and Environmental Sustainability

To date one of the major concerns related with the health of the environment is due to the presence of herbicide residues in aquatic resources. Constant anthropogenic inputs throughout industrial emission during their manufacture along with the wide spread usage in croplands, has led to the occurrence and persistence of pesticides in different environment compartments such as water, soil and biota. Indeed, total pesticides consumption for agricultural practices in the United States during 2000 and 2001 reached up to  $700 \times 10^6$  pounds of active ingredient per year (Fishel 2007). The improving trend in pesticides used in the past 10 years represents a great attempt towards agricultural productivity and generation of efficient food production systems but at the same time it is a major indicator of environmental distress.

The State of Florida has one of the biggest agricultural areas of the nation comprising about 24 % of its territory. Approximately, 42,500 commercial farms spanned 10 million acres during the reporting period from 2003-2006. Forty different kind of crops and 191 different types of pesticides (43 fungicides, 64 herbicides, 65 insecticides and 19 other pesticides) were reported in Florida during the same period of time (Shahane 2008). A total of  $78 \times 10^6$  pounds of pesticides were applied on those farms. Figure 2.1 show the estimated values for active ingredient used, where insecticides represent the highest amount used.

The 2006 Florida Agriculture Statistical Directory reported that in 2005, Florida ranked first in the U.S. in the value of production of oranges, grapefruit, tangerines, and sugarcane for sugar and seed. Florida also ranked first in the value of sales of snap beans (green beans), fresh market tomatoes, and cucumbers for fresh market and for pickles,

bell peppers, squash and watermelons with an overall economic impact of US \$ 87.6 billion in 2005 (Shahane 2008).

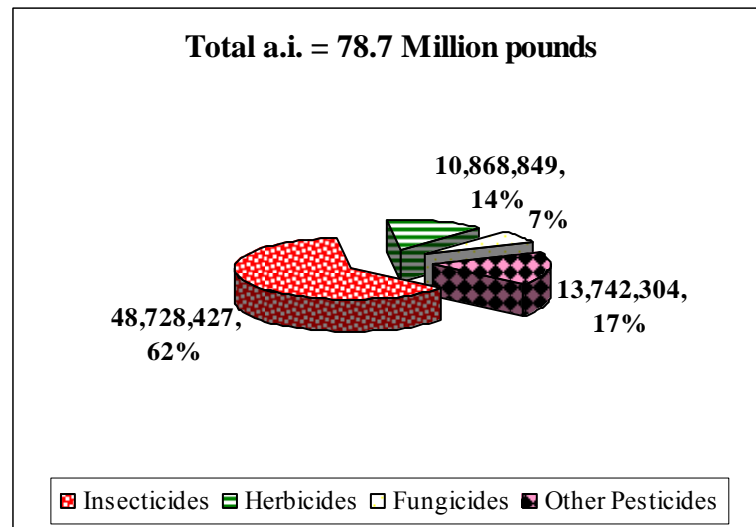


Figure 2.1. Pesticides usage for agricultural purposes in Florida during 2003-2006.  
Source: (Shahane, A. 2008)

Immerse on this superb economic buoyancy are two of the most important agricultural zones of South Florida: Everglades Agricultural Area (EAA) with 700,000 acres and Homestead Agricultural Area (HAA) with 83,000 acres, both representing approximately 9 % of the total agricultural land use in the state. These lands produce a wide array of traditional and tropical vegetables, tropical fruits, sugar cane, and seed crops. Greenhouse products and ornamental nursery characterize another two sources of incomes located in the boundaries of these agricultural areas.

Although agrochemical applications are being strictly regulated by Florida statutes, is inevitable that a portion of these chemical stressors will enter surface waters and interact with other important biological resources located in South Florida. This is particularly true for two unique and sensitive ecosystems: Everglades National Park

(ENP) and Biscayne Bay National Park (BNP) which are situated in the margins of these two agricultural areas. Moreover, the water deliveries through these protected areas, controlled by the South Florida Water Management District (SFWMD), provide a conduit for the transport of these residues to the margins at the national park limits. In spite of the primary goals: -to manage and protect water quality, flood control, ecosystem restoration and water supply- the reality has demonstrated that these complex networks of levees and canals have substantially changed the natural variability of the water flows and caused excessive drainage of wetlands, and therefore declining the general health of these ecosystems.

In response to this situation, the Comprehensive Everglades Restoration Plan (CERP) developed by a multi-agency effort, was enacted in 2000, and it recognizes that the Everglades, Florida Bay, Biscayne Bay, Big Cypress National Preserve and the Florida Keys are very complex ecosystems and the way they are interrelated are not yet fully understood by scientists. Thus a US \$ 7.8 billion plan provides for the restoration, protection and preservation of the water resources for the Central and Southern Florida and outlines a series of construction projects and water management changes to increase regional water supplies and restore the hydrological paths in the Everglades to pre-1900 conditions.

The plan is intended to capture most of 1.7 billion gallons of water per day currently lost to the ocean and gulf, and store it for later use. It also provides a way to return the right quantity and quality of water at the right time and to the right places. Although the quantity of the water has been thoroughly addressed in the restoration management plans, the quality is an issue that has generated controversy among scientists

and the agencies involved in the restoration processes (Miles and Pfeuffer 1997; Gardinali 2005).

Since the Everglades restoration plan requires the delivery of potentially anthropogenic tainted water, the South East Environmental Research Center (SERC) at FIU is currently conducting an environmental forensic investigation under the Contaminant Assessment Risk Evaluation Project (CARE) in order to get data on the occurrence, source identification, and temporal variation of chemical stressors affecting different compartments of South Florida ecosystems.

Much of the effort devoted to CARE involves the assessment of both organic and inorganic contaminants in three main protected areas: Big Cypress National Preserve, Biscayne Bay National Park and Everglades National Park. The specific objective of CARE is to conduct a survey on contaminant levels in surface water, soil and biota within these protected areas. The relevance and applicability of analytical results generated are crucial to determine possible sources of chemical contamination, the timing of releases, and spatial distribution of the toxicants.

The continuous chronic input of pollutants and their possible transport in the food chain into protected zones is considered one of the major threats to the parks. Therefore, farms and related agricultural areas are considered potential contaminant sources not only because of its geographical location but also for the intensive use of agrochemicals for crop protection.

For these reasons, the study and chemical monitoring of pesticides, as well as inorganic components, is crucial and of high priority to accomplish ecosystem sustainability.

## 2.2. Polar Herbicides: The Phenoxy Acid Family

Phenoxy acid herbicides were developed in 1940s in the course of attempts to synthesize analogs of the auxin  $\beta$ -indoleacetic acid, which has been recognized as a natural plant growth regulator (Roberts 1998). The common mode of action of auxin-type herbicides is to mimic natural auxin,  $\beta$ -indoleacetic acid, thereby producing an abnormal lethal growth. (Hereber 2000). Since their introduction at the end of the World War II as weed killers, they have been intensively used to control the growth of broad leaf weeds in a wide variety of crops. Several hundred commercial products contain chlorophenoxy herbicides in various forms, concentrations, and combinations. Sodium, potassium, and alkylamine salts are commonly formulated as aqueous solutions, while the less water-soluble esters are applied as emulsions (EPA-09).

The chemical structure of 2,4-D (2,4-dichlorophenoxy acid) and other related herbicides is presented in Table 2.1. Common to the phenoxy acid herbicides is the presence of different alkyl or halogenated substituents in the aromatic ring. For instance, the presence of 3 chlorine atoms corresponds to 2,4,5-T, while the replacement of one chlorine atom in 2,4-D for one methyl group in position 2 of the ring correspond to MCPA.

Still these two compounds are categorized as phenoxyalkanoic acid herbicides. Important to notice from the table is the presence of non-phenoxy like compounds such bentazon which holds an amide group or dinoseb with a nitro-phenol group. Two other typical acidic compounds are acifluorfen and picloram with benzoic acid and pyridine type chemical structures. All these compounds are commonly applied together in order to get a broaden range of weed control.

Table 2.1. Phenoxy acid herbicides and related compounds

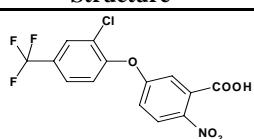
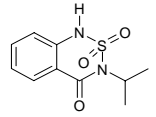
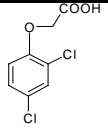
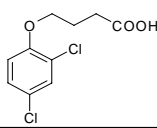
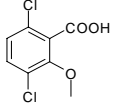
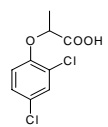
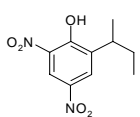
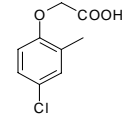
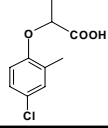
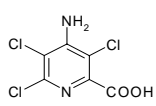
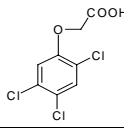
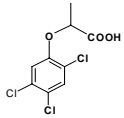
#	Compound	Structure	Molecular Weight
1	Acifluorfen $C_{14}H_7ClF_3NO_5$ CAS # 50594-66-6		360
2	Bentazon $C_{10}H_{12}N_2O_3S$ CAS # 25057-89-0		240
3	2,4-D $C_8H_6Cl_2O_3$ CAS # 94-75-7		220
4	2,4-DB $C_{10}H_{10}Cl_2O_3$ CAS # 94-82-6		248
5	Dicamba $C_8H_6Cl_2O_3$ CAS # 1918-00-9'		220
6	Dichlorprop $C_9H_8Cl_2O_3$ CAS # 120-36-5		234
7	Dinoseb $C_{10}H_{12}N_2O_5$ CAS # 88-85-7		240
8	MCPA $C_9H_9ClO_3$ CAS # 94-74-6		200
9	MCPP (Mecoprop) $C_{10}H_{11}ClO_3$ CAS # 7085-19-0		214
10	Picloram $C_6H_3Cl_3N_2O_2$ CAS # 1918-02-1'		240
11	2,4,5-T $C_8H_5Cl_3O_3$ CAS # 93-76-5		254
12	2,4,5-TP (Silvex) $C_9H_7Cl_3O_3$ CAS # 93-72-1		268

Table 2.2 shows that during 2003-2006 approximately  $10.8 \times 10^6$  pounds of active ingredients of a variety of herbicides were used in Florida, and 2,4-D was the most widely used agricultural herbicide, followed only by glyphosate and atrazine. Other acidic herbicides, such dicamba and bentazon were used to a lesser extent. The preferential application of phenoxy herbicides over other active ingredients is a clear evidence of the importance of these polar herbicides devoted for crop productivity.

Table 2.2. Herbicides used in Florida during 2003-2006 period

<b>Herbicide</b>	<b>Total lbs of AI reported</b>	<b>Percent</b>
2,4-D	2,702,917	24.9
Dicamba	864,007	7.9
Bentazon	40,860	0.4
2,4-DB	35,274	0.3
Acifluorfen	14,142	0.1
Glyphosate	1,690,282	15.6
Atrazine	1,478,834	13.6
Other Herbicides	4,042,533	37.2
<b>Total Herbicides</b>	<b>10,868,849</b>	

### 2.3. Environmental Fate of Polar Herbicides

Herbicides may enter surface waters via different pathways, of which run-off from treated fields is the most important (Reemtsma and Jekel 2006). Once these herbicides are applied into a field, both biotic (microbial) and non-biotic processes like photolysis and hydrolysis take place. Runoff to surface waters and leaching to groundwater occurs after rainfall and irrigation practices. Consequently, there is a potential for pesticide contamination of natural waters that needs to be assessed (Lopez de Alda et al., 2008).

## 2.4. Analytical Methodologies for the Determination of Phenoxy Acid Herbicides in Surface Waters

Because of their extreme usage, many efforts have been made to develop reliable extraction and quantitation procedures for determining phenoxy acid herbicides in different environmental matrices. The determination of these herbicides in real world samples is not only very challenging because of their polar and thermally-labile nature but also because of the low levels of detection often required for risk assessment purposes. Generally, clean up and enrichment steps are necessary because the contaminants are too diluted and the sample matrix is quite complex. Therefore, successful trace analysis of phenoxy herbicides requires an efficient sample preparation, sensitive detection and selective identification.

### 2.4.1. Extraction, Isolation and Enrichment Methods: Classical Methods

To a great extent, extraction, concentration and isolation of analytes from a suitable matrix will impact the reliability and accuracy of their analysis. The key objective of the sample preparation process is to provide a sample fraction enriched in all the analytes of interest, and as free as possible of matrix constituents. The ideal scenario will be to exclude the step altogether or “dilute and shoot” but for environmental samples this is not always possible (Henion et al., 1998). In the case of waters samples several linked processes are commonly followed: a) isolation (extraction) of the analytes from aqueous media; b) concentration by removing the extraction solvent, and c) elimination of matrix components which have been co-extracted and interfere in the chromatographic analysis (Barcelo 2000). Part of the design of this enrichment planning, involves the



initial consideration of physicochemical properties like pKa and Kow, along with the evaluation of the matrix characterization. This strategy may facilitate and produce a final method that accounts to be effective, practical, and economic, with reasonable processing times.

Classic methods of extraction of herbicides like liquid-liquid extraction (LLE), involve mixing the aqueous sample with a fraction volume of immiscible organic solvent for a certain period, allowing the two immiscible liquid phases to interact via manual shaking, with the objective that the analyte (s) will be extracted from the aqueous layer into the organic layer. After separation of the immiscible liquids by elution, the organic layer containing the extracted analytes is removed, concentrated to dryness, and reconstituted in an appropriate solvent (preferably the HPLC mobile phase or GC solvent) for mass spectrometric analysis (Henion et al., 1998). However, in the past 20 years LLE has been losing popularity, primarily because of its known disadvantages like the necessity of dealing with high volumes of toxic and expensive solvents, and the time required for extraction, and the cost of the waste produced. Despite the fact of its simplicity, it is also very difficult to succeed when water soluble and ionizable organic compounds are being targeted extracted (Jeannot et al., 2000).

#### 2.4.2. Modern Extraction Techniques

The Solid Phase Extraction (SPE) technique was first introduced in the mid-1970s as an alternative to LLE, due to its simplicity and economy in terms of time and solvent needs (Font et. al., 1993). It became commercially available in 1978, and now different SPE formats like cartridges and disks are available from many suppliers. Conventional

SPE is generally performed by passing aqueous samples through a solid sorbent in a column. Herbicides are eluted from the solid medium with an appropriate organic solvent. Great selectivity could be achieved depending on the selection of the sorbent chemistry, where reverse phase, mixed phase, normal phase, ion exchange, or polymeric phases are commonly used (Pico et al., 2007).

Solid phase extraction has clearly dominated the analytical processes of extraction of phenoxy herbicides from water or other complex matrices, particularly for liquid chromatography or gas chromatography separation and detection (Leon-Gonzalez 2000; Peruzzi et al., 2000; Lagana 1998; Walraevens et al., 2000; Pyrzynska et al., 2005; Geerdink et al., 1997; Wells and Yu 2000; Butz, et al., 1994; Di Corcia 1989; Li and Lee 2000; Font et al., 2007; Doble et al., 2007).

A recent publication comparing LLE with SPE have been published (Doble et al., 2007). These researchers found acceptable recoveries for the waters processed using SDB-XC Empore<sup>TM</sup>-SPE extraction disks (> 70 %) for 2,4-D and MCPA only, but not for dicamba (41%). The values for only three acidic herbicides were obtained using UV-VIS as detection system at concentration in the ppb range. Novel techniques like solid phase micro extraction (SPME) (Beltran et al., 2000; Liu 2007; Nakahara et al., 2001; Lee et al., 1998), stir bar sorptive extraction (Quintana et al., 2007), and dynamic liquid-liquid micro-extraction (Wu 2005; Saraji and Farajmand 2008) have been proposed for phenoxy herbicides quantification via gas chromatography determination with derivatization.

With SPE techniques, smaller samples volumes are needed and a large number of pesticides can be efficiently extracted in a single cartridge (Sabik et al., 2000). The

possibility of both, off line and on line connection with liquid chromatography and mass spectrometry, is one of the many conveniences of the SPE technique, which has been explored by many authors (Chiron et al., 1995; Loos et al., 2003; Marchese et al., 2002; Crescenzi et al., 1995; Geerdink et al., 1999; Crescenzi et al., 1997; Aguilar et al., 1998; Slobodnik et al., 1996; Majzik. et al., 2006).

#### 2.4.3. Typical Instrumental Methods for the Analysis of Polar Herbicides

Currently capillary GC and HPLC are two chromatographic techniques used to monitor trace residues in water and other important matrices. They are the preferred techniques because of their good separation capability and low levels of detection. GC methods offer excellent resolution capability and superior sensitivity when employed in combination with selective detectors like electron capture detectors (ECDs).

Because of their polar nature and low volatility, acidic herbicides are not suitable to direct analysis on GC and they need to be transformed into more volatile compounds by derivatization. Several derivatization strategies have been reported by different research groups (Butz and Stan 1993; Hodgeson et al., 1994; Pena and Silveira 1997; Catalina et al., 2000; Liu 2007; Nakahara et al., 2001; Lee et al., 1998). However methylation by diazomethane and derivatization using pentafluorobenzyl bromide (PFBBBr), are the most commonly used derivatizing agents for the determination of phenoxy acid herbicides and other pesticides in part per billion ranges by GC (Ollers 2001). Indeed the official method for the extraction and analytical determination of chlorophenoxy acid herbicides, EPA 8151 provides extraction, derivatization and chromatographic conditions for the analysis of chlorinated acid herbicides in water and

other matrices. Water samples are extracted with diethyl ether and then esterified with diazomethane or pentafluorobenzyl bromide. The derivatives are determined by GC with an electron capture detector (ECD). The sensitivity of the EPA method 8151 depends on the level of interferences in addition to instrumental limitations. A major disadvantage of this method is the use of diazomethane which represents a safety hazard due to its carcinogenic and explosive nature of the reagent. The complexity of generating a derivative compound from a polar compound is one of the major drawbacks of this technique. However, the likelihood of introducing interferences into the GC is also recognized as a major issue in their determination.

Fortunately, HPLC offers alternative methods in which herbicides do not require derivatization. The coupling to ultraviolet (UV) or photodiode array detector (PDA) has been frequently employed. (Balnova 1993; Pichon et al., 1996; Hogendoorn et al., 1999; Doble et al., 2007). EPA also recommended an HPLC-PDA method for the analytical determination of phenoxy acid herbicides. In this method a 100 mL water sample is adjusted to pH 12 with sodium hydroxide, and allowed to set for 1 hour to hydrolyse chlorinated esters. The sample is acidified, filtered and the chlorinated acids are extracted from a 20 mL aliquot. This aliquot is pumped in line through a C<sub>18</sub> SPE cartridge and the analytes are separated and measured by photodiode array-ultraviolet detection (PDA-UV).

In view of the chemical properties of phenoxy herbicides, HPLC would appear to be a more versatile chromatographic technique, providing added advantages over GC methods; however, the major limitation is its lack of specificity for confirming the presence of analytes in complex matrices because of inherited interferences associated

with samples containing high organic content materials. The use of only UV spectrum as a mean of identification has proven not to be sensitive or selective enough, providing insufficient information on the compounds detected. It has been found that the detected substances can only be definitively identified by coupling LC to MS using different interfaces (Niessen and Tinke 1995; Meyer and Thurman 1995).

#### 2.4.4. Hyphenated Techniques for the Analytical Determination of Polar Herbicides

With the successful coupling of liquid chromatography with mass spectrometry (LC/MS), new analytical opportunities arise to address the GC/MS limitations. Initially, these couplings were carried out with particle beam (PB) and “thermo-spray” interfaces (Cappiello and Famiglini 1995), and were of little value for environmental analysis due to their limited lack of sensitivity and repeatability (Jeannot et al., 2000). A crucial progress in LC/MS instrumentation was the design of interfaces for atmospheric-pressure ionization (API) capable of handling the high liquid flows often used in liquid chromatography (Niessen 1998; Niessen 1999). Typical modes of ionization via API are atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI) and atmospheric pressure photon ionization (APPI). They allow the soft ionization of a wide variety of substances in positive and negative mode, and have become the gold standard for LC/MS (Jeannot 2000). The selective response of the MS detector at low concentration levels, along with the structural information provided, represents the major advantages against the cumbersome derivatization procedures required by other techniques. Thus, the quantification and chemical characterization of the herbicides by mass spectrometric methods is versatile, reliable and accurate.

## 2.5. Fundamentals of Ion Trap Mass Spectrometry

The Quadrupole Ion Trap (QIT) is a three electrode device (see cross section view on Figure 2.2) where two of the electrodes are called “end caps”, while the third one (of donut shaped) is called the “ring electrode”. The ring electrode is sandwiched between the two end caps at a precise distance typically maintained by ceramic or quartz spacers (March 1997).

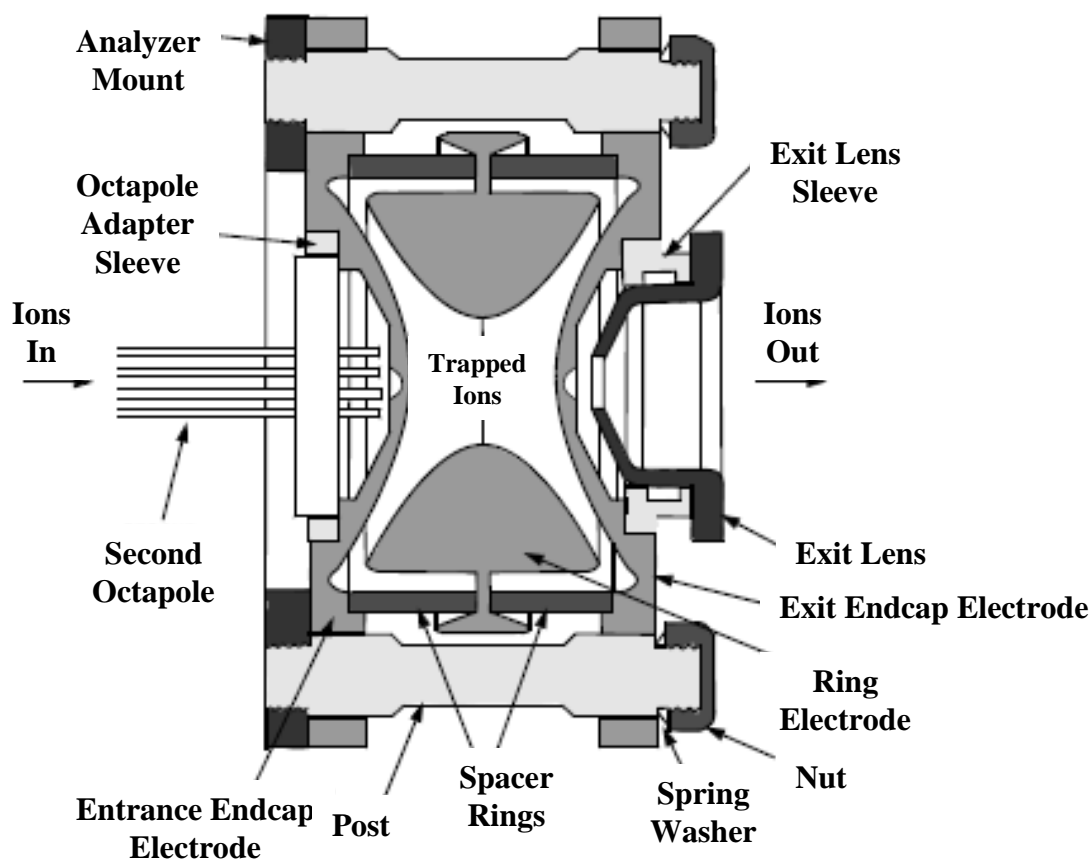


Figure 2.2. Cross sectional view of the quadrupole ion trap LCQ Advantage Max.  
(Courtesy of Thermo Electron Corporation)

The internal surface shape of these three electrodes follows a three dimensional nearly hyperbolic profile. Holes at the center of the end caps allow ions to pass in and out of the trap. A high voltage RF potential (0.78 MHz) is applied to the ring, while the end caps are held typically at ground voltage.

In LC/MS the ions are commonly formed by means of an external source like ESI, and they are guided using octapole arrays towards the trap. Ions produced in the API source enter the mass analyzer cavity through the entrance end cap electrode. Ions can be ejected through the exit end cap electrode, and are focused by the conversion dynode accelerating potential towards the ion detection system. The mass analyzer cavity is typically filled with helium through a nipple on the exit end cap electrode (1 mTorr). Collisions with helium dampen the kinetic energy of the ions and serves to quickly contract trajectories toward the center of the ion trap, enabling trapping of injected ions. Trapped ions are further focused to the center of the device by the use of an oscillating potential called the fundamental RF, applied to the ring electrode.

The motions of ions in a quadrupolar field can be described mathematically by the solutions of the second order linear differential equations described by Mathieu. Solutions to the differential equations are in terms of the Mathieu parameters  $a_z$  and  $q_z$  as shown by the following equations:

$$a_z = -2a_r = \frac{-16eU}{m(r_0^2 + 2z_0^2)\Omega^2} \quad (1)$$

$$q_z = -2q_r = \frac{-8eV}{m(r_0^2 + 2z_0^2)\Omega^2} \quad (2)$$

In equations 1 and 2,  $r$  represents the radial direction,  $z$  represents the axial direction,  $U$

is the dc amplitude,  $V$  is the rf amplitude,  $e$  is the charge of the ion,  $m$  is the mass of the ion,  $r_0$  is the inner radius of the ring electrode,  $z_0$  is the axial distance from the center of the device to the nearest point on one of the end cap electrodes, and  $\Omega = 2\pi f_{rf}$  where  $f_{rf}$  is the frequency of the main rf voltage (Bier and Schwartz 1997).

### 2.5.1. The Ion Trap Stability Diagram

The stability diagram is a two dimensional plot that indicates under what particular combination of potentials ions of a certain  $m/z$  value are stable or unstable in the quadrupolar field. This diagram is generated by plotting and overlapping the solutions to the Mathieu equation in  $(a, q)$  space for the  $r$  and  $z$  dimensions.

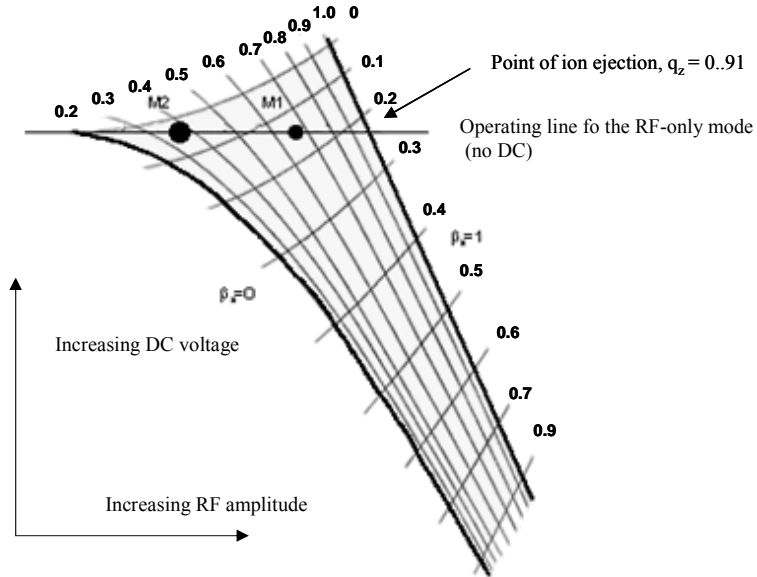


Figure 2.3. A portion of the QIT stability diagram showing the operating line at  $az=0$



The diagram is shown on Figure 2.3 and defines the areas within which the axial ( $z$ ) and radial ( $r$ ) components are stable; the region of overlap indicates the  $(a_z, q_z)$  coordinates corresponding to those ions which are held in the ion trap. The Mathieu parameters  $a$  and  $q$ , are indicative of the stability and motion of the ion in the three dimensional quadrupolar field (March and Tood 1995).

The ions trapped in the ion trap undergo periodic motions in both the radial as well as the axial direction. The axial motion in the direction of the end caps ( $z$  - axis), is of primary importance because this is the direction of ion injection and ion ejection. The classical approach involves increasing the amplitude of the fundamental RF voltage to scan out the ions. This process is shown in Figure 2.3 where the operating or scan line represents the  $(a_z, q_z)$  points that ions pass through until they are scanned out of the trap. The line starts at  $(0,0)$  and passes through a point of ejection (approx. at  $0, 0.91$ ). Since  $q_z$  is inversely proportional to mass ( $m$ ), high mass to charge ratio ions have lower  $q_z$  value than low mass to charge ratio ions, as shown by the different size circles on the Figure 2.3. The lowest mass to charge ratio ion, becomes unstable first, followed sequentially by the next higher mass to charge ratio ion, and so on (March and Todd 1995).

#### 2.5.2. Processes in the Quadrupole Ion Trap

The processes that occur in the LCQ mass analyzer can be divided into four steps: a) ion storage; b) ion isolation; c) collision induced dissociation (CID), and d) ion detection. This is presented schematically in Figure 2.4. This figure shows the events necessary to acquire a  $MS^2$  ( $MS/MS$ ) mass spectrum on an ion trap. As stated before, the storage step is generated by means of the application of an RF voltage to the ring

electrode producing a three dimensional quadrupolar field within the analyzer cavity. The isolation of the ions is performed by changing the RF voltage on the ring electrode as it is scanned in order to stimulate the motion of the lower mass ions out of the stability region of the Mathieu diagram. The rest of the ions are ejected by means of a waveform voltage which consist of a distribution of frequencies between 5 and 380 kHz containing all resonance frequencies except for those corresponding to the precursor ion (ion  $A^+$  in Figure 2.4). Once isolation of the precursor ion is achieved, the RF voltage is lowered to lower the  $q$  value of the ion.

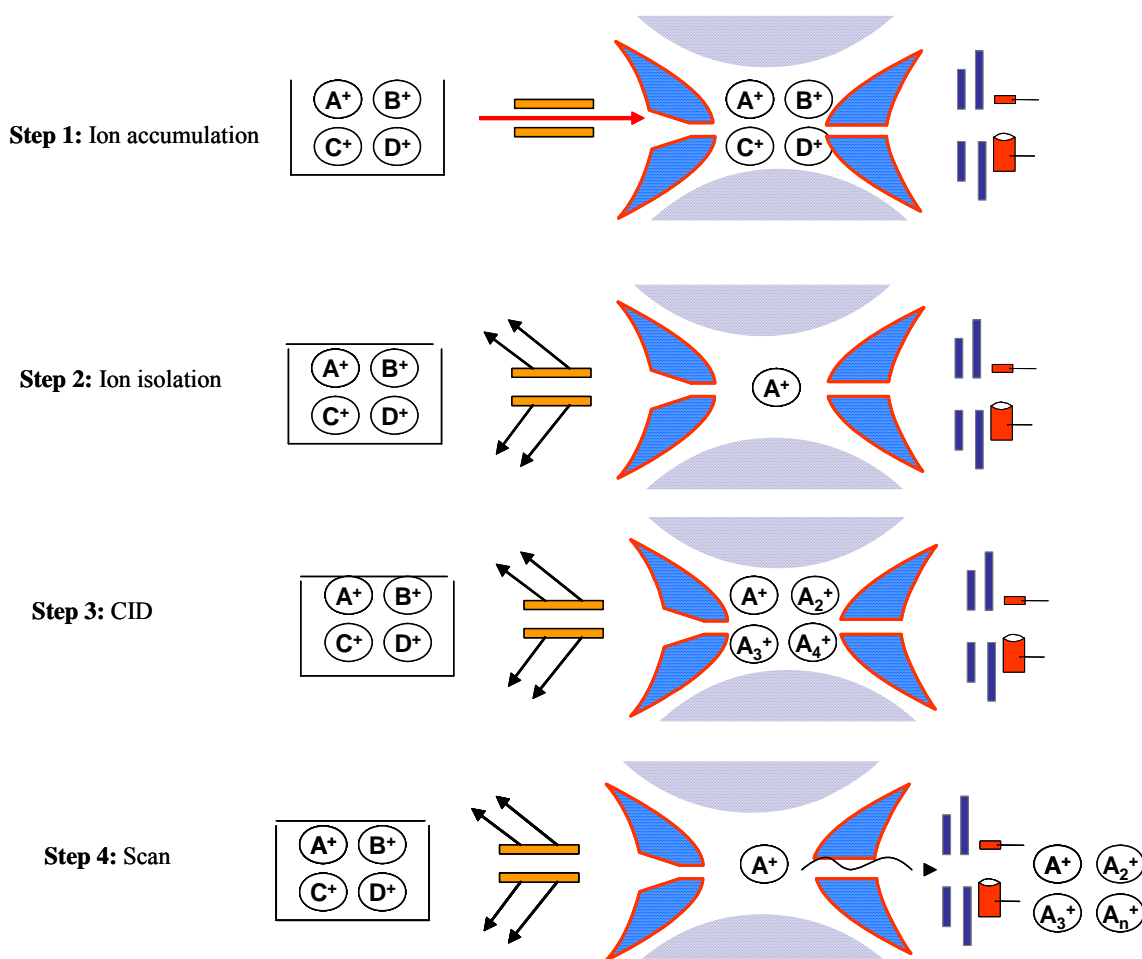


Figure 2.4. Scan events on a QIT during MS<sup>n</sup> operation

This step is necessary in order to trap the subsequent fragment ions. The other is a step to perform collision induced dissociation (CID).

The resonance excitation RF voltage is applied to the end cap electrodes to fragment parent ions to induce product ions via fragmentation. This voltage is not strong enough to eject an ion from the mass analyzer. However, the ion gains kinetic energy. After many collisions with the helium damping gas, ion gains enough internal energy to cause it to dissociate into product ions.

During the detection, the resonance ejection RF voltage facilitates the ejection of ions from the mass analyzer. This ring electrode RF voltage is ramped from low to high, while the resonance ejection RF voltage is applied simultaneously to the end cap electrodes to stimulate the ejection. When increasing the RF voltages in the ring electrodes, ions with high  $m/z$  ratios becomes unstable and are ejected from the mass analyzer. Many of these ions are focused toward the ion detection system where they are detected.

#### 2.5.3. Tandem Mass Spectrometry and Environmental Monitoring of Phenoxy Herbicides in Water Samples

The combination of liquid chromatography with tandem mass spectrometry has been shown to be very successful to achieve higher sensitivity and selectivity in the analysis of polar pesticides (Kuster et al., 2008). Table 2.3 presents a summary of the most relevant extraction and chromatographic techniques employed for the environmental water analysis of phenoxy acid herbicides in the past 10 years. As could be seen from this table, most of the reported methods are based in a combination of SPE

and a hyphenated mass spectrometric technique. Both off line and on-line SPE systems have been used with different sample volumes ranging from as low as 25 milliliters up to 10 liters. A maximum of 7 acidic target analytes have been reported for water analysis. The analytical instrumental trend is biased towards the triple stage quadrupole mass spectrometer (QqQ) probably because of its more favorable detection limit capability. However, the high cost of the triple quad instrumentation puts it out of reach for most university research and commercial laboratories doing routine analysis. Ion trap scan not only do the MS/MS process but are also the ideal for the investigation of fragmentation processes and pathways for herbicides. Also, with the novel advances in detector electronics and refined methods for ion isolation; scan functions and MS<sup>n</sup> capabilities provided by ion trap, make it ideal to run the acidic herbicides suite proposed in this environmental forensic monitoring research with high throughput.

## 2.6. Significance of the Study and Objectives.

Because of the major water management changes required by the Comprehensive Everglades Restoration Plan (CERP), the environmental forensic assessment of unpredicted anthropogenic signatures within protected areas of South Florida is a critical task in order to help maintain ecosystem sustainability. The most important questions to answers in this investigation are:

- What is the identity, spatial and temporal distribution of the acidic herbicides and main metabolites in the surface water within the protected areas?
- Is the intensive use of these acidic agrochemicals causing a real environmental threat?

Table 2.3. Overview of the methods reported for the water analysis of phenoxy acid herbicides using LC/MS/MS

Herbicides	Clean up				HPLC Separation		MS/MS	Reference
	Sample	Volume	Method	Cartridge	Column	Mobile Phase	<sup>a</sup> APCL, <sup>b</sup> ESI	
2,4-D, dicamba, bentazon, MCPA, Dichlorprop, Mecoprop, Dinoseb	Spiked tap water	1 L	SPE	Graphitized Carbon Black	Altima C <sub>18</sub> (250 x 4.6 mm, 5 µm)	0.1 % acetic acid-Methanol	<sup>b</sup> Q-IT	Baglio, D. et al. , 1999
2,4-D, Dicamba, Bentazon, MCPA, Dichlorprop, 2,4,5-T, Dinoseb	Ground and river water	1, 10 L	LLE, SPE	LLE: DCM + 1 g C <sub>18</sub> bonded silica/SPE Carboxpack B	Hypersil ODS 2 (250 x 4.6 mm, 5 µm)	Water-Acetonitrile	<sup>a</sup> Q-IT	Jeannot, R. et al. , 2000
Dicamba, Bentazone, MCPA, 2,4,5-T	Tap and river water	25, 50 mL	On-line SPE	In line: PLRPs, Hysphere-1	Hypersil BDS C <sub>18</sub> (4 x 4 mm, 5 µm)	10 mm aqueous ammonium formate (pH 3)-Methanol	<sup>b</sup> QqQ	Hogenboom, A. et al 2000
Bentazone, MCPA, Mecoprop	Spiked drinking water	0.25 - 2 mL	Direct injection		LC-LC C <sub>18</sub> /ABZ+ (100 x 2 mm, 5 µm)	25 % up to 65 % CAN in water (0.1 % formic acid)	<sup>ab</sup> QqQ	Dijkman, E. et al., 2001
2,4-D, bentazone, Dichlorprop, Dinoseb, MCPA, Mecoprop	Rain water	500 mL	SPE	Oasis HLB	Hypersil BDS C <sub>18</sub> (250 x 2.1mm, 5 µm)	Water (10% MeOH, 0.1 % acetic acid)-MeOH (0.1% acetic acid)	<sup>b</sup> QqQ	Bossi, R. et al., 2002
2,4-D, MCPA, 2,4-DB, Mecoprop	Surface and Drinking water	500 mL	SPE	Graphitized Carbon Black	Hypurity Elite C <sub>18</sub> (250 x 4.6, 5 µm)	Isocratic water-CAN (45:55 v/v), 0.3 mM tetrabutylammonium	<sup>b</sup> QqQ	Marchese, S. et al., 2002
2,4-D, MCPA, Bentazone, 2,4,5-T, Dichlorprop, Mecoprop	Surface water, waste water, rivers, streams	1 L	SPE	Isolute C <sub>18</sub> endcapped (500 mg), Oasis HLB (200 mg)	Superspher RP-18 (250 x 2 mm, 4 µm)	Acetonitrile-Water 0.1 % acetic acid (pH 3.5)	<sup>a</sup> Single Q	Loos, R. et al., 2003
2,4-D, MCPA, Bentazone	Drinking, drainage and river water	2, 1 L	SPE	Carbograph 1	Altima C <sub>18</sub> (250 x 4.6, 5 µm)	Water (1mM formic acid)-Methanol AC, 30:70 (1mM formic Acid)	<sup>b</sup> QqQ	Lagana, A. et al., 2002
2,4-D, MCPA, Mecoprop	Surface water and waste water	1 L	SPE	Oasis HLB	Nucleodur C <sub>18</sub> Gravity (125 x 2 mm, 3 µm), Gromsil C <sub>18</sub> (150 x 2 mm, 5 µm)	Water (0.1 % formic acid)-Methanol (0.6 % formic acid)	<sup>b</sup> QqQ	Freitas, L. et al., 2004
2,4-D, MCPA, Mecoprop, Bentazone	Drinking water	20 mL	On-line SPE	Hysphere GP/PLRPs (10 x 2 mm)	Purospher STAR RP <sub>18</sub> (125 x 2 mm, 5 µm)	Water-Acetonitrile	<sup>b</sup> QqQ	Kampioti, et al , 2005
2,4-D, MCPA, Mecoprop	Surface water	18 mL	On-line SPE	Oasis HLB (20 x 2.1 mm)	Nucleodur C <sub>18</sub> Gravity (125 x 2 mm, 3 µm), Gromsil C <sub>18</sub> (150 x 2 mm, 5 µm)	Water, 20 mM formic acid (pH 2.7)-Methanol 120 mM formic acid (pH 2.3)	<sup>b</sup> QqQ	Stoob, et al 2005
Dicamba, 2,4-D, MCPA, Dichlorprop, Mecoprop	Surface and ground water	500 mL	SPE	Isolute Env 200 mg	Lichrospher 60 RP Select B (125 x 3 mm, 5 µm)	Acetonitrile-Water (1:9 v/v) with 0.01 % ammonium acetate and 0.1 % formic acid, acetonitrile-water (9:1v/v) with 0.01 % ammonium acetate and 0.1 % formic acid in water	<sup>b</sup> QqQ	Majzik, E.S. et al 2006

- Is the coupling of ESI-LC/MS/MS a good screening/quantitative tool for the monitoring of acidic herbicides?

Therefore this work focused on the optimization strategies of an atmospheric pressure ionization (API) interface coupled to a state of the art ion trap instrument to evaluate the capabilities of such instrument for the environmental forensic monitoring of acidic herbicides in surface waters from the Florida Everglades.

The overarching goal for this study is to develop a new analytical protocol to determine these polar herbicides and evaluate their use in environmental forensics. The data obtained will be used for future management plans within the framework of restoration efforts.

Specific objectives of this study are:

- Develop a comprehensive enrichment trace method for the isolation and concentration of polar herbicides in complex organic rich matrices
- Develop an analytical protocol for the LC-MS/MS (tandem) separation and quantitation of acidic herbicides in surface waters from protected areas of the Greater Everglades.
- Perform an overall evaluation of the method as an environmental forensic tool by analyzing water samples from Big Cypress National Preserve, Biscayne Bay and Everglades National Parks, in order to identify potential sources and assess the prevalent transport mechanisms of these contaminants to and within the study area.
- Provide the management authorities the data generated in this research by the CARE project.

## 2.7. Experimental

### 2.7.1. Chemicals

#### 2.7.1.1. Phenoxy Acid Herbicides Analysis

Methanol ( $\text{CH}_3\text{OH}$ ), glacial acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ), water ( $\text{H}_2\text{O}$ ), acetone ( $\text{C}_2\text{H}_6\text{CO}$ ), and methyl-tert-butyl-ether (MTBE,  $\text{C}_5\text{H}_{12}\text{O}$ ) of HPLC grade or equivalent quality were obtained from Fischer Scientific (Suwannee GA, USA). Formic acid ( $\text{HCOOH}$ ) reagent grade was obtained from Fischer Scientific (Suwannee GA, USA).

The acidic herbicides 2,4-D ( $\text{C}_8\text{H}_6\text{Cl}_2\text{O}_3$ ), MCPA ( $\text{C}_9\text{H}_9\text{ClO}_3$ ), 2,4,5-T ( $\text{C}_8\text{H}_5\text{Cl}_3\text{O}_3$ ), 2,4-DB ( $\text{C}_{10}\text{H}_{10}\text{Cl}_2\text{O}_3$ ), Mecoprop ( $\text{C}_{10}\text{H}_{11}\text{ClO}_3$ ), Silvex ( $\text{C}_9\text{H}_7\text{Cl}_3\text{O}_3$ ), Dichlorprop ( $\text{C}_9\text{H}_8\text{Cl}_2\text{O}_3$ ), Dicamba ( $\text{C}_8\text{H}_8\text{Cl}_2\text{O}_3$ ), Dinoseb ( $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_5$ ), Bentazone ( $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_3\text{S}$ ), Acifluorfen ( $\text{C}_{14}\text{H}_7\text{ClF}_3\text{NO}_5$ ) and Picloram ( $\text{C}_6\text{H}_3\text{Cl}_3\text{N}_2\text{O}_2$ ), were obtained as solids at 99 % purity from Chem Service (West Chester PA, USA). The internal standards used were 3,5-dichlobenzoic acid acetic 99 % ( $\text{C}_7\text{H}_4\text{Cl}_2\text{O}_2$ ) obtained as solid from Chem Service (West Chester PA, USA) and a solution of 2,4-D  $^{13}\text{C}_6$  100 $\mu\text{g/ml}$  (Dr. Ehrenstorfer, GmbH, Augsburg, Germany).

#### 2.7.1.2. Stock, Working and Calibration Solutions

Approximately but accurately 10 mg of each one of the solid standards were weighed individually in a Cahn-28 microbalance (Cahn Instruments CA, USA), and were added to a 10 mL amber volumetric flask and dissolved with MeOH. These c.a. 1000  $\mu\text{g/ml}$  stock solutions were stored in the dark at  $-20\text{ }^\circ\text{C}$ . The intermediate working standard solution (IWS) was prepared at a concentration c.a. 20  $\mu\text{g/ml}$  by adding 1000  $\mu\text{l}$

of each individual stock solution into a 50 ml amber flask. A phenoxy spiking solution of 5 µg/ml was prepared taking 6250 µL of the IWS into a 25 ml amber flask. These spiking standard solutions were used to prepare a 9 level calibration curve between 0.050 and 1000 µg/L (see Table 2.4). The internal standard solution of 3-5-dichlorobenzoic acid or 2,4-D <sup>13</sup>C<sub>6</sub> was added to each calibration solution to a concentration of 200 µg/L. Stock solutions were replaced every 3 months.

Table 2.4. Phenoxyacid calibration solutions preparation

<b>Solution</b>	<b>Concentration of spiking solution <sup>a</sup></b>	<b>Volume of working solution <sup>a</sup></b>	<b>Volume of internal standard <sup>b</sup></b>	<b>Final volumen</b>	<b>Final concentration of analytes</b>
<b>CS<sub>0</sub></b>	0	0	150 µl	1500 µl	0
<b>CS<sub>1</sub></b>	5 ug/ml	15 ul	150 µl	1500 µl	0.050 µg/ml
<b>CS<sub>2</sub></b>	5 ug/ml	30 ul	150 µl	1500 µl	0.10 µg/ml
<b>CS<sub>3</sub></b>	5 ug/ml	60 ul	150 µl	1500 µl	0.20 µg/ml
<b>CS<sub>4</sub></b>	5 ug/ml	90 µl	150 µl	1500 µl	0.30 µg/ml
<b>CS<sub>5</sub></b>	5 ug/ml	150 µl	150 µl	1500 µl	0.50 µg/ml
<b>CS<sub>6</sub></b>	5 ug/ml	180 µl	150 µl	1500 µl	0.60 µg/ml
<b>CS<sub>7</sub></b>	5 ug/ml	210 µl	150 µl	1500 µl	0.70 µg/ml
<b>CS<sub>8</sub></b>	5 ug/ml	240 µl	150 µl	1500 µl	0.80 µg/ml
<b>CS<sub>9</sub></b>	5 µg/ml	300 µl	150 µl	1500 µl	1.00 µg/ml

<sup>a</sup>Working solution is a mix of 2,4-D; 2,4,5-T, acifluorfen, silvex, picloram, mecoprop, 2-4,D-B; bentazon, dicamba, dichlorprop, dinoseb and MCPA at 20 µg/mL.

<sup>b</sup> Internal standard solution at 2 µg/ml



#### 2.7.1.3. Spiking Solutions

Mixtures of phenoxy acid herbicides at 5 µg/ml were prepared by adding approximately 6250 µl of 20 µg/ml IWS solutions to a 25 ml amber flask and diluted with methanol. The internal standard solutions of 3-5-Dichlorobenzoic acid or 2,4-D <sup>13</sup>C<sub>6</sub> were also prepared at 2 µg/ml and added to all samples at a constant concentration of 200 µg/l.

#### 2.7.2. Samples

##### 2.7.2.1. Sample Collection

Surface water samples from the Everglades National Park (30 sites), Biscayne National Park (11 sites) and Big Cypress National Preserve (7 sites), were collected using direct sub-surface grab (30 cm) into glass containers (see table 2.5 for sampling sites details). Five different transects were sampled. The first transect follows an east-west direction across the northern boundary of the park which receives water from the Water Conservation Areas to the north of the Park and lies just south of Tamiami Trail, the major highway in this area.

The second transect follows a north-south direction across the eastern boundary of the park, this transect lies closest to the Homestead Agricultural Area (HAA). The third transect is located in the drainage basin of the C-111 canal which flows through most of the HAA. The last two transects follow the Shark Slough and Taylor Slough which flow in a south-west direction from the north and east boundaries, respectively. At each sampling site, water samples were collected from using a 1000 ml glass bottle, poured in plastic bags and store in ice coolers. All samples were refrigerated during collection, and kept in a refrigerator at < 4.0 °C until the time of analysis.

Samples from Biscayne National Park were collected from areas near land along the channel from Black Point Marina, off the channel from Turkey Point Nuclear Power Plant, near the outflows from the main inland canal.

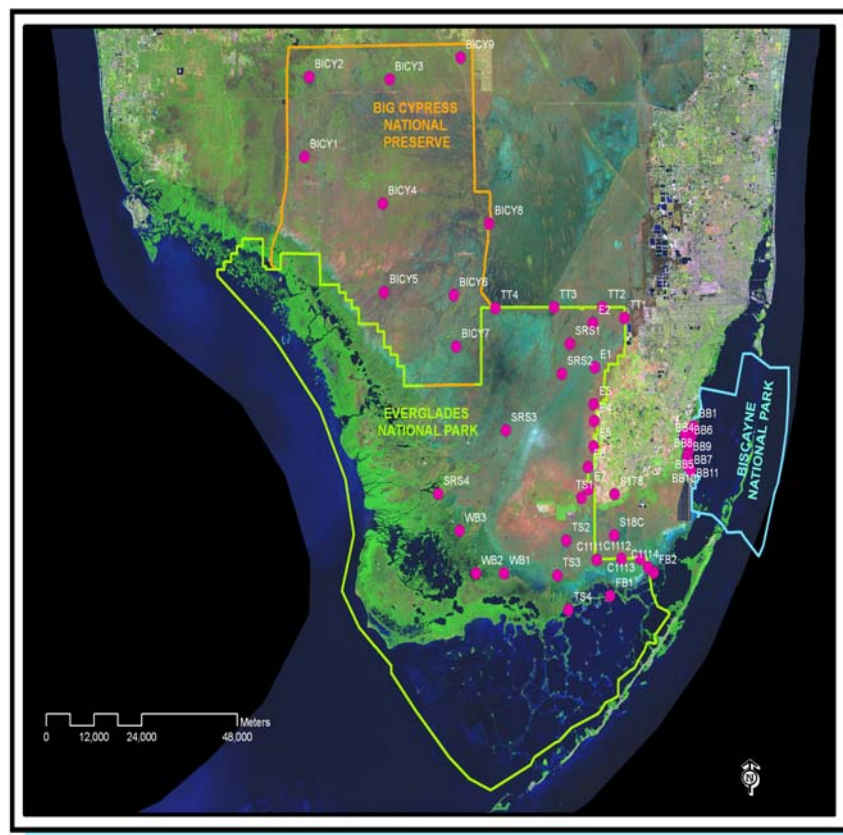


Figure 2.5. Sampling sites within the Big Cypress National Preserve, Everglades National Park and Biscayne Bay National Park under the CARE project

Table 2.5. Description of CARE Water Sampling Stations

Site ID	Descriptor	Station	Latitude (N)	Longitude (W)
BB1	Biscayne Bay	Black Creek Canal	25.5344	-80.3249
BB2	Biscayne Bay	Creek South of Black Creek Canal	25.5257	-80.3312
BB3	Biscayne Bay	Princeton Canal	25.5191	-80.3295
BB4	Biscayne Bay	Creek South of Princeton Canal	25.5077	-80.3332
BB5	Biscayne Bay	Military Canal	25.4891	-80.3380
BB6	Biscayne Bay	Creek North of Military Canal and South of Fender Point	25.5030	-80.3383
BB7	Biscayne Bay	Mowry Canal	25.4703	-80.3381
BB9	Biscayne Bay	L31-E wetlands North from Mowry canal connected	25.4733	-80.3413
BB10	Biscayne Bay	North Canal at Bayfront Park Marina	25.4630	-80.3425
BB11	Biscayne Bay	Creek North of SW344 St Fill	25.4520	-80.3330
BB12	Biscayne Bay	Intersection of Princeton and L-31E	25.5194	-80.3471
BICY1	Big Cypress	Deep Lake - SR837	26.0447	-81.2997
BICY2	Big Cypress	East Hinson Marsh - SR839	26.1963	-81.2885
BICY3	Big Cypress	Kissimmee Billy Strand	26.1916	-81.0865
BICY4	Big Cypress	Monument Road/Campsite	25.9576	-81.1036
BICY5	Big Cypress	Roberts Lake/Sweetwater Strand	25.7895	-81.0999
BICY6	Big Cypress	Pinecrest Flowway	25.7840	-80.9254
BICY7	Big Cypress	Lime Tree Hammock	25.6872	-80.9197
BICY8	Big Cypress	L-28 Tie Back Gated Culvert	25.9193	-80.8363
BICY9	Big Cypress	L-28 N of I75	26.2332	-80.9082
C-111-3	C-111 canal	C111 Basin	25.2860	-80.4603
C-111-4	C-111 canal	C111 Basin	25.2700	-80.4410

### 2.7.3. Materials

#### 2.7.3.1. Solid Phase Extraction Materials and Pre-Conditioning Process

The SPE procedure was performed with an automated SPE system AUTOTRACE SPE WorkStation with 6 column processor (Dionex Corporation, Sunnyvale, CA, USA). Two different types of SPE cartridges were used:

- a) Sep-Pak™ Environmental t-C<sub>18</sub> cartridges of 37-55µm of particle size; with a pore size of 125 Å and 900 mg of sorbent material, from Sup-Pak® Waters Corporation, Milford MA USA, and
- b) the Oasis HLB Plus cartridges (225 mg) polymeric material obtained from Waters Corporation, Milford MA, USA.

#### 2.7.3.2. Instrumentation

The liquid chromatograph-mass spectrometer system used in this investigation included a Thermo-Finnigan (Thermo-Finnigan San Jose CA, USA) Surveyor Plus, quaternary pump, a Surveyor Plus auto-sampler, and a LCQ Advantage Max quadrupole-ion trap mass spectrometer (QI-T) (50-2000 Da). The LC/MS was run under negative ion Electrospray Ionization (ESP-) for the herbicide family studied.

#### 2.8. Phenoxy Acid Sample Preparation Experiments

All water samples were filtered by using a pre-combusted 0.45  $\mu\text{m}$  GF/B glass microfibre filter attached to a filtration flask unit (Whatman Scarborough MN, USA). Approximately 100 ml of water sample are poured into a glass container and acidified using hydrochloric acid to a  $\text{pH} < 2.0$ ). The solution is homogenized using a magnetic stirrer.

The SPE procedure was performed with an automated SPE system AUTOTRACE SPE WorkStation with 6 column processor both the the Oasis HLB Plus cartridges and the Sep-Pak<sup>TM</sup> Environmental t-C<sub>18</sub>, were pre-conditioned with 10 ml of freshly mixed methyl-tert-butyl-ether (MTBE): methanol (90:10) with 0.01 % of formic acid; followed by 5 ml of methanol (0.01% formic acid); 5 ml of water and finally 5 ml of water (0.25 % sulfuric acid) (see Table 2.6). Analytes were recovered from the cartridge by using a mixture of MTBE:Methanol (90:10) with 0.01 % of formic acid (2 x 6 ml). The eluted fraction from the SPE was concentrated using nitrogen at room temperature up to 500  $\mu\text{l}$  and reconstituted in methanol to a final volume of 1000 $\mu\text{l}$ . Figure 2.6 summarizes the final extraction procedure selected for the analysis of phenoxyacid herbicides.

Table 2.6. Autotrace SPE workstation program for phenoxy acid herbicides

Operation	Description
Step 1	Process 6 samples with the following procedure
Step 2	Wash syringe with 10 mL of solvent 1
Step 3	Wash syringe with 10 mL of solvent 2
Step 4	Wash syringe with 10 mL of solvent 3
Step 5	Wash syringe with 10 mL of solvent 4
Step 6	Condition column with 5 mL of solvent 4 into solvent waste
Step 7	Condition column with 10 mL of solvent 3 into Solvent waste
Step 8	Condition column with 5 mL of solvent 2 into Aqueous waste
Step 9	Condition column with 5 mL of solvent 1 into aqueous waste
Step 10	Load 200 ml of sample onto column
Step 11	Dry column with gas for 20 minutes
Step 12	Wash syringe with 10 mL of solvent 3
Step 13	Collect 6 ml fraction into sample tube
Step 14	Collect 6 ml fraction into sample tube
Step 15	End
Solvent 1:	Acidified Water (0.25 %)
Solvent 2:	Water
Solvent 3:	Methanol
Solvent 4:	MTBE
Solvent 5:	Dichloromethane (not used)
Conditioning Flow	10 mL/min
Load Flow	10 mL/min
Rinse Flow	20 mL/min
Elute Flow	5 mL/min
Cond Air Push	10
Rinse Air Push	20
Elute Air Push	5

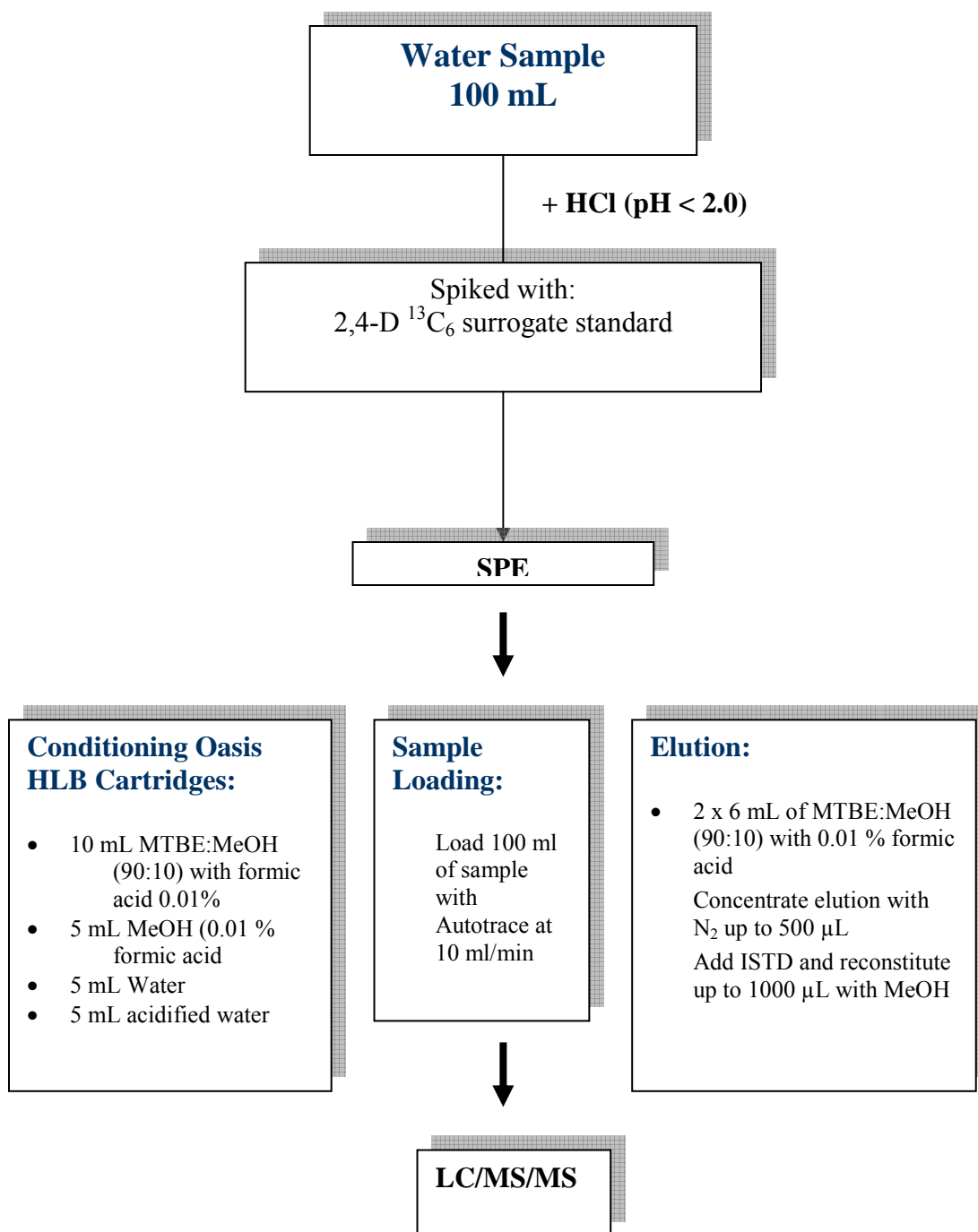


Figure 2.6. Scheme for the analysis of phenoxyacid herbicides in surface waters.

## 2.9. Results and Discussion

### 2.9.1. Optimization and Method Development

#### 2.9.1.1. LC/MS Ionization Selection and Detection Optimization

Depending on the chemical characteristics of the compounds and their physicochemical properties, selection of the ionization technique is very important for pesticides characterization (Arroyo and Gardinali 2006).

In the case of the acidic herbicides, they are expected to show good sensitivity as negative ions in electrospray mode. Phenoxy acid herbicides produce negative anions in solution and are well vaporized from the ionic state. This situation was confirmed experimentally, and concurs with the typical behavior of organic molecules as reported by Thurman in the ionization-continuum diagram, which evaluates these concept in a practical way assuming neutral pH (see Figure 2.7) (Mansoori et al., 1997; Thurman et al., 2001).

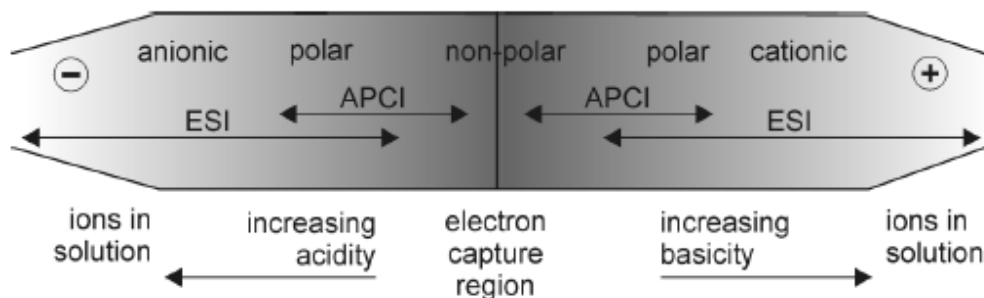


Figure 2.7. Ionization modes selected as a function of analyte properties. (Source: Thurman et al., 2001)

Several parameters affect the formation and transmission of ions from the interface to the trap itself in the LCQ quadrupole ion trap (Q-IT) mass spectrometer. This is shown graphically in Figure 2.8 where the ion formation, movement and trapping are represented. These ion source parameters should be selected and optimized in a manner that they give the best response in terms of sensitivity and selectivity. The LCQ has the capability to slowly but continuously infuse a solution into the HPLC flow and into the MS detector using an electronically-controlled syringe pump. The syringe pump delivers sample solution from the syringe into the API source at a rate of 1 % of the syringe volume per minute. Initially, the individual analytes were infused at a rate of 5  $\mu\text{l}/\text{min}$  directly to the spectrometer without HPLC separation. This mode delivers a constant amount of the analyte for the optimization.

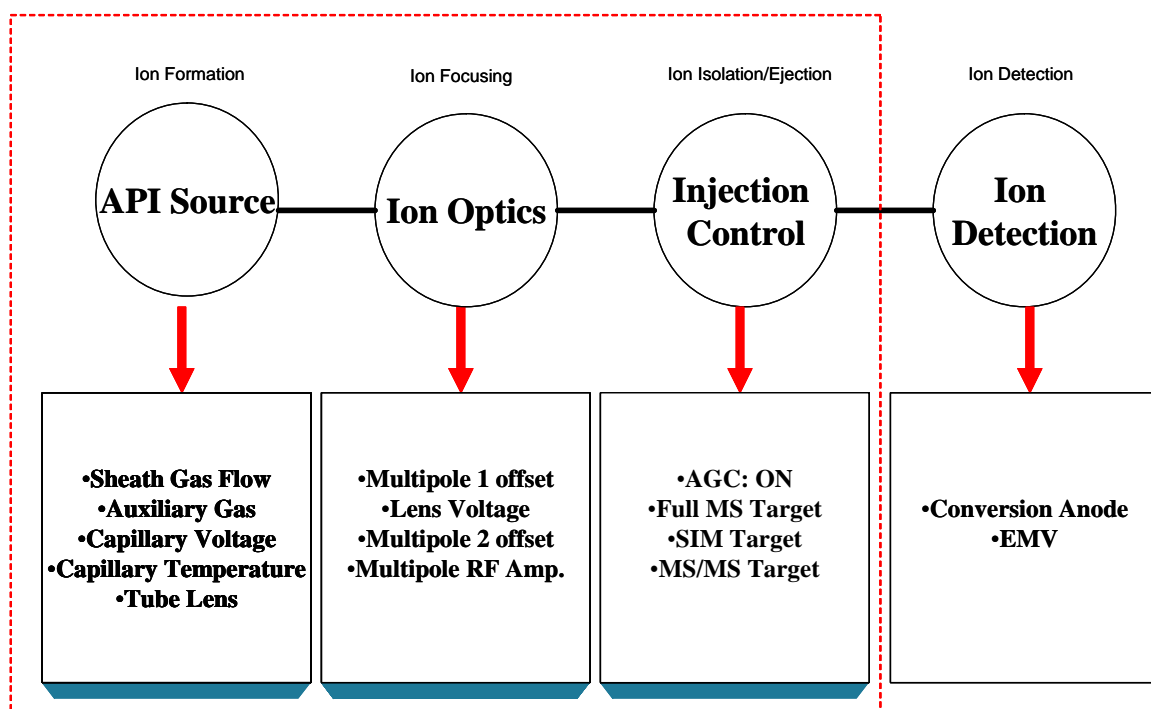


Figure 2.8. Main processes to control in a Q-IT instrument



A standard of the individual analytes, a concentration of 10 mg/l each in methanol was used to obtain the maximum population of ions resulting from the deprotonation of the acidic herbicides. The solution was electrosprayed from a stainless steel needle at 3.5 kV in the negative mode and sampled through the ion transfer capillary, which was held at 300 °C. The capillary helps to complete the desolvation process and serves as the nozzle for the supersonic expansion of the gas into the next chamber. Ions are transmitted through the skimmer using a tube lens as a gating element. The sampled ions are collected by the first RF octapole and are transmitted to the second RF octapole through an interoctapole lens and directed to the trap.

Different parameters were considered for the ion source optimization: capillary voltage, tube lens offset, and probe temperature (temperature of the ion transfer capillary). Two additional parameters evaluated were the nitrogen sheath gas, and the auxiliary gas in arbitrary units. Once generated, the ions are focused to the mass analyzer by means of a series of transmission lenses that also need to be optimized. These are: second octapole offset, first octapole offset and the inter-octapole with potential differences. The LCQ automated routine initially isolates the target ion and ramps all the values of the selected parameters. The importance of this routine is that it keeps track of the ion formation in the mass analyzer. As an example, the capillary voltage is ramped from -132 to 132 V. Once this value is adjusted (based on maximum response), it continues with the second parameter -the tube lens offset- which acts as a gating element. This process continues in a sequence until all the parameters related to the ion transmission are adjusted. The sheath gas and auxiliary gas are semi-automatically adjusted.

It is important to stress that this preliminary optimization accounts for the detection of the analyte by using direct infusion of a standard solution prepared in methanol and introduced onto the mass spectrometer, without considering the effects of the HPLC mobile phase composition and flow rate. This effect will be evaluated on a later step once the overall ion source parameters are optimized. Table 2.7 presents a summary of the optimized conditions for the different acidic herbicides. It is important to notice that the probe temperature and spray voltage were kept constant at 300 °C and 3.5 kV respectively, during the direct infusion experiments.

Table 2.7. Preliminary optimized ionization/focusing parameters for different acidic herbicides using LCQ Quadrupole Ion Trap Instrument via direct infusion

Compound	m/z	Capillary Voltage	Tube Lens Offset	Second Octapole	First Octapole	Interoctapole Lens
Picloram	239	-19.00	-15.00	21.00	1.00	30.00
Bentazon	239	-4.00	5.00	12.00	1.25	60.00
Dicamba	219	-4.00	10.00	8.50	6.00	20.00
2,4-D	219	-41.00	-45.00	6.00	1.25	14.00
MCPA	199	-38.00	-25.00	10.50	6.75	14.00
Dichlorprop	233	-19.00	-5.00	9.50	1.25	76.00
Mecroprop	213	-15.00	-15.00	15.00	1.50	76.00
2,4,5-T	253	-8.00	-15.00	14.00	1.00	16.00
2,4-DB	247	-20.00	-10.00	7.00	0.75	32.00
Acifluorfen	360	-9.00	-15.00	13.00	1.50	62.00
Silvex	267	-9.00	-10.00	5.50	3.75	54.00
Dinoseb	239	-30.00	-15.00	6.00	2.25	26.00

During the electrospray process, deprotonation of phenoxy acid and other acidic herbicides, allows the formation of a strong and abundant parent ion  $[M-H]^-$ , that retains molecular information. This could be seen on table 2.7, where the mass at m/z 219, corresponding to 2,4-D and dicamba, were produced under different ionization parameters. Indeed, each acidic herbicide shows high variability in the ionization and

focusing parameters. This variability could be an issue later when selecting a unique set of parameters that works for the detection of all the analytes during the chromatographic separation.

In order to consider the mobile phase effect on signal stability, a tee is introduced between the syringe pump and the LCQ divert valve. The mobile phase composition is selected according to the chromatographic separation. In the particular case of the phenoxy acid herbicides a mixture of methanol and acetic acid 1 % (75:25), and a flow rate of 0.5 mL/min were used in conjunction with the syringe pump. The standards are then infused into the HPLC stream at a constant flow using the syringe pump. Table 2.8 presents the same parameters obtained when the mobile phase is introduced in parallel to the infusion of the analyte standard.

Table 2.8. Ionization/focusing parameters for different acidic herbicides using LCQ Quadrupole Ion Trap Instrument via direct infusion into the HPLC flow

Compound	m/z	Change in Signal	Capillary Voltage	Tube Lens Offset	Second Octapole	First Octapole	Interoctapole Lens
Picloram	239	-8.24	-11.00	-60.00	7.00	1.25	20.00
Bentazon	239	8.32	-6.00	-60.00	7.00	1.25	14.00
Dicamba	219	-12.67	-4.00	-60.00	8.00	3.50	36.00
2,4-D	219	49.7	-22.00	-60.00	10.00	7.50	32.00
MCPA	199	5.49	-4.00	-60.00	8.50	1.00	34.00
Dichlorprop	233	-5.57	-7.00	-60.00	8.50	6.75	18.00
Mecroprop	213	26.98	-11.00	-60.00	9.00	0.75	26.00
2,4,5-T	253	33.82	-4.00	-60.00	9.00	7.50	20.00
2,4-DB	247	-1.74	-9.00	-60.00	9.00	7.00	22.00
Acifluorfen	360	-2.37	-4.00	-60.00	8.00	1.75	38.00
Silvex	267	38.38	-12.00	-60.00	9.00	7.25	20.00
Dinoseb	239	11.88	-13.00	-60.00	7.50	1.00	24.00

The values presented on Table 2.8 shows a common optimal value of -60 V for the tube lens offset, which acts as the gating element.

Also, for some compounds like 2,4-D a net increase signal of almost 50 % was found when the mobile phase composition was incorporated. However for other

compounds like picloram and dicamba, there was no net change from the initial optimized parameters.

As a final step in the optimization, the sheath gas and auxiliary gas, plus the nozzle x,y, z positions were tested. The relative intensity for the most abundant m/z was used as a criterion. Figure 2.9 presents the analyte response for a mixture of the analytes at a concentration of 150 ppb at different nozzle positions (vertical offset).

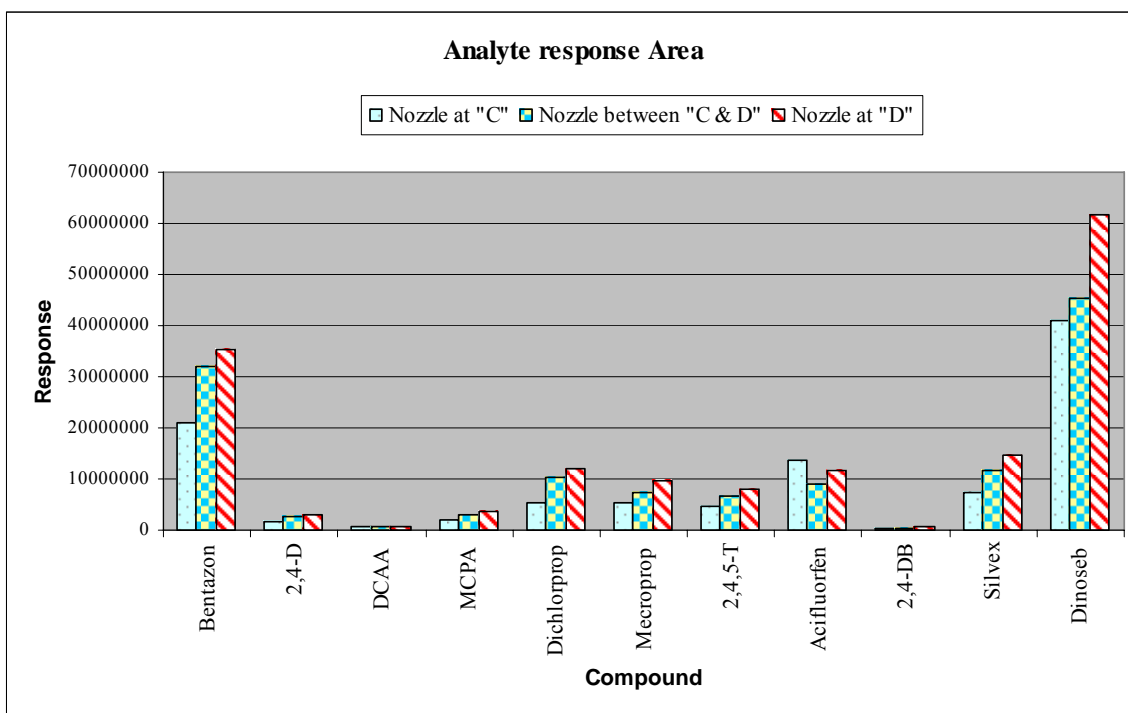


Figure 2.9. Acidic herbicide response obtained at different nozzle positions.

Position “D” on the nozzle has found to produce higher analyte responses for the majority of the analytes and was selected as the optimum position for ESI. The graphs also shows the compounds which are more easily to ionize (greater sensitivity), like bentazon and dinoseb and the least favorable ones like 2,4-DB and DCAA (2,4-

dichlorophenylacetic acid). A summary of the final optimized ESI parameters is presented in Table 2.9. The automatic gain control (AGC) is used to measured the ion signal and then adjust the ion injection time to optimize the number of ions to be store to the analytical scan. This feature of the LCQ must be “ON” ion order to get consistent results in terms of the S/N ratio of the analytes of interest (Bier and Yang 2005).

Table 2.9.Final optimized parameters for Q-IT determination of acidic herbicides

Parameter	Value
<b>ESI Source</b>	
Spray Voltage	3.5
Sheath Gas Flow Rate*	80
Auxiliary Gas Flow Rate*	20
Capillary Voltage (V)	-45
Capillary Temperature (°C)	300
Tube Lens (V)	-60
<b>Ion Optics</b>	
Multipole 1 Offset (V)	3
Lens Voltage (V)	17.5
Multipole 2 Offset (V)	6.5
Multipole RF Amplitude (V)	480
<b>Ion Detection System</b>	
AGC	On
Full MS Target	2 x10 <sup>8</sup>
Conversion Dynode	On
Dynode Voltage (kV)	14.7
Multiplier Voltage (V)	-946
HPLC Flow	0.5 ml/min
Solvents	MeOH:HOAc 1%, 75:25
x,y	0,0 mm
Nozzle Position	D

\*arbitrary units

### 2.9.1.2. Chromatographic Separation of Acidic Herbicides

Electrospray in the negative mode and in full scan will mainly generate the deprotonated species of acidic herbicides where the abundances of the signals for the pseudo-molecular ions  $[M-H]^-$ , and the isotopic cluster could be found. This is especially true for the phenoxy acid family where the presence of chlorine atoms provide additional isotopic information to characterize these herbicides. Both parent ions and qualifiers are shown in Table 2.10.

Table 2.10. Parent and main quantifying ions for phenoxy acid using full scan monitoring. Relative ion intensity, %, is reported in parenthesis

#	Compound	Formula	Retention time (s)	Nominal Mass	Parent Ion $[M-H]^-$	$[M-H+2]^-$ ( $q_1$ )	$[M-H+4]^-$ ( $q_2$ )
1	Picloram	$C_6H_3Cl_3N_2O_2$	5.99	240	239	241 (97)	243 (31)
2	Bentazon	$C_{10}H_{12}N_2O_3S$	7.30	240	239	241 (5)	-
3	Dicamba	$C_8H_6Cl_2O_3$	7.60	220	219	221 (65)	223 (10)
4	2,4-D	$C_8H_6Cl_2O_3$	9.63	220	219	221 (65)	223 (10)
5	MCPA	$C_9H_9ClO_3$	9.89	200	199	201	-
6	Dichlorprop	$C_9H_8Cl_2O_3$	11.46	234	233	235 (65)	237 (10)
7	Mecoprop	$C_{10}H_{11}ClO_3$	11.72	214	213	215 (33)	-
8	2,4,5-T	$C_8H_5Cl_3O_3$	12.57	254	253	255 (97)	257 (32)
9	2,4-DB	$C_{10}H_{10}Cl_2O_3$	12.87	248	247	249 (65)	251 (10)
10	Acifluorfen	$C_{14}H_7ClF_3NO_5$	13.03	361	360	362 (35)	-
11	Silvex	$C_9H_7Cl_3O_3$	14.89	268	267	269 (97)	271 (32)
12	Dinoseb	$C_{10}H_{12}N_2O_5$	17.54	240	239	241 (2)	-

As stated before, there are several instrumental parameters which have strong influence in the ionization efficiency. The composition of the HPLC mobile phase, the pH used, and the amount of ionic additives in the eluents are among them. The chromatographic separation was tested in two different columns: a Luna C<sub>18</sub> (100 x 2.0 mm x 2.5 µm) and a Zorbax reverse phase column C<sub>18</sub> (250 x 4.6 mm x 5 µm), and sequentially changing the composition of the mobile phase, the flow rate and the temperature of the column in order to find the best resolution between the peaks of interest in the shortest possible time of analysis. From the two columns tested, the Luna column presented high back pressure even at low flow rates like 0.2 ml/min. Indeed, under these flow rate conditions longer retentions were obtained, compromising the analysis time. The same column did not provide efficient separation capability or reasonable times, and therefore it was discarded for this separation. Therefore, the separation was tested and further optimized using the longer Zorbax Column.

In order to evaluate the effect of the modifier and the pH over the signal of the herbicides a series of experiments were conducted in the LCQ. A known concentration of a standard mixture solution (CS<sub>3</sub> = 200 ppb) was run in the Zorbax column at the optimum conditions described on Table 2.9, and using different concentrations of acetic acid (1.0 % at pH = 2.8; 0.1 % at pH = 3.08; and 0.01 % at pH= 3.55), and the areas of the analytes were determined. For example, it was found that dinoseb –(m/z = 239) the most sensitive compound in the suite- showed a decrease in its signal intensity by a factor of almost 40 % just by changing the concentration of acetic acid (and pH) from 0.1 to 0.01 % (see Figure 2.10). Indeed, as could be seen in the zoomed area of Figure 2.11 the increase on the pH, by using lower concentrations of acetic acid, affected the resolution

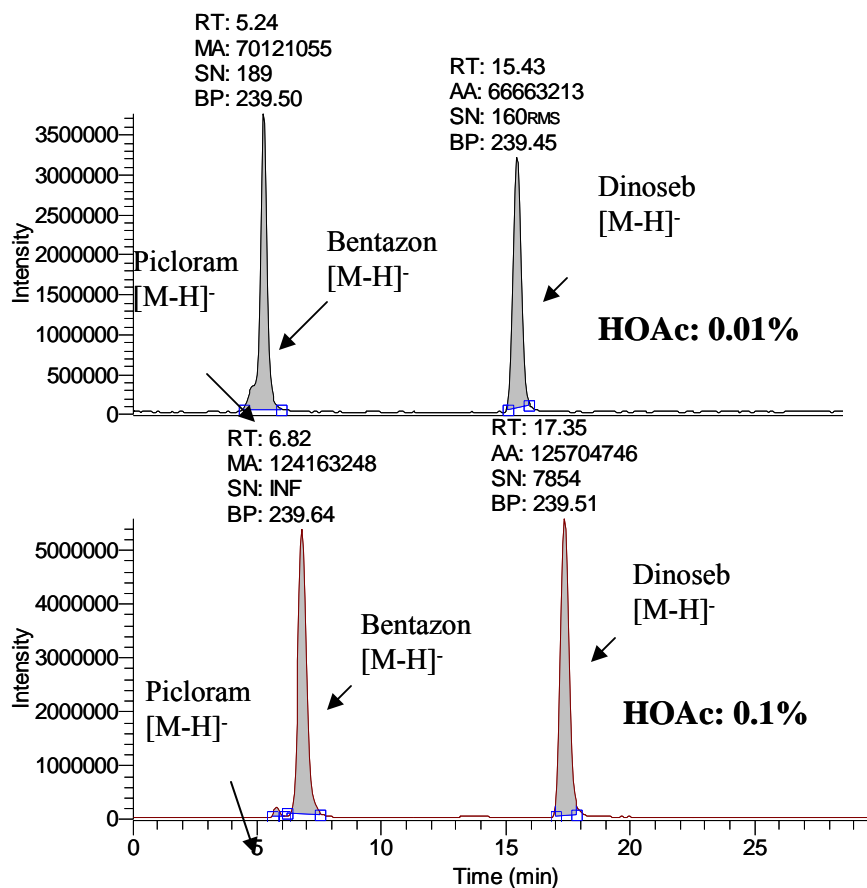


Figure 2.10. Effect of the Acetic acid concentration and pH during the chromatographic separation of some acidic herbicides. (HOAc 0.1 % pH 3.08; HOAc 0.01 %, pH = 3.55)

of picloram, which overlaps with bentazon. All the acidic herbicides just mentioned presented the same parent ion ( $m/z$ ) = 239, so their chromatographic separation is critical in order to characterize them.

The use of mobile phase modifiers like ammonium acetate or tetrabutylammonium, has been reported by several researchers as an attempt to help in



the signal stability (Solymosne et al., 2006; Marchese et al., 2002). It is important to notice, however, that a delicate balance exist between the chromatographic separation

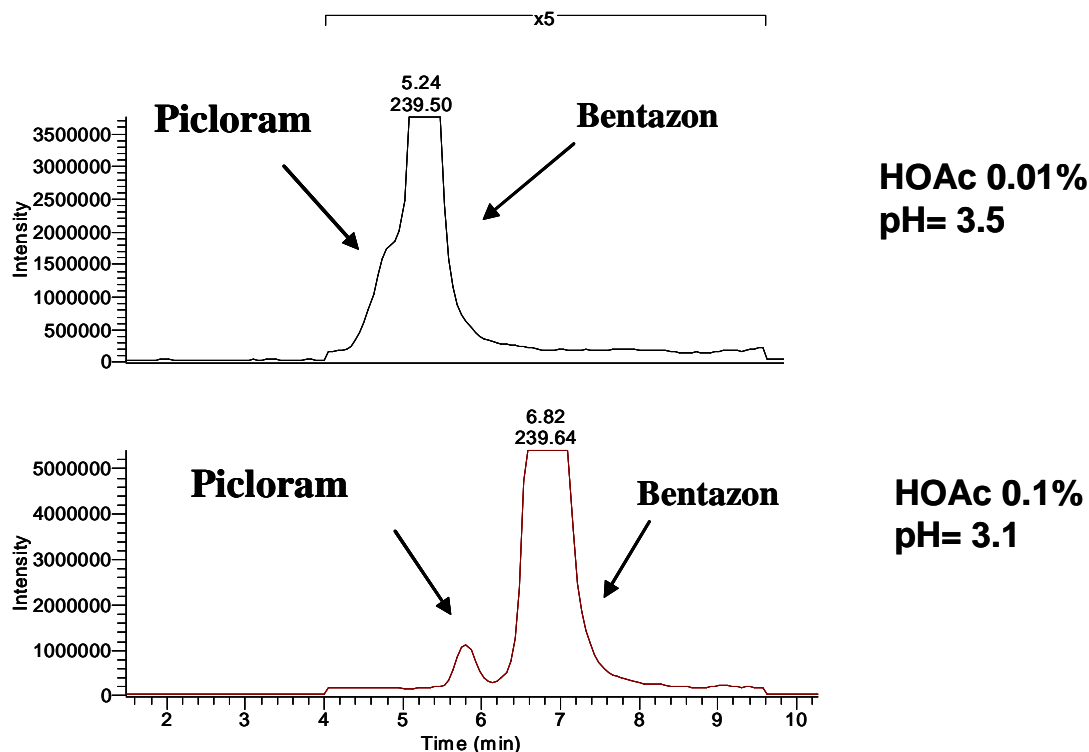


Figure 2.11. Picloram overlaps with Bentazon at lower acidic conditions with acetic acid as modifier: a) pH 3.55, b) pH 3.08

and ionization efficiency. In terms of separation, the acidic moiety of the phenoxy acid compounds and their low pKa values require separation using a slightly acidic mobile phase to selectively retain protonated-carboxylic acid groups. For instance, it was necessary to have 1% acetic acid in the mobile phase in order to keep the acid analytes stabilized in their acidic form. Also, the presence of an acidic modifier in the eluent, helped to avoid the ion suppression of the herbicides. The best electrospray performance was achieved at a flow rate of 0.5 ml/min. It was found that the combination of methanol

and acetic acid 1% (pH~ 2.8) provides the best conditions to favors both separation and ion detection, and they were chosen as the main constituents of the mobile phase. At the optimized flow rate, the mass flow of analyte is efficient and allows both a good peak shape and sensitivity for all the herbicides studied.

The approach is shown in a typical full scan chromatogram (Figure 2.12). A gradient elution program described on Table 2.11 was optimized and allowed the separation of the suite of acidic herbicides. The mass range scanned using the full scan method was from 185 to 365 Da, and the majority of the compounds were resolved in less than 20 minutes.

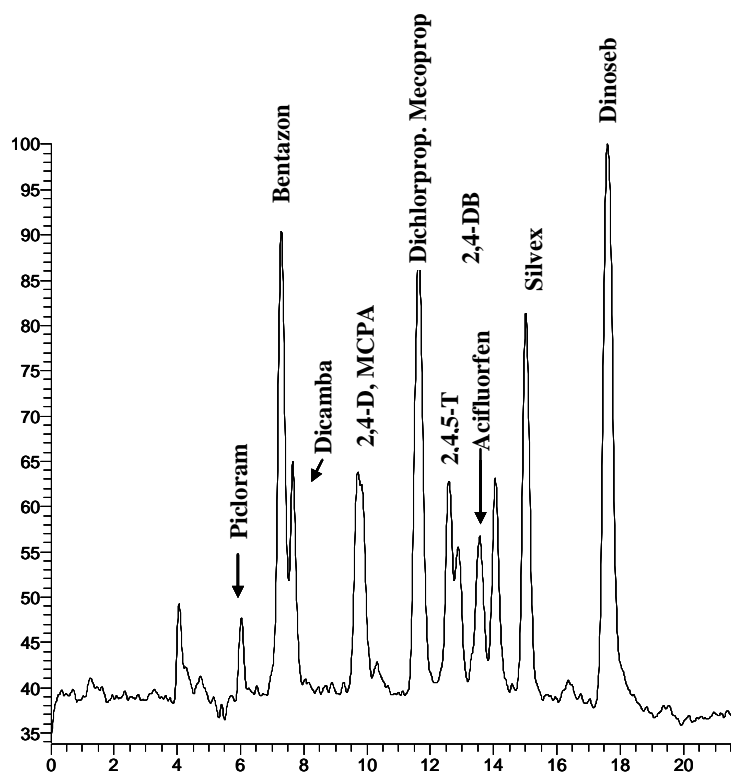


Figure 2.12. Typical chromatogram from a full scan LC-ESP-MS analysis of phenoxy acid herbicides using a Q-IT

Because of their similar structures, it was expected that some compounds will have very similar retention and therefore present co-elution. This was the case of the pairs, 2,4-D (m/z 219) and MCPA (m/z 199), and mecoprop (m/z 213) and dichlorprop (m/z 233).

Their identification however, is still possible because of the formation of different parent ions and fragments.

Table 2.11.Gradient elution program for phenoxy acid separation

#	Time (minutes)	% A (Methanol)	%B (HOAc 1%) pH 2.8
1	0	75	25
2	15	82	18
3	18	75	25
4	30	75	25

#### 2.9.1.3. Analytical Performance for Phenoxy Acid Herbicides

The analytical range was studied under the adopted chromatographic conditions. The measurement of the response by LC-ESP-MS, was performed by injecting standards solutions of all the target analytes within the range of 0.050 to 1.0 µg/ml (nine calibration points), and averaging the peaks areas of the extracted ions of interest (n=3). Satisfactory linearity ( $r^2 > 0.995$ ) was obtained for all acidic herbicides. Regression coefficients values are summarized in Table 2.12.

Table 2.12. Correlation coefficients for target analytes

Analyte	$r^2$	Analyte	$r^2$
Picloram	0.998	Dichlorprop	0.999
Bentazon	0.999	2,4,-DB	0.998
2,4-D	0.999	2,4,5-T	0.998
MCPA	0.998	Silvex	0.995
Dicamba	0.996	Acifluorfen	0.998
Mecroprop	0.995	Dinoseb	0.999

#### 2.9.1.4. Information Power of the Ion Trap

In spite of its high identification potential, a single stage of mass spectrometry (LC/MS) provides limited rate of fragmentation for the acidic herbicides, and this could be of importance when dealing with complex samples. The LCQ ion trap quadrupole mass spectrometer used in MS/MS mode, made it possible for further structural information to be obtained by selecting the deprotonated molecular ions, inducing a collision in the presence of helium buffer gas under reduced pressure in the trap, and analyzing the produced fragments. Therefore, deprotonated molecules could be chosen as precursor ions to conduct CID experiments. Product ions could be generated at different collision energies during CID, and this provides the requirements for a Single Reaction Monitoring experiment (SRM). For example, a CID experiment for acifluorfen ( $m/z$  360) will give main fragments A and B at  $m/z$  316 ( $[M-1-CO_2]$ ) and 281 ( $[M-H-CO_2-Cl]$ ), respectively as could be seen in Figure 2.13.

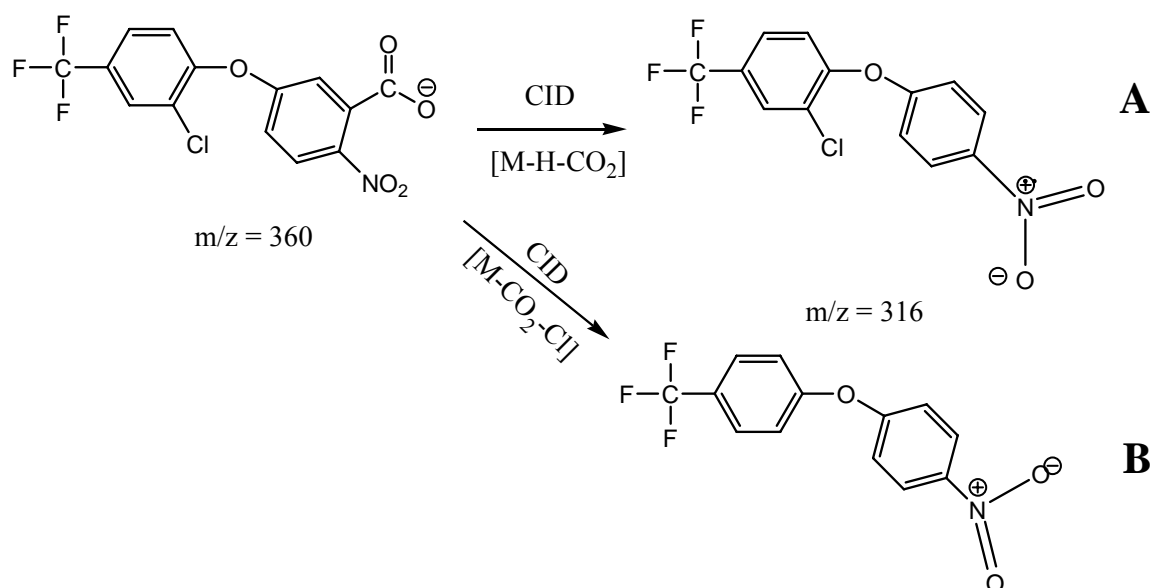


Figure 2.13. CID process for acifluorfen showing the formation of fragments A)  $[M-H-CO_2]^-$  or B)  $[M-CO_2-Cl]^-$  ions

#### 2.9.1.5. Single Reaction Monitoring (SRM) Experiments for Phenoxy Acid Herbicides

In order to improve the sensitivity of the analysis of polar herbicides and to provide a method that account for better specificity, a single reaction-monitoring program (SRM) was implemented. It was expected that this mode could help in the elimination of background interferences during the separation of the 12 peaks of interest using a gradient elution program. In fact, before conducting an SRM experiment the LCQ required optimization of the collision energy for each herbicide. This was done by direct infusion of standard solutions ranging from 1-10 mg/L. The optimized parameters presented on Table 2.9 were used as starting point during this assessment. Again, to consider the effect of the mobile phase the instrument syringe pump was interconnected to the HPLC stream using the tee diverter.

The deprotonated ions (precursors) were first isolated in the LCQ by applying an appropriate waveform across the end cap electrodes of the ion trap to eject all trapped ions except those of the  $m/z$  of interest. This process is performed automatically by the software with the aid of the analyte tuning function. Once isolated, the ions were then subjected to a supplementary AC signal to resonantly excite them and cause collision-induced dissociation (CID) to generate fragment ions. The collision energy is specified in the LCQ as a percentage of the RF potential applied to the end cap electrodes. The maximum voltage in the LCQ is 10 V. The percentage collision energy refers to the percentage of the 10 V that is actually applied. The SRM results are reported in Table 2.13 which presents a summary of the values of collision energies used along with the precursor and product ions formed during the MS<sup>2</sup>. As could be seen from this Table the values range from 20 to 40 % for the acidic herbicides experiment. This collision energy is selected to preserve the signal of the precursor about 10-20 %. It is important also to notice from this Table that analytes like 2,4-D and dicamba (which present the same  $m/z$  219 as precursor ion), clearly benefit from SRM by using their product ions ( $m/z$  161 and 175) during the MS<sup>2</sup> experiment. For compounds that presented co-elutions problems like mecoprop and dichlorprop, unambiguous detection of both product ions is attained during the SRM experiment.

#### 2.9.1.6 Fragmentation Pathways for Acidic Herbicides

The phenoxy acid herbicides MCPA, 2,4-D, mecoprop and dichlorprop have the same skeletal structure. The differences lie in the substituent in the 2-position of the ring (methyl or chlorine), and in the carbon in the  $\beta$  position of the carboxylic function

Table 2.13. Protonated molecules and product ions monitored in MS-MS experiment

	Compound	Collision Energy (CE)	Precursor Ion (m/z)	Product Ions (% relative abundance)
1	Picloram	40	239	<b>195</b> (100)
2	Bentazon	35	239	<b>197</b> (100) <b>175</b> (75)
3	Dicamba	25	219	<b>175</b> (100)
4	2,4-D	25	219	<b>161</b> (100)
5	MCPA	30	199	<b>141</b> (100)
6	Dichlorprop	20	233	<b>161</b> (100)
7	Mecroprop	25	213	<b>141</b> (100)
8	2,4,5-T	20	253	<b>195</b> (100)
9	2,4-DB	25	247	<b>161</b> (100)
10	Acifluorfen	20	360	<b>316</b> (100)
11	Silvex	20	267	<b>195</b> (100)
12	Dinoseb	40	239	<b>194</b> (100), <b>222</b> (30)
13	3,5-dichlorobenzoic Acid (ISTD)	30	189	<b>145</b> (100)
14	Surrogate	30	225	<b>167</b> (100)

(hydrogen or methyl). Therefore their fragmentation behavior in the ion trap is quite similar (Baglio et al., 1999).

The MS<sup>2</sup> spectra of the quasi-molecular ions (parent ions) show only one type of product ion corresponding to m/z 141 for mecoprop and MCPA, and m/z 161 for 2,4-D and dichlorprop. Figure 2.14 shows how the loss of the carboxylic moiety is responsible for the formation of the fragments for several herbicides (Petrovic 2003).

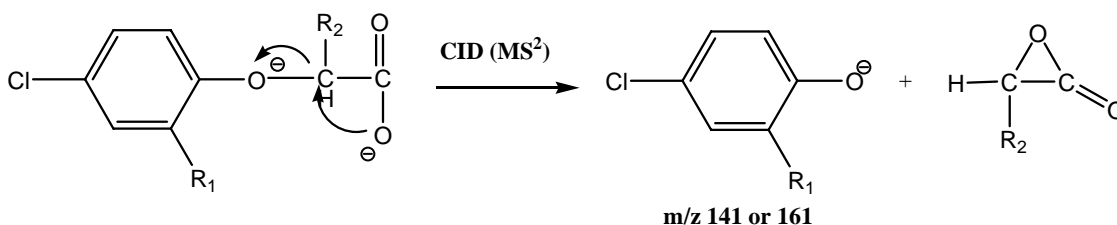


Figure 2.14. CID MS<sup>2</sup> fragmentation pathway for deprotonated phenoxy acid herbicides  
R<sub>1</sub> = Cl or CH<sub>3</sub>, R<sub>2</sub> = CH<sub>3</sub> or H

A possible pathway in the generation of these fragment ions, involves the internal electron cascade reaction by cleavage of the carboxylic group. The formed phenolate ions and the eliminated neutral lactone are shown in Figure 2.14. The mass spectrum for 2,4-D showing the formation of the  $m/z$  161 fragment is shown in Figure 2.15. A similar fragment formation is expected for 2,4-DB ( $m/z$  247 to 161).

Silvex follows a similar mechanism observed for the other classic phenoxy acids and they present a fragment at  $m/z = 195$  corresponding to the formation of the phenolate ion.

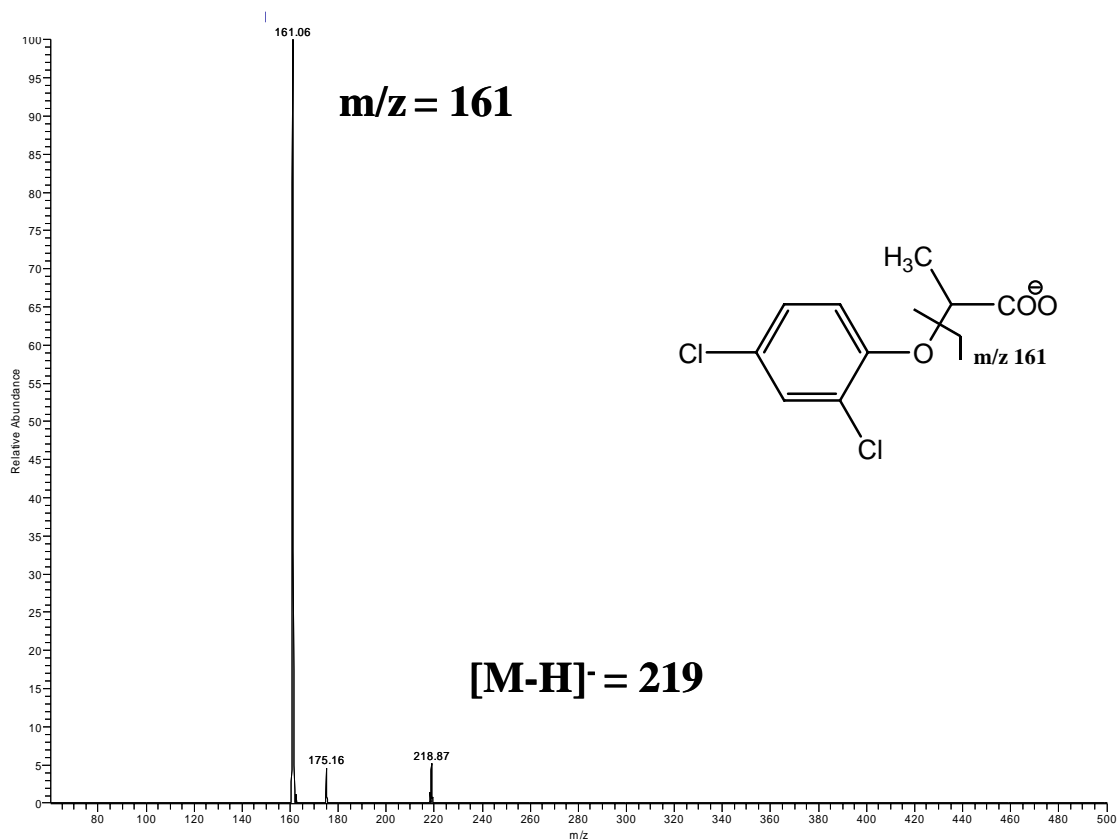


Figure 2.15. Fragmentation formation for 2,4-D during MS/MS experiment



Another compound of interesting behavior was bentazone which is not a classic phenoxy acid compound but it is included in the analytical evaluation as a target analyte as well as other acidic herbicides. The pseudo-molecular ion  $[M-H]^-$  at  $m/z$  239 dominates the ion current but during the CID process, due to the presence of hetero atoms in the chemical structure, undergoes transformation from a six-member ring into a five member ring through elimination of sulfur dioxide (cheletropic elimination) and cleavage of lateral chains. The following mechanism could explain the presence of two daughter ions: 197 and 175 (Larsen 2000) (See Figure 2.16 and 2.17).

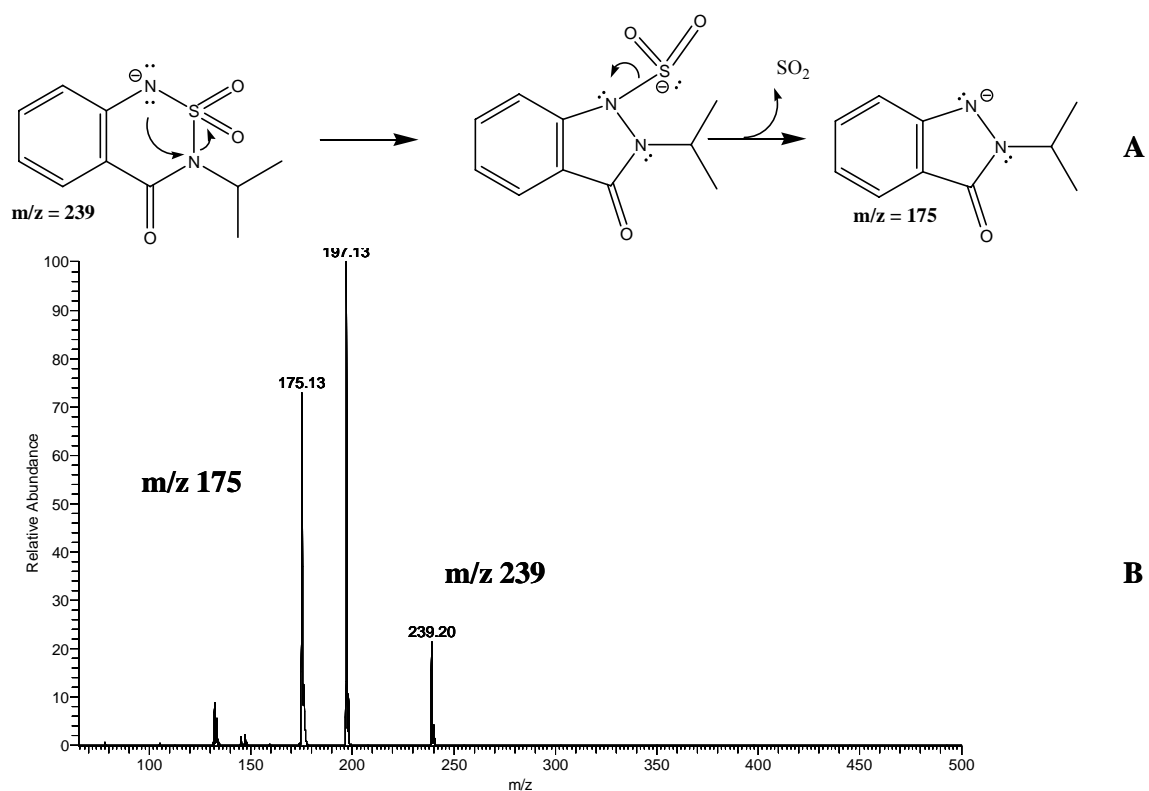


Figure 2.16. CID MS<sup>2</sup> fragmentation pathway for bentazone forming fragment at  $m/z$  175 (A) and respective mass spectra (B)

The formation of the higher intensity fragment  $m/z$  197 probably follows a McLafferty type rearrangement as could be seen in Figure 2.17:

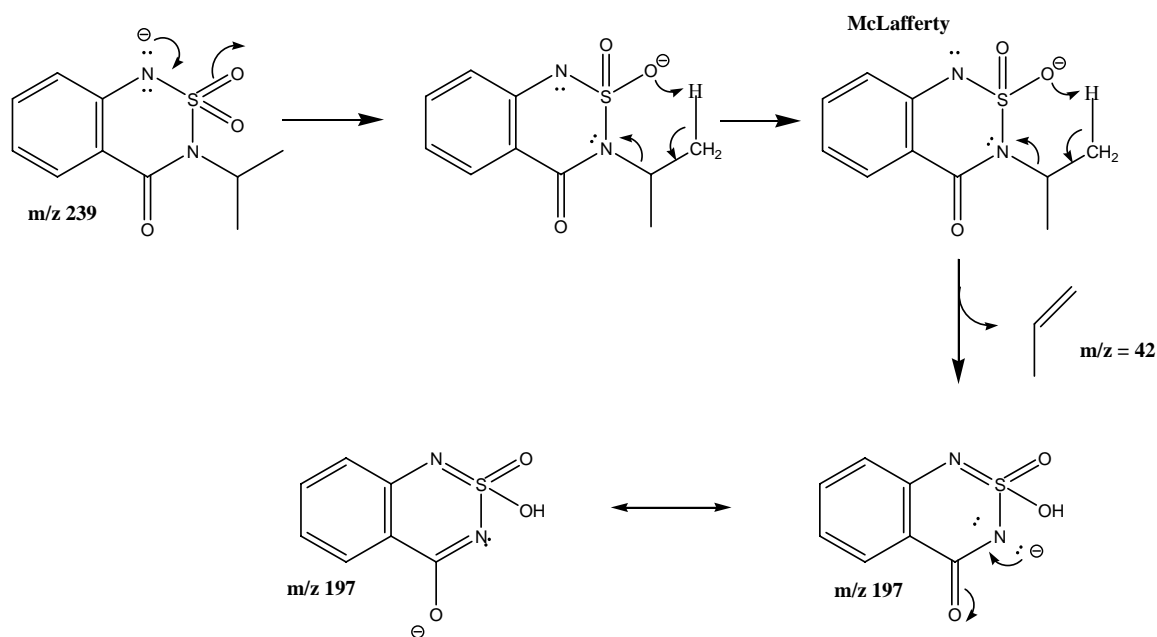


Figure 2.17. CID MS<sup>2</sup> fragmentation pathway for bentazone forming fragment at  $m/z$  197

Dinoseb is also analyzed within the suite of acidic herbicides. This compound contains two nitro groups and therefore, the MS<sup>2</sup> spectrum is very diagnostic. The fragments observed in Figure 2.18 are generated after cleavage of the lateral chain eliminating 1-propene or CH<sub>3</sub>NO, thereby yielding the corresponding nitrocresolate ions  $m/z$  197 and  $m/z$  194 (Baglio et al., 1999).

During the CID experiment, however, the  $m/z$  194 was more intense than the  $m/z$  197 at the optimized collision energy and thus used for quantitation. The multiple stages

offered by the LCQ ion trap instrument to provide MS<sup>2</sup> gives the alternative to get additional structural information of these polar compounds.

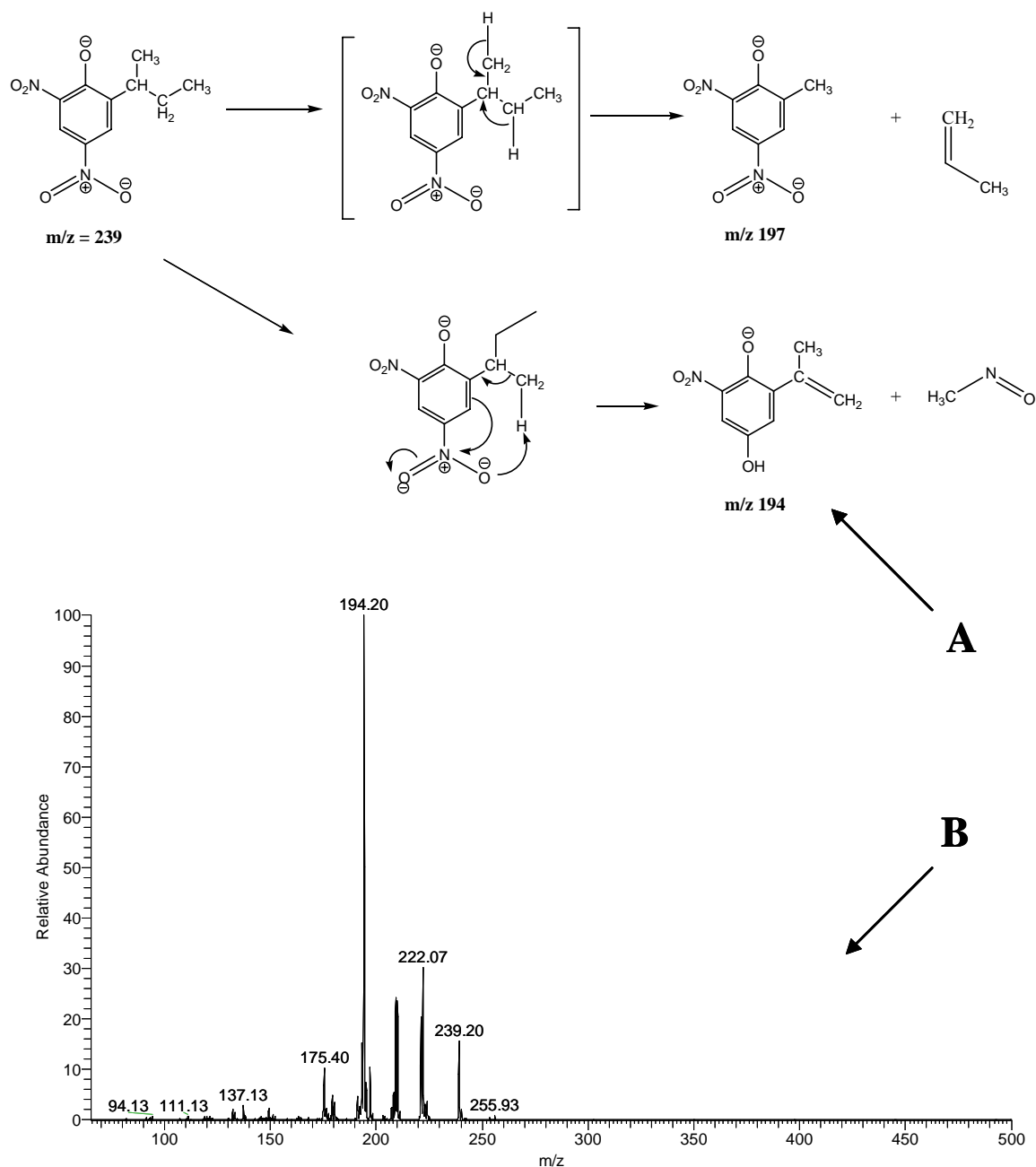


Figure 2.18. CID MS<sup>2</sup> fragmentation pathway for dinoseb (A) and respective mass spectra (B)

The main advantage of using this feature consists in considerably reducing the background noise as could be seen in the reconstructed chromatogram for some individual fragments of the acidic herbicides in Figure 2.19. The isolation of individual fragment ions for each compound accounts for an accurate quantification and identification. The assessment of these herbicides in surface waters is therefore feasible with this technique.

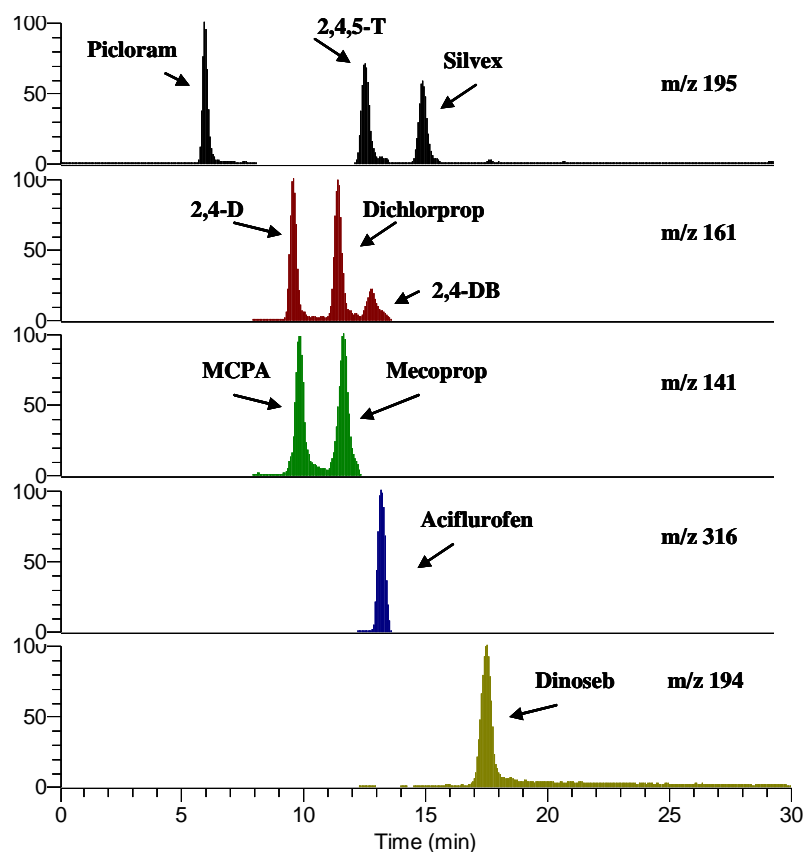


Figure 2.19. Typical chromatogram for an SRM experiment using LC-ESP-MS<sup>2</sup> analysis of phenoxy acid herbicides using a Q-IT

## 2.10. Method Development for Extraction and Clean up Procedures

### 2.10.1. Analyte Enrichment

The Environmental Protection Agency (EPA) provides several methodologies to extract and analyze phenoxy acid herbicides from aqueous matrices by HPLC and GC. The method 8321 B involves both extraction via LLE and detection using, UV or MS, MS<sup>n</sup> by turboionspray (single quadrupole and triple quadrupole), and also the EPA 3535 method, which involves only to the solid phase extraction. The updated version for the Method Detection Limits (MDL's) reported by the EPA given in the LC-001-2.1 standard operating procedure (EPA LC-011-2.1 2008), are in the ppb range (0.2 to 0.4  $\mu\text{gL}^{-1}$ ) for the majority of the acidic herbicides. These guidelines were followed in order to determine the MDL values for the herbicides of interest (Analytical Detection Limit Guidelines 1996).

In order to determine the most suitable conditions for performing the off-line SPE of the tested herbicides from surface waters, several preliminary experiments were run in order to comply with the detection range proposed by the EPA. As a preliminary assessment, the trials were performed using deionized water (Milli-Q water). For selection of both the sample volume and the SPE sorbent, 300 mL aliquots of deionized water spiked with the analytes at 0.25 and 5  $\mu\text{gL}^{-1}$  levels, were analyzed by n= 4 replicates using Oasis HLB Plus, Sep-Pak<sup>TM</sup> Environmental t-C<sub>18</sub> and Oasis MAX cartridges.

All these cartridges were conditioned using an automated SPE system AUTOTRACE and the conditions are given in the experimental section but and an additional hydrolysis step was conducted in the Oasis Max cartridge as discussed later.

Table 2.14. Comparison of percent recoveries from off line SPE analysis of 300 mL sample aliquots of spiked DI water at  $0.3 \mu\text{g} \cdot \text{L}^{-1}$

Cartridge	Oasis HLB Plus 225 mg (n=4)			SEP PAK SPE C <sub>18</sub> , 900 mg (n=4)			Oasis MAX 500 mg (n=4)		
Sample Volume	300 mL			300 mL			300 mL		
Spike Level	0.3 ppb			0.3 ppb			0.3 ppb		
Sample treatment	pH < 2.0			pH < 2.0			NaOH 1 hour, pH < 2.0		
	Average	SD	%RSD	Average	SD	%RSD	Average	SD	%RSD
Picloram	*	*	*	*	*	*	79.0	22.6	28.6
Bentazon	11.9	3.2	26.7	11.0	1.8	16.0	8.4	2.5	29.4
Dicamba	32.4	4.7	14.5	30.1	11.7	38.7	*	*	*
2,4-D	43.2	11.8	27.3	32.6	7.3	22.2	27.4	8.9	32.4
MCPA	27.5	12.0	43.5	21.7	3.7	17.0	23.9	8.1	34.0
Dichlorprop	45.0	7.5	16.6	44.5	9.1	20.5	31.0	5.5	17.7
Mecroprop	35.3	8.5	24.0	39.3	9.1	23.1	28.9	5.3	18.3
2,4,5-T	59.9	11.0	18.4	41.0	8.6	21.0	106.4	39.8	37.4
2,4-DB	136.1	29.3	21.5	87.6	8.4	9.6	52.0	20.0	38.5
Acifluorfen	81.7	3.7	4.5	65.8	15.3	23.3	31.9	4.8	15.0
Silvex	62.5	8.9	14.3	52.4	10.3	19.6	28.0	6.0	21.4
Dinoseb	40.4	2.7	6.7	35.0	4.5	12.9	13.1	7.4	56.5
Overall Average	52.3	9.4	19.8	41.9	8.2	20.4	39.1	11.9	29.9

\* not detected

Table 2.14 shows the recovery percentages obtained for three different cartridges used in this study a low level of spike. The recoveries were calculated from the peak areas obtained for each analyte compared to the peak areas obtained from standards in a calibration curve.

As shown on Table 2.14, different extraction recoveries are obtained depending on the cartridge used. However, both the Oasis HLB and the Sep Pak cartridges, showed fairly similar results for all the analytes. Because of the acidic properties of the compounds, the water sample was acidified to a pH < 2.0 before loading it into the cartridges. Considering the physicochemical properties shown on Table 2.15 for these compounds and based on preliminary experience in the laboratory, this step was considering crucial as a way to improve the recoveries. In spite of these analytical

Table 2.15. Chemical properties of the phenoxyacid herbicides

Compound	Formula	Log K <sub>ow</sub>	K <sub>oc</sub>	log K <sub>oc</sub>	pka	Aqueous solubility (mg/l)
Acifluorfen	C <sub>14</sub> H <sub>7</sub> ClF <sub>3</sub> NO <sub>5</sub>	3.70	3125	3.49	1.93	120
Bentazon	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> S	2.81	37.5	1.57	3.30	500
2,4-D	C <sub>8</sub> H <sub>6</sub> Cl <sub>2</sub> O <sub>3</sub>	2.83	29.4	1.47	2.60	620
2,4-DB	C <sub>10</sub> H <sub>10</sub> Cl <sub>2</sub> O <sub>3</sub>	3.53	100	2.00	4.80	46
Dicamba	C <sub>8</sub> H <sub>6</sub> Cl <sub>2</sub> O <sub>3</sub>	0.54	28.8	1.46	1.87	4500
Dichlorprop	C <sub>9</sub> H <sub>8</sub> Cl <sub>2</sub> O <sub>3</sub>	3.00	48.6	1.69	2.86	350
Dinoseb	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>5</sub>	2.29	3544	3.55	4.50	52
MCPA	C <sub>9</sub> H <sub>9</sub> ClO <sub>3</sub>	3.25	29.4	1.47	3.12	825
(Mecoprop)	C <sub>10</sub> H <sub>11</sub> ClO <sub>3</sub>	3.13	48.6	1.69	3.11	620
Picloram	C <sub>6</sub> H <sub>3</sub> Cl <sub>3</sub> N <sub>2</sub> O <sub>2</sub>	1.92	18.1	1.26	1.97	430
2,4,5-T	C <sub>8</sub> H <sub>5</sub> Cl <sub>3</sub> O <sub>3</sub>	3.31	48.6	1.69	2.20	238
2,4,5-TP (Silvex)	C <sub>9</sub> H <sub>7</sub> Cl <sub>3</sub> O <sub>3</sub>	3.80	80.4	1.91	3.00	140

considerations, both the Oasis HLB and the Sep Pak<sup>TM</sup> did not lose picloram at the volume tested and the recoveries for bentazon, dicamba, MCPA and dinoseb were marginally low. The poor results obtained for these compounds are probably related to the breakthrough volume (Lagana, et. al., 1998).

Even though the acidic herbicides under study hold similarities in their chemical structures, the physical and chemical properties vary according to the main functional groups. A variety of acid functional groups in the suite of acidic herbicides like phenol, benzoic acid, acetic acid, pyridine-carboxylic acid and different phenoxyacetic acids are

targeted during the SPE process. Indeed, the surface chemistry, and the sorption properties together with the chemical properties of the acidic herbicides, influences directly the way these compounds are extracted and also the recovery efficiencies. Sample overload is one of the main causes of low recoveries and poor reproducibility for SPE.

An estimation of the load capacity of the SPE sorbents is given by the calculation of the breakthrough volume ( $V_B$ ). The breakthrough occurs when a solute is no longer retained by the sorbent, because the capacity of the sorbent has been reached. This is especially important in solid phase extraction because the sample is being continuously applied to the sorbent and this material must retain all the solute (Pawliszyn 2002). Different models have been developed to estimate the  $V_B$  based on solute and analyte properties. In this study the model proposed by Thurman, was used to estimate this volume (Thurman et al., 2001).

The model uses the octanol –water partition coefficients ( $\log K_{ow}$ ) to estimate the sorbent-water retention factor ( $\log K_w$ ) using the following equations:

$$\log k_w = 0.988 * \log K_{ow} + 0.02$$

$$V_r = V_o (1 + K_w)$$

$$V_B = V_r - 2.3 \delta$$

$$\delta = V_o (1 + k_w) / \sqrt{N} \quad \text{where:}$$

- $\log K_{ow}$  is the water-octanol distribution constant for each analyte
- $V_o$  is the void volume estimated from the total pore volume
- $\delta$  is the standard deviation depending on the axial dispersion of the analyte and
- $N$  is the plate number for the sorbent



As an example, according with the certificate of analysis from the HLB extraction cartridges, the reported total pore volume was 1.28 cm<sup>3</sup>/g, the cartridges employed for this study have 225 mg of sorbent weight and therefore the void volume can be estimated as follows:

$$V_o = (1.28 \text{ mL / g}) \times 0.225 \text{ g}$$

$$V_o = 0.288 \text{ mL per cartridge}$$

The plate number used for the calculations has been estimated to be ~20 regular C<sub>18</sub> or polymeric SPE cartridges (Poole 2002).

Using the reported water-octanol constants the calculated breakthrough volumes are summarized and compared on Table 2.16 for the cartridges used. From this table, dicamba, bentazon, picloram and dinoseb presented a very low breakthrough volume, which is below the sample volume added into cartridges for enrichment, which was of 300 mL.

This low  $V_B$  could contribute to the low recoveries values obtained for those compounds because that could limit the retention of analytes into the cartridge and produce some losses during the loading step. It is interesting to notice that Sep Pak<sup>TM</sup> cartridges provided larger breakthrough volumes mainly because of its higher sorbent capacity but still presented the same low recoveries as the HLB cartridge.

A different approach was tried with the Oasis MAX cartridges. In this case, the water sample was spiked with the analyte mixture at the same concentration level tested before (0.3 µg L<sup>-1</sup>), and then the sample was hydrolyzed at pH 12.0 with sodium hydroxide 0.1 M for 1 hour, followed by decreasing the pH < 2 with hydrochloric acid. The elution was done using only MeOH with 2% TFA acid. The rationale of introducing

Table 2.16. Estimated breakthrough volumes for the acidic herbicides using Oasis HLB, Sep Pak t-C<sub>18</sub> and Oasis MAX cartridges

Compound	HLB 225 mg V <sub>B</sub> (mL)	Sep Pak t-C <sub>18</sub> , 900 mg V <sub>B</sub> (mL)	Oasis MAX, 500 mg V <sub>B</sub> (mL)
Acifluorfen	663	2590	1439
Bentazon	88	342	190
2,4-D	92	358	199
2,4-DB	450	1759	977
Dicamba	22	88	49
Dichlorprop	135	527	293
Dinoseb	27	105	58
MCPA	238	931	517
Mecoprop	181	708	394
Picloram	12	46	25
2,4,5-T	273	1067	593
Silvex	832	3251	1806

this step is based on the way the acidic herbicides are manufacture. The formulations can include free acids, alkaline salts or esters and in herbicides combinations. As could be seen on Table 2.13, different solubility's of the unionized acidic herbicides are present, but the acidic herbicides most frequently exist in ionized form at the environmental pH values (Wells and Yu, 2000). Because some formulation may exhibit an ionized form it is important to convert all the analytes to their free acid form before the SPE process (by decreasing the pH). However, the recovery values obtained and presented in Table 2.14 demonstrated poor performance for the same type of compounds that in the case of the other sets of cartridges. The main difference is that picloram was successfully recovered from the water sample and dicamba was not detected. The overall precision for these three sets is also similar. In order to avoid the breakthrough of target compounds, and as a way to improve the extraction efficiency a lower volume of sample was selected (100 mL) and also a significantly higher spiked amount (5 µg<sup>-1</sup>) was tested by using only the HLB Plus. This is because the HLB cartridges offered similar results than those obtained with the Sep-Pak<sup>TM</sup> and Oasis MAX cartridges, and also because of the wetabiliting

properties of these cartridges which makes them very versatile while consumes less solvent. The results are presented in Table 2.17. An overall improvement in both recovery and precision was obtained for most of the analytes with the exception of dinoseb. The percent recovery ranged from 11 to 109 %. Dinoseb, along other compounds like dicamba, picloram and bentazon has been reported by several authors as “difficult to recover by SPE” (Wells and Yu, 2000; Balinova, 1996). Further attempts were used to try to recover this compound. The introduction of sodium chloride to improve ionic strength and small amounts of methanol in the water sample as a helper were tried without further success for dinoseb. Therefore, the SPE protocol was flagged for dinoseb as a consistent low-end recovered analyte.

Table 2.17. Recovery study for phenoxy acid herbicides on spiked DI water samples

<b>Cartridge</b>	<b>Oasis HLB Plus 225 mg</b>		
<b>Sample Volume</b>	<b>(n=4)</b>		
<b>Spike Level</b>	<b>100 mL</b>		
	<b>5 ppb</b>		
	<b>Average</b>	<b>SD</b>	<b>% RSD</b>
Picloram	67.7	8.9	13.1
Bentazon	109.2	8.7	8.0
Dicamba	66.4	10.3	15.5
2,4-D	88.4	8.4	9.5
MCPA	76.6	10.9	14.2
Dichlorprop	78.5	6.0	7.6
Mecroprop	65.6	8.6	13.0
2,4,5-T	84.1	6.4	7.6
2,4-DB	94.0	5.1	5.4
Acifluorfen	89.5	3.1	3.5
Silvex	90.2	7.0	7.8
Dinoseb	11.7	5.5	47.4
<b>Overall Average</b>	<b>76.8</b>	<b>7.4</b>	<b>12.7</b>

### 2.10.2. Method Detection Limit Study (MDL)

In analytical mass spectrometry there are a few basic parameters that need to be assessed to determine the robustness of the analytical procedure. The method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified and measured in a real matrix with the complete analytical method, including in this case, all the steps involved in the SPE extraction/clean up and LC/MS determination (Budde 2001). A more comprehensive definition is given by the EPA Guidelines: “the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, and is determine from analysis of a sample in a given matrix containing the analyte” (Analytical Detection Limit Guidance 1996).

Therefore, the MDL is based upon the variability, or precision, between seven or more replicates run at identical concentrations. Two types of MDL are required for compliance purposes: reagent water MDL and the real matrix specific MDL. The US EPA guidelines require that the ratio of spiking concentrations to estimate MDL should be lower than 5:1 (EPA LC-011-2.1 2008).

Five different check points are evaluated to determine if the MDL meet all the necessary criteria:

- Does the spike level exceed 10 times the MDL? If so, the spike level is high
- Is the MDL higher than the spike level? If so, the spike level is low
- Does the calculated MDL meet regulatory requirements for the necessary program(s)?

- Is the signal/noise (S/N) in the appropriate range?, ( $> 2.5$ )
- Are the replicate recoveries reasonable?

The EPA and NOAA (National Oceanographic Atmospheric Administration), provides data related to the Maximum Concentration Level (MCL) for some acidic herbicides in surface water. The MCL is defined as the highest level permitted of a contaminant in drinking water. They are presented in Table 2.18 for those herbicides reported by these agencies. A suitable method for determining these herbicides should be able to account for this MCL levels.

Table 2.18. Maximum contaminant level for acidic herbicides

	<b>Contaminant</b>	<b>MCL (<math>\mu\text{g}\cdot\text{L}^{-1}</math>)</b>
1	2,4-D	70
2	Dinoseb	7
3	Picloram	29
4	Silvex	50
5	Dicamba	10
6	Bentazon	200

Source: US EPA Drinking Water Standards, NOAA SquiRT tables, 2008

The method limit of detection was calculated spiking both, the reagent laboratory water and real surface water samples at  $n = 11$  and  $n = 7$  replicates, respectively.

This strategy was followed to account for variability of the method in both “best case limits” in clean samples, and more confident real surface water samples. The MDL recovery study was conducted at a targeting spike level of  $5 \mu\text{g}\cdot\text{L}^{-1}$  level and the recovery results are presented in Table 2.19.

Table 2.19. Method Detection Limits Study for Acidic Herbicides using laboratory reagent water (DDI) as matrix (n=11)

	<b>MDL= <math>t*s</math> (<math>\mu\text{gL}^{-1}</math>)</b>	<b>LOQ = <math>10*s</math></b>	<b>s/n = mean/std</b>	<b>% Recovery</b>	<b>Meet EPA Criteria?</b>
Picloram	2.9	10.6	4.1	87	YES
Bentazon	4.1	14.9	3.3	99	YES
Dicamba	4.7	17.1	2.6	89	YES
2,4-D	3.3	11.9	3.9	92	YES
MCPA	3.8	13.8	3.0	83	YES
Dichlorprop	3.0	10.8	4.0	87	YES
Mecoprop	3.7	13.5	2.8	74	YES
2,4,5-T	2.4	8.7	4.9	85	YES
2,4-DB	2.5	9.2	4.7	85	YES
Acifluorfen	3.3	12.0	3.5	83	YES
Silvex	2.5	9.2	4.9	90	YES
Dinoseb	1.1	3.8	2.0	15	NO

LOQ: Lower limit of Quantitation

Each one of the check points criteria describe before are evaluated individually to determine if they met the EPA requirements. The first MDL for assessment is the reagent water as matrix replacement. The MDL values obtained were below the spike level selected, meeting the criteria. Ten times the standard deviation of the individual herbicides were higher than the spike level of  $5 \mu\text{gL}^{-1}$  which complies with the guidelines. The S/N for all the herbicides are within the acceptance criteria ( $S/N > 2.5$ ) except for dinoseb. Probably, the MDL estimated for this herbicide was high and therefore, the sample should be spiked at a higher level to increase the signal. The S/N criterion is also a good estimate of the instrument detection limit. The average percent recoveries are acceptable for all the acidic herbicides except dinoseb that consistently present a low recovery value. The regulatory requirements were met for all the herbicides but special caution needs to be given to dinoseb, which present a very low MCL.

In order to determine the “real” MDL a set of surface water samples collected from different areas within the Everglades and Biscayne National Parks and Big Cypress National Preserve were spiked at the same level than the reagent laboratory water ( $5 \mu\text{g} \cdot \text{L}^{-1}$ ) for a total of  $n = 7$  replicates (See Table 2.20).

Table 2.20. Method Detection Limits Study for Acidic Herbicides using real surface water matrix ( $n=7$ )

	MDL= $t \cdot s$ ( $\mu\text{g} \cdot \text{L}^{-1}$ )	LOQ = $10 \cdot s$	s/n = (mean/std)	% Recovery	Meet EPA Criteria?
Picloram	4.5	16.3	1.8	60	NO
Bentazon	2.6	8.2	3.8	63	YES
Dicamba	2.5	8.5	4.5	76	YES
2,4-D	2.3	7.4	6.2	92	YES
MCPA	2.4	7.6	5.2	80	YES
Dichlorprop	1.5	5.1	9.3	94	YES
Mecoprop	2.5	8.6	4.8	82	YES
2,4,5-T	1.9	6.4	7.6	97	YES
2,4-DB	5.5	17.5	2.4	85	NO
Acifluorfen	2.9	10.2	4.9	100	YES
Silvex	1.3	4.7	10.7	101	YES
Dinoseb	1.9	6.5	1.5	20	NO

LOQ: Limit of quantitation

From Table 2.20 it is interesting to notice the effect of the matrix over the MDL values. Almost all the herbicides met the requirement of being lower than the spike level. An exception to this was 2,4-DB which presents a higher number of  $5.5 \mu\text{g} \cdot \text{L}^{-1}$  and did not met the criteria. When applying the S/N criterion only three of the herbicides did not comply with the requirements, meaning that the contribution of matrix affects influences the sensitivity of the signal for herbicides like picloram, 2,4-DB and dinoseb.

When comparing the values obtained with both, the laboratory reagent water and the real spiked surface water, the recoveries were similar for some of the herbicides.

However, Picloram, Bentazon and Dicamba presented low recovery values. Specifically, Dicamba present very similar and consistent low recoveries as found with the reagent DDI water MDL study. Compounds like silvex and acifluorfen showed slightly better values probably because of their greater hydrophobicity.

### 2.10.3. Sample Considerations when Assessing Recovery Data

Surface waters from the protected areas studied presented several types of matrix constituents like dissolve organic matter (DOM), suspended materials, peryphyton, plants and organism debris, and in some areas, changing rain and salinity regimes. All of these variables together in the water sample, compromise the efficient enrichment of the herbicides.

The use of a glass fiber 0.45  $\mu\text{m}$  filters, helped to remove part of these suspended solid materials present in the surface waters, although many samples required several filters changes to achieve the desire volume before SPE processing. Therefore, this filtration step was vital to avoid the physical clogging of the SPE cartridges.

It is also critical to stress that the most likely reason for the low recoveries in the real samples is related to the presence of variable amounts of humic materials dissolved in the water samples. The humic acids consist of a mixture of complex macromolecules that have polymeric phenolic structures with the ability to chelate with metals, forming aggregates with the target herbicides (Doble et al., 2007). Indeed, the sensitivity of the method is affected by these materials that tend to adhere to the surface of the HLB polymeric cartridge forming a coating during the pre-concentration process. This new layer acted as a new sorbent material “extracting” the analytes of interest. Since the



bonding properties to this humic phase is stronger than bonding to the polymeric phase of the cartridge, the analyte recoveries are lower (Geerdink et al., 1997).

Because of the necessity to have protonated species of the acidic herbicides in the surface water for its proper extraction, the presence of the humic materials was enhanced. This condition imposed by a decreasing in the pH of the natural water before the SPE process, caused that the humic materials were co-extracted and retained through the rest of the analytical protocol until their mass spectrometric determination and therefore affecting the recoveries values.

#### 2.10.4. Precision Evaluation

It is very important to consider the complexity of the matrix when evaluating the precision of the proposed analytical methodology. Fortified laboratory blanks were run with every set of samples analyzed. The Laboratory Blank Spiked (LBS) was made using DI water as matrix replacement, the pH was adjusted, and they were spiked with a mixture of the acidic herbicides at  $5\ \mu\text{gL}^{-1}$  level. The LBS are then processed through the whole analytical procedure.

Figure 2.20, shows the variation of the recoveries of a laboratory blank spike (LBS) within 11 different extraction batches for a total of 96 samples (each batch of sample required at least one LBS). Acceptable precision (overall  $< 30\%$  RSD) was obtained for the inter-batch quantification of the different herbicides. Also, an overall average of  $80\%$  recovery was found for the majority of the herbicides. It is also notorious the lack of extraction capability for dinoseb but also its consistency throughout different batches.

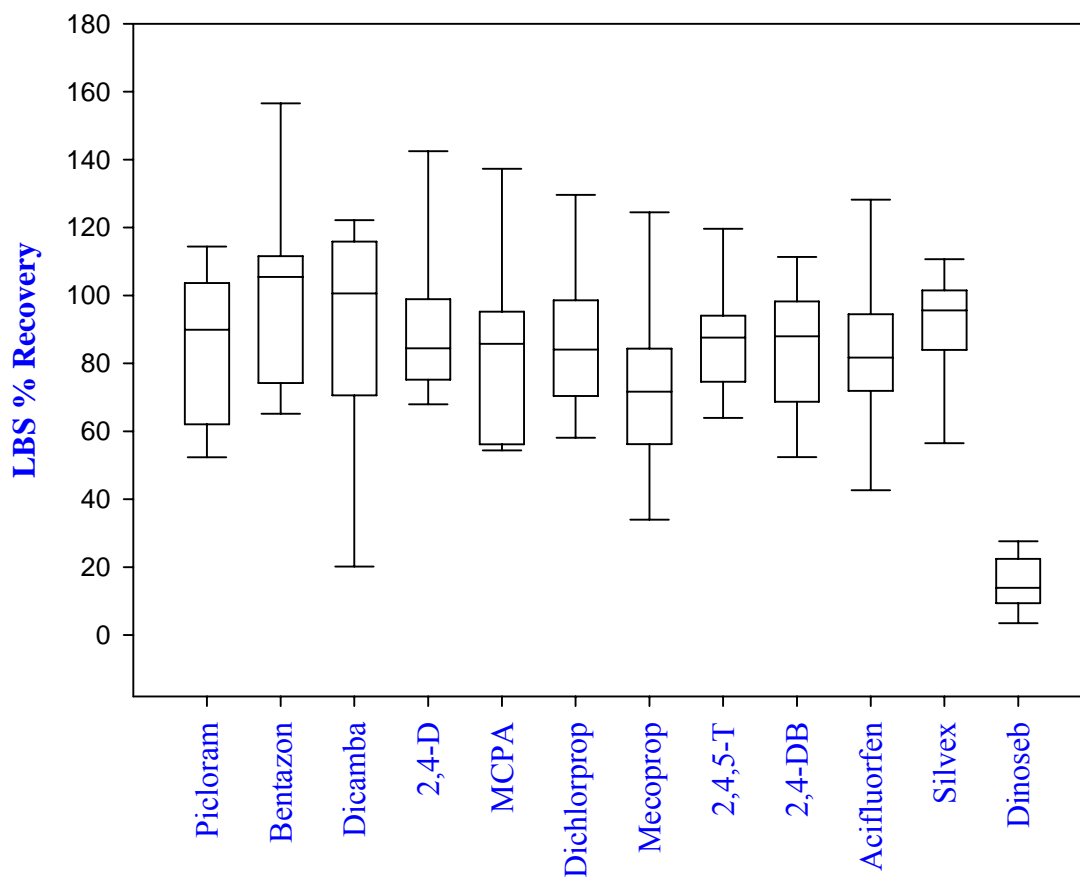


Figure 2.20. Interday variation of the LBS for acidic herbicides (n=11)

To assess both precision and accuracy, a matrix spike (MS) was analyzed during for the same batches where the LBS was run. The matrix spike consisted in the use of real fortified water sample, selected randomly the day of the analysis and spiked with a mixture of the herbicides of interest. Once again, the whole analytical procedure was followed and the behavior of this control sample is shown in Figure 2.21. The matrix spike parameter was used as an indicator of the effect of the sample matrix on the recovery of the target analytes. Based on the results obtained, the precision of the method was evaluated showing global values of below 35 % of RSD with the exception

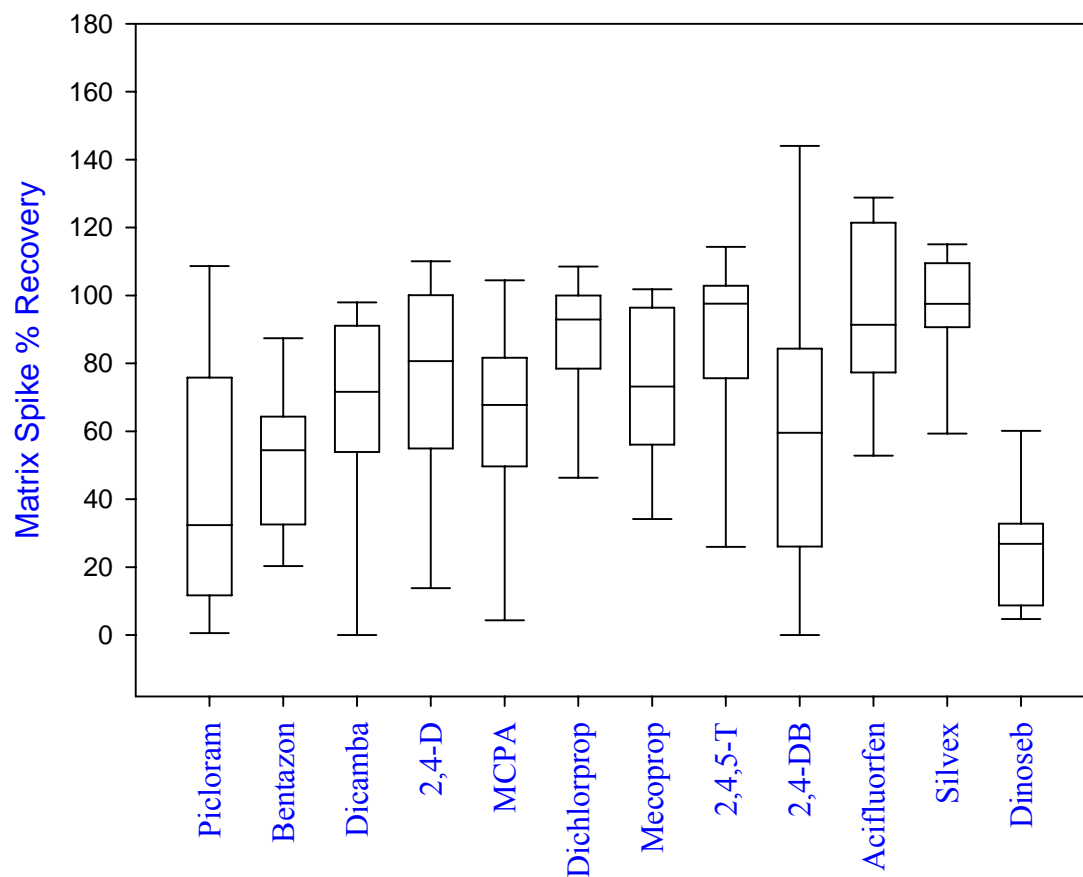


Figure 2.21. Inter-day variation for MS for acidic herbicides (n=11)

of picloram, 2,4-DB, and MCPA. A lower recovery trend was observed for the acidic herbicides demonstrating a pronounced effect of the matrix.

Although these inter-day values for the matrix spike were high, they are in agreement for what could be expected and reported by the EPA method 8321B. Table 2.21 presents a comparison of the precision of the acidic herbicides with the EPA method with one proposed in this investigation.

The values offered by EPA are for drinking water while the values offered in the proposed method are for natural waters.

Table 2.21. Accuracy and Precision for Chlorinated Phenoxy Acid Herbicides Spiked into natural water at 5 µgL<sup>-1</sup> level using different Methodologies

Method Matrix Spike Concentration (µg*L-1) # of analysis Enrichment Mass Spectrometric Technique	EPA METHOD 8321 B Drinking Water 5 µg*L <sup>-1</sup> 9 SPE Single Q (SIM) or QqQ			This Study Natural Water 5 µg*L <sup>-1</sup> 11 SPE Q-IT		
Compound	% Recovery	Std. Dev.	% RSD	% Recovery	Std. Dev.	% RSD
Picloram	*	*	*	41	37	89
Bentazon	*	*	*	50	22	43
Dicamba	63	22	35	62	34	55
2,4-D	26	13	50	72	33	46
MCPA	60	23	38	62	31	51
Dichlorprop	43	18	42	87	19	22
Mecoprop	78	21	27	74	23	31
2,4,5-T	72	31	43	85	31	36
2,4-DB	29	24	83	60	46	76
Acifluorfen	*	*	*	95	25	26
Silvex	62	14	23	97	17	18
Dinoseb	73	11	15	25	18	73
<b>Overall Average</b>	56	20	40	67	28	47

\* not reported

The values presented in this table demonstrate the complexity of the analysis of this type of herbicides and the range of variability expected. The values reported in this investigation are considered as acceptable for the majority of herbicides if considering the broad physicochemical variation of the real samples. However, the suite of possible candidates for reporting options cannot include picloram, 2,4-DB or Dinoseb because of the high variability and poor performance obtained.

In spite of this complex matrix scenario, Table 2.22 shows a comparison of the MDLs results reported for 7 acidic herbicides using a similar Ion Trap System (Baglio et al., 1999). The values are express as injected amounts and as could be notice from this table, there is a good agreement for the majority of the polar herbicides included in the comparison.

Table 2.22.MDL Comparison data for the analysis of Acidic Herbicides

Sample Volume Spike Level Cartridge Amount injected	This Study		Baglio et al.**	
	100 mL 5 µg*L <sup>-1</sup> Oasis HLB 225 mg 25 µL MDL (pg)***	LOQ	1000 mL 10 µg*L <sup>-1</sup> Carbon Black 50 µL MDL (pg)***	LOQ
Picloram	113	408	*	*
Bentazon	65	205	110	340
Dicamba	62	213	330	1100
2,4-D	58	184	390	1240
MCPA	60	191	130	420
Dichlorprop	37	127	90	290
Mecoprop	62	214	100	320
2,4,5-T	46	161	*	*
2,4-DB	137	437	*	*
Acifluorfen	72	254	*	*
Silvex	33	119	*	*
Dinoseb	47	162	100	320

\* not reported;

\*\* Baglio et al., 1999. ;

\*\*\*method detection limit defined as 10 standard deviation

### 2.11. Application to Real Samples

Surface waters samples were collected from Everglades National Park, Biscayne Bay National Park, and Big Cypress National Preserve in South Florida, and they were analyzed to assess the presence of these polar herbicides. The proposed method was applied to 48 different sampling sites. Due to the SPE advantages and the nature and characteristics of the waters samples from the Parks, solid phase extraction (SPE) is probably the best method available for simultaneously carrying fractionation and concentration of the studied organic contaminants.

Among the different compounds studied, picloram was the most frequently detected, was found above the method detection limit at different sampling sites, as shown in Table 2.23. The ranges of concentration for this compound were from 0.6 to 35.0  $\mu\text{g/L}$  (although the reporting value is above the MDL of 4.5  $\mu\text{g/L}^{-1}$ ). Also, 2,4-D was found on 5 stations at Biscayne Bay area at concentrations below MDL of 2.3  $\mu\text{g/L}^{-1}$  but the data was presented as “detected” for illustration purposes, but none of the values were reported to the management authorities. The variation throughout the CARE Stations is shown on Figure 2.22 for picloram. This chromatogram shows the effectiveness of the proposed methodology for clean up and detection capabilities of the mass spectrometer even for complex matrices.

## 2.12. Conclusions

The complex nature and diverse physical-chemical characteristics, together with the high content of organic matter of the waters studied, made the isolation, concentration and fractionation of pollutants by SPE a difficult task.

The polymeric cartridges Oasis HLB exhibited a fair performance for elimination of the background interferences presented in the water samples. The easy conditioning steps and the rapid setup, allowed for a straightforward pre-treatment of water samples by the autotrace system.

The chromatographic separation of the phenoxy acid herbicides was successfully accomplished with gradient elution program using methanol and acetic acid 1% in a  $\text{C}_{18}$  column and by electrospray ionization in the negative mode (ESI). This ionization technique with selected reaction monitoring, was effective for the detection and

Table 2.23. Summary of concentration of acidic herbicides found within South Florida Protected areas

				Concentration (µg/L)											
Collection Date	FTU ID	Site ID	Description	Picloram	Bentazon	Dicamba	2,4-D	MCPA	Dichlorprop	Mecoprop	2,4,5-T	2,4-DB	Acifluorfen	Silvex	Dinoseb
6/14/2007	07-00087	BB1	Black Creek Canal	*	*	*	*	*	*	*	*	*	*	*	*
4/20/2007	07-00061	BB1	Black Creek Canal	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00166	BB1	Black Creek Canal	*	*	*	0.71	*	*	*	*	*	*	*	*
6/14/2007	07-00091	BB10	North Canal at Bayfront Park Marina	*	*	*	*	*	*	*	*	*	*	*	*
4/18/2007	07-00060	BB10	North Canal at Bayfront Park Marina	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00173	BB10	North Canal at Bayfront Park Marina	*	*	*	0.91	*	*	*	*	*	*	*	*
6/14/2007	07-00092	BB11	Creek North of SW344 St Fill	*	*	*	*	*	*	*	*	*	*	*	*
4/18/2007	07-00059	BB11	Creek North of SW344 St Fill	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00174	BB11	Creek North of SW344 St Fill	*	*	*	0.95	*	*	*	*	*	*	*	*
4/20/2007	07-00062	BB12	Intersection of Princeton and L-31E	*	*	*	*	*	*	*	*	*	*	*	*
6/28/2007	07-00110	BB12	Intersection of Princeton and L-31E	*	*	*	*	*	*	*	*	*	*	*	*
3/19/2008	08-00056	BB12	Intersection of Princeton and L-31E	*	*	*	*	*	*	*	*	*	*	*	*
4/18/2007	07-00055	BB2	Creek South of Black Creek Canal	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00167	BB2	Creek South of Black Creek Canal	*	*	*	0.86	*	*	*	*	*	*	*	*
6/13/2007	07-00086	BB3	Princeton Canal	*	*	*	*	*	*	*	*	*	*	*	*
4/20/2007	07-00063	BB3	Princeton Canal	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00168	BB3	Princeton Canal	*	*	*	*	*	*	*	*	*	*	*	*
6/13/2007	07-00086	BB4	Creek South of Princeton Canal	*	*	*	*	*	*	*	*	*	*	*	*
4/18/2007	07-00056	BB4	Creek South of Princeton Canal	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00169	BB4	Creek South of Princeton Canal	6.19	*	*	1.1	*	*	*	*	*	*	*	*
6/14/2007	07-00088	BB5	Military Canal	*	*	*	*	*	*	*	*	*	*	*	*
4/20/2007	07-00064	BB5	Military Canal	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00170	BB5	Military Canal	*	*	*	*	*	*	*	*	*	*	*	*
6/14/2007	07-00089	BB6	Creek North of Military Canal and South of Fender Point	*	*	*	*	*	*	*	*	*	*	*	*
4/18/2007	07-00057	BB6	Creek North of Military Canal and South of Fender Point	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00171	BB6	Creek North of Military Canal and South of Fender Point	*	*	*	1.05	*	*	*	*	*	*	*	*
6/14/2007	07-00085	BB7	Mowry Canal	*	*	*	*	*	*	*	*	*	*	*	*
6/14/2007	07-00090	BB7	Mowry Canal	*	*	*	*	*	*	*	*	*	*	*	*
4/18/2007	07-00058	BB7	Mowry Canal	*	*	*	*	*	*	*	*	*	*	*	*
10/11/2007	07-00172	BB7	Mowry Canal	*	*	*	1.05	*	*	*	*	*	*	*	*
4/20/2007	07-00066	BB9	L31-E wetlands North from Mowry canal connected	*	*	*	*	*	*	*	*	*	*	*	*
6/28/2007	07-00109	BB9	L31-E wetlands North from Mowry canal connected	*	*	*	*	*	*	*	*	*	*	*	*
8/8/2007	07-00120	BICY1	Deep Lake - SR837	*	*	*	*	*	*	*	*	*	*	*	*
5/14/2007	07-00073	BICY2	East Hinson Marsh - SR839	*	*	*	*	*	*	*	*	*	*	*	*
8/8/2007	07-00119	BICY3	Kissimmee Billy Strand	*	*	*	*	*	*	*	*	*	*	*	*
8/8/2007	07-00121	BICY4	Monument Road/Campsite	*	*	*	*	*	*	*	*	*	*	*	*
3/18/2008	08-00050	BICY5	Roberts Lake/Sweetwater Strand	7.1	*	*	*	*	*	*	*	*	*	*	*
5/14/2007	07-00074	BICY5	Roberts Lake/Sweetwater Strand	*	*	*	*	*	*	*	*	*	*	*	*
3/18/2008	08-00049	BICY6	Pinecrest Flowway	34.9	*	*	*	0.78	*	*	*	*	*	*	*
5/14/2007	07-00075	BICY6	Pinecrest Flowway	*	*	*	*	*	*	*	*	*	*	*	*
8/10/2007	07-00132	BICY6	Pinecrest Flowway	*	*	*	*	*	*	*	*	*	*	*	*
8/8/2007	07-00123	BICY7	Lime Tree Hammock	*	*	*	*	*	*	*	*	*	*	*	*
8/8/2007	07-00122	BICY8	L-28 Tie Back Gated Culvert	*	*	*	*	*	*	*	*	*	*	*	*
8/8/2007	07-00130	BICY9	L-28 N of I75	*	*	*	*	*	*	*	*	*	*	*	*
5/2/2007	07-00067	C-111-3	C111 Basin	*	*	*	*	*	*	*	*	*	0.38	*	*
8/24/2007	07-00133	C-111-3	C111 Basin	*	*	*	*	*	*	*	*	*	*	*	*
3/18/2008	08-00052	C-111-3	C111 Basin	0.52	*	*	*	*	*	*	*	*	*	*	*
5/2/2007	07-00066	C-111-4	C111 Basin	*	*	*	*	*	*	*	*	*	*	*	*

\* < MDL

Table 2.23. Summary of concentration of acidic herbicides found within South Florida Protected areas (Cont.)

Collection Date	FIU ID	Site ID	Description	Concentration (µg/L)											
				Picloram	Bentazon	Dicamba	2,4-D	MCPA	Dichlorprop	Mecoprop	2,4,5-T	2,4-DB	Acifluorfen	Silvex	Dinoseb
6/28/2007	07-00104	E1	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00115	E2	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00158	E2	East Boundary	7.6	*	*	*	*	*	*	*	*	*	*	*
6/28/2007	07-00100	E3	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
6/28/2007	07-00106	E4	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
6/28/2007	07-00107	E5	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00143	E6	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
5/2/2007	07-00070	E7	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
6/28/2007	07-00108	E7	East Boundary	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00150	FB1	Florida Bay	*	*	*	*	*	*	*	*	*	*	*	*
5/31/2007	07-00079	FB1	Florida Bay	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00151	FB2	Florida Bay	*	*	*	*	*	*	*	*	*	*	*	*
6/1/2007	07-00080	FB2	Florida Bay	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00140	S178	Special Interest	*	*	*	*	*	*	*	*	*	*	*	*
5/2/2007	07-00069	S178	Special Interest	*	*	*	*	*	*	*	*	*	0.49	*	*
3/19/2008	08-00053	S178	Special Interest	0.87	*	*	*	*	*	*	*	*	*	*	*
5/2/2007	07-00068	S18C	Special Interest	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00138	S18C	Special Interest	*	*	*	*	*	*	*	*	*	*	*	*
3/19/2008	08-00054	S18C	Special Interest	0.32	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00157	SRS1	Shark River Slough	0.6	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00113	SRS1	Shark River Slough	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00156	SRS2	Shark River Slough	*	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00112	SRS2	Shark River Slough	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00155	SRS3	Shark River Slough	9.6	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00111	SRS3	Shark River Slough	*	*	*	*	*	*	*	*	*	*	*	*
6/4/2007	07-00081	SRS4	Shark River Slough	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00144	SRS4	Shark River Slough	5.1	*	*	*	*	*	*	*	*	*	*	*
5/4/2007	07-00072	TS1	Taylor Slough	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00142	TS1	Taylor Slough	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00134	TS2	Taylor Slough	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00135	TS3	Taylor Slough	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00149	TS4	Taylor Slough/Florida Bay	0.64	*	*	*	*	*	*	*	*	*	*	*
5/31/2007	07-00078	TS4	Taylor Slough/Florida Bay	*	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00118	TT1	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00159	TT2	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
5/14/2007	07-00077	TT3	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00117	TT3	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
3/18/2008	08-00052	TT3	Tamiami Trail	15.2	*	*	*	*	*	*	*	*	*	*	*
5/14/2007	07-00076	TT4	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
7/12/2007	07-00116	TT4	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
3/18/2008	08-00051	TT4	Tamiami Trail	*	*	*	*	*	*	*	*	*	*	*	*
5/4/2007	07-00071	WB1	South West Boundary	*	*	*	*	*	*	*	*	*	*	*	*
8/24/2007	07-00141	WB1	South West Boundary	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00146	WB2	South West Boundary	6.4	*	*	*	*	*	*	*	*	*	*	*
6/4/2007	07-00083	WB2	South West Boundary	*	*	*	*	*	*	*	*	*	*	*	*
9/20/2007	07-00145	WB3	South West Boundary	12.1	*	*	*	*	*	*	*	*	*	*	*
6/4/2007	07-00082	WB3	South West Boundary	*	*	*	*	*	*	*	*	*	*	*	*

\* < MDL



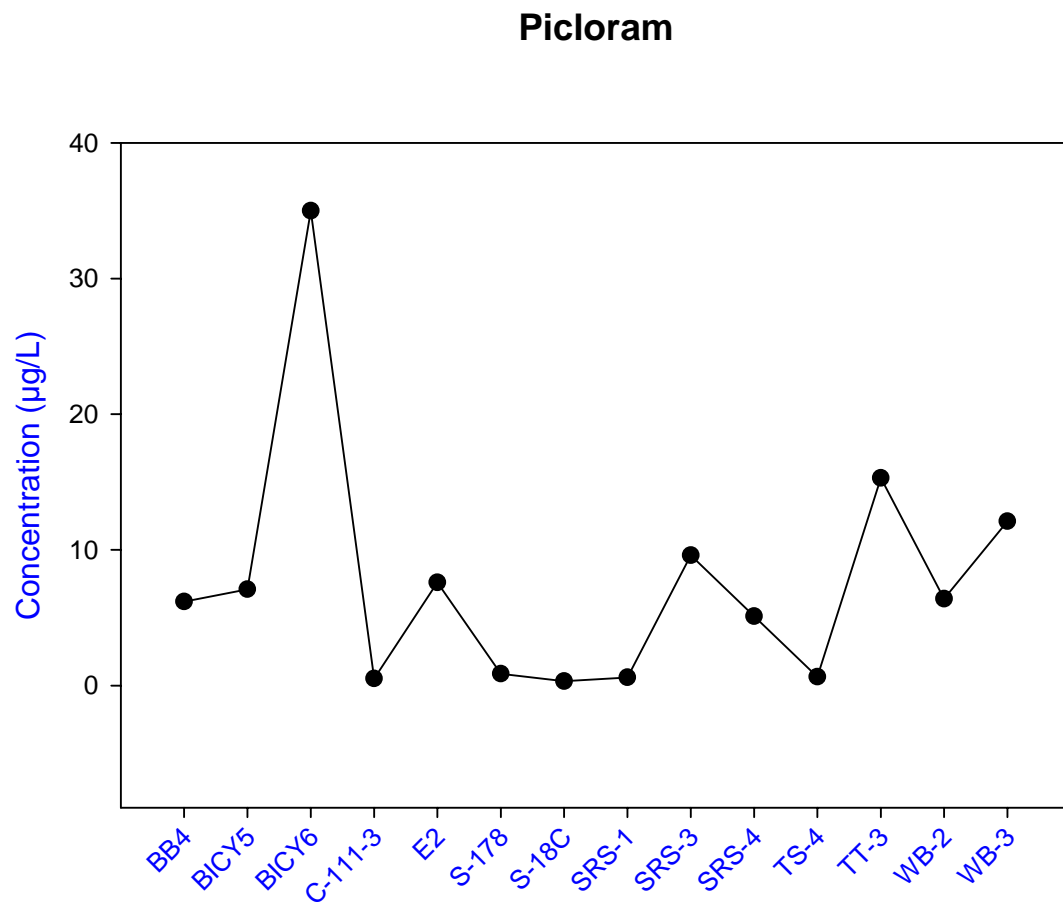


Figure 2.22. Picloram distributions through some CARE Station during 2007-2008 sampling period

quantification of target phenoxy herbicides.

Different parameters showed a great influence in the ionization process of the mass spectrometer. Focusing voltages, probe temperatures, and mobile phase composition to avoid ion suppression, were of primary importance as variables that control the detection, sensitivity and selectivity.

The analytical sensitivity of the phenoxy acid quantification method was evaluated through the use of internal calibration curves. The accuracy of the method was measured by running spiked replicates. The interday variation was tested for precision showed values below 30 % RSD. The MDL ranged from 1.9 to 4.5  $\mu\text{gL}^{-1}$  for the compounds tested.

The novelty of the proposed method for the screening analysis of phenoxy acid herbicides was to combine a polymeric SPE phase with LC/MS/MS for the simultaneous analysis of 12 acidic herbicides. Advantages over conventional extraction and detection methodologies include compatibility with aqueous matrices, minimization of solvents and simplification of the analysis for water samples with a high content of dissolved organic matter. Nine compounds were efficiently extracted and analyzed using the proposed methodology. However, picloram, 2,4-DB and dinoseb were not recovered from the natural matrix). Hyphenated chromatographic techniques, such as HPLC/ESI/MS in both full scan mode and SRM offer a good alternative for the screening and quantification of the analytes at screening concentrations in surface waters.

This method required only 100 ml of sample to achieve limits of detection as low as 1.9  $\mu\text{gL}^{-1}$  for the suite of herbicides studied, which is at least 10 times below of the MCL requirements.

## CHAPTER 3

### 3.0. Laser Ablation ICP-MS of Soils and Sediments

### 3.1. Introduction

#### 3.1.1. Elemental Analysis of Soils in Environmental Forensics

The forensic examination of soil is receiving increasing attention in legal proceedings. Soil analysis is an important part of the services provided by forensic laboratories and law enforcement agencies since they are matrices frequently encountered and transferred at crime scenes (Murray and Solebello 2002).

The value of the information that the analysis of soil and sediments may provide depends on several factors such as: a) adequate sampling, b) amount of sample, c) methods of analysis and d) final purpose of the analysis.

The amount of soils and its constituents varies a lot from case to case, soils can be encountered in minute quantities as “trace evidence”, for example when transferred to the sole of a suspect’s shoe or when found as residue under the victim’s nail. On the other hand, soil can be also found in larger amounts, for example in some environmental crimes where large land areas are the target of the study.

Forensic analysis of these matrices is usually conducted for identification or comparison purposes. The identification of the elemental profile is typically performed to identify contaminants and to establish levels of toxic elements. Comparison of questioned and control samples is performed with the aim of determining provenance.

Some of the methods typically used in forensic laboratories for the analysis of soil include the measurement of physical properties such as color, moisture content, pH, ash content, and particle size distribution. Chemical analysis of the organic matter is usually conducted by chromatographic techniques (GC, HPLC) and micro-FTIR. The inorganic analysis is performed using x-ray fluorescence (XRF), inductively coupled plasma (ICP)

and Scanning Electron Microscope with Energy Dispersive x-ray detector or Wavelength Dispersive x-ray detector (SEM/EDX, SEM/WDX). Detection limits vary depending on the element and instrument of choice (Saferstein 2002).

From an environmental perspective, the accurate and rapid determination of soil pollutants at presumptive contaminated sites is desirable for regulatory, remediation or characterization purposes.

Trace elements in environmental science are usually analyzed by sensitive methods such as Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Optical Emission (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry ICP-MS. The communality of these spectrochemical methods is that they usually require the digestion of the soil by microwave, alkaline fusion or open-vessel acid digestions, which are complex, time consuming and in many cases incomplete (Chen and Ma 2001; Chen and Ma 1998; Trimm et al., 1998).

Recoveries and precision of pseudo-total and total digestion methods vary with many factors, including the mineral composition and origin of the soil, the digestion method, as well as the elements of interest (Chen et al., 2001). Moreover, the determination of some elements in soil is challenging and may require the development of various specific methods for a complete multi-element analysis (Kubravova 1997; Wu et al., 1996; Hosick et al., 2002; Chen et al., 1999).

These drawbacks, have driven the search for alternative techniques that do not require digestion of the sample and much less sample preparation such as X-ray Fluorescence (XRF) (Goldstein et al., 1996; Kalähne et al., 2006; Buttler et al., 2007), Laser Induced Breakdown Spectroscopy (LIBS) (Eppler et al., 1996), Scanning Electron

Microscopy with Energy Dispersive X-ray detector (SEM/EDX) or Wavelength Dispersive X-ray detector SEM/WDX) (Kennedy et al., 2002; dos Anjos et al., 2000; Butler et al., 2006; Butler et al., 2007). Each technique has its own advantages and limitations. In general terms, XRF and LIBS are useful for bulk analysis, particularly as screening tools for field analysis and for rapid assessment of contaminated sites but they lack the required sensitivity and/or precision for most environmental applications.

Due to the combined advantages of laser ablation and ICP methods, there has been a growing attention towards the study of fundamentals and methodology development for the analysis of soils. About a dozen manuscripts have been published in the last 13 years discussing the use of LA-ICP-MS and, to a lesser extent, LA-ICP-OES for the analysis of soils and sediments (Litche 1995; Hoffman 1997; Baker et al., 1999; Musil et al., 2000; Klem et al., 2001; Tao et al., 2002; Mikolas et al., 2002; Boulyga et al., 2004; Gilon 2005; Beccaglia et al., 2006; Hubova et al., 2007; Fernandez et al., 2008).

A typical LA-ICP-MS system consists of a laser, an ablation cell and the ICP-MS. A solid sample is placed in the ablation cell where a laser beam is focused into the surface of the sample. The interaction of the laser beam with the sample removes fine particles that can be swept to the ICP plasma by aim of a carrier gas to be further atomized, ionized and analyzed (Butler et al., 2007). More details about this technique are given in the following section.

Although significant contributions and improvements have been reported in the last decade, this technique is still being developed. Some limitations remain on the existing methods; such as: a) these are either optimized for single element determinations

(Tao et al., 2002; Boulyga et al., 2004) or for a limited suite of elements (Hubova et al., 2007; Mikolas et al., 2002; Boulyga et al., 2004; Fernandez et al., 2004), b) sample preparation compromises the quantitation of volatile compounds (Litche 1995), c) sample preparation remains labor intensive (Hubova et al., 2007; Musil et al., 2000; Mikolas et al., 2002), and/or requires the use of binders (Musil et al., 2000; Beccaglia et al., 2006; Mikolas et al., 2002; Mason et al., 2006; Boulyga et al., 2004), d) complex or expensive quantitation strategies are required to improve accuracy and precision (Baker et al., 1999; Hoffman et al., 1997; Hubova et al., 1997; Boulyga et al., 2004) or e) the effect of sample heterogeneity has not been effectively addressed (Hofmman et al., 1997; Klem et al., 2001; Boulyga et al., 2004; Fernandez et al., 2008).

Soils represent a matrix with inherent heterogeneity and variable mineral composition leading to analytical complexity. This challenge has been recognized by the soil community and consequently, acceptable precision and accuracy for digestion methods has been reported as typically  $\leq 20\%$  (Chen et al., 2001).

Likewise, since laser ablation is a micro-sampling technique, the need for homogenization of the sample prior to analysis has been a major issue for the accurate and precise measurement of soils. Although the importance of grinding soils has been stressed in different publications, the range of particle size of the sample reported by some authors still ranges from 30 to  $>80\ \mu\text{m}$ . The presence of large particles requires the use of either large laser spot sizes (of about 1 -2 mm) which may not be available in some UV-lasers; or the ablation of numerous craters (30-100 craters) to achieve a representative sampling of the bulk soil, which may not be practical in terms of time of analysis (Hofmman et al., 1997). Reported accuracy on laser ablation of soils has been

generally  $\leq 20\%$  and ranging from 5 to 100% depending on many variables such as elements under study, sample preparation, concentration range, standard reference materials and calibration strategies (Litche 1995; Hoffman 1997; Baker et al., 1999; Musil et al., 2000; Klem et al., 2001; Tao et al., 2002; Mikolas et al., 2002; Boulyga et al., 2004; Gilon 2005; Beccaglia et al., 2006; Hubova et al., 2007; Fernandez et al., 2008).

Alternative sample preparation methods have also been recommended to improve homogeneity, such as the use of fusion with lithium borate (Litche 1995), methacrylate glue (Klemm et al., 2001) and the use of a sol-gel technique (Hubova et al., 2007).

Although the fusion method is convenient due to the fast batch sample preparation, samples are heated to 1040°C producing losses of volatile compounds. Litche reports the use of individual internal isotopic standards to account for fractionation during laser sampling and/or sample preparation, which increases the cost and complexity of analysis. The reported overall accuracy of this method is  $<5\%$  for major and rare earth elements, although a 1 to 112 % bias is also reported for several trace elements depending on the element and the reference standard used (Litche 1995).

The glue casting method allows for the addition of either liquid or solid spikes. This sample preparation technique was also applied for rare earth elements. Despite the fact that calibration curves showed  $r^2 > 0.97$ , this homogenization method still produces precision figures ranging from 7 to 35% RSD and accuracy ranging from 0.7 to 52% bias for the reported soil reference standard (Klemm et al., 2001).

Silica sol-gel was recently studied to assist the pellet preparation of agricultural soils. Hubova's research group applied this sample preparation scheme for the successful



analysis of 5 elements by using LA-ICP-OES and compared the results versus XRF and solution ICP-OES. Bias for a set of four soil samples was below 15% for most of the elements (ranging from 1 to 31% depending on the soil and the reference technique) (Hubova et al., 2007). The main disadvantage of this method is extensive sample preparation, since soils still have to be ground and pressed into pellets after the gel is created and dried overnight.

Most of the work reported to date has been conducted with IR-ns lasers (1064nm) which is generally associated to larger fractionation effects than UV-ns lasers (Jefries et al., 1996; Figg et al., 1998; Mao et al., 1998; Russo et al., 2000; Montelica-Heino et al., 2001).

The mechanical strength of the soils with respect to the laser interaction and cohesion problems have been largely solved using a wide variety of binders. However, care must be taken on the amounts added and the binder selection, because the mass removed by the laser varies with the binder-to-sample ratio. The method proposed in this manuscript combines the advantages of using a UV-ns laser (266nm) with homogenization procedures that reduces the size of laser-induced particles transported to the plasma, and the particle size of the test sample to  $<1\mu\text{m}$  scale, improving consequently not only precision but also cohesion and resistance of the pellet (test sample) without requiring the use of binders.

Unconventional calibration techniques have also been explored by Baker et al. They reported the use of a solution-based calibration, where a flow of a nebulized solution is combined with the laser ablation carrier gas and then directed to the ICP-MS torch (Baker et al., 1999). One disadvantage of this method is that knowledge of the

concentration of the internal standard is required in advance. Reasonable accuracy is reported for most of the elements (typically <20%) using a single solution calibration but the accuracy of elements such as rubidium, strontium, barium and yttrium was compromised (by around a factor 2-3). Baker provides in this study a complete discussion about the effects of organic content and particle size in the sample.

Isotope dilution has also been used as the quantitation technique to determine plutonium in soils at ultratrace levels by laser ablation coupled to a sector field ICP-MS (Boulyga et al., 2004).

Although good agreement with a certified reference material is reported, low precision is attributed to heterogeneity of the sample. Solid-spiking isotope dilution has been recently published by Fernandez et al. for the quantitation of copper, zinc, lead and tin in soils and sediments, achieving precisions lower than 10% RSD (Fernandez et al., 2008).

Despite of the significant studies conducted up to date on laser ablation of soils, most of them have been focused on optimization on standard reference materials as well as fundamental studies of the interaction of the laser with the solid sample. Relatively few applications have been reported on “real” samples and they are often related to a single or few analytes (Tao et al., 2002; Seltzer et al., 2003; Boulyga et al., 2004, Becaggia et al., 2006).

To the best of our knowledge, our research group was the first one to report a method for routine environmental forensic analysis of soils covering an extensive list of target analytes as traditional solution work (Arroyo et al., 2009). This method will be also applied to multiple sets of samples to test its applicability to soils and sediments located

in pristine areas as well as in contaminated areas, both with broad chemical and physical properties.

### 3.2. Principles of Laser Ablation Inductively Coupled Plasma Mass Spectrometry

#### 3.2.1. Laser Ablation Fundamentals

Laser is an acronym for Light Amplification by the Stimulated Emission of Radiation. Stimulated emission occurs when an atom or molecule holds onto excess energy until it is “stimulated” to emit it as light (Silvfast 1996).

The existence of stimulated emission was first suggested by Albert Einstein in 1917. Up to date, lasers have been used for many areas, such as medicine, geology, reading and writing information, measurement and inspection, environmental science and others. There are different types of lasers, such as a) gas lasers, b) semiconductor lasers and c) solid state laser. Each type of laser emits a characteristic wavelength or range of wavelengths. The wavelength depends on the type of material that emits the laser light, the lasers optical system and the way the laser is energized (Hecht 1992).

A laser has to have a population inversion in order to produce the stimulated emission. In order to produce population inversion, energy should be put into the laser medium to selectively excite atoms or molecules to certain higher levels (Hecht 1992).

In addition, to extract energy efficiently from a medium with a population inversion and make a laser beam, it will be necessary to have a resonant cavity that helps build up (or amplify) stimulated emission by feedback-reflecting some of it back into the laser medium. Stimulated emission can amplify light. One photon with energy

corresponding to a laser transition can stimulate the emission of a cascade of other photons at the same wavelength. The amount of amplification increases sharply with the distance light travels through the laser material (Hecht 1992).

Solid state lasers are the ones more commonly used for laser ablation of solid materials. In solid- state lasers, light is emitted by atoms immersed in a crystal or glassy material. The crystal is shaped into a rod, with mirrors placed at each end. Light from an external source enters the laser rod and excites the light-emitting atoms. The cavity mirrors from a resonant cavity provide the feedback needed to generate a laser beam that emerges through the output mirror (Silfvast 1996).

The lasers used in this study are Nd-YAG laser. In neodymium-YAG, the neodymium is an impurity that takes the place of some yttrium atoms in the YAG crystal. YAG's chemical formula is  $Y_3Al_5O_{12}$ , its crystalline structure is similar to that of garnet. The crystal has good thermal, optical and mechanical properties. The laser could use accessories that change lasers wavelength and pulse duration. The near- infrared wavelength of Nd-YAG lasers is fine for some purposes, but visible or UV light is better for many others (as for LA-ICP). Using a harmonic generator the frequency could be changed and therefore these lasers could emit light at 532, 355 or 266nm for different applications (Hecht 1992).

A typical LA-ICP-MS setup consists of a laser, an ablation chamber and the ICP-MS, which is used as an ionization source and analyzer. A solid sample is placed inside the ablation chamber and a laser beam is focused on the surface of the sample (See Figure 3.1). When the laser is fired, the interaction between the laser and the sample surface produces the removal of material in the form of small particles. These particles are then

transported to the ICP plasma by a carrier gas, usually argon or helium, for further atomization, ionization and subsequent analysis (Russo 1998).

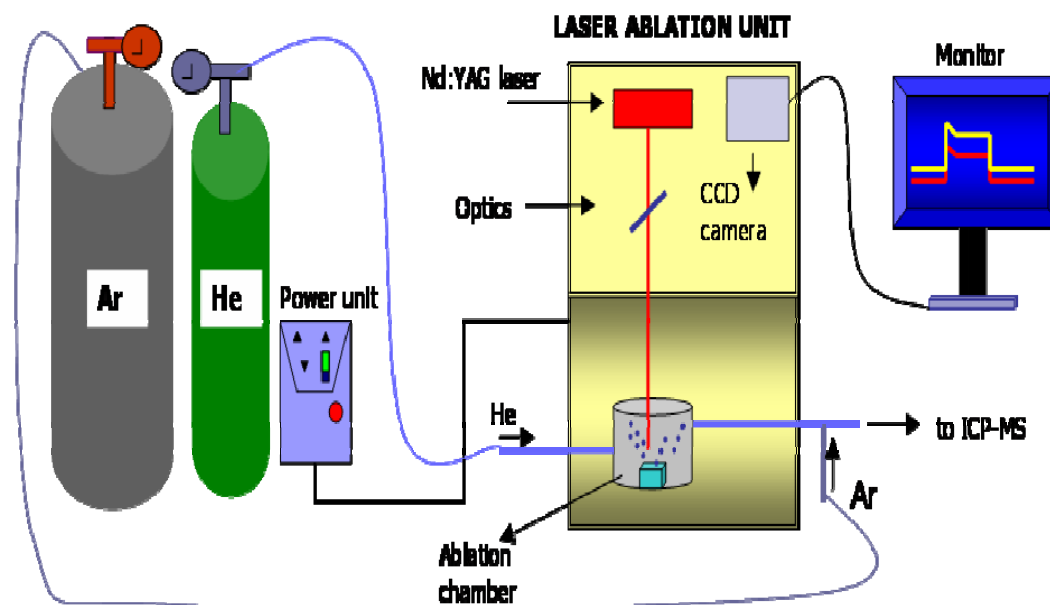


Figure 3.1. Diagram of a typical LA-ICP-MS system.

A CCD camera and a z-translation stage are also provided to aid focusing the laser to the sample surface.

This technique enables the direct introduction of the particles, minimizing the risks of contamination and the use of hazardous reagents typically needed for acid digestions. Laser ablation has the added advantages of providing excellent absolute detection limits due to the small amounts of sample introduced in the ICP-MS, normally in the order of few hundreds nanograms.

Some disadvantages of LA sample introduction include matrix dependence and the lack of solid calibration matrix-matched standards, which makes the quantification less straightforward than with solution analysis.

Another well-known disadvantage to LA is elemental fractionation. Ideally, the ablation process should eject particles from the matrix only by mechanical means. Nevertheless, the ablation process could be accompanied by undesirable processes such as melting and vaporization, which cause fractionation (Trejos et al., 2003).

Fractionation is defined as the process that produces a stoichiometry of laser products that is not representative of the sample composition (Beauchemin 2000). Its effects on glass and other matrices have been investigated. Some authors have attributed fractionation to several factors including: laser wavelength (Figg 1997; Montelica-Heino 2001), depth of the craters (Borisov 2000), mineralogical, crystallographic and chemical compositions of the matrix (Montelica-Heino 2001), mode of ablation, laser densities and energy profiles (Borisov 1998; Mao 1998), transport system, carrier gas and the ionization in the plasma (Chen 1999; Eggins 1998; Figg, 1998; Guillong 2002; Horn 2003). The effect of this event is element-dependent and appears to be related to thermal properties of a particular element in different matrices and needs to be determined for individual matrices.

Laser ablation has been studied extensively for matrices such as glass because this matrix is considered a matrix model for many reasons such as: a) there are matrix matched standards available on the market, b) sample homogeneity is appropriate for micro-sampling, c) there are elements present at constant concentration that can be used as internal standards, such as silicon and calcium and d) fractionation for UV nanosecond

and femtosecond lasers is not significant and does not affect the analytical performance (Almirall 2003; Trejos 2003; Almirall 2004; Trejos 2005; Latkoczy 2005; Bajic 2005; Smith 2006; Umpierrez 2006, Almirall 2006; Berends-Montero 2006; Bridge 2006; Ito 2007; Castro 2008).

The analysis of soils is not as straightforward as glass matrices because the composition of soil may vary over time and sample heterogeneity may be a limitation for micro-sampling techniques. Fortunately, the powder nature of soils facilitates the homogenization of the sample and the addition of internal standards. Sample preparation strategies for soils and sediments will be discussed in detail in the results section.

### 3.3. Statistics and Chemometrics for Elemental Composition of Soils

#### 3.3.1. Chemometrics in Environmental Sciences and the Use of Non Parametric Methods

Most of the data required for environmental forensics is quantitative, since the concentration levels play an important role in any ecosystem. An element can be vital for an organism at a specific concentration but the same element can be toxic at higher concentration levels. For this reason, statistical analysis is of utmost importance in this field since the correct reduction and treatment of the data is a key factor for interpretation of the analytical results.

Descriptive statistics will be used during this study to evaluate the quality of the analytical measurements of the methods, including mean values, standard deviations, relative standard deviations, significance tests and estimations of accuracy and precision. The fundamental behind this type of statistics is given elsewhere and therefore won't be discussed in detail (Miller and Miller 2000).

Analytical chemists commonly deal with data that is normally distributed. Nevertheless, the concentration of trace elements in environmental matrices such as soil and sediments may have a quite different distribution, requiring non-parametric chemometrics for the analysis of the data. The term non-parametric statistic refers to a statistic whose interpretation does not depend on the population fitting any parametrized distributions. Generally, the geochemical data follows a log normal distribution because of the presence of either high or low values for element concentration within a population.

The Saphiro-Wilk is one of the methods that can be conducted to test the normality of concentration distribution of elements. The Shapiro-Wilk tests, proposed in 1965, calculates a  $W$  statistic that tests whether a random sample comes from a normal distribution. Small values of  $W$  are evidence of departure from normality. The  $W$  statistic is calculated as follows:

$$W = \frac{\left( \sum_{i=1}^n a_i x_{(i)} \right)^2}{\sum_{i=1}^n (x_i - \bar{x})^2}$$

where the  $x_{(i)}$  are the ordered sample values ( $x_{(1)}$  is the smallest) and the  $a_i$  are constants generated from the means, variances and covariances of the order statistics of a sample of size  $n$  from a normal distribution . More information about the Shapiro-Wilk tests can be found elsewhere (Shapiro and Wilk 1965; Miller and Miller 2000).

In statistical analysis is very important to characterize the location and variability of a dataset. This information is given by the mean and standard deviations presented as



arithmetic mean and arithmetic standard deviation, but in order to have a further characterization of the data two other moment coefficients -skewness and kurtosis- included, and they determine how the shapes of sample frequency distribution curves differ from ideal Gaussian (normal) curves (Miller and Miller 2000).

Skewness is a measure of symmetry or more accurately lack of symmetry. It describes the asymmetry of the upper and lower halves of the curve around the mean, and it is calculated as the third moment of the population mean, according to the following formula:

$$Skewness = \sum z_i^3 \frac{N}{(N-1)(N-2)}, \text{ where, } z_i = \frac{x_i - \bar{x}}{std(x)}$$

In this formula N represents the number of data points. The skewness for a normal distribution is zero and any symmetric data should have a skewness value near zero. Negative values for skewness indicate data that are skewed left and positive values for skewness data that are skewed right.

Kurtosis is a measure of whether the data is peaked or flat relative to a normal distribution (Miller and Miller 2000). It is calculated as fourth moment of the population mean minus 3, according to the following formula:

$$kurtosis = \sum z_i^4 \frac{N(N-1)}{(N-1)(N-2)(N-3)} - 3 \frac{(N-1)^2}{(N-2)(N-3)}, \text{ where, } z_i = \frac{x_i - \bar{x}}{std(x)}$$

Data sets with high kurtosis tend to have a distinct peak around the mean, decline rather rapidly and have heavy tails. Data sets with low kurtosis tend to have a flat top near the mean rather than a sharp peak.

If the dataset follows a log-normal distribution, the data can be log-transformed to further fit the normality assumption. Skewness and kurtosis can be calculated on the data before and after log conversion as a mean to confirm the distribution followed by the population.

For datasets that do not follow a normal distribution, non parametric comparison tests should be employed for comparison studies between data obtained by two different methods of analysis. Ranking methods can be used for such correlation studies.

The Spearman Rank correlation coefficient method also known as Spearman's rho, was first described in 1904. Like other ranking methods, it is based on expressing one or both sets of observations under investigation in terms of a rank order rather than quantitative units. This test provides a non parametric measure of correlation; it assesses how well an arbitrary monotonic function could describe the relationship between two variables, without making any assumptions about the frequency distributions of the variables. Some of the advantages of this test is that is easy to determine and interpret (Miller and Miller 2000).

### 3.3.2. Non Detects and Data Analysis

Measurements whose values are known only to be above or below a threshold are called “censored” data. In environmental studies, censored data is commonly encountered as values below detection limit and called “non-detects”. These low values for contaminants such as trace elements are known inexactly (Helsel 2005).

Non-detects are an issue in environmental sciences, particularly for population studies, databases, determination of baseline levels for toxic components and comparison

among groups of data between and within studies. Statistics using only detected observations overstate the values actually encountered, and are difficult to put into context for the entire distribution (Helsel 2005). Non-detect values can also provide a great deal of information, but care must be taken in order to report the values properly.

Some authors choose to “delete” censored values but this has several disadvantages, including differences in results among studies due to differences in detection limits which may also lead to bias.

Another approach is called “substitution” and consists on assigning an arbitrary fraction of the detection limit to each censored observation. In numerous studies over the years, one half of the detection limit has been used; however this method can induce a signal not present in the original data, or obscure a signal that was actually there. Substitution methods are most often used because it is easy and no special software is required.

Maximum likelihood estimation (MLE) can be also applied; it uses three pieces of information: a) numerical values above detection limits, b) the proportion of data below each detection limit and c) the mathematical formula for an assumed distribution. Data both above and below detection limit is assumed to follow a distribution such as the lognormal.

Parameters are computed that best match a fitted distribution to the observed values above detection limit and to the percentage of data below detection. Nevertheless, this method is not efficient for datasets with less than 50 observations (Helsel 2005).

Finally, non parametric methods that use the relative positions (ranks) of data and are especially useful for censored data because they efficiently use the available

information. Non-detects is known to be lower than values above their detection limit and therefore is ranked lower. These methods do not require estimates of the unknown distances between non-detects and detected values, but only their relative order. These methods are now more commonly used in environmental sciences.

### 3.4. Sediment Quality Assessment

Trace metals represent a potential hazard for the environment because they are widely used in many commercial products and they can be easily transported into the different environmental compartments. In addition, some metals can persist in the ecosystems for long periods of time.

Soils and sediments are widely used as matrices for environmental monitoring because they are the ultimate repository for metals. For these reason, there are guidelines available, such as the sediment quality assessment guidelines (SQAGs) that may be used to identify the probability for sediment of being associated with adverse effects on aquatic organisms. (MacDonald 1994) The SQAGs assist ecological risk assessments by defining contaminant concentrations that are unlikely or usually associated with adverse effects.

For example, threshold effect levels (TEL's) are formulated to define concentrations of contaminants below which biological effects are not expected. Likewise, probable effect levels (PELs) for each substance are developed to define ranges of concentrations above which biological effects are likely. When contaminant concentrations exceed one or more PELs, sediment samples are predicted to be toxic. (McDonald 1994). These levels are useful for designing monitoring programs,

supporting regulatory decisions, developing sediment quality objectives, conducting risk assessments and developing remediation objectives at contaminated sites.

Although in South Florida trace metal concentration are relatively uncontaminated (Fernandez 2004), high concentrations of certain elements may be present in sediments. The sources of these metal contaminations can be local, regional and/or trans-oceanic. Examples of local sources include municipal solid waste, emission from cement industries, emissions from municipal and medical waste incinerations.

In this study, TEL and PEL levels were used in conjunction to other information, to assist the CARE environmental monitoring program. Comparison of existing elemental sediment data with the SQAGs will help to identify high priority areas and priority contaminants. This monitoring program will aid as a basis to conduct further investigations to identify contaminant sources, assess the extent and severity of the contamination, and determine the need for remediation measures.

Although TEL and PEL information is useful, it should not be used in isolation and therefore additional methods such as background levels and evaluation of natural vs. anthropogenic sources should be also conducted.

### 3.5. Background Concentration and Baseline Concentration of Trace Elements

The determination of background concentration levels of trace metals in soils and sediments and its variability is crucial for the assessment of a potential contaminated site within a specific region and is widely utilized in environmental sciences. This survey on trace metal concentration is needed to provide a scientific databases in order to address how clean is a region and to determine clean up criteria (Beck et al., 2005). According

to the USEPA, background concentrations represent natural elemental concentrations in soils without human activity (FDEP 2008). They are attributable initially to the natural geological and hydro-geological characteristics of the area under study. However, this is an ideal scenario where other variables like anthropogenic addition, long-range transport of contaminants, chemical, and biological processes are also playing their role and are affecting this background values. Consequently, the background concentration values can consider both naturally occurring and local or regional man-made contributions.

The geochemical baseline concentrations are usually express as an expected range of elemental concentrations around a mean in a normal sample medium, and are defined as 95 % of the expected range of background concentrations (Adriano 2001). Based on the normal distribution theory, the expected range can be expressed as the arithmetic mean  $\pm 2$  arithmetic standard deviations ( $AM \pm 2 ASD$ ). The arithmetic means are best used as estimates of geochemical abundance (Gough et al., 1998). Because the geochemical trace metal concentrations is generally positively skewed, and the expected range followed a log-normal distribution, then the expected range can be express as the average of logarithms  $\pm 2$  standard deviations (Dudka et al., 1995). Geometric means are therefore better “maximum likelihood estimators” for most geological data because of the tendency for concentrations of elements to have positively skewed frequency distributions. Therefore the analytical data is transformed to logarithms, and the geometric mean (antilogarithm of the mean of logarithmic values) is reported as the best estimator of central tendency (Gough et al., 1998).

The baseline value has been recognized as the only means to established reliable worldwide elemental concentration in natural materials, and some researchers

recommend its use for interpreting contamination in soils, since the log transformation of the data minimize the distorting effects of few high values (Beck et al., 2005; Kabata Pendias and Pendias 1992; Dudka et al., 1995).

### 3.6. Normalization Methods for the Identification of Anthropogenic Sources

Interpretation of environmental metals data can be challenging because absolute metal concentrations in coastal sediments are influenced by a variety of factors, including sediment mineralogy, grain size, organic content, and anthropogenic enrichment (Schropp et al., 1990). This combination of factors results in metal levels that can vary over several orders of magnitude at uncontaminated sites in Florida. Therefore, it is important to consider the natural background levels of sediment associated metals when conducting sediment quality assessments.

Normalization is the attempt to compensate for the natural variability of trace metals in sediments so that any anthropogenic contribution may be detected and quantified (Loring 1991). Grain size and provenance are significant parameters that should be compensated by these approaches. Granulometric and geochemical methods can be used for this purpose, but the latter is superior because it compensated for both mineralogical and natural granular variability.

The Schropp's method for identifying anthropogenic sources is a geochemical method that has been widely used. The original study was conducted on sediments originated from Florida and is based on normalization of metal concentrations to concentrations of aluminum (Schropp et al., 1990). This normalization approach is valid for southeastern US geographical regions.

### 3.7. Significance of the LA-ICP-MS Analysis of Soils and Sediments and Sample Description.

The significance of the current study is to provide a standard UV-ns-LA-ICP-MS method for the routine quantitative analysis of a large suite of elements of interest to the environmental forensics, which provides analytical data comparable to typical digestion procedures with the convenience and other advantages of laser sampling. Enough scientific support will be reported to facilitate the incorporation of this technique into the environmental sciences.

The proposed LA-ICP-MS method incorporates some of the main improvements previously reported by other authors in this field, offering the following additional advances: a) the validation and optimization of the method is extended for a larger number of elements of interest to the environmental and forensic sciences, b) superior homogenization strategies for micro-sampling reduces the particles of the soil to  $<1\mu\text{m}$  improving the precision of measurements and cohesion of soils during pellet formation without requiring binders, c) simpler and faster sample preparation and quantitation methods are proposed to allow routine monitoring of contaminated sites and determination of background levels in protected areas, d) lower limits of detection are reported for elements of environmental concern.

Figures of merit such as precision, accuracy, limits of detection, reproducibility and repeatability were also studied not only for reference standard materials but also on soil proficiency tests to evaluate the laser ablation results versus digestion methods currently adopted by the soil analysis community. Matrix effects were studied and different calibration methods were also proposed to make laser ablation feasible for



routine elemental quantitation of these matrices.

Moreover, in this work we also studied the applicability of this previously reported method to different sets of real samples with broad chemical and physical properties. Soil and sediments were collected from a variety of impacted and non-impacted sites.

The analytical protocol was evaluated under different scenarios like a) screening and quantitative analysis of potential contaminated sites, b) screening of background levels in pristine areas and c) environmental monitoring of sediment samples as a tool to later assessment for enrichment.

Two data sets are described herein and illustrate the robustness of the method. The first group of samples corresponds to a subset of litter soil collected as part of 80 soil cores from a residential area adjacent to an automobile battery manufacturing facility. Samples were analysed for lead, antimony and tin by digestion-ICP methods as part of a larger study to determine soil lead variability to make remediation decisions (Hosick et al., 2002; Machemer and Hosick 2004; Machemer et al., 2007). Laser ablation ICP-MS analyses are compared to those results reported by ICP methods.

The second data set contains sediments collected from forty-eight different sampling sites in environmentally sensitive areas in South Florida. The samples are part of a larger study that is being conducted within the framework of the Comprehensive Everglades Restoration Project (CERP) to assess the background levels of toxic metals. Two methods were used to assess the presence of trace elements in these sites: leachate by acid digestion (nitric acid) ICP-MS and exhaustive analysis by LA-ICP-MS. The leachate approach is intended to evaluate the availability of the trace metals to biota,

while the total elemental profile will be provided by laser ablation. The combined information obtained from both measurements will be compared in terms of the informative power and it will help in the creation of a database that accounts for the soil elemental profile variability and its spatial distribution in samples collected across the Everglades National Park, Biscayne Bay National Park and Big Cypress National Preserve. This “in house” database will help to determine both background and baseline concentration of trace metals in these important protected ecosystems.

Non-parametric statistical studies were conducted to determine if there was a correlation between the elemental composition obtained by leachate-digestion and laser ablation methods. Aluminum normalization models were also employed on this sediment samples to compensate for the natural variability of trace elements with the aim of detecting possible anthropogenic contributions.

Due to the complexity of this second dataset, which comprises information for multiple elements in 48 sediment samples, the data was reduced by principal component analysis (PCA).

Methods of multivariate data analysis have proved to be suitable tool for interpretation of the elemental composition of soils and sediments. (Kalähne et al., 2000; Slavkovic et al., 2004; Chen et al., 2005; Buccolieri et al., 2006; Praveena et al., 2008). PCA analysis was used with the purpose of gaining further sight into the chemistry of the sediment samples as well as information about variances of the samples. This statistical analysis was also useful to detect whether or not there is correlation between data resulting from the different measuring methods (LA-ICP-MS and leachate digestion ICP-MS).

In a later stage, these scientific data will help to develop future management to prevent or correct anthropogenic inputs.

### 3.8. LA-ICP-MS of Soils and Sediments: Objectives

As mentioned before, whilst direct solid sampling of materials by laser ablation has increased in popularity as an analytical method, only a few studies have been published describing the application of LA-ICP-MS to the forensic analysis of soil and sediments.

The general objective of this work is to provide a simple and rapid UV-ns-LA-ICP-MS method for the analysis of soils and sediments and evaluate its applicability for the routine quantitative analysis of a large suite of elements of interest to the environmental and forensic sciences.

The specific objectives of this work are divided into two main areas:

1) Development and optimization of a LA-ICP-MS method for the routine analysis of soils and sediments

Develop and optimize a LA-ICP-MS method for the analysis of soils and sediments using an UV-ns-Nd:YAG laser ablation system coupled to a quadrupole ICP-MS. Parameters of interest will be: sample preparation, spot size, ablation mode, ablation time, use of internal standard, quantification strategies and elemental menu for characterization and quantification of samples.

Evaluate the advantages of LA-ICP-MS over typical ICP-MS digestion methods for soils and sediments. The results will be compared with solution work in terms of time of analysis, inter-day variation, precision and accuracy.

Soil and sediment reference standard materials will be used for the evaluation of the analytical performance of the method.

Validate the LA-ICP-MS methodology by participating in proficiency tests designed for the analysis of trace metals in soils and comparing the analytical performance versus other methodologies commonly used in the scientific community.

Conduct a homogeneity study of soil and sediment reference materials in order to optimize and determine sampling strategies.

2) Evaluation of the applicability of the method to real samples with broad chemical and physical properties.

Evaluate the capabilities of the method for the analysis of a set of real soil samples. A set of ten soil samples from contaminated sites will be monitored for tin, lead and antimony, and the results will be compared to total-total digestion methods followed by ICP measurements. The results will be compared in terms of ease of analysis, precision and accuracy. Regression statistics will be employed to compare the quantitative results of both methodologies.

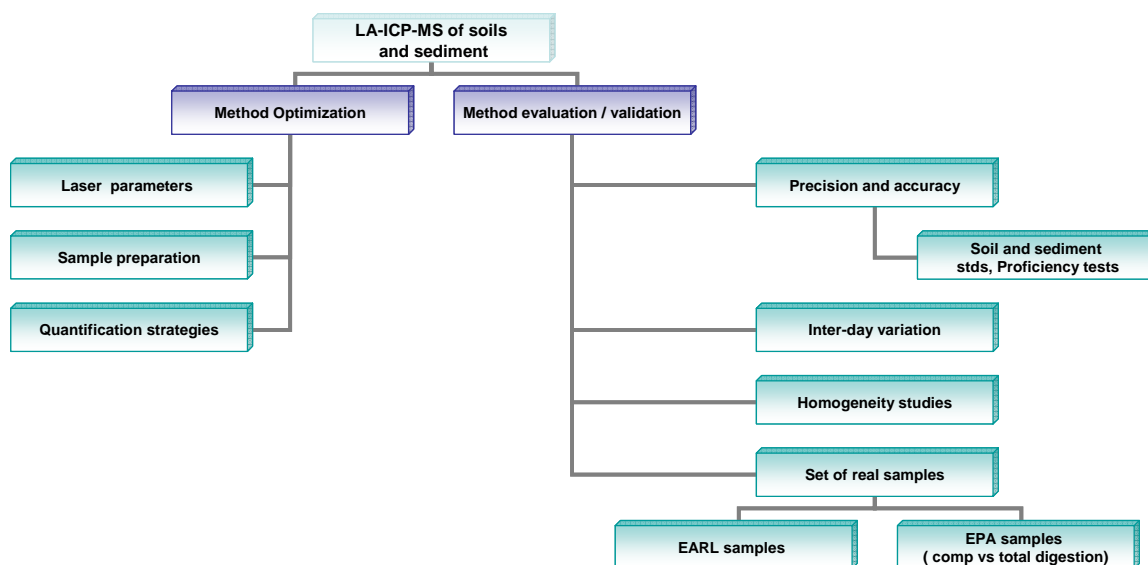
Evaluate the capabilities of the method for the analysis of a set of sediment samples. A set of 48 sediments from South Florida protected areas will be used for this study. Analytical results obtained by LA-ICP-MS will be compared to partial digestion ICP-MS methods (leachate) since this digestion protocol are currently used in to monitor bioavailability of trace elements in the sampling areas.

Employ different statistical and chemometric tools to determine whether or not there is a correlation between partial digestion methods and exhaustive sampling method such as laser ablation.

Report concentrations and baseline concentrations for 16 trace elements for the sediment samples collected from South Florida.

Figure 3.2 shows the strategic plan followed to accomplish these tasks.

Figure 3.2. Flow chart of the strategy for the optimization and evaluation of LA-ICP-MS for soil and sediments analysis.



### 3.9. Methodology.

The experimental design for the analysis of soils and sediments will be divided in two main areas as previously shown in Figure 3.2:

- Method Optimization and Evaluation of the analytical performance of the method
- Application of the method to real samples

### 3.9.1. Method Optimization and Evaluation of the Analytical Performance

#### 3.9.1.1. Instrumentation and Measurement Parameters

ICP-MS analysis were conducted on a quadrupole ELAN DRC II (Perkin Elmer LAS, Shelton CT USA), used in the standard operation mode. A 266 nm Nd-YAG laser (LSX 500, CETAC, USA) was used for this work. Optimization of laser parameters included the study of different repetition rates (5 Hz, 10 Hz, 20 Hz), ablation modes (single spot, depth profile, line, raster), spot sizes (100, 200, 250  $\mu\text{m}$ ). Best ablation results, evaluated as the best precision and accuracy for reference standards, were obtained using depth profile ablation mode with 200  $\mu\text{m}$  spot size and 10 Hz. The laser was focused at the surface of the sample and energy ranging from 3.1 to 3.2 mJ was used for all samples. The element menu for major, minor and trace analytes of interest includes the following isotopes:  $^9\text{Be}$ ,  $^{29}\text{Si}$ ,  $^{42}\text{Ca}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{57}\text{Fe}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{73}\text{Ge}$ ,  $^{75}\text{As}$ ,  $^{89}\text{Y}$ ,  $^{103}\text{Rh}$ ,  $^{107}\text{Ag}$ ,  $^{111}\text{Cd}$ ,  $^{117,118, 120}\text{Sn}$ ,  $^{121,123}\text{Sb}$ ,  $^{205}\text{Tl}$ ,  $^{206,207,208}\text{Pb}$ ,  $^{232}\text{Th}$  and  $^{238}\text{U}$ . The EPA methods 3050B and 6020A, were followed for the digestion and measurement of trace elements in soils and sediments by solution ICP-MS, respectively.

A Scanning Electron Microscope Philips XL 30 with EDX detector (Philips, The Netherlands and EDAX, USA, respectively) was used for the imaging of craters on soil standards and for the determination of particle size and elemental distribution of the internal standard. The particle size distribution was evaluated using the particle analysis EDS 2008 software (IXRF systems, V1.01 rev F Beta 1).

A piezoelectric microbalance model 3521 (KANOMAX, Japan) was used to measure the mass of the ablated particles from the laser ablation cell. This system was employed to conduct experiments of matrix effects on the analysis of different soil and

sediment standards. The piezoelectric microbalance provides an on-line mass measurement independent of particle size and shape. A detailed description of the system set up can be found in the literature (Houck et al., 2003). The mass was acquired under the same ablation parameters used during the LA-ICP-MS analyses. Blanks were measured between samples and each sample analysis was conducted in four replicates.

#### 3.9.1.2. Reagents and Standards

Germanium dioxide and Yttrium (III) oxide (99.999%, Acros Organics), were used as the internal standards. The following soil and sediment standards were used for the optimization and evaluation of the analytical performance of the method: a) Marine sediment reference material, PACS-2 (National Research Council of Canada, Ottawa, Canada); b) soil reference material, SRM NIST 2710 (Montana Soil), US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA); c) Two proficiency test samples, trace elements in soil (Analytical Products Group Inc, lot # 45961 and lot #46798, Belpre, Ohio, USA); d) quality control sample, trace elements in soil (Analytical Products Group Inc, lot # 43697, Belpre, Ohio, USA).

For the calibration curves, single element solutions of lead, tin and antimony at  $1000 \mu\text{g mL}^{-1}$  (Peak performance, CPI International, USA) were used to spike sand (NEIC sand) and graphite (graphite powder,  $<45\mu\text{m}$ ,  $>99.99\%$  purity, Sigma Aldrich, USA).

#### 3.9.1.3. Sample Preparation for LA-ICP-MS Analysis

Germanium and Yttrium were used for the experiments as powder standard by mixing 20 mg of the Germanium standard and 10 mg of the Yttrium standard with one

gram of the dried material. Samples were then ground and homogenized in a tungsten carbide ball mill. Two ball mills were used for this study: a SPEX 8000 ball mill BM (NJ, USA) and a high speed mixer mill HS (Glenmills, USA). Each of the samples was homogenized for 40 minutes on the SPEX mill or for 20 minutes on the high-speed mill. Ball mill parameters such as time of grinding (10-40min), size and number of grinding balls (1 or 2 WC balls, 9mm vs 12mm diameter) and speed (10-25 Hz), were carefully selected in order to obtain the smaller particle size in the powder in the least time possible. The optimized parameters were evaluated based on the precision of the test sample by LA-ICP-MS and mechanical resistance of the pellets.

Optimum parameters were found using a single 9mm WC ball on each sample container with a frequency of 25Hz for 20 minutes. Preliminary grinding studies were also conducted on a manual agate mortar (MM). Test samples in the form of pellets of ~12 mm of diameter and 2mm of thickness were prepared using a manual press (Carver Benchtop Pellet Press, Model 4350L (IN, USA).

The pressure applied to the milled powder was 44 kPa for 2 minutes. After cleaning the ball mill components, sand blank controls were run between samples to avoid cross contamination. The laser-induced particle size studies were performed for 3 replicates of test samples of PACS-2 prepared by MM, BM and HS.

On-line filters (Versapor 450R, 0.45 $\mu$ m, PALL Life Sciences, USA) were installed at the exit of the laser ablation cell and particles were collected while conducting laser ablation of the soil test samples under the normal operational parameters. Filters were then imaged in low vacuum SEM (BSE) at 200 and 1000 magnification and 20KV of accelerating voltage.



#### 3.9.1.4. Quantitation Strategies for Laser Ablation

All samples were acquired in transient mode (intensity vs. time) with a 25 second gas blank (helium gas through the ablation cell) followed by 50 seconds of ablation of the corresponding sample and then a 30 second of blank to purge. Each sample was placed separately on the ablation cell to avoid any cross contamination. Calibration was conducted using one of the following methods:

##### 3.9.1.4.1. Single Point Calibration using Soil or Sediment Standards as Calibrators

Solid sediment standard PACS-2 or the soil reference material NIST 2710 was used as the external calibrator and/or control standard respectively to check for accuracy and precision. Germanium and Yttrium were used as internal standards.

##### 3.9.1.4.2. External Calibration Curves

External calibration curves were built for a smaller group of elements to determine if there was an improvement in accuracy versus the single point determinations. Two different solid materials were used as a “matrix” to create the calibration curves: a) *Sand matrix*: approximately 1 g of sand (NEIC) was weighed and transferred to a polypropylene tube (Falcon, USA). Approximately 20 mg of germanium oxide was added to each tube and vortexed for 2 minutes. The mix was then spiked with liquid standards of lead, tin and antimony. The spiked standards were then placed into a heater block at 80°C until complete dryness and then homogenized in a HS mill. The calibration curve was composed of 5 data points ranging from 0-100  $\mu\text{g g}^{-1}$  for tin and antimony, respectively and from 0-1000  $\mu\text{g g}^{-1}$  for lead. b) *Graphite matrix*: a curve using

graphite was created using the same procedure as in *a* but weighing approximately 0.5 g of graphite instead of sand.

The LA data acquired was processed with GLITTER software (GEMOC, Macquarie University, Australia) or with GEOPRO software (1999, v 1.0, CETAC, USA).

### 3.10. Evaluation of the Analytical Performance

The analytical performance of the LA-ICP-MS method was evaluated in terms of precision, accuracy, repeatability, reproducibility, inter-day variation, linear dynamic range and method limits of detection.

#### 3.10.1. Micro-Homogeneity Study

A homogeneity study was conducted on 4 different test samples of one of the proficiency tests and quality control soil, respectively. Ten replicates per sample, each one consisting of a crater of 200  $\mu\text{m}$  diameter, were randomly conducted on the surface of the pellet. Homogeneity studies were conducted within a single test sample and between different test samples. All statistical analyses were performed by either the use of SYSTAT for windows 8.0 (SPSS Science, Chicago, IL) or Excel 2000 (Microsoft Corp., v9.0.2719).

### 3.11. Application of the LA-ICP-MS Method to Real Soil and Sediment Samples

Two main set of samples were used for the evaluation of the applicability of the LA-ICP-MS method: a) contaminated residential soils and b) sediments collected from semi-pristine areas.

The following sections described the procedure followed for the analysis of each set by 1) Laser ablation ICP-MS and 2) Acid digestion methods followed by ICP measurements.

### 3.11.1. Laser Ablation ICP-MS Analysis:

#### 3.11.1.1. Instrumental Parameters

LA-ICP-MS analyses were conducted on a quadrupole ELAN DRC II (Perkin Elmer LAS, Shelton CT USA), used in the standard operation mode and coupled to a 266 nm Nd-YAG laser, LSX 500 (CETAC, USA). Table 1 lists the optimized parameters for the ablation experiments.

#### 3.11.1.2. Reagents and Standards

The following soil and sediment standards were used for either quantitation or as quality control standards to verify the analytical performance a) Marine sediment reference material, PACS-2 (National Research Council of Canada, Ottawa, Canada) b) soil reference material, SRM NIST 2710 (Montana Soil), US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA). Germanium dioxide (99.999%, Across Organics), was used as the internal normalization standard.

#### 3.11.1.3. Sample Preparation for LA-ICP-MS analysis

Twenty milligrams of the germanium dioxide were mixed with one gram of the dry soil or sediment. Samples were then ground and homogenized in a tungsten carbide ball mill (High Speed Mixer Mill, Glenmills, USA) for 20 minutes until a final particle

diameter of less than 1µm was achieved (Arroyo et al., 2009). Pellets of ~12 mm of diameter and 2mm of thickness were prepared using the homogenized sample by manual press (Carver Benchtop Pellet Press, Model 4350L (IN, USA). After cleaning the ball mill components, sand blank controls are run between samples to prevent cross contamination.

#### 3.11.1.4. Analysis of Contaminated Residential Soils

A set of 10 contaminated residential soils was received from the Environmental Protection Agency (EPA) as a blind test to conduct LA-ICP-MS analysis and to compare the results to total-total digestion ICP methods. Each sample consisted of approximately 5g of ground and dried soil litter.

Approximately 1 gram of each sample was mixed with the internal standard, homogenized and pressed in a pellet as described above. Four replicates (ablation) per pellet were analyzed.

The analytes of interest for this blind test were lead, tin and antimony. The following isotopes were monitored during the analysis  $^{72}\text{Ge}$ ,  $^{73}\text{Ge}$  (IS),  $^{116}\text{Sn}$ ,  $^{117}\text{Sn}$ ,  $^{118}\text{Sn}$ ,  $^{119}\text{Sn}$ ,  $^{120}\text{Sn}$ ,  $^{121}\text{Sb}$ ,  $^{123}\text{Sb}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$ . Different isotopes were measured for each element of interest to assess possible matrix interferences.

Quantitative results are reported based on the intensities of  $^{120}\text{Sn}$ ,  $^{121}\text{Sb}$  and the sum of all three lead isotopes. For quantitation purposes, single point calibration was conducted with the sediment standard PACS-2 while the soil reference material NIST 2710 was evaluated as quality control standard to assess for accuracy and precision of the method.

#### 3.11.1.5. Analysis of Sediments from South Florida

A set of sediments collected from 48 environmentally sensitive areas of South Florida was also analyzed by LA-ICP-MS.

The selected areas were known to contain both significantly different contents of organic matter and several distinct inorganic matrices, useful to evaluate the robustness of the previously reported LA-ICP-MS method.

Figure 3.3 shows a map of the sampling stations with their corresponding identifiers. Samples from Everglades National Park were collected from 5 transects using a small Eckman dredge or a polycarbonate-coring device. The first transect follows an east-west direction across the northern boundary of the park which receives water from the Water Conservation Areas to the north of the Park. The second transect follows a north-south direction across the eastern boundary of the park, this transect lies closest to the Homestead Agricultural Area (HAA). The third transect is located in the drainage basin of the C-111 canal which flows through most of the HAA. The last two transects follow the Shark Slough and Taylor Slough which flow in a southwest direction from the north and east boundaries, respectively. The specific description of the sites is given in Table 3.1.

At each sampling site, 2.5 x 12" cores were collected from a 10 m<sup>2</sup> area and consolidated as one representative sample (composite sample) in a plastic bucket and transferred by means of a plastic spoon onto a clean 125 mL high density polypropylene bottle. All samples were refrigerated during collection, and kept frozen at < 20.0 °C until the time of analysis. Approximately 15 grams of the soil samples were homogenized, and individual one-gram pellets were produced and four ablation replicates were

analyzed as described before.

The following extended element menu, similar to the acid digestion method, was used for this set of samples to monitor background levels of those protected areas and to characterize their elemental profile  $^9\text{Be}$ ,  $^{27}\text{Al}$ ,  $^{29}\text{Si}$ ,  $^{42}\text{Ca}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{73}\text{Ge}$ ,  $^{75}\text{As}$ ,  $^{89}\text{Y}$ ,  $^{98}\text{Mo}$ ,  $^{107}\text{Ag}$ ,  $^{111}\text{Cd}$ ,  $^{117,118,120}\text{Sn}$ ,  $^{121,123}\text{Sb}$ ,  $^{205}\text{Tl}$ ,  $^{206,207,208}\text{Pb}$ ,  $^{232}\text{Th}$  and  $^{238}\text{U}$ .

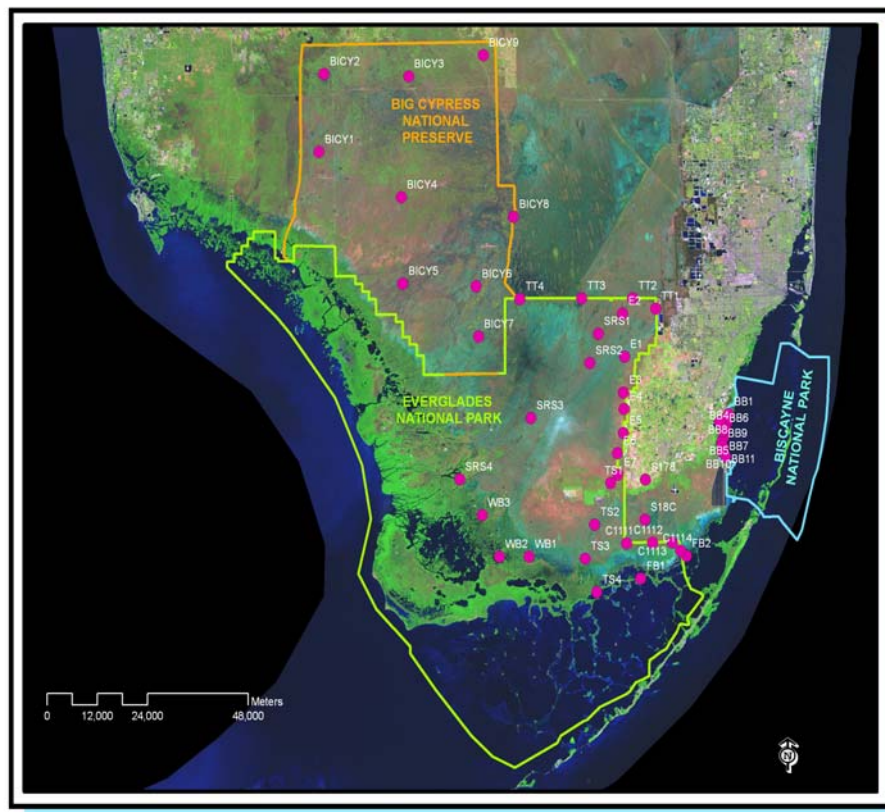


Figure 3.3. Map of the sampling 48 sampling stations monitored in this study.

Table 3.1.Description of CARE Sampling Stations

Station	FIU-ID	Description	Latitude (N)	Longitude (W)
CARE-C111-1	06-00053	C111 Basin	25.2852	-80.5687
CARE-C111-2	06-00054	C111 Basin	25.2863	-80.5072
CARE-C111-4	06-00055	C111 Basin	25.2700	-80.4410
CARE-E1	06-00025	East Boundary	25.6471	-80.5724
CARE-E2	06-00060	East Boundary	25.7310	-80.5770
CARE-E4	06-00027	East Boundary	25.5460	-80.5743
CARE-E5	06-00028	East Boundary	25.4995	-80.5755
CARE-E6	06-00057	East Boundary	25.4600	-80.5900
CARE-E7	06-00030	East Boundary	25.4180	-80.5888
CARE-FB1	06-00047	Florida Bay	25.2164	-80.5354
CARE-SRS-1	06-00058	Shark River Slough	25.6920	-80.6348
CARE-SRS-2	06-00059	Shark River Slough	25.6352	-80.6552
CARE-S18C	06-00020	Special Interest	25.3310	-80.5250
CARE-TT1	06-00037	Tamiami Trail	25.7404	-80.4979
CARE-TT2	06-00036	Tamiami Trail	25.7608	-80.5527
CARE-TT3	06-00035	Tamiami Trail	25.7611	-80.6738
CARE-TT4	06-00034	Tamiami Trail	25.7600	-80.8211
CARE-TS1	06-00040	Taylor Slough	25.4021	-80.6065
CARE-TS2	06-00051	Taylor Slough	25.3216	-80.6443
CARE-TS3	06-00052	Taylor Slough	25.2554	-80.6675
CARE-TS4	06-00046	Taylor Slough/Florida Bay	25.1905	-80.6400
CARE-WB2	06-00045	South West Boundary	25.2600	-80.8700
CARE-WB3	06-00044	South West Boundary	25.3401	-80.9112
CARE-BICY1	06-00232	Deep Lake - SR837	26.0447	-81.2997
CARE-BICY2	07-00020	East Hinson Marsh - SR839	26.1963	-81.2885
CARE-BICY3	06-00233	Kissimmee Billy Strand	26.1916	-81.0865
CARE-BICY4	06-00231	Monument Road/Campsite	25.9576	-81.1036
CARE-BICY5	07-00021	Roberts Lake/Sweetwater Strand	25.7895	-81.0999
CARE-BICY6	07-00022	Pinecrest Flowway	25.7840	-80.9254
CARE-BICY7	06-00229	Lime Tree Hammock	25.6872	-80.9197
CARE-BICY8	06-00230	L-28 Tie Back Gated Culvert	25.9193	-80.8363
CARE-BICY9	07-00019	L-28 N of I75	26.2332	-80.9082
CARE-BB1	07-00048	Black Creek Canal	25.5344	-80.3249
CARE-BB2	07-00047	Creek South of Black Creek Canal	25.5257	-80.3312
CARE-BB3	07-00018	Princeton Canal	25.5191	-80.3295
CARE-BB4	07-00045	Creek South of Princeton Canal	25.5077	-80.3332
CARE-BB5	07-00008	Military Canal	25.4891	-80.3380
CARE-BB6	07-00007	Creek North of Military Canal and South of Fender Point	25.5030	-80.3383
CARE-BB7	07-00009	Mowry Canal	25.4703	-80.3381
CARE-BB8	07-00016	L31-E wetlands North from Mowry canal non-connected	25.4801	-80.3430
CARE-BB9	07-00015	L31-E wetlands North from Mowry canal connected	25.4733	-80.3413
CARE-BB10	07-00010	North Canal at Bayfront Park Marina	25.4630	-80.3425
CARE-BB11	07-00006	Creek North of SW344 St Fill	25.4520	-80.3330
CARE-BB12	07-00017	Intersection of Princeton and L-31E	25.5194	-80.3471
CARE-BB13	07-00041	C-100 canal, northern boundary BNP	25.6110	-80.3060
CARE-BB14	07-00042	Canal at SW 196 St	25.5840	-80.3070
CARE-BB15	07-00043	Creek at SW214 St	25.5650	-80.3060
CARE-BB16	07-00044	Small Bay North from Black Point	25.5470	-80.3120

### 3.11.2. Digestion ICP-MS Analysis

#### 3.11.2.1. Set of Contaminated Residential Soils

##### 3.11.2.1.1. Instrumental Parameters

Lead was determined in KOH fusions by ICP-OES, (Thermo Jarrel Ash ICAP 61-E) and in nitric acid and hydrochloric acid digestions by ICP-MS (Machemer and Hosick 2004), antimony was determined in hydrochloric acid digestions by ICP-MS (Machemer et al., 2007), and tin was determined in KOH fusions by ICP-MS via hydride generation (Hosick et al., 2002). ICP-MS measurements of this set of samples were conducted on a Perkin-Elmer Sciex Elan 6000.

##### 3.11.2.1.2 Reagents and Standards

The reagents used were as follows: nitric acid (J.T. Baker Instra-analyzed), hydrochloric acid (J.T. Baker Instra-analyzed), sodium borohydride (J.T. Baker ACS reagent grade), potassium hydroxide (J.T. Baker ACS reagent grade) and hydrogen peroxide (J.T. Baker ACS reagent grade). Standard reference materials PACS-2, NIST 2710 and NIST 1944 were used for quality control purposes to evaluate the accuracy and efficiency of the digestion method.

##### 3.11.2.1.3. Sample Preparation for Residential Soil Analysis

Samples of soil litter were dried to a constant weight at 50 °C in a convection oven and were ground using a Spex Shatterbox grinder with a steel ring and puck mill. A detailed description of the methods used for the analysis can be found in previous papers (Machemer and Hosick 2004; Machemer et al., 2007; Hosick et al., 2002).



Batch QC triplicates were run to evaluate the precision of the methods.

#### 3.11.2.1.4. Determination of Lead and Tin in KOH Fusions

KOH fusions were conducted on 0.2 gram of soil with 2.0 grams of KOH pellets in a 15 mL vitreous carbon crucible. Crucibles were heated to 160°C for at least 1 hour with rotary agitation until all material was molten. Crucibles were heated to 460°C for an additional hour with rotary agitation. After cooling, fused pellets were rinsed from the crucibles to plastic beakers using distilled-deionized (DDI) water, and the crucibles were rinsed again with 10 mL of concentrated HNO<sub>3</sub>. One mL of 30% hydrogen peroxide was added to sample solutions before dilution to 100 mL with DDI water. After overnight rotary agitation, solutions were vacuum filtered through Nalgene™ disposable, 0.8 micrometer, cellulose nitrate membrane filtration units. Samples were then measured by ICP-OES. Spectral background and inter-element interference corrections were applied, as appropriate.

Solutions were also analyzed for tin by ICP-MS with continuous hydride generation. Solutions were brought to 3.0 M HCl by the addition of concentrated HCl, then mixed at a rate of 2.2 mL/min in line with a solution of 2.4 %NaBH<sub>4</sub> in 0.25 M KOH at a rate of 1 mL/min.

The mixture was introduced into a gas/liquid separator where stannane gas was separated from the solution for introduction into the ICP-MS. The RF power and nebulizer gas flow rate were optimized to give the maximum Sn signal at 1200 W and 1.1 L/min, respectively. Multiple tin isotopes (<sup>116</sup>Sn, <sup>117</sup>Sn, <sup>119</sup>Sn, <sup>120</sup>Sn,) were measured to observe and avoid isobaric or molecular interferences.

#### 3.11.2.1.5. Determination of Lead and Antimony in Acid Digestions

Nitric acid digestions were conducted on 0.25 gram of soil with 10 mL of 50%  $\text{HNO}_3$  in a 30 mL Teflon<sup>TM</sup> tube with screw cap. Solutions were heated to 95°C overnight in a convection oven.

After cooling, 20 mL of DDI water were added to each solution prior to vacuum filtration through 0.45 micrometer pore size, cellulose nitrate membranes in disposable Nalgene<sup>TM</sup> filtration units. After filtration, solutions were diluted to 100 mL with DDI water. Nitric acid digestions were analyzed for lead by ICP-MS with cross-flow nebulization.

Hydrochloric acid digestions were conducted using 1.0 gram of soil with 25 mL of concentrated HCl acid and 0.1 mL of 30%  $\text{H}_2\text{O}_2$  in 50-mL, glass, and culture tubes with screw caps. Culture tubes were loosely capped and heated for 1 hour at 95°C. After cooling, an additional 0.1 mL of 30%  $\text{H}_2\text{O}_2$  was added before bringing the final volume to 50 mL with DDI water.

Hydrochloric acid digestions were analyzed for lead and antimony by ICP-MS with cross-flow nebulization.

#### 3.11.2.2. Set of Sediments from South Florida:

##### 3.11.2.2.1. Instrumental parameters

Acid digests of the sediments were measured by ICP-MS using a Agilent-4500 (Agilent Technologies, Palo Alto, USA). The digestion method for soils and sediments was conducted using a hot block digestion system (Environmental Express, SC, USA) following EPA 3050B guidelines and EPA 6020A for measurement in the ICP-MS.

#### 3.11.2.2.2. Reagents and Standards

All solutions were prepared using high purity water ( $\geq 18 \text{ M}\Omega\text{cm}^{-1}$ ) obtained with a Millipore direct Q VV3 system (Millipore, MA, USA). Trace elemental grade (optima grade) nitric acid and hydrogen peroxide (Fisher, Pittsburg, PA, USA) were used throughout the analysis. Two standard mixtures of elements in concentration of 10 ppm from AccuStandard (CT, USA) were used to prepare element calibration solutions for the following monitored isotopes:  $^9\text{Be}$ ,  $^{27}\text{Al}$ ,  $^{42,44}\text{Ca}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{59}\text{Co}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{75}\text{As}$ ,  $^{98}\text{Mo}$ ,  $^{107}\text{Ag}$ ,  $^{111}\text{Cd}$ ,  $^{120}\text{Sn}$ ,  $^{121}\text{Sb}$ ,  $^{137}\text{Ba}$ ,  $^{205}\text{Tl}$ ,  $^{232}\text{Th}$ ,  $^{206, 207, 208}\text{Pb}$ ,  $^{238}\text{U}$ . Spiked solutions of 10 ppm were prepared from the same standard mixtures. A standard solution of 10 ppm for Yttrium, Scandium, Rhodium and Indium from AccuStandard (CT, USA) was used as an internal standard at a measurement concentration of 50 ppb in the calibration curve.

#### 3.11.2.2.3. Sample Preparation for South Florida Sediments

The sediment samples were digested using the acid digestion method EPA 3050B with an adaptation for the hot block. An amount of 0.25 grams of dried samples were measured in a 68 ml polypropylene digestion vessel. An aliquot of 5 mL (1+1) nitric acid trace metal grade was added to the vessel and cover with a watch glass and heated at  $95^\circ\text{C}$  for 15 minutes without boiling. Once the sample was cool, concentrated nitric acid (trace metal grade) was added and the solution was refluxed for 30 minutes. This procedure was repeated until no brown fumes are given off the sample. The sample was heated for an additional 1.5 hours avoiding boiling the sample or taking the sample to dryness. After this period of time, the sample was cooled to room temperature. 2-5 mL of

water and 0.5 mL of 30 % H<sub>2</sub>O<sub>2</sub> was added and the sample was placed in the hot block. The H<sub>2</sub>O<sub>2</sub> was continuously added until the sample color remained unchanged while heating for a total of 2 hours. Samples were then diluted to 50 mL with DDI water and a Filtermate® (Environmental Express, SC, USA) was used for filtration. The samples were later divided in three groups; trace, minor and major elements.

Digestion blanks, laboratory blanks, and procedural blanks were also used to assess the introduction of contaminants during the digestion of the samples. In order to determine the ruggedness of the analytical method, fortified laboratory blanks were analyzed with every set of samples analyzed (LBS). The LBS are made of laboratory water, and they are spiked with a mixture of the elements of interest in the digestion vessel. The LBS are then processed through the whole analytical procedure. To assess both precision and accuracy, matrix spikes (MS) and duplicates (D) are analyzed with each batch of sediments samples. Both consisted of the use of real fortified sediment or soil sample, spiked with a mixture of the elements of interest.

### 3.12. Statistical Analysis and Data Processing

All statistical analysis was performed using JMP 5.01 (SAS Institute, 2002); Microsoft® Excel 2002 (10.2614.2625), and Sigma Plot® v11.0.0.67 (Systat Software Inc, 2008) and Systat 11® (Systat Software Inc, 2006).

Descriptive statistics were used during this study to conduct the optimization and to evaluate the quality of the analytical measurements of the methods, including mean values, standard deviations, relative standard deviations, significance tests and estimations of accuracy and precision.

For the set of residential soils, regression methods at 95% confidence level were used to do comparison of the results obtained by laser ablation and by total-total digestion methods.

All major, minor, and trace metal concentrations are based on dry matter basis. For the set of 48 sediments, the values were log transformed before analysis to meet the assumption of normality.

Geometric mean and geometric standard deviation ( $GM \pm GSD$ ) were calculated using  $GM/GSD^2$  to  $GM \times GSD^2$  of the samples, where  $GM \times GSD^2$  corresponds to the upper baseline limit (UBL) and  $GM/GSD^2$  is the lower baseline limit (LBL), which includes the expected range of 95 % of the population. The range of concentrations between the calculated  $GM/GSD^2$  and  $GM \times GSD^2$  are considered the baseline values.

Saphiro-Wilk tests ( $\alpha = 0.05$ ) was used to test the normality of the concentration distribution within each element in both sets of data (laser and leacheate) (Miller and Miller 2000).

Non parametric statistics was used to compare two analytical schemes followed in this investigation. Spearman Rank analysis was used to compare these two analytical schemes (leacheate and Laser Ablation) by their element concentrations. Also Spearman Rho coefficients were calculated to evaluate the main factor affecting the leacheate method by correlation of the sediment properties like the total organic carbon, salinity, total aluminum concentration, total iron concentration, particle size distribution and the corresponding log transformed elemental concentration. A factor was interpreted as a physical or chemical process based on the loadings of the variables. Each factor contains all variables but only variable with loadings above 0.5 was considered to be important for

interpreting a factor (Dudka 1992). A principal component (PCA) analysis was conducted as an attempt to classify the sediments sets, also to observe local and temporal variations within the areas of study.

### 3.13 Results and Discussion

#### 3.13.1. Method Optimization and Evaluation of the Analytical Performance

##### 3.13.1.1. Selection of the Ablation Parameters

At the beginning of this study two different ablation systems were employed for the method development, one operating at 213nm and the other one at 266nm. The laser systems that were studied have different options for ablation mode such as single spot, depth profile, single line and raster. In single spot the laser drills a hole into the surface of the sample at certain specified conditions. In depth profile a hole is also drilled into the sample but in contrast with the single point mode the surface of the sample is constantly moved in the “z” axis while the ablation takes place.

The depth profile mode is only necessary on the 266nm laser since in the 213nm laser the focal point remains stable while the crater is drilled. Using single lines, the laser moves in the “x or y” axis, drawing a “line” into the surface of the material. Finally, using the raster option the laser can draw lines into different directions (a square shape was selected for this experiment). Selection of the ablation mode for both lasers was based on four critical factors: a) precision between runs, b) shape of the craters, c) shape and stability of the transient signal and d) accuracy for reference standard materials. All ablation modes were initially conducted at 100 $\mu$ m spot size. Figures 3.4 and Figure 3.5, show the effect of these ablation modes on accuracy and precision for the NIST 2710

reference standard material. Raster showed better precision (overall %RSD 6.5%), but spot size presented better accuracy (overall % error < 15 %).

Table 3.2 shows the individual values. Although laser lines and raster sampled larger surface areas than spot mode, previous studies on glass have demonstrated that the majority of particles produced using single spot and depth profile were in the lower range of 0.1-0.2  $\mu\text{m}$  and very few were above 1  $\mu\text{m}$  (Trejos 2003), while during line ablations the particles produced are typically greater than 0.7  $\mu\text{m}$ . Bigger particles are more difficult to ionized in the plasma, they can produce fractionation and affect the accuracy of the analysis.

For the above reasons, spot mode was chosen as the ablation mode since it produces better accuracy, and precision was improved later by increasing the spot size to 200 $\mu\text{m}$ . Further experiments conducted using spot mode at different spot sizes (100 $\mu\text{m}$ , 200 $\mu\text{m}$ , 250 $\mu\text{m}$ ) showed that a spot of 200 $\mu\text{m}$  gave good repeatability between runs and account for natural heterogeneity at this scale range in the pellet, which was later confirmed by SEM imaging.

The influence of the repetition rate of the laser and its impact on the surface of the ablated material was studied at 10 and 20 Hz at a fixed spot size of 200  $\mu\text{m}$ . This is shown in Figure 3.6. Better precision (6% vs. 16%) and less destruction into the sample were noticed at 10 Hz.

#### 2.13.2. Sample Preparation and Homogenization

Soil and sediments are complex matrices in that the grain size and mineral composition of major elements varies widely between sample sites. The main challenge

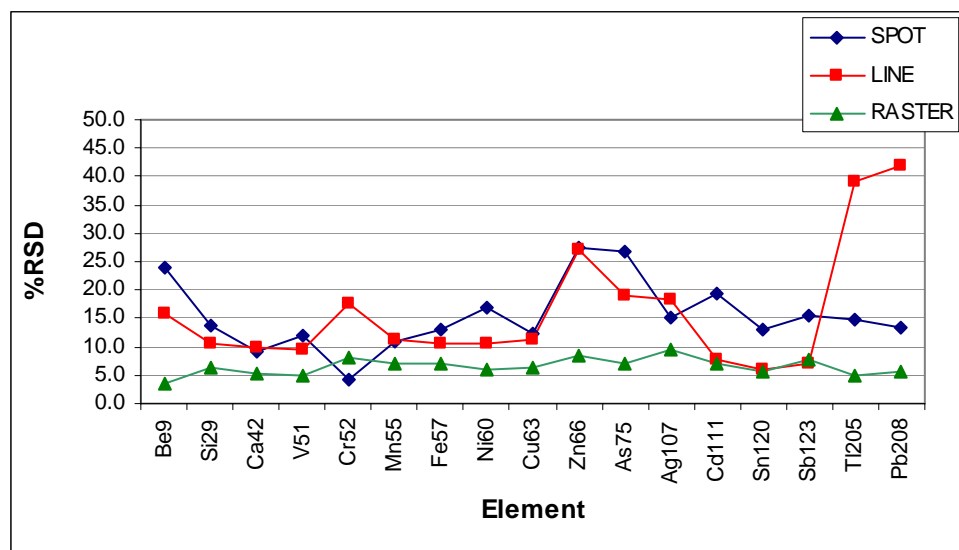


Figure 3.4. Comparison of precision (as %RSD) obtained on soil reference material NIST 2710 for different ablation modes.

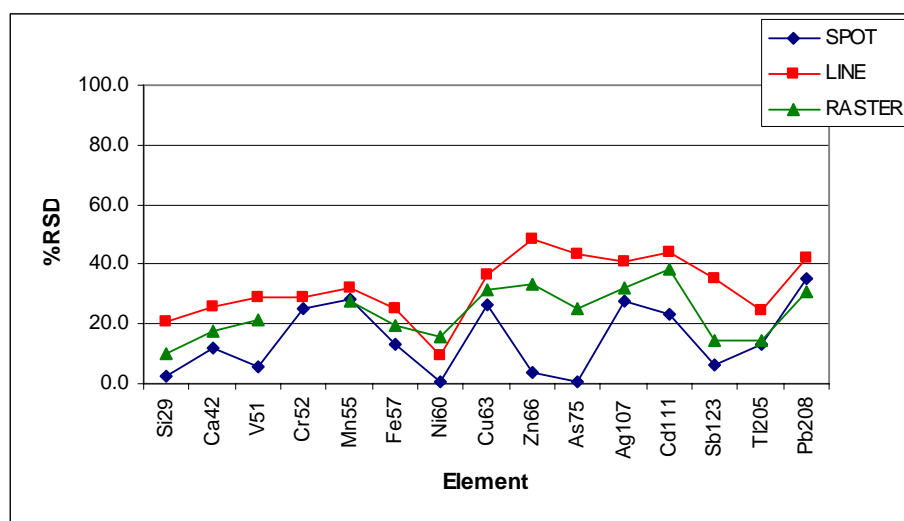


Figure 3.5. Comparison of accuracy (as %error) obtained on soil reference material NIST 2710 for different ablation modes.



Table 3.2. Comparison of Precision and accuracy for different ablation modes on NIST 2710.

Element	% RSD				% error		
	SPOT	LINE	RASTER		SPOT	LINE	RASTER
Be9	23.9	16.0	3.4				
Si29	13.7	10.5	6.3	Si29	2.5	20.5	10.4
Ca42	9.0	9.8	5.2	Ca42	11.8	25.6	17.5
V51	12.0	9.4	5.0	V51	5.7	29.2	21.5
Cr52	4.3	17.5	7.9	Cr52	25.3	28.6	26.3
Mn55	10.9	11.1	7.0	Mn55	28.3	32.1	27.5
Fe57	13.2	10.6	7.1	Fe57	13.4	25.3	19.5
Ni60	16.8	10.7	6.1	Ni60	0.7	9.3	15.5
Cu63	12.3	11.3	6.3	Cu63	26.7	36.2	31.4
Zn66	27.5	27.2	8.5	Zn66	3.6	48.7	33.2
As75	26.8	18.9	7.2	As75	0.6	43.1	25.0
Ag107	15.0	18.2	9.4	Ag107	27.5	40.7	32.0
Cd111	19.3	7.7	7.1	Cd111	23.5	43.9	38.5
Sb123	15.4	7.1	7.9	Sb123	6.5	35.1	14.3
Tl205	14.8	38.9	4.9	Tl205	13.1	24.8	14.3
Pb208	13.4	41.9	5.7	Pb208	35.0	41.9	30.9
<b>Overall % RSD</b>	<b>15.5</b>	<b>16.7</b>	<b>6.5</b>	<b>Overall % error</b>	<b>14.5</b>	<b>32.3</b>	<b>23.7</b>

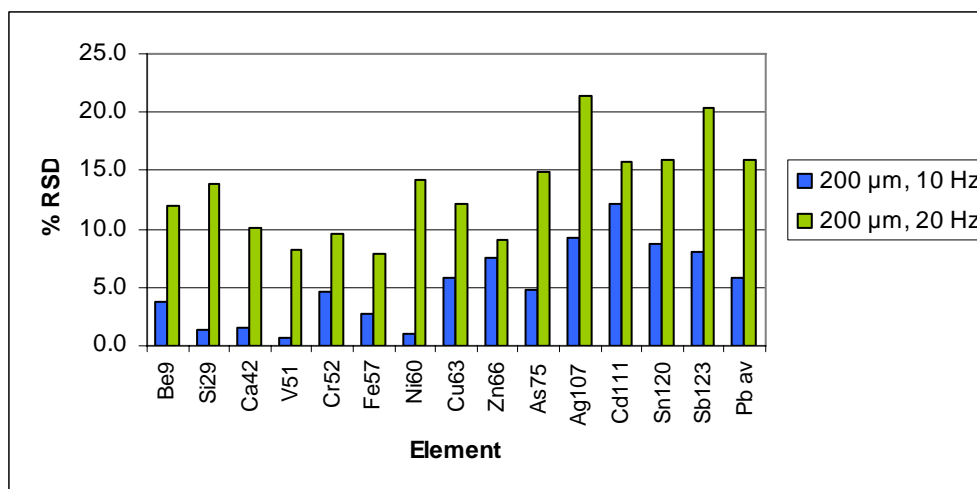


Figure 3.6. Effect of the laser repetition rate on the precision of the measurements on NIST 2710.

for sample preparation of soils is to provide a homogeneous distribution of particles and elemental composition on a micro scale ( $\sim 200\text{ }\mu\text{m}$  diameter \*  $200\text{ }\mu\text{m}$  depth craters).

The easiest way to handle a sediment sample for laser ablation is to create a pellet that could be stored for long term use, with enough surface area, high impact resistance to the laser and with homogeneous distribution of the elements and/or internal standards. The test sample formation was achieved by using a manual pellet press. In this study, two different approaches were followed to determine the proper homogenization of “in-house” matrix matched solid standards. This is a critical aspect because to date, there is no such commercially available standard for laser ablation sampling.

In the first approach, a manual agate mortar (MM) was used to grind the soil/sediment sample. In the second procedure a ball mixer/mill (BM) or a high-speed mixer (HS) mill was employed. The main difference between these two mills is that the HS mill works at a higher speed and it can process two samples at a time. The ball mill requires about 40 minutes per sample and reduces the average particle size to  $< 10\mu\text{m}$ , while the High Speed Mill requires about 20 minutes per two samples and reduces the average particle size to  $< 1\mu\text{m}$ .

The overall precision for a PACS-2 standard improved from 44% to 9% when the ball mill grinding procedure was used instead of the manual mortar. SEM images were used to measure the particle size of the soil after manual homogenization, typically  $\sim 100\text{ }\mu\text{m}$  diameter particles, which represent significant heterogeneity at the micro-scale. Samples homogenized with the ball mill presented particle sizes  $\sim 10\text{ }\mu\text{m}$  in diameter. The use of the high-speed mill produced particle sizes of  $\sim 1\text{ }\mu\text{m}$  in diameter, which also improved the precision of the measurements to less than 4% RSD, for most of the cases.

The cohesion of the test sample played an important role in precision because the more compact the pellet, the more reproducible the laser – soil interaction. The particle size was evaluated at the surface of the pellets using particle analysis software (EDS 2008), which combines SEM image analysis with EDS quantitative analysis to locate and identify particles of different size on a sample. The particle diameters reported above correspond to the most frequent value found for a total of ~200000 particles analyzed.

Additionally, the size distribution of the particles transported to the ICP-MS was also studied in order to evaluate whether or not the test sample preparation method and the particle size on the surface of the pellet had an effect on the laser-induced particles that reach the plasma. The production of smaller particles is well known to enhance the ionization into the plasma (Figgs et al., 1998; Horn and Gunther 2003; Kuhn and Gunther 2004; Guillong and Gunther 2002).

Ablated particles were collected on 0.45µm filter and imaged by SEM. Figure 3.7 shows that the particle size transported to the plasma decreased with the efficiency of the grinding method. The most frequent diameter of particles collected on the filter were 1µm (range 0.45-2µm), 4µm (range 1-18µm) and 12µm (range 1-40µm) for the test samples prepared by HS, BM and MM, respectively. Although the particles were collected using the same ablation conditions, Figure 3.7 depicts that the density of particles collected from the HS-pellet is much less than the density of particles collected from the BM-pellet, suggesting that a majority of the particles were not retained at the filter (<0.45µm). This particle size studies are in good agreement with the observations of accuracy and precision enhancement obtained by LA-ICP-MS when the test samples were prepared by HS-mill.

Furthermore, the grinding process with the high speed mill also had a positive effect in the accuracy, particularly for some volatile elements such as lead and tin. This improvement in accuracy may be a consequence of the faster sample preparation time which avoids the heating of the sample. In most of the cases, the container of the regular mill was very hot after 40 minutes of grinding and that may have caused some loss of those volatile elements. This heating effect was not experienced with the high speed mill.

Additionally, the smaller particle size powder ( $\sim 1\mu\text{m}$ ) also contributed to improve the cohesion and mechanical resistance of the test sample, while soils homogenates of grain sizes of  $\sim 10\mu\text{m}$  tend to break more easily not only during pellet formation but also after manipulation of the pellets during LA analysis.

Figure 3.8 shows the comparison of morphology of laser ablation craters on a PACS-2 pellet formed with homogenized sediments of  $\sim 10\mu\text{m}$  and  $1\mu\text{m}$  of particle size, respectively.

Craters produced on the sediment of larger particle sizes not only present more signs of fractures around the crater but also more re-deposition of ablated material. Moreover, the combined use of UV-ni lasers and the advantage of improving cohesion as a consequence of particle size of the powder, avoids the need to use binders as required by other authors (Musil et al., 2000; Becaggia et al., 2006; Mikolas et al., 2002; Mason and van Elteren 2006; Boulyga et al., 2004).

This not only simplifies the sample preparation but also prevents the dilution of elements into the sample, and reduces sources of contamination or interferences from the walls of the mixer.

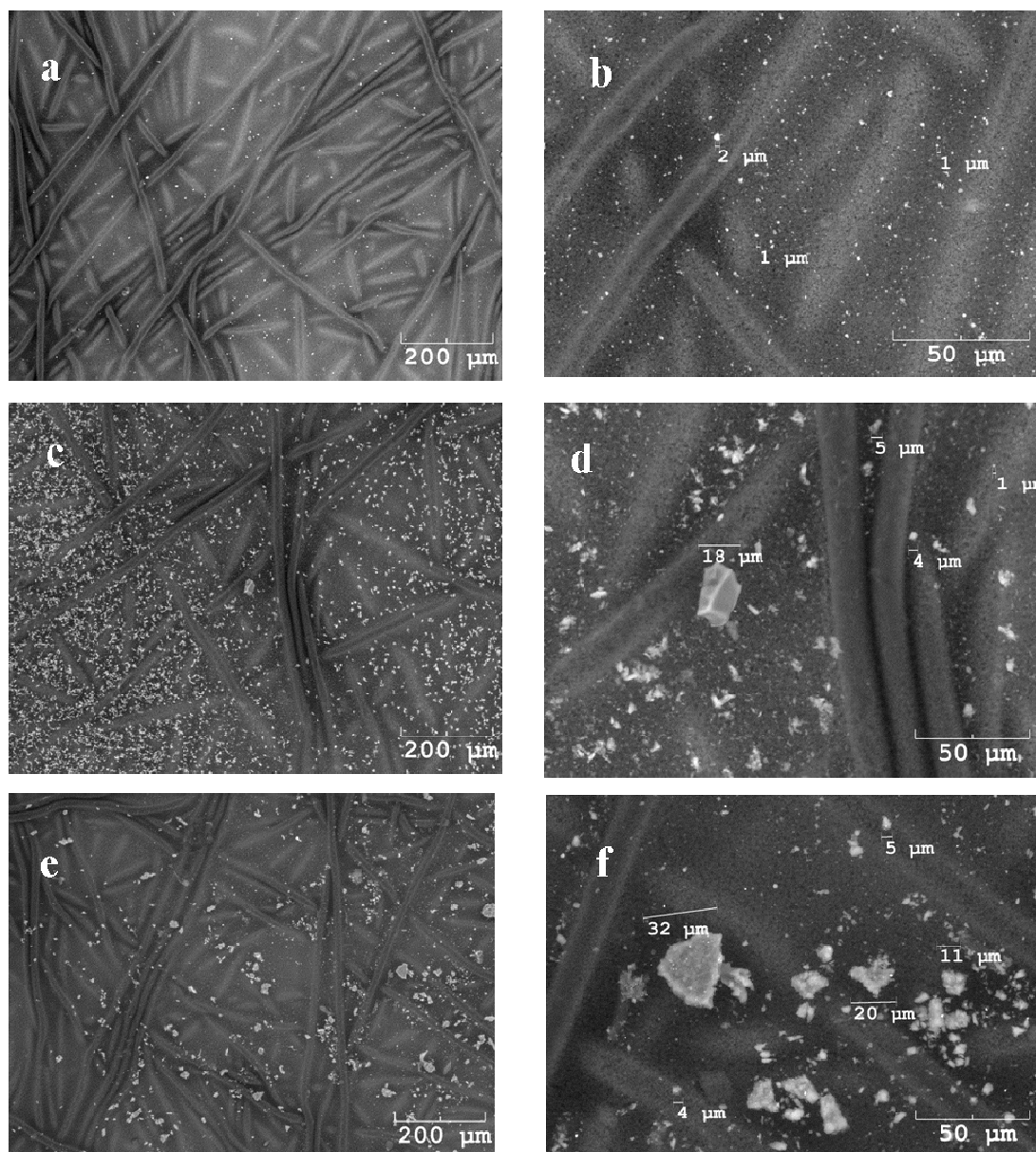


Figure 3.7. SEM image of laser-induced particles collected on a filter paper during ablation of a PACS-2 test sample homogenized by HS (a,b), BM (c,d) and MM (e,f). Images were taken at 200x (left) and 1000x (right).

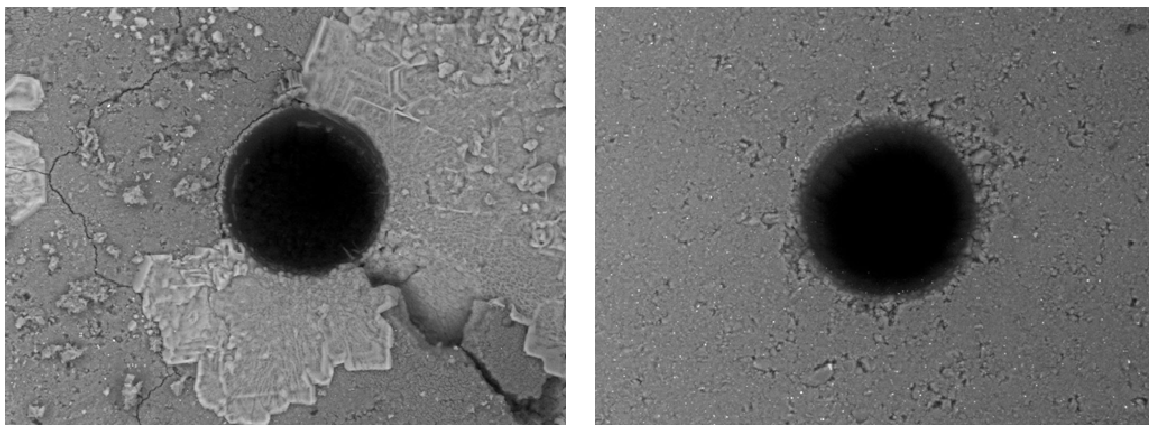


Figure 3.8. SEM morphology image for the laser ablation crater and surroundings on PACS-2. Left: pellet formed with soil particulate  $<10\mu\text{m}$ . Right: pellet formed with soil particulate  $<1\mu\text{m}$ . Each ablation crater is of  $200\mu\text{m}$  diameter, magnification 400x.

SEM imaging was conducted to assess the crater morphology of different soil samples. Figure 3.9 shows the crater morphology for NIST 2710 (3a) demonstrating that the interaction of the laser at the wavelength of 266nm does not produce major destruction on the surface.

Similar crater profiles were observed for other soil types (sediment PACS-2, Proficiency tests, Sand test samples (quartz) and Soil test samples ranging from whitish to dark black colors), suggesting the cohesion and strength of the soil pellets did not varied significantly between soils of different composition.

Nevertheless, for materials of different composition than soil, the ablation rate was significantly matrix dependent as observed by the different profile of graphite pellets.

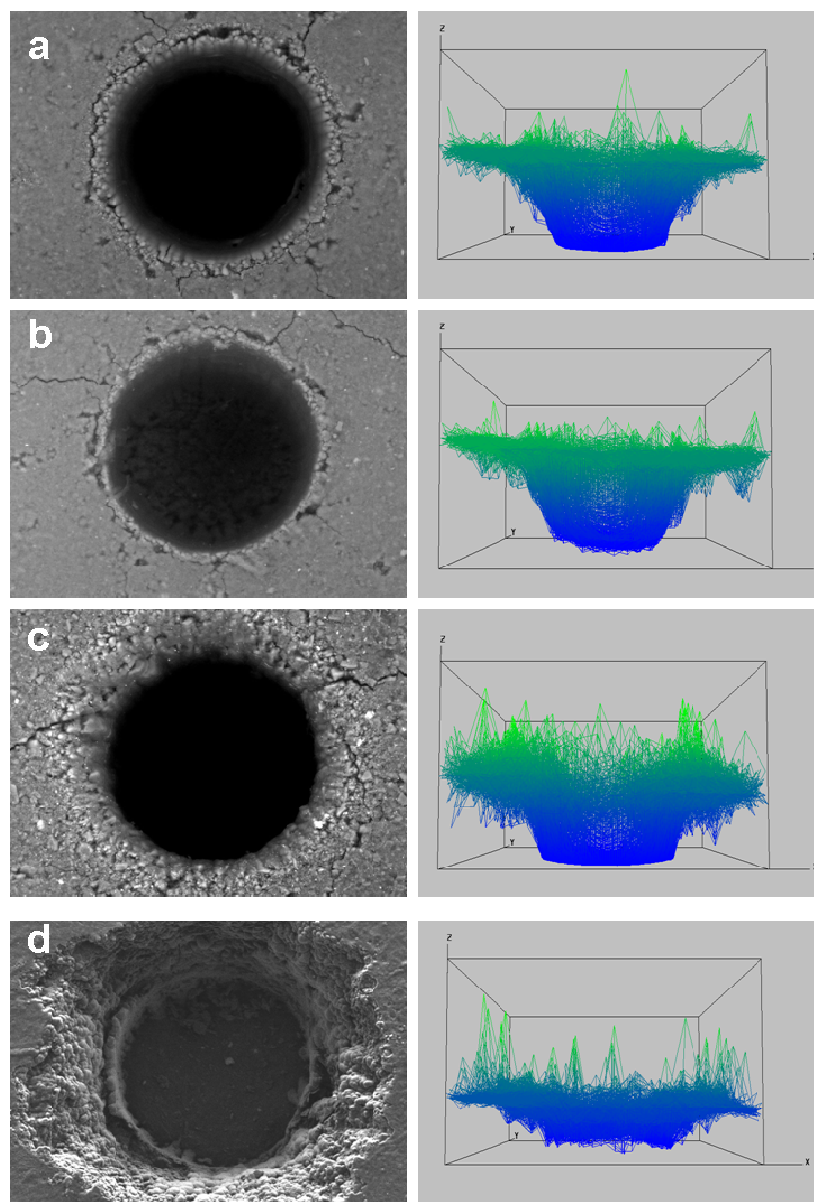


Figure 3.9. SEM image (left) and 3D SEM images (right) of the morphology of laser ablation craters on different materials: a) NIST 2710, b) Soil proficiency test, c) Sand standard (quartz), d) Graphite standard. All images are at 700x magnification.

### 3.14. Quantitation Strategies

#### 3.14.1. Internal Standardization

The use of an internal standard and its dispersion and distribution in the solid material is important because it compensates for the differences in the amount of ablated material and efficiency of transportation into the plasma.

Since Calcium and Silicon are major components in soil and sediment samples, they were initially considered as possible internal standards. As an alternative, powdered Germanium and Yttrium oxides were spiked in the sample. Germanium and Yttrium resulted in a more convenient internal standard than Calcium or Silicon. Germanium and Yttrium are added at known concentrations, not requiring prior knowledge of the natural amount of the element in each of the samples. Another advantage of the use of Germanium and Yttrium is that the concentration of the internal standard remains constant in both samples and standards.

SEM/EDX mapping and semi-quantitative analyses were conducted to evaluate the particle size distribution (SEM) and the elemental distribution (SEM/EDX) of the internal standard and matrix.

Figure 3.10 shows the homogeneous distribution of germanium into the sample in a 200  $\mu\text{m}$  scale to simulate the size of the crater diameter used during each ablation. Table 3.3 shows the average composition of Germanium at this micro-scale, obtained by SEM/EDX, was 1.8 %, which is in agreement with the amount added to the samples (2.0 % added).

Similar results were obtained using Yttrium as internal standard and therefore, only Germanium was later spiked on the samples.



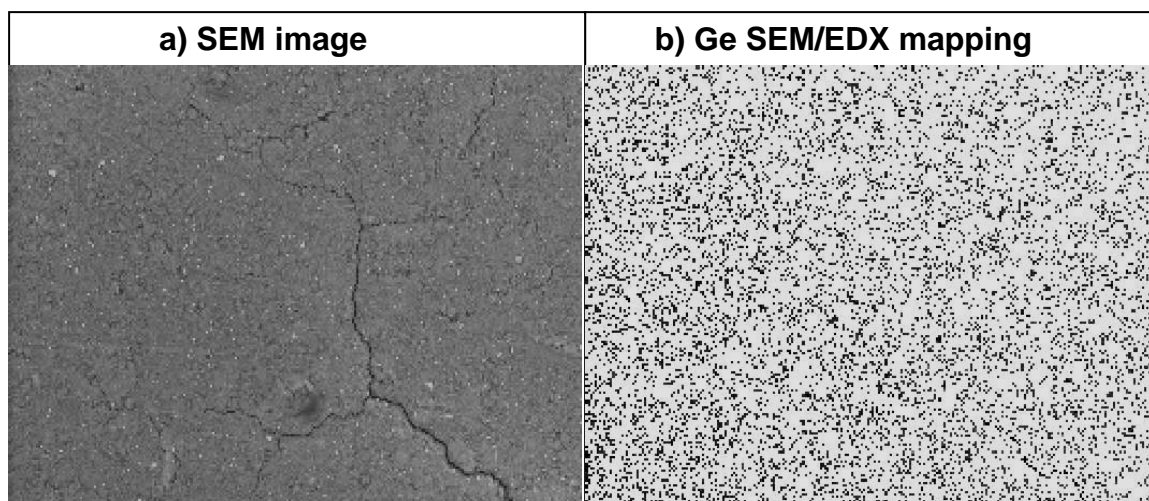


Figure 3.10. Particle size and elemental distribution of germanium on a NIST 2710 pellet:  
a) SEM image and b) SEM/EDX mapping of Ge (dark spots) for an area of 200  $\mu\text{m}$  by 200  $\mu\text{m}$ .

Table 3.3. SEM/EDX semiquantitative determination of Germanium on a surface area of 200  $\mu\text{m}$ \* 200  $\mu\text{m}$ . Germanium concentration reported as % wt.

Replicate	NIST 2710	PACS-2	PT
1	2.55	2.16	1.89
2	2.61	1.92	1.87
3	2.23	1.95	1.84
<b>mean value</b>	<b>2.46</b>	<b>2.01</b>	<b>1.87</b>
stdev	0.20	0.13	0.03
%RSD	8.3	6.5	1.3
<b>expected value</b>	<b>2.45</b>	<b>2.43</b>	<b>2.18</b>

### 3.14.2. Single Point Calibration Using Soil or Sediment Standards as Calibrator:

The sediment standard PACS-2 and the soil reference material NIST 2710 were spiked with Germanium and Yttrium and homogenized to create “matrix matched” standards for LA-ICP-MS. Although these standards are not designed and manufactured for micro-analysis, the homogenization process reduced the particle size to less than 1  $\mu\text{m}$  making the distribution of the elements on the test samples appropriate for laser ablation sampling. One advantage of soil matrices is that the physical characteristics of their components made possible the creation of pellets that compact the material and facilitate the manipulation of the sample.

The use of single point calibration provides a simple quantitation method, which is convenient for routine determinations. Precision was evaluated as the percentage of relative standard deviation while accuracy (trueness) was evaluated as absolute percentage of bias. The mean relative standard deviation and bias, reported as the mean values for the suite of 16 elements, was 4 % RSD and 8 % bias, for PACS-2 when NIST 2710 was used as calibrator (see Table 3.4).

### 3.14.3. External Calibration Curves:

External calibration curves were determined for a smaller group of elements to determine any improvement of accuracy versus single point quantitations. Although good precision and low bias was observed with both methods ( $\leq 10\%$ ), the multi-point calibration improved the accuracy (Table 3.5). Calibration curves obtained for the spiked sand showed good linearity for all three analytes ( $r^2 \geq 0.9934$ ). The precision between replicates, accuracy and linearity of the calibration curve suggests that aqueous-based

Table 3.4. Precision and bias of LA-ICP-MS determinations on standard reference marine sediment PACS-2.

<b>PACS-2</b>	<b>Certified(<math>\mu\text{g g}^{-1}</math>)</b>	<b>Found (n=4)</b>	<b>%RSD</b>	<b>Bias (% error)</b>
Be	$1.0 \pm 0.2$	$1.1 \pm 0.2$	10	10
Si	280000*a	$289835 \pm 9466$	2.0	3.5
Ca	$19600 \pm 1800$	$20868 \pm 864$	3.0	6.5
V	$133 \pm 5$	$145 \pm 2$	1.0	8.9
Cr	$90.7 \pm 4.6$	$105 \pm 4$	4.0	15.8
Fe	$40900 \pm 600$	$44902 \pm 986$	2.0	9.8
Ni	$39.5 \pm 2.3$	$35.5 \pm 1.4$	4.0	10.1
Cu	$310 \pm 12$	$346 \pm 24$	7.0	11.5
Zn	$364 \pm 24$	$407 \pm 9$	2.0	11.8
As	$26.2 \pm 1.5$	$30.4 \pm 0.6$	2.0	16.0
Ag	$1.22 \pm 0.14$	$1.30 \pm 0.06$	5.0	6.4
Cd	$2.11 \pm 0.15$	$2.27 \pm 0.07$	3.0	7.3
Sn	$19.8 \pm 2.5$	$21.8 \pm 1.3$	6.0	10.1
Sb	$11.3 \pm 2.6$	$12.2 \pm 0.7$	6.0	8.2
Tl	$0.6^{\text{*a}}$	$0.67 \pm 0.04$	6.0	10.8
Pb	$183 \pm 8$	$196 \pm 11$	4.0	7.2
mean			4.2	8.0

Table 3.5. Comparison of accuracy for PACS-2 obtained using single point vs. multiple point calibration.

<b>Element</b>	<i>NIST 2710 as calibrator</i>			<i>SAND curve as calibrator</i>		
	<b>Mean (<math>\mu\text{g g}^{-1}</math>)</b>	<b>%RSD</b>	<b>%error</b>	<b>Mean (<math>\mu\text{g g}^{-1}</math>)</b>	<b>%RSD</b>	<b>%error</b>
<b>Sn</b>	21.8 $\pm$ 1.3	6	10.1	21.6 $\pm$ 2.9	9	9.1
<b>Sb</b>	12.2 $\pm$ 0.7	6	8.2	10.5 $\pm$ 0.8	5	7.0
<b>Pb</b>	196 $\pm$ 11	4	7.2	175 $\pm$ 9	3	4.4

spikes are a good choice for the preparation of calibration standards. It also demonstrated that the drying and the homogenization processes of the standards were efficient. The low temperature used for the drying process prevented losses of volatile compounds.

Figure 3.11 shows the correlation obtained between concentrations of a set of eight soil samples when PACS-2 was used as single calibrator (x-axis) and when the sand curve was used for quantitation (y-axis). The concentration on those samples ranged from 25 to 4850  $\mu\text{g g}^{-1}$  in lead, from 1.5 to 25  $\mu\text{g g}^{-1}$  in tin, and from 0.5 to 40  $\mu\text{g g}^{-1}$  in antimony. A statistical regression analysis resulted in no significant difference between the two quantitation strategies at the 95% confidence level. This demonstrated that sand (quartz) is a good matrix for calibration purposes by LA-ICP-MS, although is more time consuming than single point calibration and therefore it may be useful in determinations of single or few elements rather than multi-element routine analysis and if the improved accuracy is necessary.

A similar experiment was conducted using graphite as the matrix for the calibration curve. Although good linearity was observed within the calibration range

( $\geq 0.9991$ ), the quantitative data obtained for the control standards PACS-2 and NIST 2710 was not accurate (bias above 90%). These results show that there is a significant matrix effect when graphite is used as a matrix. This effect can be due to differences in the ablation parameters, transport and ionization conditions in the plasma.

In order to further study the matrix effects of graphite on the quantitation of soils and sediments, a piezoelectric balance was coupled to the laser ablation system. The laser was operated under the same optimal conditions used for LA-ICP-MS analysis but the output of the carrier gas was connected into the balance instead of the ICP-MS.

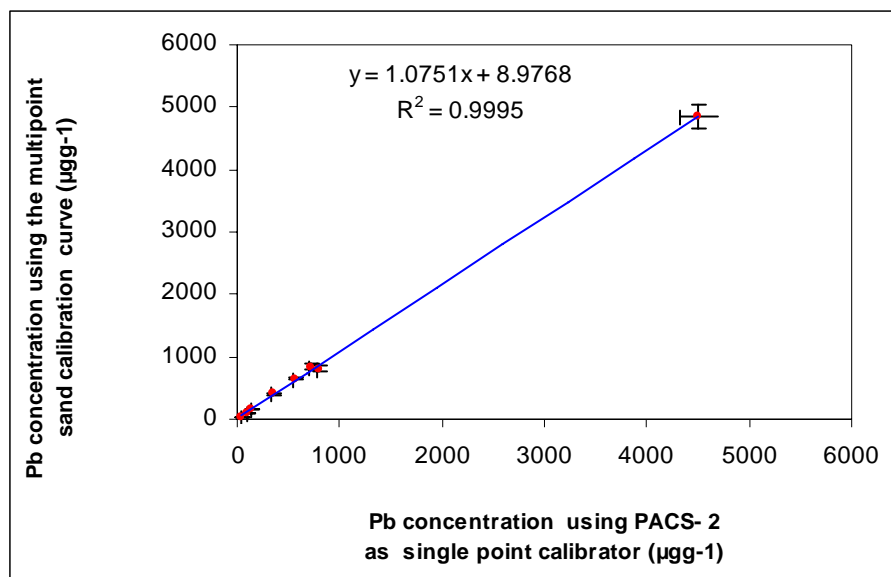


Figure 3.11. Regression line for the comparison of the quantification of lead on eight soil samples using single point calibration (PACS-2) and multiple point calibration (sand matrix).

The mass removed by the laser (500 shots) from the sand pellet and from the standards is similar, on the order of  $\sim 6 \mu\text{g}$  (Table 3.6). The interaction of the laser with graphite matrix remove 94.5% less mass than the mass removed from soils. This significant change may be explained due to the differences in ablation interaction,

absorption of the laser by the material, the transport efficiency and density of the materials, so for similar ablation parameters, the amount of mass removed and transported is significantly lower for graphite.

The reduced mass transported can be partially explained by the lower laser-to sample interaction efficiency that reflects a less deep crater (Figure 3.9) and also by observed depositions of the graphite into the ablation cell walls and tubing. The matrix effect using graphite was significant and was not even corrected by the use of internal standard.

These results stress the importance of using matrix matched standards for soil analysis and also demonstrate that careful considerations are necessary if graphite is used as a matrix or as a binder for soil samples. The results also corroborate that sand is a convenient matrix for the “in-house” manufacture of matrix matched standards. The agreement on the mass removed from soil and sediment from different origins (Montana soil, marine sediment, sand, QC and PT soils) is also evidence that the ablation rate does not change significantly between these samples, which also agrees with the morphology studies of the craters conducted by SEM (Figure 3.10).

#### 3.14.4. Micro-Homogeneity Study

The homogeneity studies presented here are intended to evaluate whether the small amount of sample removed from each of the 200  $\mu\text{m}$  diameter craters produced by laser ablation ( $\sim 6 \mu\text{g}$  of sample) is representative of the elemental composition of the test sample and of the original composite sample. Analysis of variance was employed to compare the mean value given by the instrument variation (10 replicates or test portions

Table 3.6.Total mass ablated measured with the piezoelectric balance during the laser ablation of different solid materials.

<b>Standard</b>	<b>Ablated mass (µg, n=4)</b>
PACS 2 pellet	5.6 ± 0.9
NIST 2710 pellet	6.3 ± 0.3
Sand pellet	6.1 ± 0.7
Proficiency test soil pellet (PT1)	5.9 ± 0.6
QC soil pellet	6.0 ± 0.8
Graphite pellet	0.3 ± 0.3

from a single test sample) and the mean value of the 10 replicates of each of the other 3 pellets (30 replicates), which represents the variation given by the natural heterogeneity within the pellets. The statistical analysis was performed for each of the elements reported on this manuscript and in all cases they were not significantly different with a 95% of confidence ( $p > 0.05$ ). Figure 3.12 shows a comparison of the elemental profile of replicates from different pellets of the same soil proficiency test.

The homogeneity study represents important evidence that the microanalysis of soils, with the proposed method is representative from the composite sample and also supported by the agreement with certified values. Nevertheless, for quality control purposes it is very important to provide a good sampling of the soil in order to arrive to accurate conclusions. It is recommended to sample the known material from different areas to account for natural heterogeneity within the soil and to conduct at least four replicates per test sample.

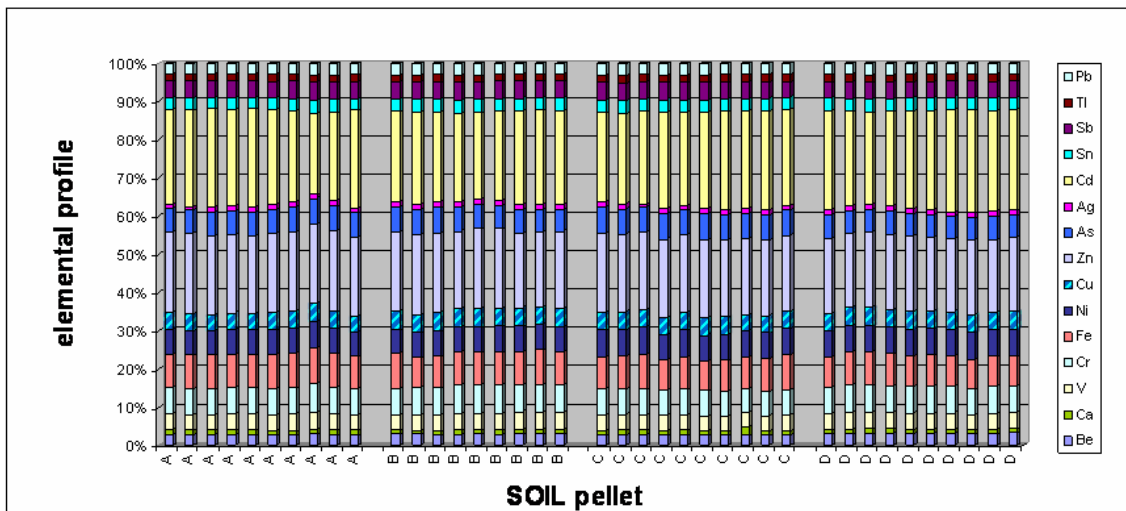


Figure 3.12. Comparison of elemental profile of replicates from different soil pellets of the same proficiency test.

### 3.15. Precision, Accuracy and Reproducibility

Precision and accuracy were evaluated on the reference standard sediment PACS-2 and in three soil samples, QC and two PT. The overall precision obtained ranged from 2 to 6 % depending on the sample. Tables 3.7 to 3.9 show the individual results respectively. The overall accuracy, evaluated as absolute percentage of bias of the test results versus certified or assigned values, was 8 % for the PACS-2 standard, 15 % for the quality control test sample, and 8 to 9 % for the proficiency tests.

As part of the validation study, two individual proficiency test soil samples were analyzed by LA-ICP-MS. These proficiency tests are designed for determination of trace metals in soils by conventional digestion methods followed by AA or ICP analysis. LA-ICP-MS results were compared to values obtained by typical acid digestion methods from a total of 24 participant laboratories (PT1) and 33 laboratories (PT2).



The evaluation criterion for this test is expressed as a “z score”. This factor is calculated subtracting the proficiency test mean value minus the participant laboratory mean value and divided by the standard deviation of the study. Figure 3.13 shows an example of the graphical representation of the Z scores obtained by our laboratory for Arsenic using the digestion method and the laser method.

The results obtained for LA-ICP-MS are within the acceptance criteria ( $z \leq 3$ ) for all but one of the elements monitored in one of the aforementioned round robin tests (see Table 3.11), demonstrating that the laser ablation method provides good accuracy and agreement to generally accepted digestion methods for atomic absorption (AA) and inductively coupled plasma (ICP) analysis of trace metals in soils.

Laser ablation of solids can be accompanied by undesirable processes such as fractionation or non-stoichiometric generation of vapor species. This has long been recognized and reported elsewhere as a potential problem in LA-ICP-MS (Figgs et al., 1998; Borisov et al., 2000).

Although the study of this phenomenon is out of the scope of the current work, based on the good accuracy obtained on the soil reference standard materials and the proficiency tests, there is evidence that the effect of fractionation does not represents a major problem in the proposed methodology.

The reproducibility of the method was also studied by analyzing the samples in three non-consecutive days. The results of this study are presented in Table 3.11 which shows that the inter-day variation was lower than 10 % for most of the elements (overall 5.8%).

Table 3.7. Precision and bias of LA-ICP-MS determinations obtained in the soil quality control sample (QC) from Analytical Products Group (APG).

QC	Certified( $\mu\text{g g}^{-1}$ )	Found (n=4)	%RSD	Bias (% error)
Be	179	$167 \pm 12$	5.4	6.5
Si	nr	$435269 \pm 23795$	3.9	na
Ca	nr	$37605 \pm 2333$	4.5	na
V	105	$124 \pm 4$	2.3	18.2
Cr	113	$144 \pm 26$	13.2	27.1
Fe	14200	$19202 \pm 2572$	9.6	35.2
Ni	141	$153 \pm 14$	6.7	8.8
Cu	128	$159 \pm 21$	9.6	23.9
Zn	550	$634 \pm 22$	2.4	16.2
As	239	$244 \pm 5$	1.4	1.9
Ag	145	$138 \pm 3$	1.7	4.9
Cd	194	$218 \pm 6$	1.8	12.2
Sn	115	$120 \pm 1$	0.7	4.4
Sb	84.9	$92 \pm 4$	3.2	9.7
Tl	114	$87 \pm 14$	11.7	24.1
Pb	120	$103 \pm 5$	3.2	14.4
mean			5.1	14.8

Table 3.8. Precision and Bias for LA-ICP-MS in the first proficiency tests study.

soil PT 1	Assigned ( $\mu\text{g g}^{-1}$ )	Found (n=4)	%RSD	Bias (% error)	z score
Be	120	123 $\pm$ 5	2.7	2.3	-1.8
Si	nr	564575 $\pm$ 16300	2.1	na	-1.6
Ca	nr	6359 $\pm$ 258	2.9	na	-0.2
V	120	129 $\pm$ 3	1.5	7.5	0.6
Cr	181	185 $\pm$ 5	2.1	2.4	-1.6
Fe	15400	13215 $\pm$ 410	2.2	14.2	-2.2
Ni	190	191 $\pm$ 3	1.1	0.7	-2.1
Cu	139	161 $\pm$ 13	5.9	15.5	-0.4
Zn	656	721 $\pm$ 13	1.3	9.9	2.3
As	203	191 $\pm$ 2	0.7	5.8	-0.1
Ag	124	126 $\pm$ 6	3.2	1.6	-5.4
Cd	228	273 $\pm$ 14	3.7	20.0	-1.0
Sn	139	138 $\pm$ 5	2.4	0.4	-2.5
Sb	152	169 $\pm$ 5	2.0	11.7	1.2
Tl	147	162 $\pm$ 9	4.0	10.5	-0.7
Pb	86.2	71 $\pm$ 1	1.3	18.1	2.2
mean			2.4	8.6	-0.8

Table 3.9. Precision and bias for LA-ICP-MS in the second proficiency tests study.

soil	PT 2	Assigned ( $\mu\text{g g}^{-1}$ )	Found (n=4)	%RSD	Bias (% error)	z score
Be		133	$138 \pm 6$	6.0	3.6	0.3
Si		nr	$565075 \pm 8365$	2.0	na	na
Ca		3560	$2174 \pm 129$	8.3	39.0	na
V		152	$155 \pm 5$	4.9	1.8	-0.1
Cr		79	$89 \pm 3$	4.6	12.5	0.2
Fe		15200	$16100 \pm 772$	6.7	5.9	-2.2
Ni		227	$197 \pm 3$	4.3	13.2	0.1
Cu		171	$173 \pm 4$	3.1	1.3	-1.7
Zn		784	$800 \pm 30$	5.2	2.1	1.6
As		266	$263 \pm 5$	2.7	1.0	0.7
Ag		203	$214 \pm 5$	3.3	5.4	-0.9
Cd		127	$131 \pm 12$	12.7	3.1	1.4
Sn		190	$198 \pm 4$	3.0	4.5	0.5
Sb		253	$266 \pm 2$	1.3	5.2	-0.4
Tl		108	$109 \pm 6$	8.2	1.2	1.0
Pb		119	$135 \pm 2$	1.8	13.8	0.7
<b>mean</b>				<b>4.9</b>	<b>7.6</b>	<b>0.1</b>

Table 3.10.Comparison of Z-Score for the soil proficiency tests using solution-ICP-MS and LA-ICP-MS.

Element	SOLUTION						
	APG			ICP-MS		LA-ICP-MS	
	Mean Value	Low	High	Average	Z		Z
	* <sup>1</sup>	Accep * <sup>2</sup>	Accep * <sup>2</sup>	µg g <sup>-1</sup>	score	mean	score * <sup>3</sup>
Be	107.0	80.99	133.01	106	0.09	123	-1.8
V	113.0	82.70	143.30	109	0.41	129	-1.6
Cr	181.0	131.80	230.20	182	-0.07	185	-0.2
Fe	15100.0	5770.00	24430.00	10607	1.44	13215	0.6
Ni	166.0	118.90	213.10	161	0.29	191	-1.6
Cu	128.0	82.70	173.30	108	1.29	161	-2.2
Zn	579.0	380.10	777.90	669	-1.35	721	-2.1
As	181.0	109.00	253.00	206	-1.04	191	-0.4
Mo	138.0	102.90	173.10	139	-0.08	111	2.3
Ag	124.0	10.30	237.70	105	0.49	126	-0.1
Cd	200.0	158.90	241.10	206	-0.41	273	-5.4
Sn	121.0	68.50	173.50	210	-5.11	138	-1.0
Sb	118.0	55.30	180.70	120	-0.08	170	-2.5
Hg	19.8	6.87	32.73	21.9	-0.49	15	1.2
Tl	147.0	81.90	212.10	162	-0.69	162	-0.7
Pb	86.2	64.42	107.98	95.1	-1.23	70	2.2

Note: \*1: APG mean value: is the calculated mean of reported results in the proficiency test study, \*2: Low accep and high accep: are the low and high acceptance range reported by the proficiency test provider, they are defined as the study mean  $\pm$  three times

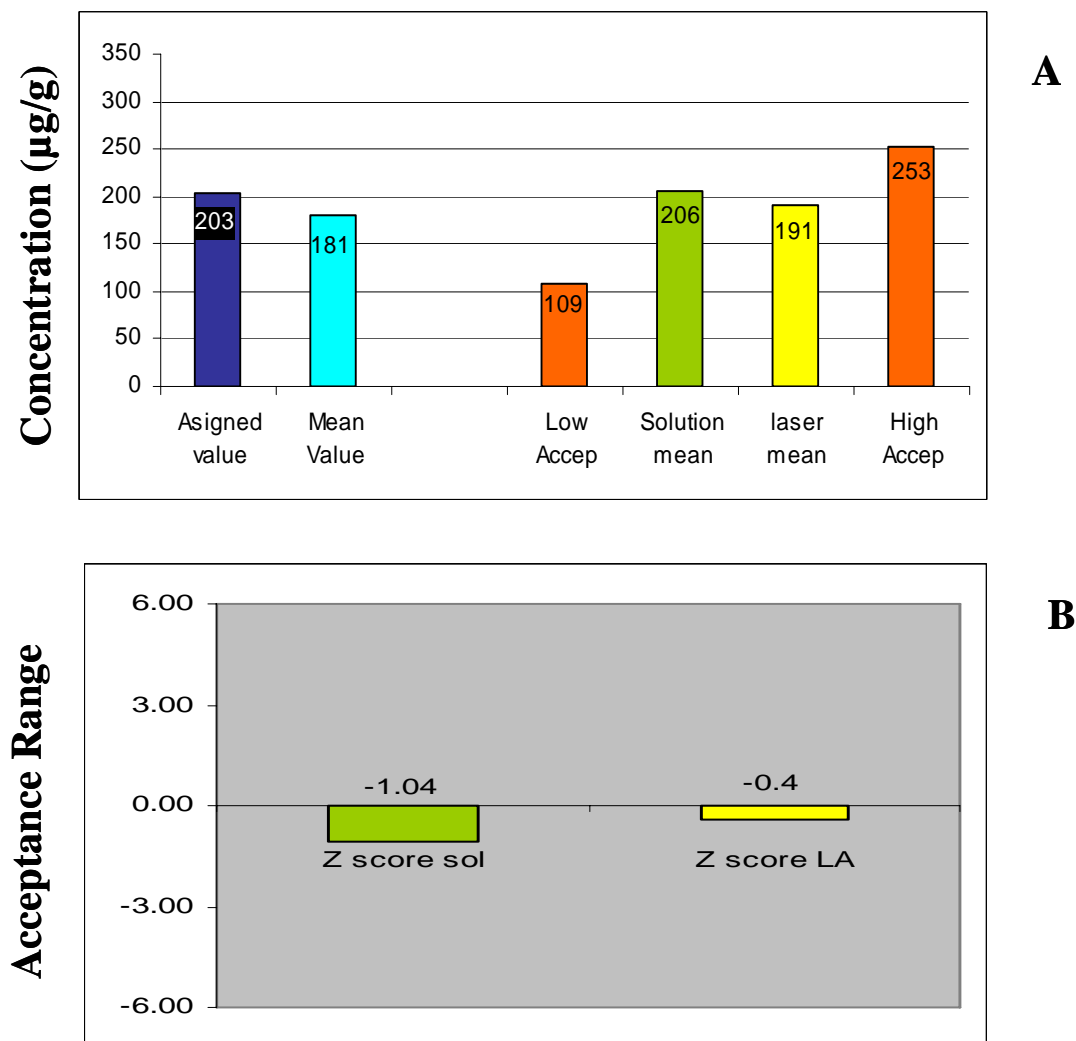


Figure 3.13.A) Comparison of quantitative results for Arsenic on the first proficiency tests by acid digestion-ICP-MS (green) and LA-ICP-MS (yellow); B) Z scores values comparison for Arsenic in the same inter-laboratory study

### 3.16. Limits of Detection

The LA-ICP-MS detection limit (MDL) was estimated by the GLITTER software at the 99 % confidence level, determined by 3.25 times the signal to noise ratio. The MDL reported is a mean value for 7 replicates of the MDL obtained after running the soil standards, samples QC and PT, as well as sand blank spiked at low levels.

Limits of detection in sediments after acid digestion (EPA 3050B) were estimated on the treated solutions of sediments spiked at low levels of concentration following recommendations from the literature (WDNR 1996).

The results obtained are shown in Table 3.12. The use of UV-ns-laser ablation ICP-MS improved the limit of detection (LOD) of metals in about 1 to 2 order of magnitude versus previous reported work conducted by IR-ns-LA-ICP-OES (Musil et al., 2000) and LA-MS (Beccaglia et al., 2006).

The detection limits obtained with LA were similar or lower than obtained after partial acid digestion –ICP-MS. This demonstrates that LA-ICP-MS can be used to monitor these elements at low trace levels. It is important to highlight that although the reported MDL in  $\text{mg kg}^{-1}$  are similar for both methods (acid digestion and LA) the amount removed by the laser is about 40000 times less ( $\sim 0.25$  g for digestion vs.  $6 \mu\text{g}$  for LA).

Moreover, the limits of detection (LODs) obtained with the proposed UV-ns-LA-ICP-MS method were similar or slightly better than the ones recently reported for tin, zinc, copper and lead in soil using a fs-LA-ID-ICP-MS method (Fernandez et al., 2008), which demonstrates that reliable soil analysis can be conducted with fairly cheap ns-266nm lasers compared to fs-IR laser technology.

Table 3.11.Reproducibility of the LA-ICP-MS determinations conducted in three different days by different analysts. All mean values are reported in  $\mu\text{g g}^{-1}$ .

	Day 1	Day 2	Day 3			
				Overall	Overall	Overall
Element	mean	mean	mean	Average	SD	%RSD
Be	2.1	2.0	2.0	2.0	0.1	3.8
S	275744	271315	278879	275313	3800	1.4
Ca	10280	10075	10400	10251	165	1.6
V	62.6	61.7	62.2	62.2	0.5	0.8
Cr	30.0	28.7	27.4	28.7	1.3	4.6
Fe	30175	29101	30679	29985	806	2.7
Ni	16.8	16.5	16.6	16.6	0.2	1.0
Cu	2413	2645	2377	2478	146	5.9
Zn	5025	5791	5213	5343	399	7.5
As	447	482	489	473	23	4.8
Ag	24.4	24.6	28.7	25.9	2.4	9.2
Cd	16.3	13.5	17.0	15.6	1.9	12.1
Sn	6.1	5.6	6.7	6.1	0.5	8.7
Sb	31.7	33.2	36.9	33.9	2.7	8.0
Pb	5834	6922	5140	5965	898	15.1



Table 3.12.Comparison of sediments and soils method limit of detection obtained from partial digestion ICP-MS and LA-ICP-MS methods.

<b>Element</b>	<b>Acid digestion ICP-MS</b>	<b>Laser ablation -ICP-MS</b>
	<b>MDL (mg kg<sup>-1</sup>)</b>	<b>MDL (mg kg<sup>-1</sup>)</b>
Be	0.04	0.03
V	0.38	0.10
Cr	1.12	0.15
Co	0.04	0.01
Ni	0.36	0.02
Cu	0.33	0.05
Zn	3.10	0.81
As	0.15	0.24
Mo	0.26	0.07
Ag	0.08	0.01
Cd	0.03	0.07
Sn	0.26	0.04
Sb	0.03	0.04
Pb	0.57	0.01

### 3.17. Application of the LA-ICP-MS Method to Real Soil and Sediment Samples

The previous section demonstrated good analytical performance of the proposed method for a series of soils and sediment standards, as well as two independent proficiency tests for trace metals in soils. Results obtained by LA-ICP-MS were comparable to partial digestion ICP-MS methods in terms of accuracy, precision and limits of detection, with the adding advantage of reducing time and complexity of sample preparation (Arroyo et al., 2009).

The second part of this study was designed to evaluate the applicability of this method on real soil and sediment samples with diverse physical and chemical composition. Two data sets were used for this purpose; the first one consists of soils from contaminated sites. The LA-ICP-MS composition of this dataset was compared to total-digestion ICP methods. The second set consists on sediments collected from pristine areas. Different statistical tools were employed to determine if there is correlation between the elemental composition obtained by LA-ICP-MS and by non-total digestion methods (partial digestion).

#### 3.17.1. Analysis of contaminated residential soils:

In order to evaluate the use of LA-ICP-MS as an alternative tool for rapid monitoring of inorganic contaminants on soil, a set of ten residential soil samples were received from the Environmental Protection Agency, National Enforcement Investigation Center (EPA-NEIC), and run as a blind test. These soils samples consisted of litter soil collected as part of 80 soil cores from a residential area adjacent to an automobile battery manufacturing facility. Samples were previously analyzed for lead, antimony and tin by

digestion-ICP methods as part of a larger study to determine soil lead variability in order to make remediation decisions.

Because of the legal implications involved in the study, these three analytes required accurate quantification. In order to achieve good analytical performance for all 3 elements, multiple cumbersome digestion schemes were required (Hosick et al., 2002; Machemer and Hosick 2004; Machemer et al., 2007). For this reason, the LA-ICP-MS protocol was used on this study to determine whether or not comparable analytical data can be obtained for lead, tin and antimony in a single and rapid method.

Soil samples sent to our laboratory were pelletized and quantified using PACS-2 as single point calibrator. Table 3.13 shows the results of LA-ICP-MS analysis in comparison to digestion, followed by ICP-OES and ICP-MS detection. Good precision was obtained by LA-ICP-MS for all samples ( $< 10\%$  RSD,  $n=4$ ), except for the sample identified as “101” which higher RSD’s are likely related to the low concentration in the soil at levels close to the method detection limit. The precision obtained between replicates samples by LA-ICP-MS was comparable to precision obtained for QC triplicates on the digestion methods (7-15% RSD).

Since LA-ICP-MS is a new method for the determination of trace elements in soils, this method needs to be validated amongst other techniques such as total-digestion ICP-methods. The aim of this comparison was the identification of systematic errors, if any. In cases where an analysis is repeated several times over a limited range of concentrations, such comparison can be done by paired-t test. However, when two methods are to be compared at different analyte concentration the regression method is preferred (Miller and Miller 2000).

In regression methods, one axis of the regression graph is used for the results obtained by the new method and the other one for the comparison or reference method. This test is used to calculate the slope, intercept and correlation coefficient ( $r^2$ ) of the regression line. In the ideal case that both analytical methods give identical results for each of the samples, the intercept will be zero and the slope and  $r^2$  will be equal to one. In practice, however this does not occurs because even random errors will produce differences in the data points. Therefore, the regression method measures how much the intercept and slope differs from the idea values of zero and one, respectively. Such test is conducted by calculating the confidence limits for intercept and slope at 95% confidence level.

Comparable results for all these methods are shown on Table 3.13. Lead concentration on the soil samples ranges from approximately  $5 \mu\text{g g}^{-1}$  to approximately  $3000 \mu\text{g g}^{-1}$ , while tin and antimony range from below detection limit ( $< 0.4 \mu\text{g g}^{-1}$ ) to approximately  $25 \mu\text{g g}^{-1}$ . Due to this large concentration ranges, regression statistics were used to evaluate if any systematic errors are present in the LA-ICP-MS data when compared to total digestion ICP methods.

Figure 3.14 shows the correlation of these complementary techniques for all three elements. Each point of the graph represents a single sample analyzed by two separate methods (digestion ICP-MS and LA-ICP-MS). In the case of lead, the mean concentration of the alkaline digestion ICP-OES and the acid digestion ICP-MS was plotted against the LA-ICP-MS method.

Statistical regression analysis showed good correlation and no significant systematic errors at 95% significance level. Both ICP and LA-ICP-MS methods produced

similar precision ( $<10\%RSD$ ), with few exceptions where the LA method showed better precision than digestion methods as observed on Figure 3.14. This clearly demonstrates that the laser ablation method provide similar results to total digestion methods and can be applied to the quantitative determination of these three elements in soil samples.

From the three elements of interest, tin showed a lower correlation value (see Figure 3.14) which is mainly caused by the poor agreement of concentration values from one of the samples (sample #102). Nevertheless, the overall results obtained for tin by the two methods were not significantly different at 95% confidence level. Moreover, the accuracy and precision of the reference material NIST 2710, used as quality control, was good even for elements at low concentration such as tin (below 15% and 7% respectively).

These values are considered acceptable for environmental monitoring purposes and reveal the potential use of the laser method in complex matrices. Although this reference standard does not have a certified value for tin, the accuracy was evaluated against a reported value (Hosick et al., 2002).

So far, it has been demonstrated that the recoveries and precision for the analysis of lead, tin and antimony in soils widely vary within the digestion method and that soils do require the development of specific techniques, such the ones applied to these samples. However, one of the main advantages offered by the laser method consist in the capability of measuring all these trace metals by using a single protocol, which simplifies the sample preparation and analysis. The precision obtained corroborates the efficiency of the laser ablation method to deal with complex soil samples with varying element concentration.

Table 3.13. Lead, antimony and tin determinations by LA-ICP-MS compared to ICP-OES and ICP-MS in soil samples. All concentrations are reported in  $\mu\text{g g}^{-1}$ .

Sample ID	Pb <sup>1</sup> (LA-ICP-MS)	Pb <sup>2</sup> (HCl/ICP-MS)	Pb <sup>3</sup> (KOH / ICP-OES)	Sb <sup>1</sup> (LA-ICP-MS)	Sb <sup>4</sup> (HCl/ICP-MS)	Sn <sup>1</sup> (LA-ICP-MS)	Sn <sup>5</sup> (KOH/ICP-MS hydride gen)
101	5 ± 2	< 3	< 20	< 0.4	< 0.06	< 0.4	1
102	350 ± 34	320	400	2.5 ± 0.4	1.8	9.7 ± 2.3	17
104	96 ± 4	74	120	0.5 ± 0.06	0.6	2.6 ± 0.3	4
105	697 ± 40	686	668	5.6 ± 0.7	5.22	12.4 ± 1.7	12
106	795 ± 26	568	530	< 0.4	0.39	6.4 ± 0.4	8
107	138 ± 1	114	140	1.4 ± 0.03	1.0	3.1 ± 0.7	3
108	39 ± 1	30	50	< 0.4	0.34	1.8 ± 0.4	3
109	545 ± 22	471	481	4.3 ± 0.1	3.75	7.0 ± 0.7	9
110	1107 ± 47	973	874	6.9 ± 0.7	5.62	6.6 ± 0.5	7
103	2636 ± 151	2810	2910	24.7 ± 0.8	22.9	16.0 ± 0.3	18

1: this study: confidence interval estimated at 95%, n=4;

2: Batch QC triplicates of HCl digestions were below 15% RSD for Pb.;

3: Batch QC triplicates of KOH fusions were below 7% RSD for Pb.;

4: Batch QC triplicates of HCl digestions were below 15% RSD for Sb.;

5: Batch QC triplicates of KOH fusions were below 10% RSD for Sn.

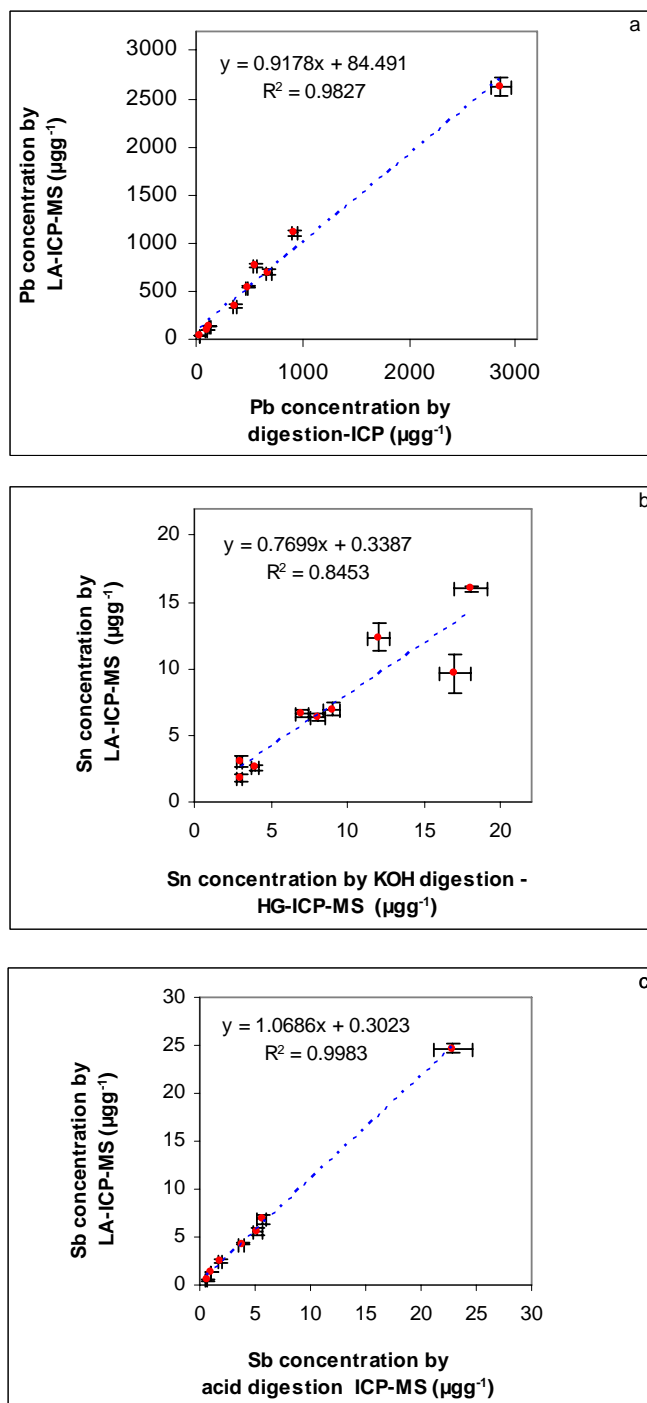


Figure 3.14. Regression line for the comparison of the analytical results obtained for a) lead, b) tin and c) antimony by LA-ICP-MS (y-axis) and ICP methods (x-axis). Error bars are reported as standard deviations for each method.

### 3.17.2. Analysis of South Florida Sediments:

As mentioned earlier, LA-ICP-MS provides good accuracy against certified values of reference soil and sediment standards or when compared to soil samples analyzed by total digestion methods. Nevertheless, the comparison of concentration values between a pseudo-total acid digestion method and LA-ICP-MS may not be straightforward and therefore different statistical models were used in this study to evaluate if there is correlation between these methods.

An extended elemental menu for LA-ICP-MS was employed for this set of sediments with two main purposes: a) to evaluate the use of laser ablation on the determination of the background/baseline concentration levels of a full suite of elements in non impacted areas and b) to determine if the variability of the elemental profile of major, minor and trace elements in different sampling areas by the laser ablation approach is comparable to that of partial digestion (bio-available fraction).

Under this scope, forty-eight sampling stations along protected areas in South Florida were included for the present study. Each station was analyzed using two different analytical approaches: laser ablation ICP-MS (direct method) and pseudo total digestion (leacheate) followed by ICP-MS.

In 1999, Chen's research group published a comprehensive study to determine 8 trace elements in Florida surface soils comparing total-total metal concentration (total digestion) and total-recoverable (leacheate) metal concentration by two different digestion protocols (Chen et al., 1999). One of the main contributions of this report relies on the creation of a database that accounts for the background concentration of metals in common Florida soils. However, some important areas of South Florida where not



included and the results presented here will attempt to fill this gap of information. Due to the affinity of Chen's study and our data set, this paper will be used as a reference within this discussion. In specific, the resemblance to our dataset relies on: a) comparison of a total-total (in our case laser ablation, in their case total-total acid digestion) to total recoverable data (leachate digestion methods) and b) analysis of soils from South Florida region.

Each one of the analytical schemes that are presented in our study provides information about the elemental characterization of the sampled areas. Nevertheless, a direct comparison of these values cannot be done as in the previous battery facility case. The reason for that relies on the fact that laser ablation provides analytical information as an exhaustive sampling method and therefore can be directly compared to either total-total digestion methods and/or certified values of standards. On the other hand, leachate methods are non exhaustive and recoveries of elements depends on many variables including physical and chemical characteristics of matrices, preventing a direct association to total-total values.

Regardless of this limitation, one of our goals was to use statistical tools to find if there is a correlation between the leachate content and the actual elemental content on the sediments. This is of importance because leachate methods are widely used in environmental science. If this correlation can be predicted, both data sets can be further employed to assess bioavailability, background and enrichment levels in protected areas.

Due to the nature of the large number of multielemental data generated on these 48 samples, the following approaches were employed for the statistical analysis:

- a) Tests for normality of the data,

- b) Estimation of baseline levels,
- c) Correlation analysis between leachate and laser ablation methods,
- d) Estimation of sediment quality assessment (TEL and PEL),
- e) Normalization of the data, and
- f) Data reduction by multivariate analysis.

#### 3.17.2.1. Normality Test of the Data

The correct interpretation of geochemical information is based on the right selection of statistical tools that could lead to meaningful comparison of a dataset. Generally the geochemical data follows a log normal distribution because of the presence of either high or low values for element concentration within a population. The Saphiro-Wilks test was conducted to test the normality of concentration distribution within each element on each set of data, and it was demonstrated that the element concentration in both sets followed a log-normal distribution. Therefore, the data was log transformed before further analysis to meet the assumption of normality.

Tables 3.14 to 3.17 show basic statistical information, non parametric information and concentrations of the different elements within the study area in South Florida obtained by the laser ablation approach and the leacheate method, respectively. As could be seen from these tables the concentration of trace elements varied greatly within all different sampling areas due to many variables including, physical and chemical properties, mineralogy, vegetation, climate, environmental deposition, among others. The use of statistical tools is of utmost importance to understand how this and other physical-chemical mechanism are related and could be affecting the recovery of trace

metals in the sets of sediments under assessment. In statistical analysis is very important to characterize the location and variability of a dataset. This information is given by the mean and standard deviations presented as arithmetic mean and arithmetic standard deviation, but in order to have a further characterization of the data two other moment coefficients -skewness and kurtosis- are included, and they determine how the shapes of sample frequency distribution curves differ from ideal Gaussian (normal) curves (Miller and Miller 2000). Skewness is a measure of symmetry or more accurately lack of symmetry. It describes the asymmetry of the upper and lower halves of the curve around the mean. The skewness for a normal distribution is zero and any symmetric data should have a skewness value near zero. Negative values for skewness indicate data that are skewed left and positive values for skewness data that are skewed right. Kurtosis is a measure of whether the data is peaked or flat relative to a normal distribution (Miller and Miller 2000). Data sets with high kurtosis tend to have a distinct peak around the mean, decline rather rapidly and have heavy tails. Data sets with low kurtosis tend to have a flat top near the mean rather than a sharp peak.

It is clearly observed on Table 3.16 and 3.17 that the majority of the distributions of the elemental concentrations were strongly positively-skewed and heavily tailed. As an example, using the laser approach, the log transformation reduced significantly the original values for skewness and kurtosis for zinc from 4.84 and 26.80 to 0.33 and 0.38 respectively. Tables 3.16 and 3.17 show the data for the leachate method. As an example, copper showed an original value for skewness and kurtosis of 6.58 and 44.66, once log transformed the values were reduced to 0.79 and 1.71. Both results are consistent with those reported by Chen's research group for 8 trace metals in soils of

Table 3.14. Concentration of trace elements in South Florida Sediments under CARE project (mgkg-1) using Laser Ablation (Part A)

Test	Cr	Ni	Cu	Zn	As	Cd	Pb	Mn	Mo	Ag	Sn
<b>Minumum</b>	6.72	2.07	0.71	1.23	0.55	0.07	1.00	8.35	0.17	0.01	0.32
<b>Median</b>	21.65	9.28	4.46	10.18	4.69	0.07	2.14	80.21	0.84	0.04	0.89
<b>Maximum</b>	129.24	40.10	221.0	198.1	26.81	0.46	2048	210.5	10.26	0.55	3.17
<b>original data</b>											
<b>AM</b>	33.20	11.29	12.18	17.60	5.28	0.09	53.93	85.82	1.82	0.06	1.04
<b>ASD</b>	30.00	7.60	31.98	30.60	4.14	0.06	295.60	55.58	2.22	0.08	0.59
<b>Skewness</b>	1.82	1.73	6.19	4.84	3.14	4.18	6.81	0.47	2.15	4.71	2.11
<b>Kurtosis</b>	2.89	4.25	40.75	26.80	14.89	21.04	46.86	-0.77	4.79	26.77	5.13
<b>log 10 transformed data</b>											
<b>GM</b>	24.00	9.33	5.01	9.39	4.17	0.01	6.05	64.56	1.01	0.04	0.93
<b>GSD</b>	2.19	2.00	3.22	2.89	1.98	1.49	3.71	2.35	2.91	2.44	1.58
<b>Skewness LOG</b>	0.41	-0.48	0.66	0.33	-0.19	2.30	2.20	-0.807	0.42	0.60	0.51
<b>Kurtosis LOG</b>	-0.61	0.35	0.98	0.38	0.96	5.78	7.92	-0.16	-0.80	0.38	0.77
<b>Baseline Low (LBL)</b>	5.00	2.33	0.48	1.12	1.06	0.00	0.44	11.69	0.12	0.01	0.37
<b>Baseline High (UBL)</b>	115.11	37.32	51.95	78.43	16.34	0.02	83.27	356.53	8.55	0.22	2.32

Table 3.15. Concentration of trace elements in South Florida Sediments under CARE project (mgkg-1), using Laser Ablation (Part B)

Test	Sb	Hg	U	Be	V	Sr
<b>Minumum</b>	0.04	0.13	0.30	0.03	2.13	13.80
<b>Median</b>	0.20	0.56	2.05	0.14	12.85	1854
<b>Maximum</b>	18.15	15.80	11.10	2.13	61.09	8742
<b>original data</b>						
<b>AM</b>	0.64	1.52	2.74	0.34	16.70	2249
<b>ASD</b>	2.60	2.75	2.43	0.47	12.70	1950
<b>Skewness</b>	6.76	3.60	1.61	2.32	1.52	0.98
<b>Kurtosis</b>	46.42	15.60	2.68	5.56	2.31	1.04
<b>log 10 transformed data</b>						
<b>GM</b>	0.20	0.57	1.86	0.15	13.06	1047.10
<b>GSD</b>	2.88	3.92	2.57	3.89	2.12	5.24
<b>Skewness LOG</b>	1.62	0.52	-0.30	0.14	-0.29	-1.25
<b>Kurtosis LOG</b>	6.06	-0.627	-0.49	-1.26	0.12	0.62
<b>Baseline Low (LBL)</b>	0.02	0.04	0.28	0.01	2.91	38.14
<b>Baseline High (UBL)</b>	1.66	8.76	12.29	2.28	58.70	28751

Table 3.16. Concentration of trace elements in South Florida Sediments under CARE project (mgkg-1), using the Leacheate method (Part A)

Test	Cr	Ni	Cu	Zn	As	Cd	Pb	Mn	Mo	Ag	Sn
<b>Minumum</b>	0.80	0.36	0.33	3.10	0.15	0.03	0.63	6.40	0.26	0.04	0.26
<b>Median</b>	7.66	2.47	2.80	8.08	2.02	0.06	6.33	34.54	0.48	0.08	0.26
<b>Maximum</b>	58.10	8.47	194.0	204.8	26.40	0.48	377	207.0	9.87	0.40	1.15
<b>original data</b>											
<b>AM</b>	11.58	2.76	8.72	19.53	2.89	0.08	17.30	42.55	1.42	0.09	0.42
<b>ASD</b>	11.26	1.73	27.70	36.30	3.85	0.08	56.00	37.39	2.00	0.05	0.26
<b>Skewness</b>	2.15	1.15	6.58	4.10	5.16	2.79	6.03	2.24	2.46	5.30	1.43
<b>Kurtosis</b>	4.94	1.55	44.66	17.90	30.40	9.39	38.30	7.21	6.54	31.40	0.74
<b>log10 transformed data</b>											
<b>GM</b>	7.76	2.24	3.16	9.55	2.10	0.06	6.02	30.20	0.71	0.09	0.37
<b>GSD</b>	2.75	2.00	3.23	2.82	1.74	2.10	3.10	2.40	3.02	1.35	1.66
<b>Skewness LOG</b>	-0.25	-0.6	0.79	0.96	0.44	0.72	0.90	-0.3	0.79	3.30	-0.43
<b>Kurtosis LOG</b>	-0.04	0.28	1.71	0.70	0.78	0.16	0.35	-0.49	-0.62	16.00	0.26
<b>Baseline Low (LBL)</b>	1.03	0.56	0.30	1.20	0.69	0.01	0.63	5.24	0.08	0.05	0.13
<b>Baseline High (UBL)</b>	58.69	8.96	32.97	75.95	6.36	0.28	57.85	173.95	6.48	0.15	1.02

Table 3.17. Concentration of trace elements in South Florida Sediments under CARE project (mgkg-1), using the Leacheate method (Part B)

Test	Sb	Hg	U	Be	V	Sr
<b>Minumum</b>	0.03	n/d	n/d	0.04	0.38	n/d
<b>Median</b>	0.06	n/d	n/d	0.09	6.88	n/d
<b>Maximum</b>	0.74	n/d	n/d	0.72	26.09	n/d
<b>original data</b>						
<b>AM</b>	0.12	n/d	n/d	0.18	7.81	n/d
<b>ASD</b>	0.16	n/d	n/d	0.19	5.42	n/d
<b>Skewness</b>	2.81	n/d	n/d	0.47	1.33	n/d
<b>Kurtosis</b>	7.62	n/d	n/d	-0.98	2.16	n/d
<b>log 10 transformed data</b>						
<b>GM</b>	0.07	n/d	n/d	0.12	6.61	n/d
<b>GSD</b>	2.51	n/d	n/d	2.57	2.00	n/d
<b>Skewness LOG</b>	1.01	n/d	n/d	0.47	-0.46	n/d
<b>Kurtosis LOG</b>	0.39	n/d	n/d	-0.98	0.24	n/d
<b>Baseline Low (LBL)</b>	0.01	n/d	n/d	0.02	1.65	n/d
<b>Baseline High (UBL)</b>	0.45	n/d	n/d	0.77	26.44	n/d

Florida including the mentioned elements (Chen et al., 1999). Based on the non-normality behavior, log-transformed data and non-parametric methods were used herein.

### 3.17.3. Baseline concentration of elements in South Florida Sediments

The geochemical baseline concentrations are usually expressed as an expected range of elemental concentrations around a mean in a normal sample medium, and are defined as 95 % of the expected range of background concentrations (Adriano 2001). The baseline value has been recognized as the only means to established reliable worldwide elemental concentration in natural materials, and some researchers recommends its use for interpreting contamination in soils (Bech et al., 2005; Kabata Pendias and Pendias 1992; Dudka et al., 1995).

Based on the normal distribution theory, the expected range can be expressed as the arithmetic mean  $\pm$  2 arithmetic standard deviations ( $AM \pm 2 ASD$ ). The arithmetic means are best used as estimates of geochemical abundance (Gough et al., 1994; Gough et al., 1998). As could be seen from Tables 3.14-3.17, the values of AM on the set of samples presented higher values for the laser ablation for the majority of the elements, which is in agreement with previous studies conducted in Florida soils, where higher recoveries were obtained by the total-total than by total-recoverable digestion methods. It is recognized that these leacheate (total-recoverable) digestion procedures do not recover all metals in soils samples (Chen et al., 1998).

Although, AM and ASD are useful to describe the central tendency and variation of the data, geometric means (GM) are better “maximum likelihood estimators” for most geological data because of the tendency for concentrations of elements to have positively

skewed frequency distributions as it was shown before for our dataset. Therefore the analytical data is transformed to logarithms, and the geometric mean (antilogarithm of the mean of logarithmic values) is reported as the best estimator of central tendency (Gough et al. 1998). All major, minor, and trace metal concentrations are based on dry matter basis.

Geometric mean and geometric standard deviation ( $GM \pm GSD$ ) were used in our dataset to calculate the upper baseline limit (UBL) and the lower baseline limit (LBL). The UBL is calculated as  $GM \times GSD^2$  while the LBL is calculated as  $GM/GSD^2$ , which includes the expected range of 95 % of the population. The range of concentrations between UBL and LBL are considered the baseline values and they are shown on Table 3.14 and Table 3.15. The laser ablation data presented on these tables, show that the observed maximum concentration ranges were greater than the upper baseline concentration limits -with the exception of manganese, beryllium, uranium and strontium - which may indicate either contamination or influence from soil formation factors as was also found for a similar elemental menu in Florida soils in the Chen study (Chen et al., 1999). On the other hand, the leachate data presented in Table 3.16 and Table 3.17 indicates that the observed concentration ranges were greater than the upper baseline concentration limits -with the exception of chromium, nickel, beryllium and vanadium. Therefore, a careful interpretation should be addressed when assessing the sediment samples depending on the method of digestion employed.

It is also important to stress that independently of the analytical approach used, the geometric mean values were much closer to the median values than the arithmetic mean for all the elements evaluated which corroborates that the data were strongly

positively skewed as discussed before. Additionally the baseline concentration of trace elements provides a better representation of the natural concentration because of the distorting effects of a few high concentrations is minimized by the use of logarithmic values.

#### 3.17.4. Correlation Analysis for trace elements in South Florida Sediments

Due to the non-normal distribution of the data, a non parametric correlation test, such as Spearman Rank Correlation, was used in order to determine if there is correlation of the 48 sampling stations between laser ablation and the leacheate approach. Table 3.18 shows a summary of the values along with regression statistics figures for this study and data from Chen et al. reported as comparative data (Chen et al., 1999). From the 16 elements of interest, 3 were present above LOD for LA-ICP-MS only. From the 13 remaining elements, four were present at concentration below LOD in the majority of the sampling stations, consequently only 9 elements were used for the correlation analysis.

Eight of the nine studied elements correlated with the total-recoverable concentration in the sediments at 95 % confidence level ( $\rho > 0.447$ ).

For the majority of elements, a positive correlation between both analytical approaches is clearly observed, their Spearman Ranked coefficients (Rho) ranged from 0.61 up to 0.90. Nevertheless, for nickel the situation was different, it neither shows a correlation *per se* because its Spearman Rho value was below the critical value of 0.447 (Miller and Miller 2000), nor good correlation coefficient. This could be attributable to the presence of insoluble minerals that affect the recovery in the leacheate method.



Similar correlations were obtained by Chen (Chen et al., 1999) for 5 out of 8 elements when comparing the total-total concentration and the total-recoverable concentration data.

Table 3.18. Correlation coefficients and regression statistics between total recoverable and laser ablation for trace elements in South Florida sediments

Element	Laser Ablation vs Leacheate		Regression *		Spearman Ranked Coefficients (rho) *** (Chen et al)
	Spearman Ranked Coefficients (rho)	Slope m	Intercept b	r <sup>2</sup>	
Cu	0.90	0.93 ± 0.06	-0.15 ± 0.05	0.842	0.97
Pb	0.89	0.75 ± 0.04	0.28 ± 0.04	0.890	0.56
Mn	0.87	0.90 ± 0.05	-0.11 ± 0.10	0.865	N/A
Zn	0.83	0.97 ± 0.07	0.002 ± 0.008	0.798	N/A
Mo	0.83	1.00 ± 0.01	-0.16 ± 0.02	0.837	N/A
Cr	0.82	1.03 ± 0.07	-0.53 ± 0.11	0.803	0.85
As	0.78	0.65 ± 0.01	-0.11 ± 0.005	0.624	0.99
V	0.61	0.67 ± 0.08	0.08 ± 0.01	0.617	N/A
Ni	0.25	0.56 ± 0.13	-0.196 ± 0.13	0.293	0.14
Cd	0.60	**	**	**	0.33
Sn	0.21	**	**	**	N/A
Ag	0.08	**	**	**	N/A
Sb	0.05	**	**	**	N/A

\* [Leacheate concentration] = m x[Laser Ablation concentration] + b

\*\* Below LOD

\*\*\* Data from Chen et al. 1999, University of South Florida, Report # 99-7, Table 1-18

In the case of nickel, Chen reported a Spearman Rho value of 0.14. Our data is not only consistent with the behavior for nickel as reported by Chen but also supports the hypothesis that the laser ablation could be considered a total-total digestion for many elements in the list. The reason for that relies on the fact that laser ablation is an exhaustive sampling technique, removing micro amounts of soil that are representative of the bulk composition and where elements are removed efficiently regardless of mineral composition and without considerable loss of volatile compounds.

#### 2.17.5. Correlation between Physical and Chemical Properties of the Sediments and the Recovery of Trace Elements.

Both physical and chemical properties of sediments have to be considered in order to account for the variability in the elemental concentration of the trace metals within the studied area. Soil and sediment properties like organic content, silt, salinity, total aluminum and total iron are among the most significant environmental variables influencing adsorption, desorption and ion exchange in soils and sediments. All of these parameters could affect the extraction efficiency in non-exhaustive procedures.

Figures 3.15 and 3.16 show the variability in properties such as salinity and TOC for samples collected in this study. For example, TOC values in samples collected from the Big Cypress area (BICY) ranged from 0.8 to 38.3%, while salinity in Taylor Slough (TS) stations ranged from 0.1 to 29.4 ppt. These significant differences in physical and chemical properties even for sampling stations that are in close vicinity adds complexity to the interpretation of results originated from non-total digestion methods.

Spearman Rho correlation coefficient comparing total-recoverable (leacheate) concentration and physical sediment characteristics was calculated in order to gain further sight into the chemistry of the sediments and its possible effects in recovery efficiency. The values are summarized on Table 3.19. Bold numbers represent the most influent factor that could affect the recovery of the metals. As could be seen from the table, total Aluminum concentration is one of the most important properties correlating total-recoverable concentrations for refractory elements like chromium and vanadium. Interesting to note is that the total organic content affects important elements like

Table 3.19.Spearman Rho Ranked Correlation coefficients between Leacheate concentrations of trace metals and sediment properties in South Florida Sediments

<b>Metals</b>	<b>Total Al</b>	<b>Total Fe</b>	<b>TOC</b>	<b>Salinity</b>	<b>% Silt</b>
<b>Be</b>	<b>0.81</b>	<b>0.75</b>	0.49	-0.50	-0.03
<b>V</b>	<b>0.65</b>	<b>0.44</b>	0.37	-0.21	-0.04
<b>Cr</b>	<b>0.81</b>	<b>0.54</b>	0.38	-0.10	-0.08
<b>Ni</b>	<b>0.72</b>	<b>0.62</b>	0.54	-0.18	0.01
<b>Zn</b>	<b>0.21</b>	0.10	<b>0.21</b>	0.11	-0.02
<b>Cd</b>	<b>0.32</b>	0.20	<b>0.39</b>	0.07	0.05
<b>Pb</b>	0.47	<b>0.48</b>	<b>0.57</b>	-0.19	-0.04
<b>Sb</b>	-0.35	-0.31	-0.19	<b>0.34</b>	-0.11
<b>Cu</b>	0.15	-0.01	<b>0.19</b>	<b>0.33</b>	-0.06
<b>Mo</b>	-0.24	-0.30	0.04	<b>0.70</b>	-0.01
<b>Ag</b>	0.01	0.00	-0.13	<b>0.34</b>	<b>0.20</b>
<b>Mn</b>	0.06	<b>0.61</b>	-0.15	-0.12	<b>0.62</b>
<b>Sn</b>	-0.01	0.30	0.22	0.02	<b>0.36</b>
<b>As</b>	0.11	<b>0.25</b>	0.22	0.16	<b>0.28</b>

cadmium, lead and zinc, the latter equally affected by the total aluminum concentration. The silt content is positively correlated with concentration of arsenic as well as total iron and organic matter. These results are in agreement to previous studies conducted by Chen et al. (Chen et al., 2001).

#### 3.17.6. Sediment Quality Assessment: TEL and PEL criteria

According to Macdonald (McDonald 1994), there are essential considerations that should be addressed in conducting site-specific sediment quality assessment programs (SQAGs):

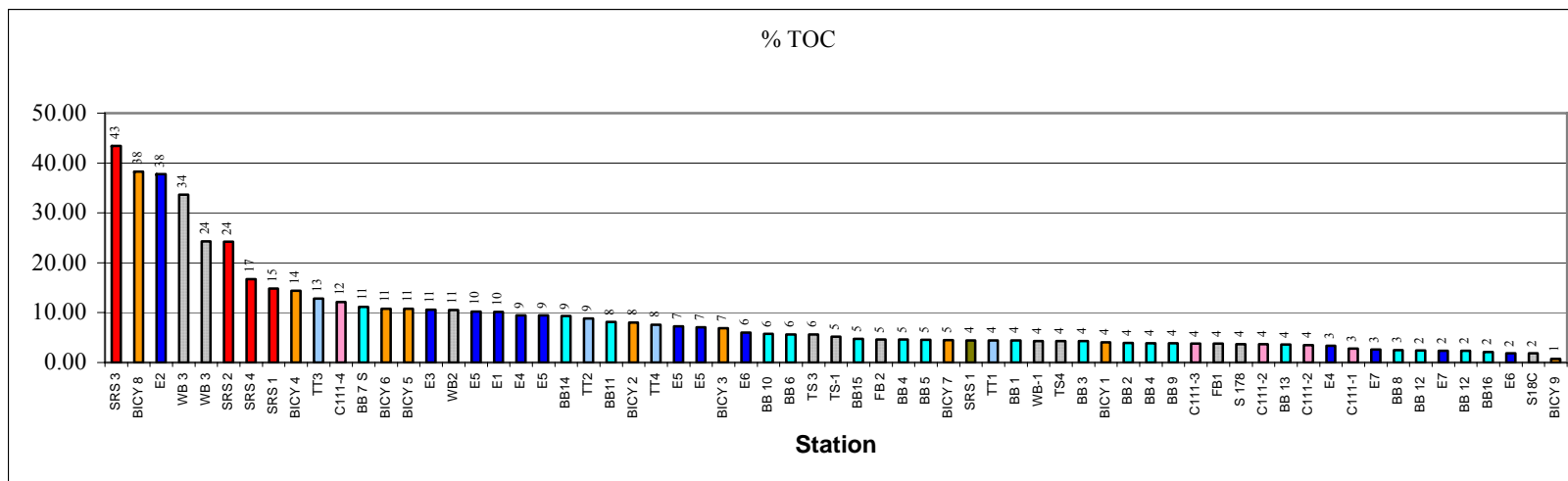


Figure 3.15. Ranked TOC values for the 48 sediment samples collected. Similar color bars represent s stations from same geographical area.

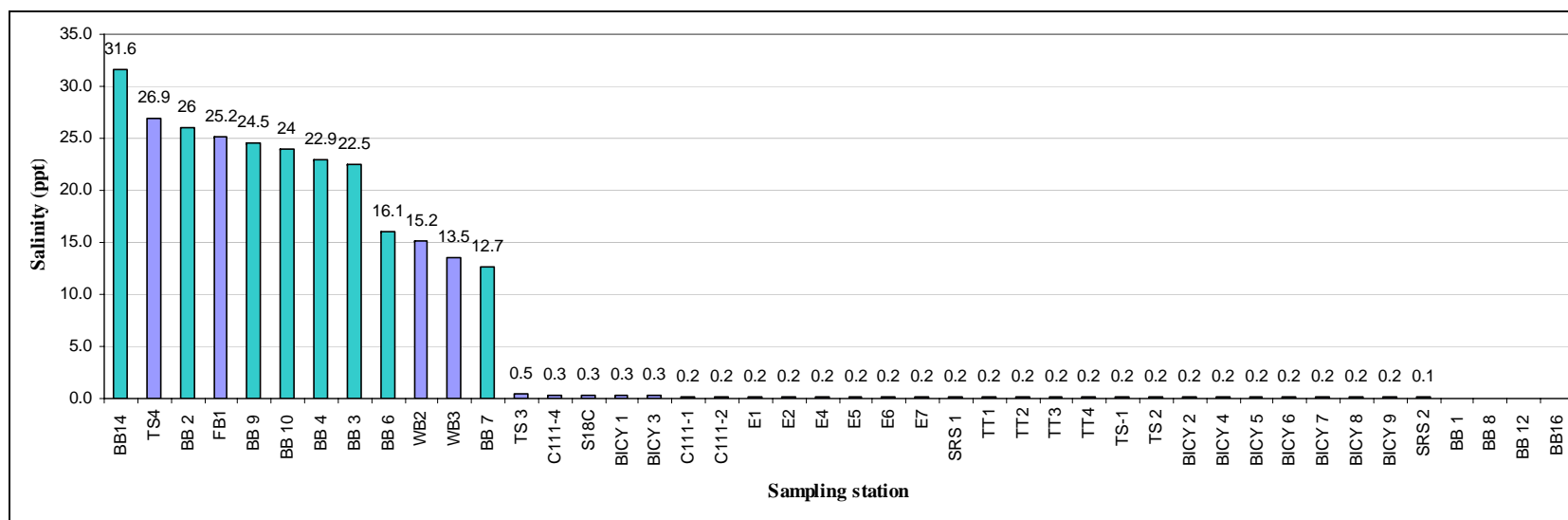


Figure 3.16. Ranked salinity values for 44 sediment samples. Salinity data is not available for the last four stations represented in the graph.

- Collect Historical Land and Water Use Information;
- Collect and Evaluate Existing Sediment Chemistry Data;
- Collect Supplemental Sediment Chemistry Data;
- Evaluate the Origin of Sediment-Associated Contaminants;
- Conduct Preliminary Assessment of the Potential for Biological Effects of Sediment-Associated Contaminants;
- Conduct Biological Assessment of Sediment Quality; and,
- Implement Management of Sediment Quality.

Collection and evaluation of existing sediment chemistry data are critical components of the site-specific sediment quality assessment process. Concerns regarding data quality may be resolved by evaluating the quality assurance/quality control measures that were implemented during collection, transport, and analysis of sediment samples. A number of conventions have now been established which provide guidance on the field aspects of sediment sampling programs (ASTM 1994a; EPA and ACE 1991) and they were followed under this study.

While a diversity of analytical procedures have been developed to quantify concentrations of contaminants in sediments, a number of standard methods have been recommended (for example by EPA and ACE 1991; ASTM 1994a). McDonald stresses the importance of using total- total digestion techniques (using strong acids, such as hydrofluoric acid) for metal analysis (McDonald 1994). Nevertheless, some historical data is reported based on leachate acid digestion methods to account for elements that are bio-available.

As a consequence, 48 stations were monitored in this study using both leachate ICP-MS methods as well as LA-ICP-MS methods in order to collect and evaluate sediment chemistry data (steps a to c). The list of elements monitored was selected based on existing and historical contaminant sources from land and water use activities in the area (Fernandez 2004). For example, copper and zinc are monitored because they are associated to their use in antifouling paints for boats.

TEL and PEL values were used as preliminary criteria for assessment of the potential biological effects of trace metals found in sediments. Concentrations of the elements analyzed by two different methods (acid leachate digestion and LA-ICP-MS) were ranked and plotted in reference to SQAGs for which TEL and PEL criteria is available (MacDonald 1994).

Regardless of the differences in removal efficiency between laser ablation and leachate methods, similar interpretation of the samples above or below PEL values were obtained by both analytical methods. Figures 3.17 to 3.23 show the agreement of both methods, where none of the stations monitored presented concentrations of arsenic, cadmium, nickel or zinc above PEL criteria. Similarly, of the elements determined, only Cu and Pb exceeded the PEL criteria in 2 and 8% of the samples, respectively.

Chromium represents an exception where the PEL criterion reflects different results for stations analyzed by laser ablation and by acid leachate. However, higher levels on stations analyzed by LA are expected because the strength of the bond to insoluble minerals -such as chromspinels and chromite- does not affects the effectiveness of an exhaustive method but does difficult the recovery of pseudo-total digestions, as reported by Chen (Chen et. al., 2001) and by our correlation studies.

For the same reason, more sampling stations lie between the TEL and the PEL criteria when analyzed by laser ablation than when analyzed by leachate. Yet, this information provided by leachate and laser ablation can be complimentary, since one provides values related to elements that are bioavailable to aquatic organisms and the other one represents data from what is on the sediment.

It is important to emphasize, though, that this TEL and PEL criteria is just one model for interpretation of data and the possibility of arriving at erroneous conclusions still exists if they are used in isolation.

According to this rationale, the natural background levels of metals in sediments were also estimated and other models such as the Schropp's normalization approach were used to do an overall evaluation on whether these concentrations of elements present at levels above PEL could have an anthropogenic origin.

#### 3.17.7. Normalization of Elemental Data

Additionally, in order to assess if both techniques –laser ablation and leachate– can provide similar enrichment information under the areas of study, a normalization of the data using ratios of the metal/aluminum was performed. This normalization was initially proposed by Schropp and Windom in 1988 and it helps to identify if the concentration of metals is natural or anthropogenically enriched (Schropp and Windom 1988). A complete discussion about the reasons to select aluminum as normalizing element is given elsewhere (Loring 1991; Lin et al., 2008). The interpretative tool designed by Schropp encompassed a variety of sediment types, ranging from terrigenous, aluminosilicate-rich sediments in northern Florida, to biogenic, carbonate-rich sediments



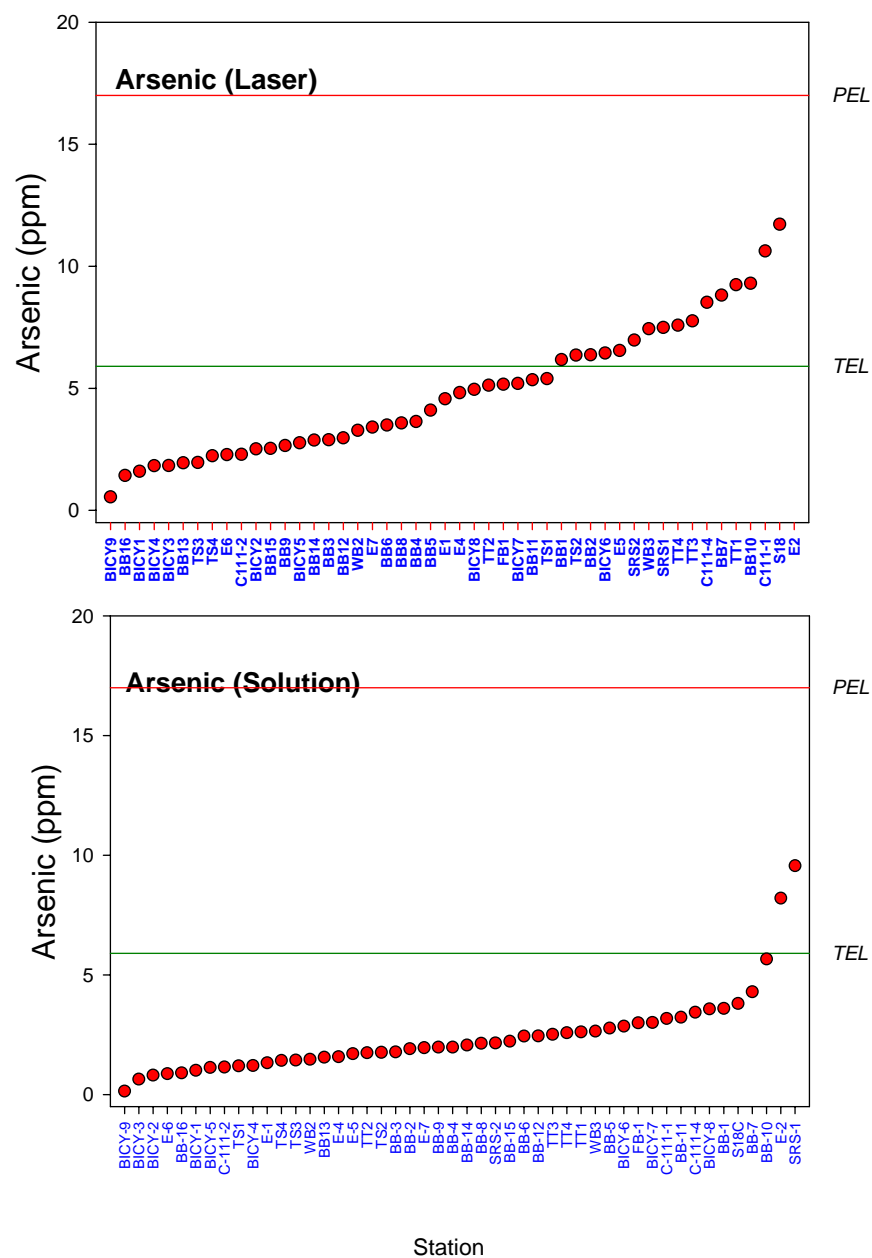


Figure 3.17. Ranked concentrations of arsenic for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

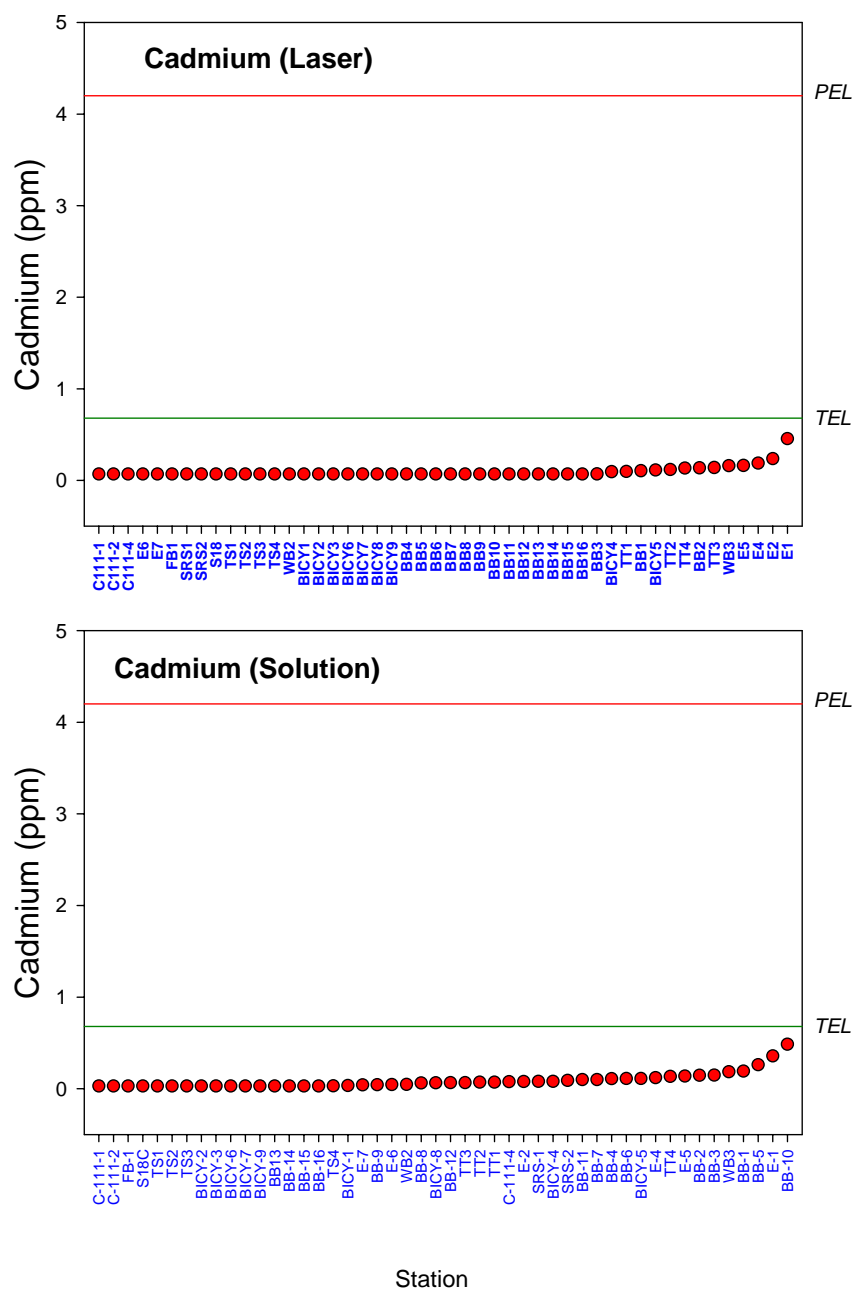


Figure 3.18. Ranked concentrations of cadmium for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

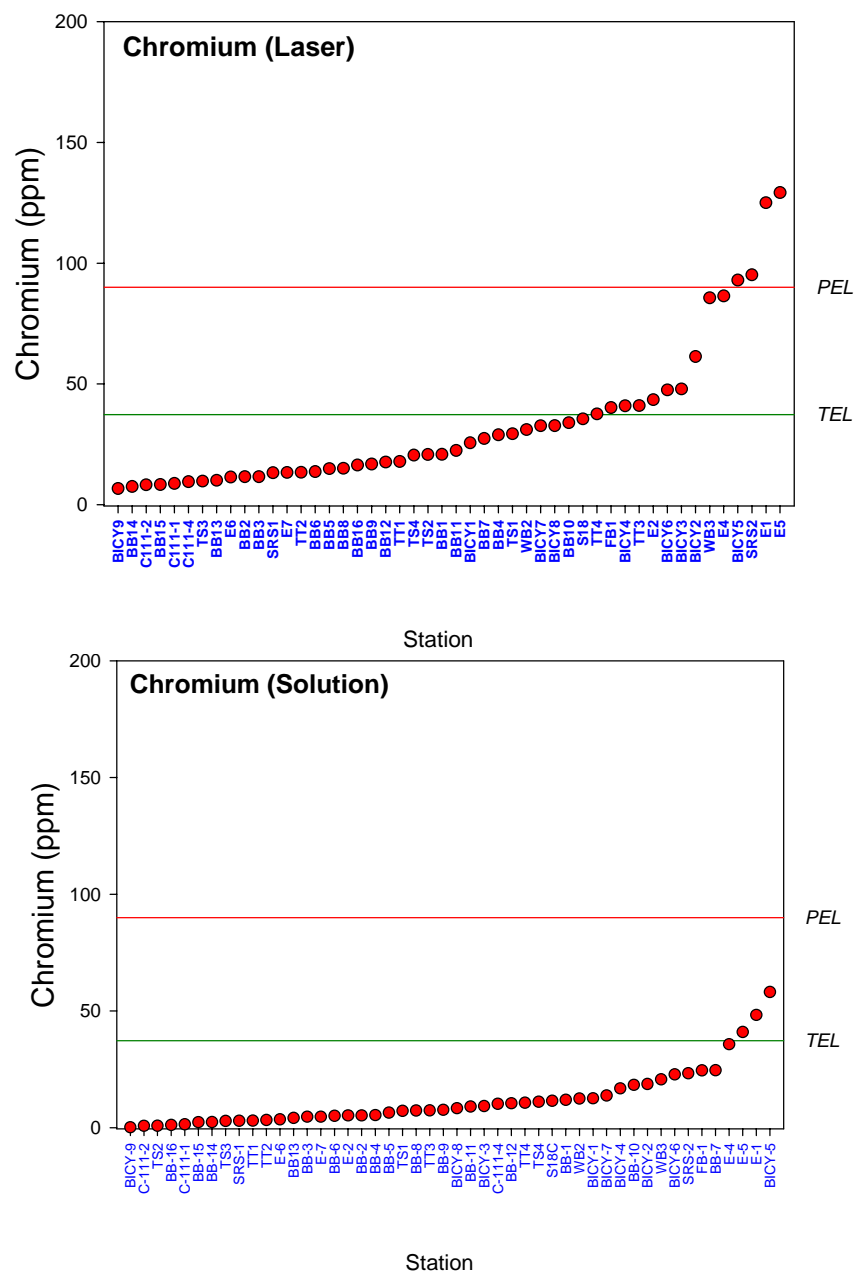


Figure 3.19. Ranked concentrations of chromium for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

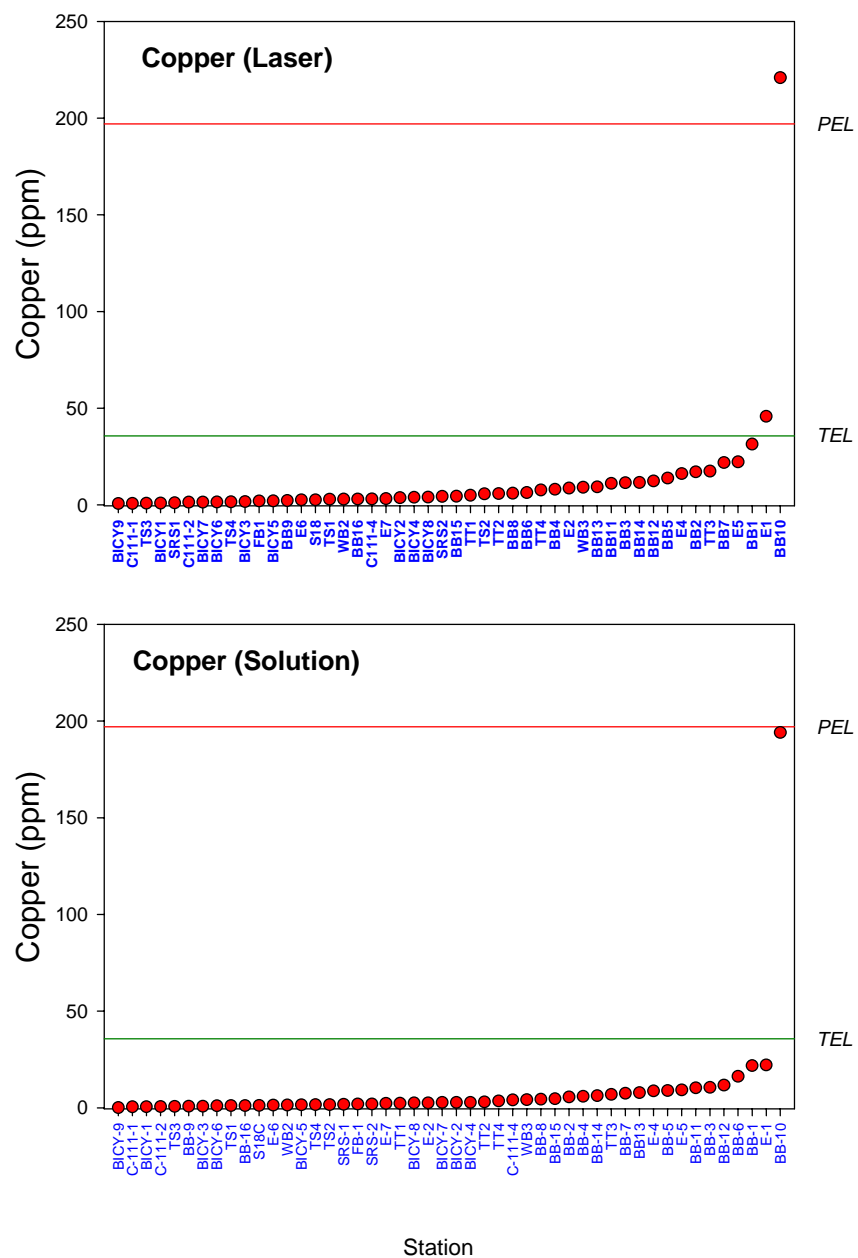


Figure 3.20. Ranked concentrations of copper for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

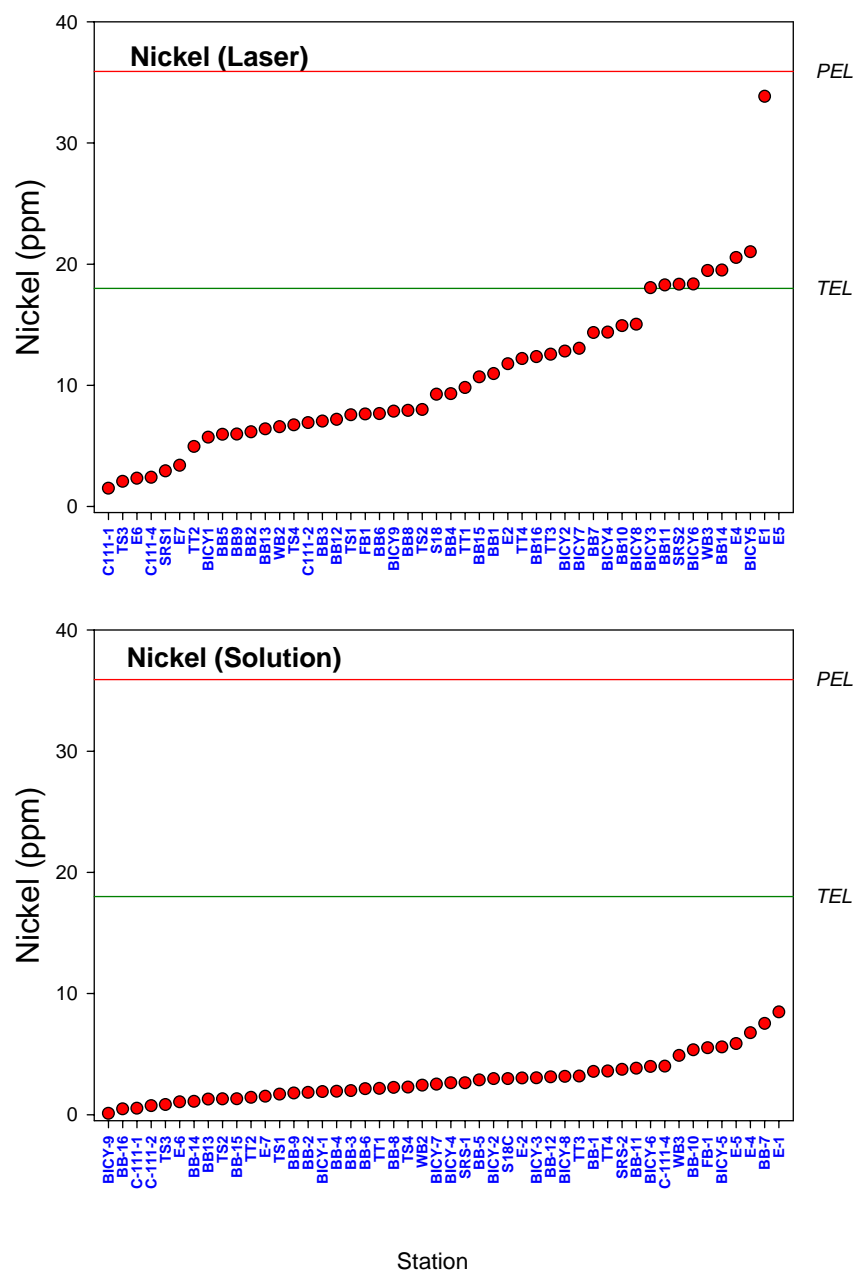


Figure 3.21. Ranked concentrations of nickel for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

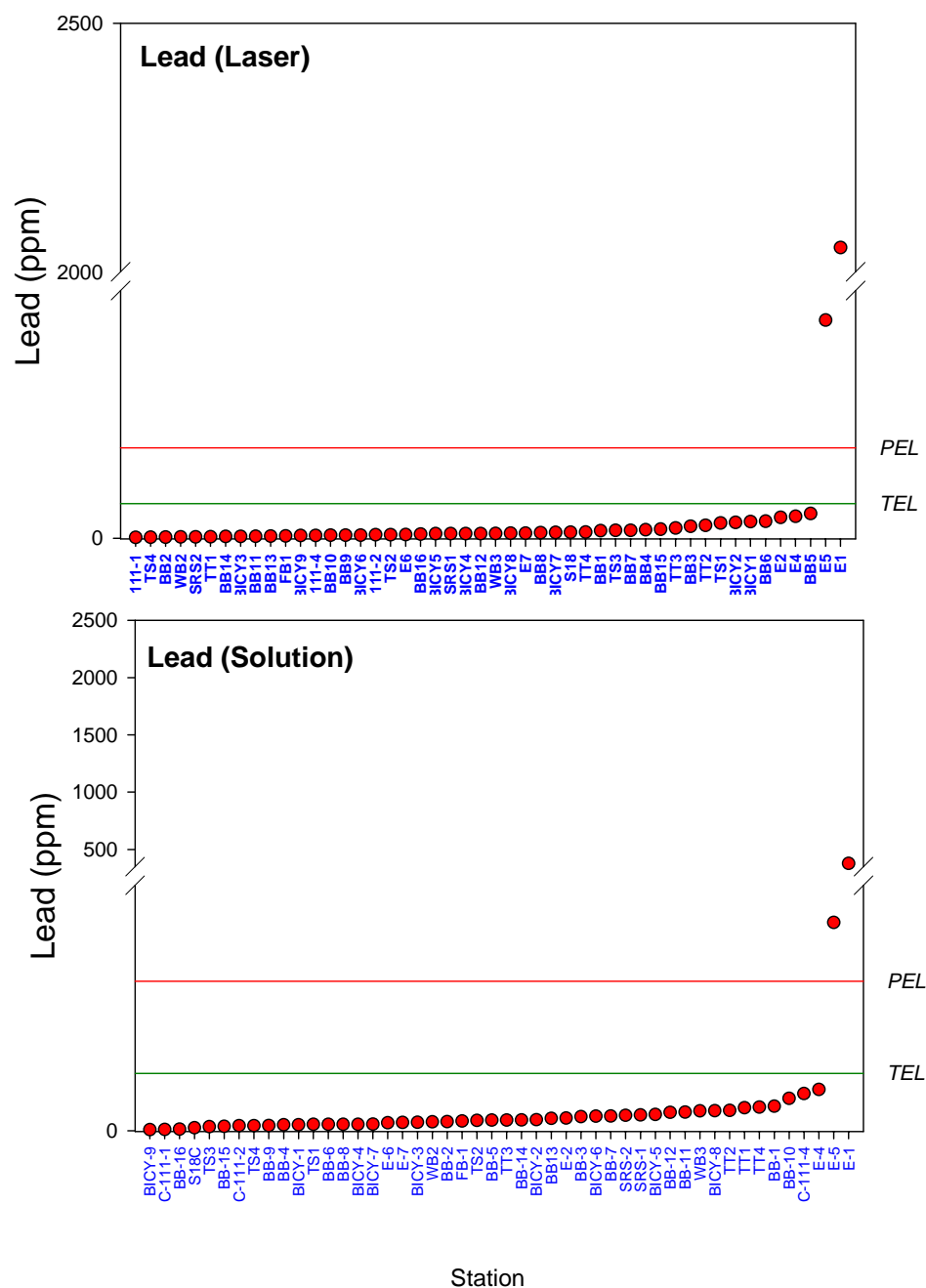


Figure 3.22. Ranked concentrations of lead for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

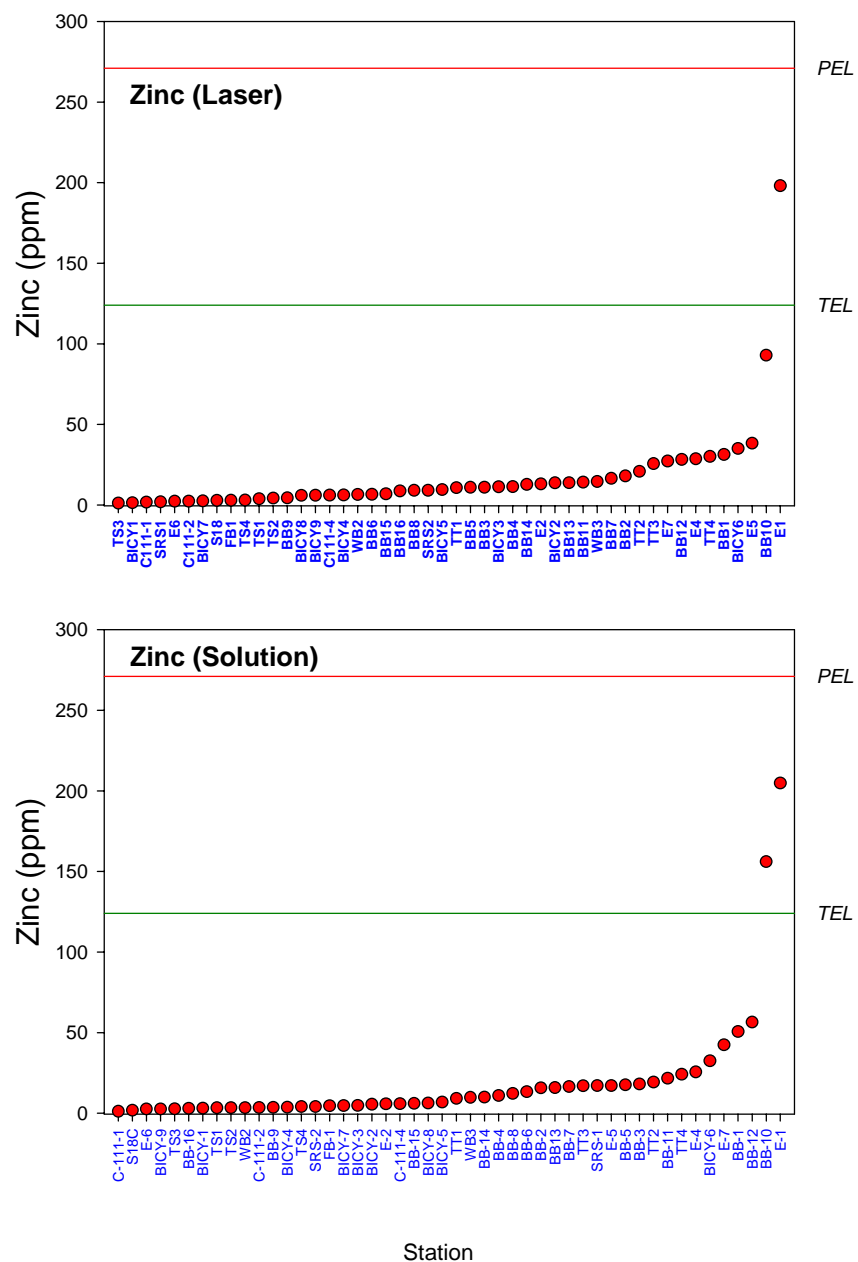


Figure 3.23. Ranked concentrations of zinc for CARE sediment samples using two analytical methodologies (top: laser, bottom: leacheate)

in southern Florida (Schropp and Windom 1988; Carvahlo and Schropp 2002).

The concentration of trace elements in sediments using both analytical approaches were plotted against aluminum concentrations in reference to the 95 % confidence level of the regression line determined by Schropp and Windom for non-anthropogenically enriched sediments. The results of some metals are represented in figures below. Similar enrichment graphs were observed within the monitored stations. For example, in the case of copper, stations BB10, BB1, BB2, BB3, BB6 and BB14 were above the 95 % confidence limit, which may indicate anthropogenic inputs on those sites (Figure 3.26). The majority of these stations were just above or very close to the upper limit while station BB10 was far away from the confidence limit, as evidenced by the high concentration levels of copper ( $\sim 200\mu\text{gg}^{-1}$ ). These elevated levels of copper are associated to contamination originated from the high boating activity in the marine located at the Biscayne Bay.

On the other hand, both arsenic and chromium were within the confidence limits and therefore they did not show enrichment for any of the monitored stations (Figure 3.24 and Figure 3.26). Elements that were found at concentrations above the confidence limits at some sites were copper, zinc, cadmium and lead.

Regardless of the fact that laser ablation removes more efficiently trace elements from sediments, the normalized data was comparable to the leacheate and they both identified the same “enriched” elements at the same sampling stations. There were some exceptions were samples were enriched only by one of the methods, but the corresponding samples on the second analytical method were just at the upper confidence limit.



It is important to stress the fact that this enrichment graphs do not provide exposure-based interpretation which is out of the scope of this paper, but they indicate the likelihood that sediments are enriched for that element over its natural abundance (Fernandez 2004).

Additionally, is significant to mention that this Schropp graphs were designed for total-total digestions and therefore laser ablation result may be more accurate. This information also corroborates the potential use of laser ablation in providing environmental forensic data that could be used in a later stage to assess bioavailability for these protected areas.

#### 3.17.8. Principal Component Analysis for the Elemental Composition of the Sediments

PCA was applied to this dataset to untangle the complexity of the multi-elemental composition of the 48 sampling sites. In particular, PCA was used on these samples with the aim of: a) detecting similarities and differences between samples according to their sampling site, b) visualize sites that may be potentially enriched, c) gain further sight into the chemistry of the sediment samples and d) determine whether or not there is correspondence between both methods -laser ablation and digestion leachate- with respect to the grouping and separation of sampling sites based on the multi-elemental composition. Figure 3.31 shows the 3D plot of the first, second and third principal components (x, y and z-axis respectively), which results from the set of the 48 sediments. Only those elements found above detection limit for the majority of the sampling sites were included for PCA (11 of the 16 elements monitored in this study). The sampling points are labeled according to the sampling station.

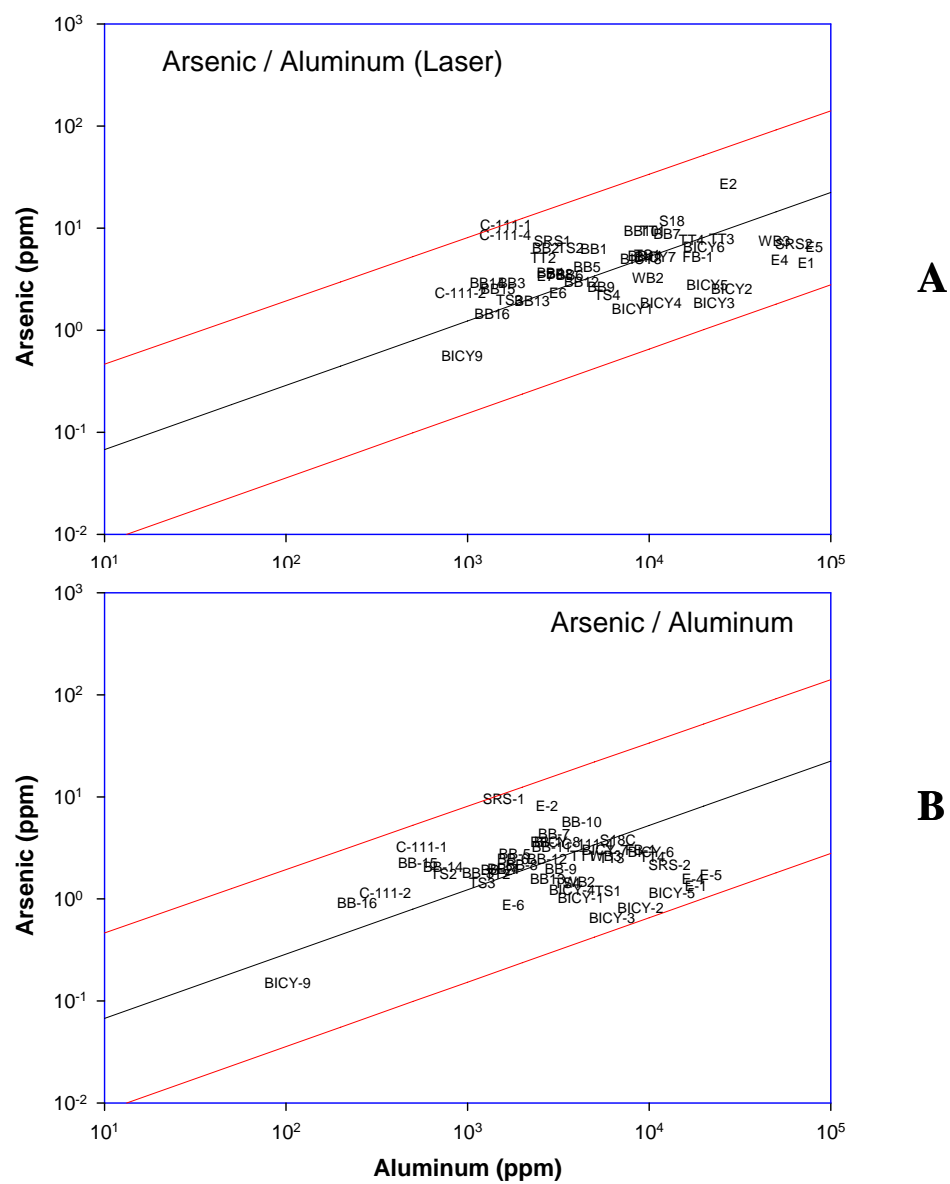


Figure 3.24. Concentration of arsenic vs. aluminum for CARE samples using two analytical methodologies. A) laser, B) leachate

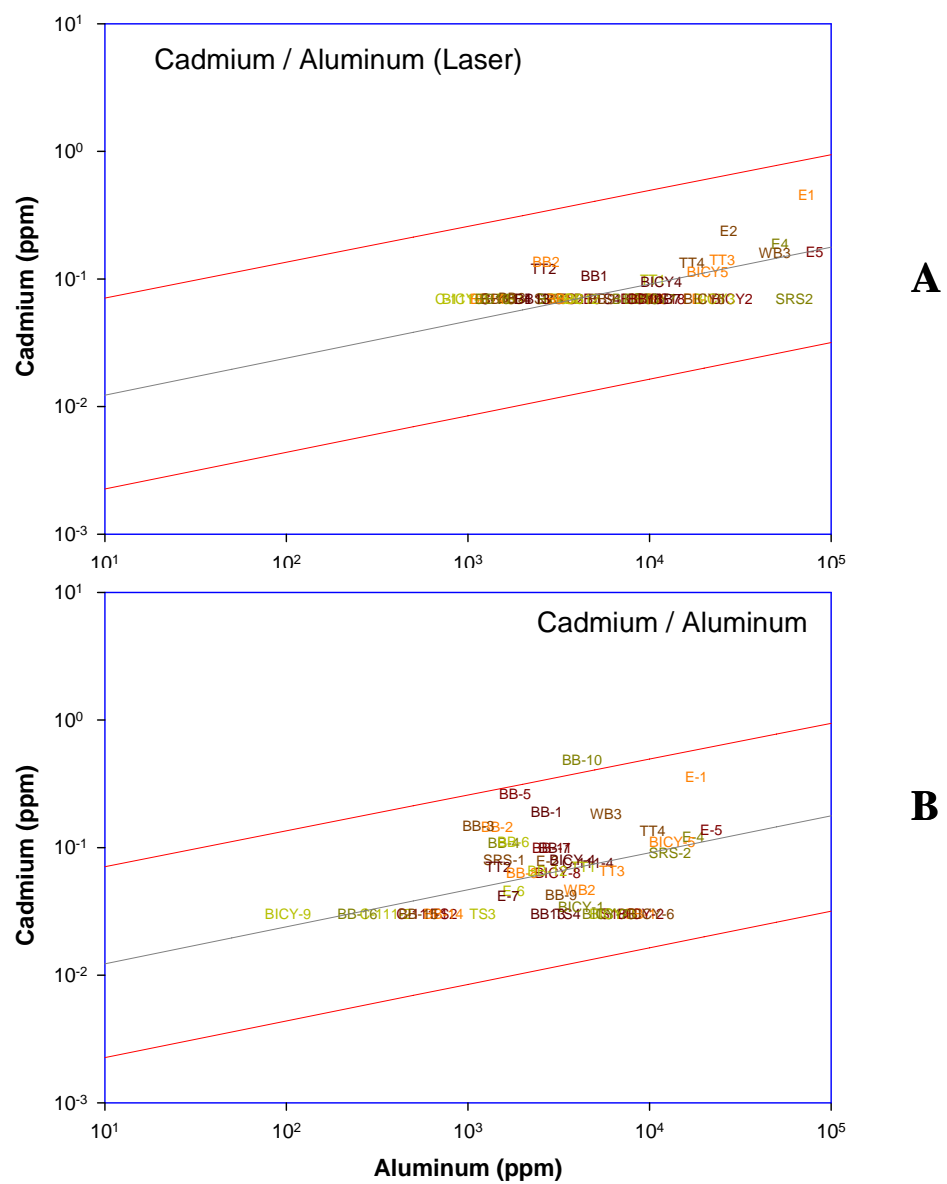


Figure 3.25. Concentration of cadmium vs. aluminum for CARE samples using two analytical methodologies . A) laser, B) leacheate

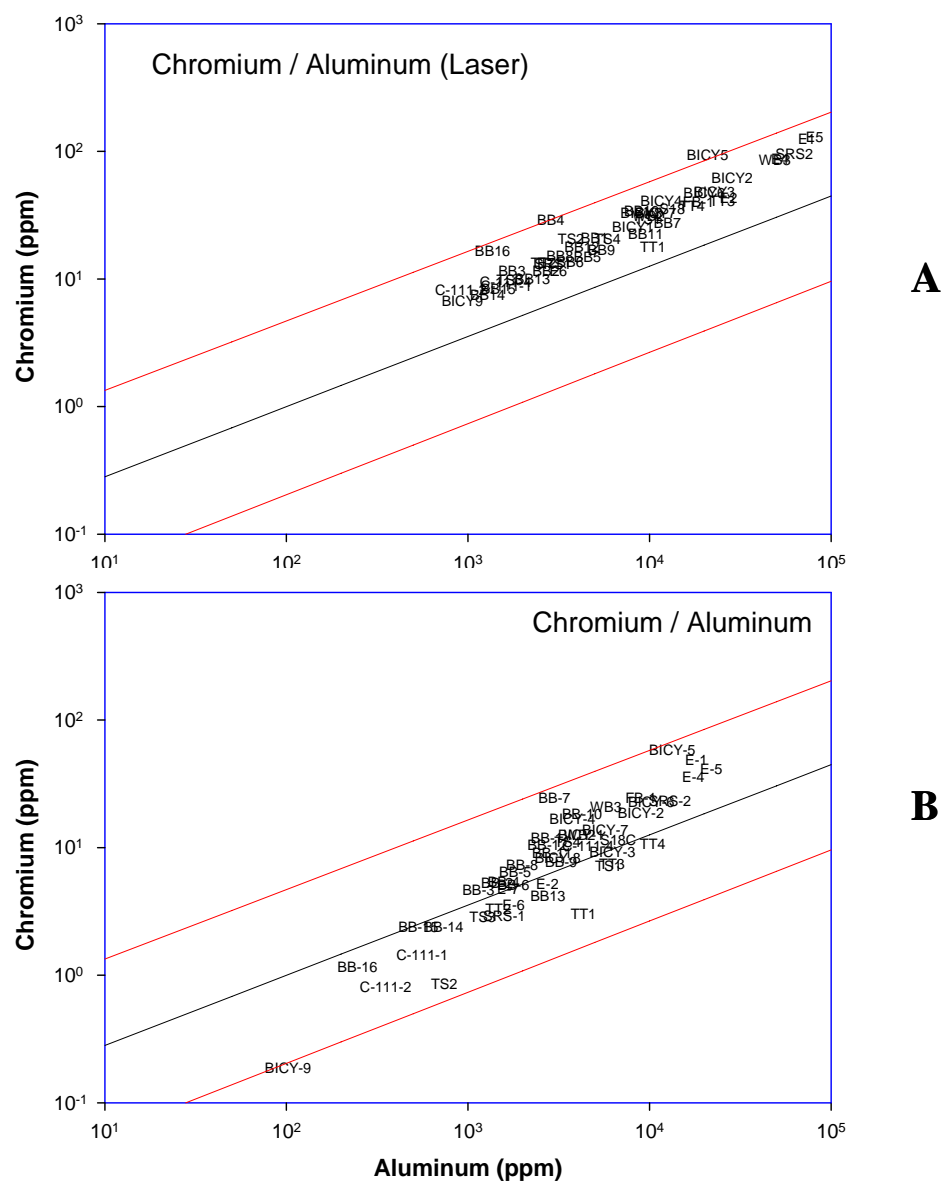


Figure 3.26. Concentration of chromium vs. aluminum for CARE samples using two analytical methodologies. A) laser, B) leacheate

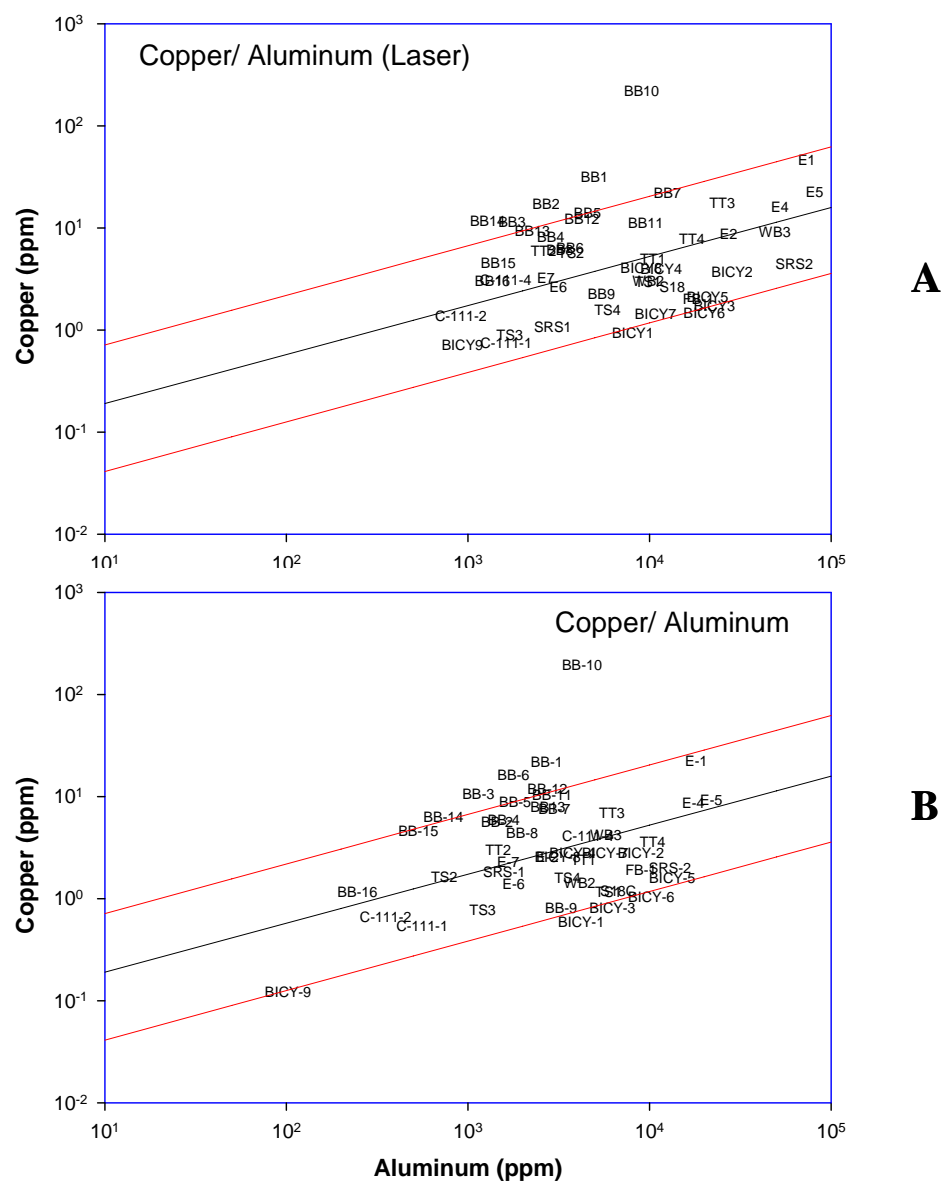


Figure 3.27. Concentration of copper vs. aluminum for CARE samples using two analytical methodologies. A) laser, B) leachate

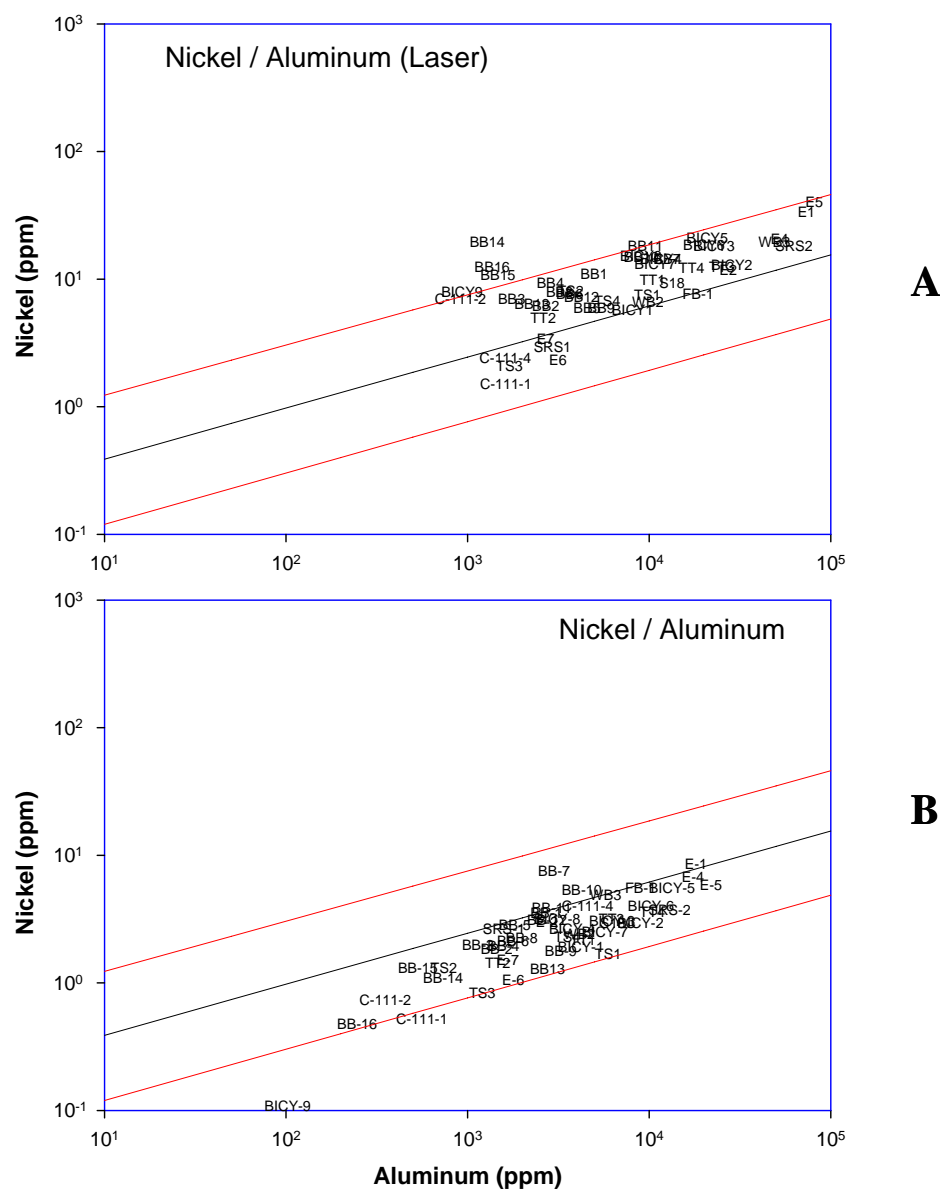


Figure 3.28. Concentration of nickel vs. aluminum for CARE samples using two analytical methodologies. A) laser, B) leacheate

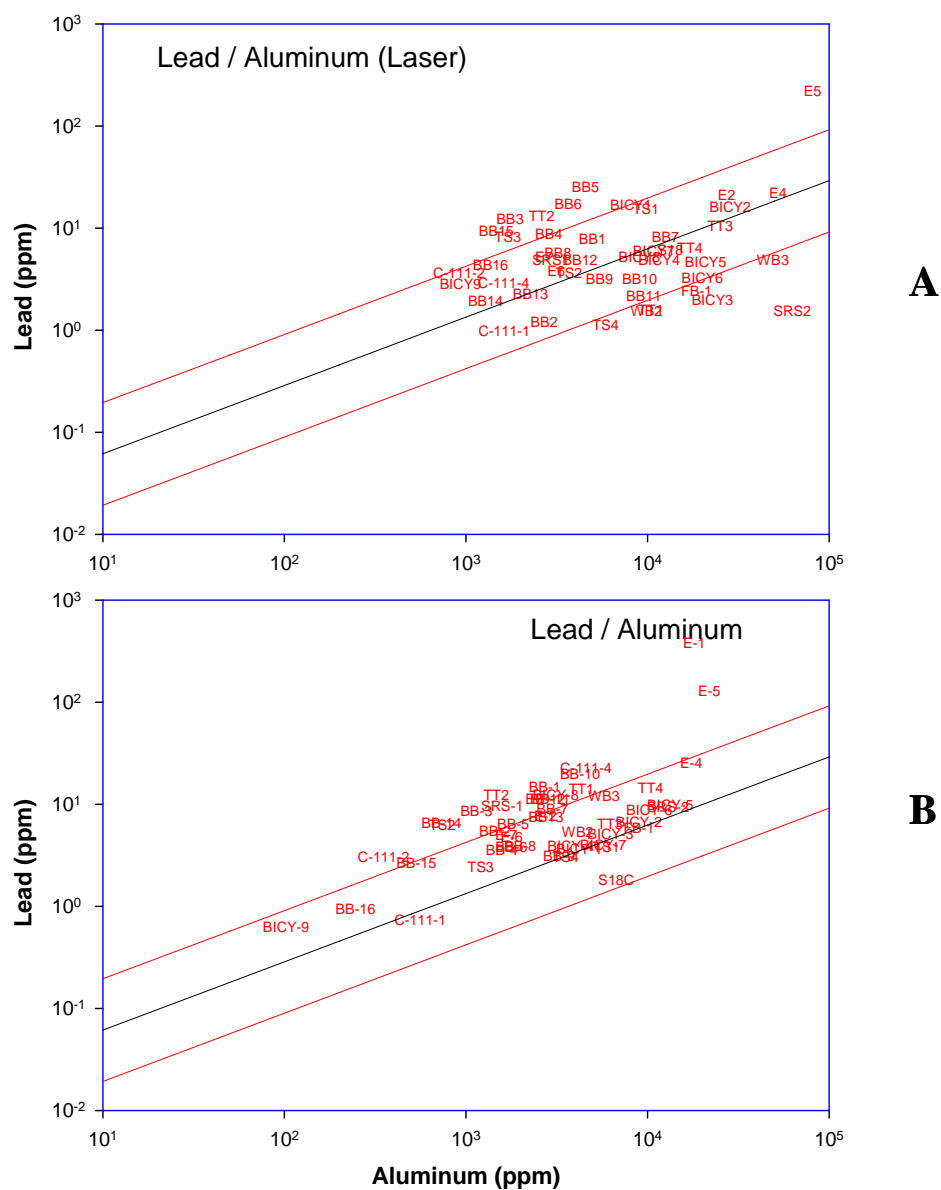


Figure 3.29. Concentration of lead vs. aluminum for CARE samples using two analytical methodologies. A) laser, B) leacheate

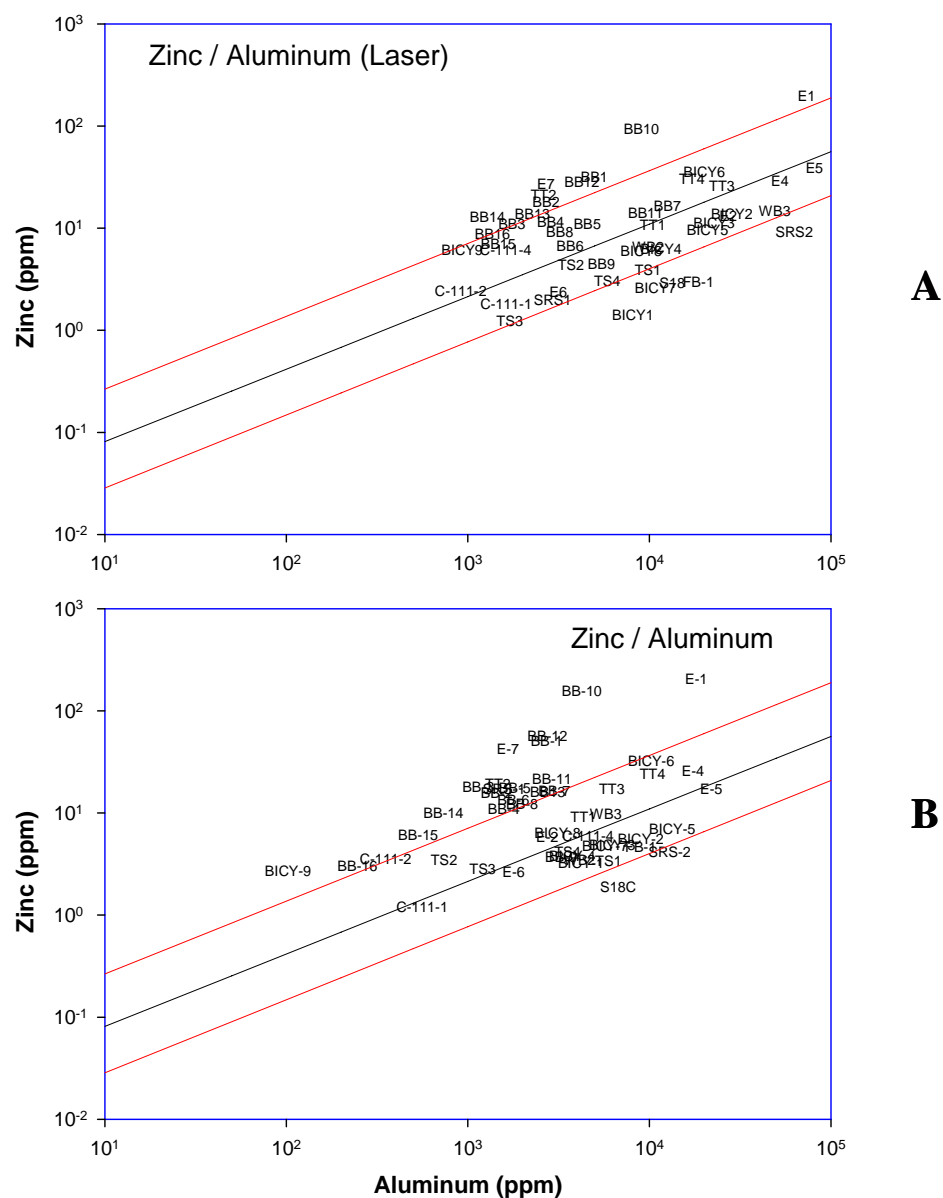


Figure 3.30. Concentration of zinc vs. aluminum for CARE samples using two analytical methodologies. A) laser, B) leacheate



The ellipses are drawn to show the distribution of the groups more clearly. PCA of both, laser ablation and leachate digestion methods provided similar grouping and separation of the samples. The ellipses show three major groups of the sediments according to the location of their sampling sites.

Sediments from the Biscayne Bay area (BB) grouped together (group “A”). Their elemental profile clearly differentiates from the rest of the sampling sites, with the exception of the sampling site TS4. Although TS4 is not located in the immediate vicinity of the BB, this site shows similar salinity and total organic content which may also influence the chemical composition and interaction of elements.

This TS4 station was expected to have a different composition to the other TS stations (Taylor Slough) and therefore is not surprising they are not grouped together. Differences between this stations are not only influenced by their natural environmental variables (i.e differences in TOC, salinity) but also by anthropogenic activities (i.e. vicinity to agricultural areas (TS1) vs. vicinity to coastal water and related activities such boating). The salinity and TOC distributions in the different sampling stations are observed in the maps shown on Figures 3.32 and 3.33 respectively. These maps allow a better visualization of the difference in salinity and TOC values between the stations.

Other important information drawn from the PCA of this first group “A” is the fact that sampling station BB10 differs from the rest of the BB sampling sites. Although, in general terms the Biscayne Bay samples presented elevated concentrations of elements that may be associated to boating activities (such as copper, zinc and cadmium), sampling station BB10 showed considerable higher concentrations for these elements. This pattern was observed with PCA graphs from both laser and solution based methods.

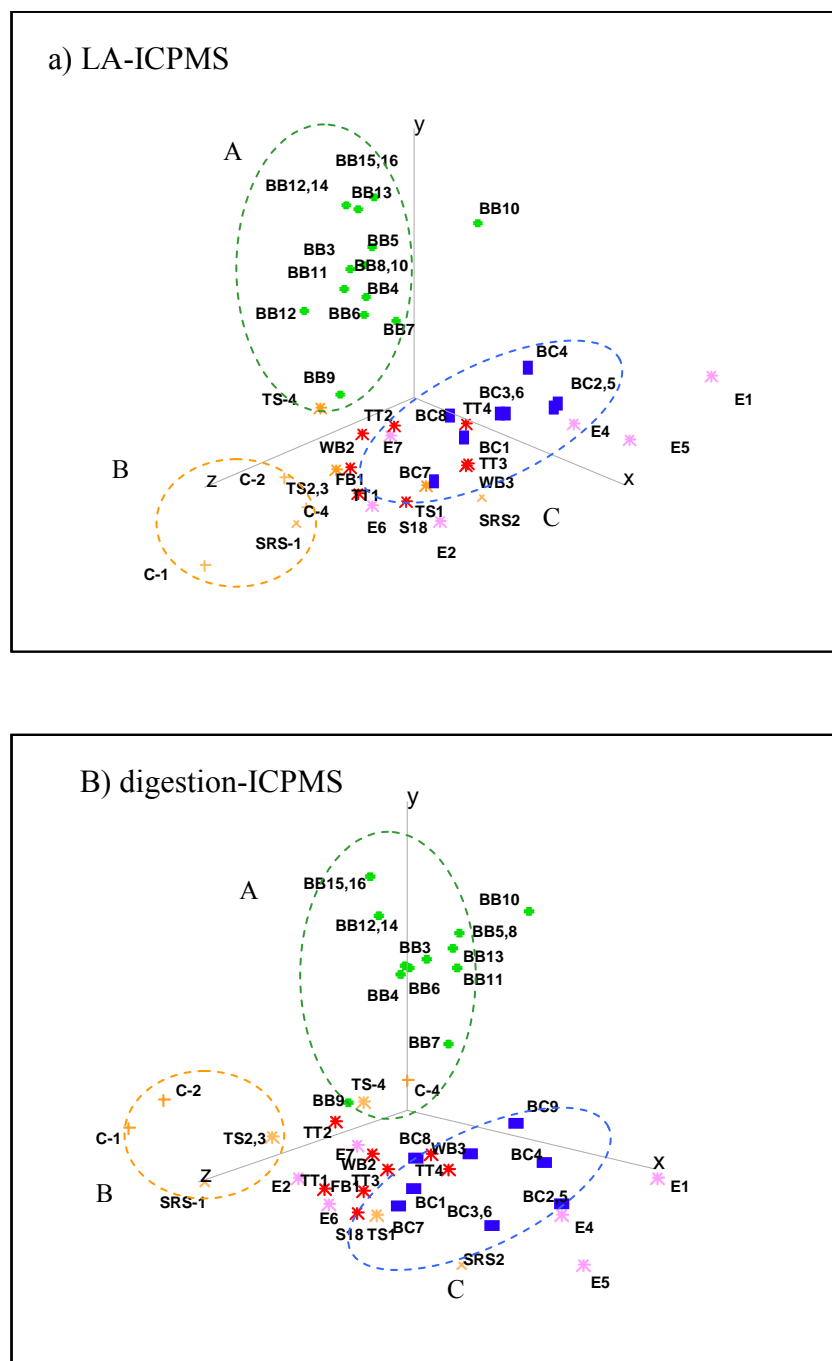


Figure 3.31. Principal component analysis of LA-ICP-MS data (top) and digestion leachate (bottom) of sediments from 48 sampling sites in South Florida protected areas.

The ellipse labeled as “B” represents another well separated group containing samples from the C stations (C1, C2), TS2, TS3 and SRS-1. The first four sampling sites came from a close geographical area. Although SRS1 is not adjacent to the rest of the stations in this group, they all have in common relatively low concentrations of the monitored elements.

Samples from Big Cypress National Preserve grouped together and are separated from the previous sampling groups (A and B). Nevertheless, there is also some sediment samples from the stations Tamiami Trail (TT) and Eastern boundary (E) that also fall into this group. The wide spread of sampling sites on the x-z plane is not unexpected because reflects the large variability of geochemical composition and anthropogenic inputs even for samples located next to each other in the map.

This variable elemental composition is influenced by natural composition of the sites such as salinity, mineralogy, organic matter, atmospheric deposition, sedimentation after major events such as hurricanes, and vicinity to areas with high agricultural activity. This variability is clearly detected by both methods (laser and solution) and is easily observed in “surface” sediments like the ones collected from this dataset.

Figure 3.31 also shows clearly the evident separation of sediment collected from stations of the East Boundary, E1 and E5, which presented elevated concentrations of lead by solution and laser ablation methods. This high concentration of lead may be associated to the proximity of these sites to abandoned shooting ranges, since lead is one of the major components in gunshot residues.

It is interesting to stress the fact that regardless of the differences between an exhaustive method (such LA) and a leachate method, there were no significant

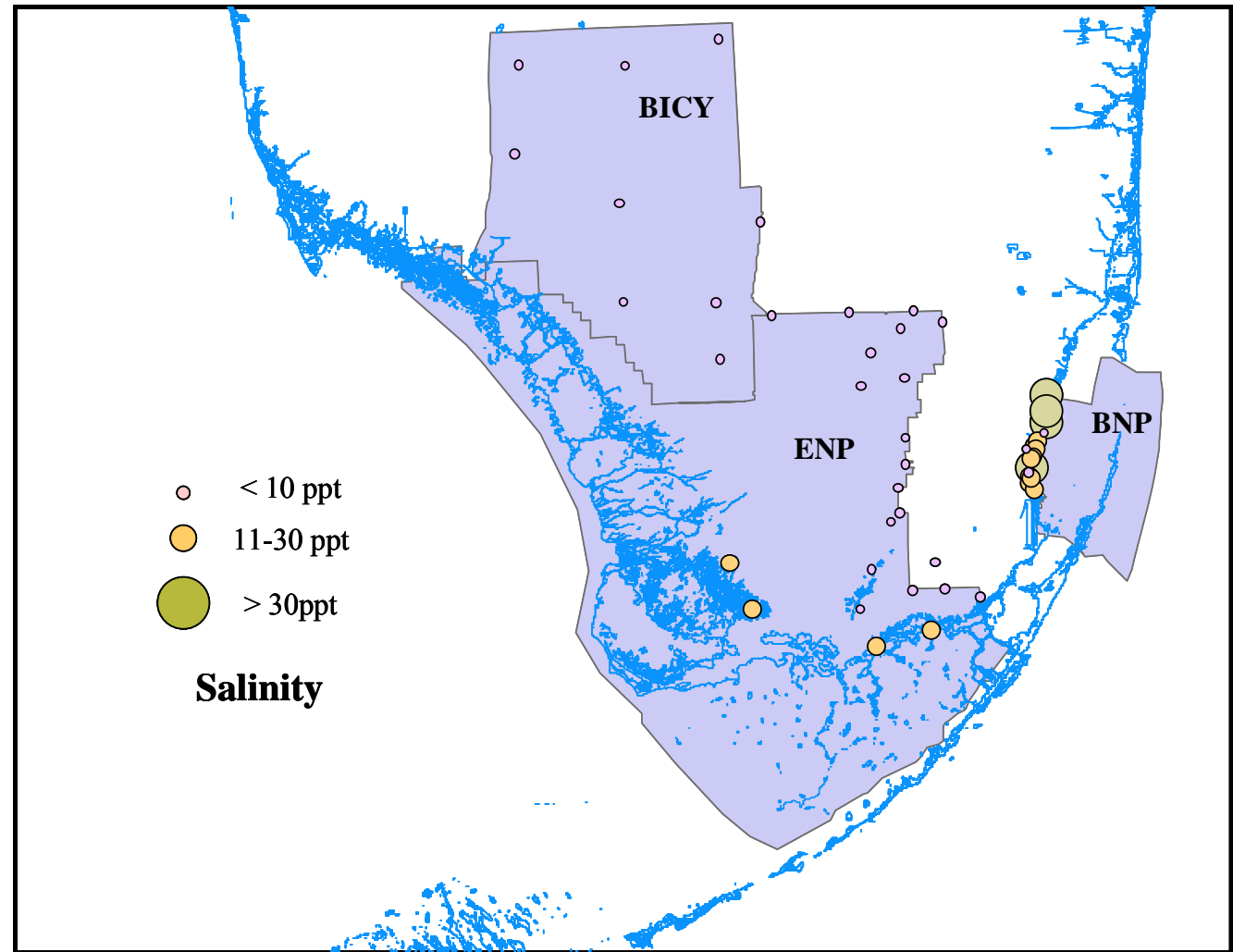


Figure 3.32. Salinity spatial distribution in stations under the CARE project (water samples)

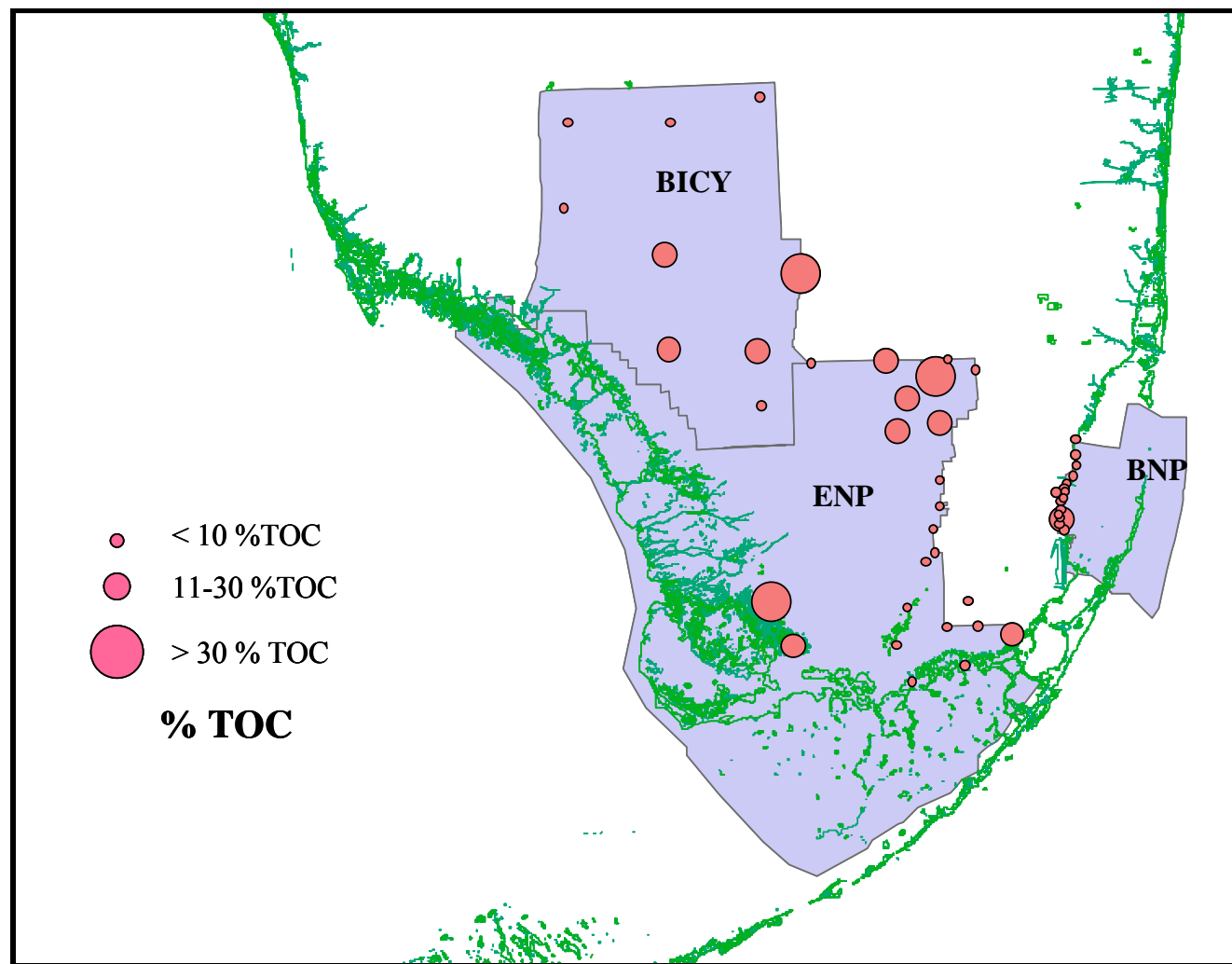


Figure 3.33. Total organic carbon (TOC) spatial distribution in stations under the CARE project (sediment samples)

differences between the main grouping of sediments according to their geographical origin and/or chemical composition influenced by anthropogenic activities.

Both PCA outputs provided similar information with regards to a) grouping and separation of sampling sites and b) visualize potential contaminated sites, which was the main purpose of the multivariate treatment of the data.

This correspondence between the informative value of both methods is also reinforced on Table 3.20 which shows the PCA loadings for each component and element. It is observed for both, LA and leachate digestion, that the first, second and third component already contain about 80% of the variance of the data. Such a distribution of the total variance means that it is possible to compress the information provided in the dataset onto the first three components without losing any substantial information.

There is agreement for both methods on the most significant factors on each component, which are highlighted in bold. The first factor with ~44% of the variance comprises aluminum, vanadium, chromium, lead and cadmium with high loadings. Some of these elements such as lead were good indicators of possible anthropogenic sources. The second factor comprises ~60% of the variance with copper, zinc and molybdenum being the higher loadings.

The contents of copper, zinc and molybdenum on this component was the most influencing vector for the separation of the BB samples, were high copper and zinc concentrations were observed. The third factor comprises an overall variance of ~80%, with manganese, arsenic and iron as the most significant factors which may relate more to natural composition rather than anthropogenic inputs.

Table 3.20.Principal component loadings for trace elements data found in sediment samples.

	PCA loadings for LA-ICP-MS			PCA loadings for leachate-digestion ICP-MS		
Component	PC1/ x	PC2 / y	PC3 / z	PC1/ x	PC2 / y	PC3 / z
<b>Eigenvalue</b>	4.8160	2.2607	1.3929	4.2854	2.6371	1.7401
<b>Percent</b>	43.78	20.55	12.66	38.96	23.97	15.82
<b>Cum Percent</b>	43.78	64.33	76.99	38.96	62.93	78.75
<b>Eigenvectors</b>						
<b>Log Al</b>	<b>0.4064</b>	-0.1478	-0.2108	<b>0.3358</b>	-0.3999	-0.0628
<b>Log V</b>	<b>0.3669</b>	0.0561	-0.3700	<b>0.3705</b>	-0.2204	-0.1665
<b>Log Cr</b>	<b>0.3911</b>	-0.1077	-0.3133	<b>0.3676</b>	-0.2987	-0.1865
<b>Log Mn</b>	0.1329	-0.4561	<b>0.4103</b>	-0.0166	-0.0523	<b>0.6523</b>
<b>Log Cu</b>	0.249	<b>0.4608</b>	0.2629	0.3080	<b>0.3852</b>	-0.1088
<b>Log Zn</b>	<b>0.3072</b>	<b>0.3631</b>	0.0811	<b>0.3540</b>	<b>0.2918</b>	0.0363
<b>Log As</b>	0.2318	-0.1006	<b>0.5899</b>	0.1160	0.2692	<b>0.42501</b>
<b>Log Mo</b>	0.0615	<b>0.5099</b>	0.2505	0.07671	<b>0.5242</b>	-0.1157
<b>Log Cd</b>	<b>0.3573</b>	0.0201	0.0918	<b>0.3723</b>	0.2545	0.0410
<b>Log Pb</b>	<b>0.3405</b>	0.0911	-0.1660	<b>0.4111</b>	-0.0475	0.0258
<b>Log Fe</b>	0.2686	-0.3639	0.1722	0.21938	-0.2269	0.5463

### 3.18. Conclusions for LA-ICP-MS Results for the Analysis of Soils and Sediments

A simple sample preparation and quantitation method by LA-ICP-MS was optimized and validated for soils and sediments, as a viable alternative for routine monitoring of contaminated sites and determination of background levels.

The novelty of the proposed method relies on the application of UV-laser ablation-ICP-MS for the simultaneous analysis of a large variety of elements of interest to the environmental sciences with limits of detection as low as 0.01 mg/kg. The micro-homogeneity and particle size studies showed that the milling methods and pellet formation provide efficient homogenization at the micro-scale to produce accurate chemical representation of the bulk sample. This sample preparation procedure improved not only the precision and accuracy of measurements, but also the cohesion of soils during pellet formation avoiding the use of binders that dilute the sample.

Despite the added steps, sample preparation times are more than adequate for routine applications. With a batch of three high speed mill systems, one technician can prepare ~72 samples/day. The LA-ICP-MS analysis can determine trace, minor and major elements simultaneously without requiring the use of multiple dilutions as often required in typical digestion methods. Laser ablation demonstrated to be an efficient sampling method for the elemental menu of interest (19 elements). The recoveries did not vary significantly by element or among soil type.

Spot size, grain size and the use of an appropriate internal standard were found to be the key factors, controlling the analytical performance. Assessment of analytical parameters produced good accuracy and precision for the reported elements for certified



soil and sediment standards. Additionally, the LA-ICP-MS method performs very well in comparison to other ICP and AA digestion methods typically used by the soil community as established by the participation on the proficiency test exercises.

LA-ICP-MS also allows direct characterization of solids with minimum consumption of the sample (~ micrograms), which is critical in some of the forensic application where this method is intending to be used. Micromilling and pellet formation is also a valuable method for specimen preservation.

The robustness and applicability of the method was also tested for a large set of real sediment and soils with broad chemical and physical properties. From this second part of the study, the following conclusions can be drawn:

The proposed methodology can be applied for the rapid quantitative assessment of contaminated soils for major, minor and trace levels, including analytes such as lead, tin and antimony which are of particularly importance to determine anthropogenic sources and to establish remediation or clean up efforts.

When compared to traditional methods, Laser ablation can be considered an exhaustive sampling method that recovers significantly higher amounts of analytes than leachate or pseudo-total digestion protocols. Despite of the inherent differences between leachate digestions and exhaustive methods such as laser ablation, different statistical models such as non-parametric correlation tests, aluminum normalization and principal component analysis, demonstrated that there is good agreement between the informative value of the data acquired by both methods and therefore LA-ICP-MS can be also used to establish baseline concentrations and to establish enrichment levels.

The application of the LA-ICP-MS method to environmental forensics has several

advantages over the digestion methods discussed in this study, such as: providing reduced time of analysis and complexity, offering a single rapid alternative to conduct multi-elemental quantitative analysis of soils without requiring multiple digestion and independent measurement procedures.

## CHAPTER 4

### 4.0. Screening for Airborne Trace Elements by in situ Laser Ablation ICP-MS: Method Development and Preliminary Assessment

#### 4.1. Introduction

Pollutants such as heavy metals, low molecular weight hydrocarbons, and other organic compounds are transported via long range atmospheric processes reaching marine environments worldwide.

Both local and long-range transport (LRT) of pollutants has been heavily investigated in the past 30 years because of their effects on receiving ecosystems. Global transport of microorganisms, pollutants and or nutrients from as far as Africa and China have been singled out as sources on environmental and health problems along both coasts of the US and the Greater Caribbean Basin (Windom et al., 1976; Griffin et al., 2004; Griffin et al., 2007; Holmes et al., 2007).

Airborne particulate matter is a complex mixture of dust, dirt, soot, smoke and liquid droplets emitted into the air by mechanical processes that are small enough to be suspended in the atmosphere. They contain both organic and inorganic components as well as biological particles. Airborne particulate can be characterized by their physical properties such as mass concentration and size distribution that directly influence their transport and deposition. They can also be characterized by their chemical composition, which ultimately dictates their effect on human health and the environment (World Bank Group 1998).

Volatile “metalloids” can be transported in a gaseous form or enriched on particles (e.g. selenium, mercury, arsenic and antimony), whereas other metals are transported only in the particle phase (e.g. cadmium, lead and zinc) and may travel long distances before deposition to land (Adriano 1992). There is strong evidence that atmospheric particulates contribute to pollution of coastal areas, and in some instances

this contribution could become more significant than water discharge of wastes. For example, high lead content in Greenland ice has been attributed to burned tetraethyl lead from vehicle fuel in populated areas in the northern hemisphere. Also, the presence of persistent pollutants of concerns (POPs), mainly hexachlorobenzene (HCB) and hexachlorocyclohexanes (HCHs) were reported in the arctic atmosphere (Windom et al., 1976; Halsall et al., 1998).

In particular, “African Dust” is thought to be a major player as source of anthropogenic iron in tropical coral reef systems and a potential source of airborne pathogens and toxic substances that could potentially act as major stressors in these delicate environments (Goudie et al., 2001; Zhu et al., 1997; Gyan et al., 2005; Muhs et al., 2007, Harvel et al., 2007). For example, Walsh and Steidinger linked a phytoplankton blooms (red tide) in the Gulf of Mexico directly to iron delivered by Saharan dust (Walsh and Steidinger, 2001). Also, Prospero reported the presence of fungi and bacteria only when African dust was present (Prospero et al., 2005). Weir-Brush presented a relationship between a coral disease and African Dust Storms (Weir-Brush et al., 2004).

Acting as primary reservoirs, the Sahara and the Sahel deserts in Africa, are a major source of mobilized top soils that travel great distances through the atmosphere in a continuously but chronic fashion (Prospero et al., 1999; Prospero et al., 2003). Of the about 2.0 billion metric tons of dessert dust that is globally spread per year, 50 to 75 % comes from these two dessert areas. These atmospheric conveyor belts had been transporting fine soil particles for thousand of years to the Caribbean and the Southeastern USA. The majority of the studies relating the atmospheric deposition in the

Caribbean and Florida had been conducted by the research group of Dr. Joseph Prospero in the Rosenstiel School of Marine and Atmospheric Science (RSMAS) at the University of Miami in Florida. Part of his research has provided scientific evidence in different areas, e.g. an extensive monitoring relating the African drought and dust transport into the Caribbean was published in 2003, where the quantities of dust from 1965 to 1998 were presented and discussed in terms of the implications of climate change in the region (Prospero and Lamb 2003). Also, geochemical evidence for African Dust inputs to soils in Barbados, the Bahamas and Florida Keys was established, in which, the use of trace metals like scandium, chromium, thorium, zirconium and rare earth elements, were use to distinguish between parent materials as external sources of the carbonate substrate of these three regions (Muhs et al., 2007).

The long term variability of African dust transport across the Atlantic was determined in Barbados surface concentrations using Total Ozone Mapping Spectrometer (TOMS). This study has been done since 1966 and they provide dust optical thickness that was used to assess the characteristics of the impact of climate factors, e.g. North Atlantic Oscillations (NAO) and Sahel drought, on dust emission and export as a function of season (Chiapello, Moulin and Prospero 2005). The determination of iron in South and Central Florida was related to the LRT of African mineral dust (Prospero et al., 2000). They concluded that the iron concentrations were low during most of the years 1995-1996, but the concentrations increase dramatically during the summer when African dust was advected into Florida.

African and Asian dust consists primarily of clay soil minerals such as illite, quartz, kaolinite, chlorite, microcline, plagioclase, and calcite (Prospero, 1981), which

may undergo chemical change during aerosol transport (Ravishankara 1997). Some elements (e.g. manganese, iron, scandium, cobalt) occur on African dust particles in concentrations similar to average crustal abundance, whereas other elements (e.g. mercury, selenium, lead), accumulate via scavenging, at concentrations three orders of magnitude greater than mean crustal abundance (Garrison et al., 2003, Duce et al., 1976).

The amount of dust that crosses continents varies as a result of global climate, meteorology, geomorphology of the sources areas and anthropogenic signatures. For instance the quality of the transoceanic dust is of primary interest for researchers because of the variety of human related stressors coming from biomass and waste burning, (e.g. airborne contaminants including plant detritus, microorganisms, soils and nutrients, and toxic metals, pharmaceuticals, and POPs) that could be carried as a cloud of stable aerosols (Griffin and Kellogg 2004).

However, one of the main challenges in the assessment of these parameters is differentiate between local and transoceanic contributions. In general, contributions from power plant, vehicular and industrial emissions represent a relatively constant source and could be figured out if long term monitoring of dust data is collected at the same time.

African Dust clouds are easily detected using remote sensing via the SeaWiFS satellite (see Figure 4.1). They tend to cross the Atlantic Ocean during the summer months and particles are deposited along the southeast coast of the US, the Caribbean Sea and the Gulf of Mexico on a regular basis. In this regard, a relatively precise timeline is available to coordinate the aerosol sampling or to determine the window of influence. One of the major challenges sampling African dust events is the location of the air samplers, the limited sample loading, and the analytical approach for detecting multiple

elements in very low concentrations. This investigation plans to address some of these problems.

Sampling of airborne particulate can be conducted in the ocean from a ship or by coastlines. There are always great risks of cross contamination from boat emissions and residues; thus land sampling is a more practical way to obtain values of atmospheric concentration and fluxes of pollutants over the ocean.

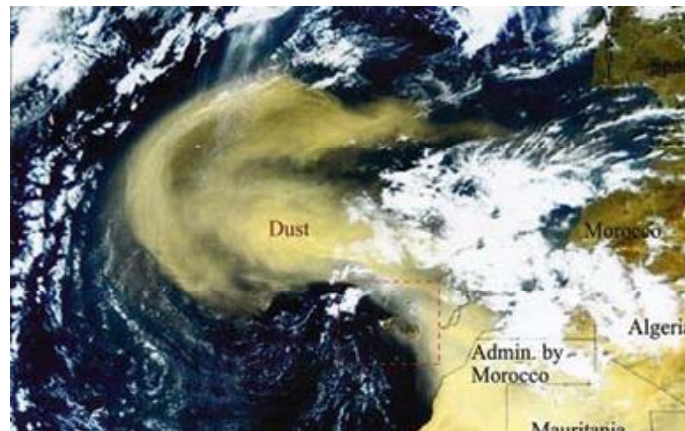
Since natural and anthropogenic inputs are collected together by sampling devices, collection of samples should be done over relatively extended periods of time to be able to differentiate the local vs. the global signatures. Indeed, aerosol research has focused it's interested in determining the relative abundance of trace elements carried in the dust. In a recent study, Marx presented a method for determining the concentration of trace metals and rare earth elements, as a way to determine provenance of loess samples in Australia from New Zealand glaciers (Marx et al., 2005).

Due to the low mass loadings and low total concentrations of trace metals, sensitive methods have been used in the past to detect inorganic compounds in airborne particulate, such as Atomic Absorption Spectrometry (AAS Windom et al., 1976), Neutron Activation (NAA; Windom et al., 1976), UV-Vis spectroscopy (Zhu 1997), Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Espino 1998) and High Resolution ICP-MS (Chu-Fang Wang, 1999).

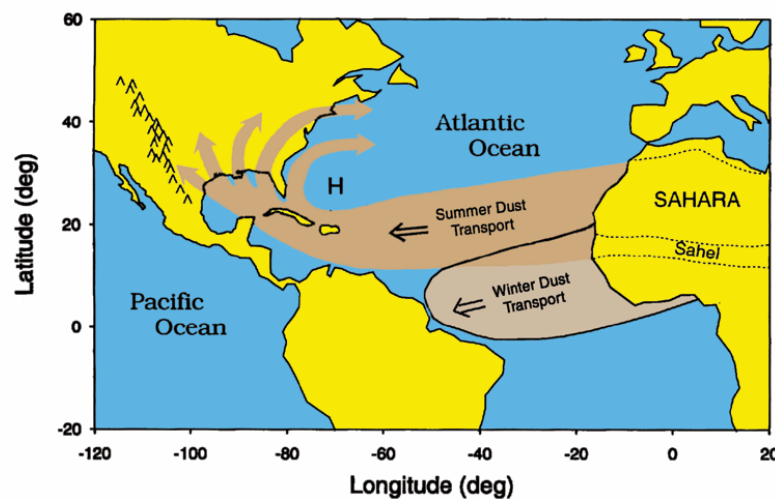
Although evidence of atmospheric input of African dust in South Florida has been previously reported (Griffin 2004; Muhs et al., 2007; Holmes et al., 2007; Harvel et al., 2007), up to date there is no information on the composition and variability of metals in airborne particulate associated to African dust nor even for local emissions.



For this reason, this study intends to develop an analytical method for the monitoring of airborne particulate in South Florida coastal areas as a first step to identify inorganic pollutants originated specifically from African dust storms.



A



B

Figure 4.1.A) Example of a SeaWIFS satellite picture (source: [http://science.nasa.gov/headlines/y2001/ast26jun\\_1.htm](http://science.nasa.gov/headlines/y2001/ast26jun_1.htm)) B) Diagram of the trajectory of African Dust (from Perry et al., 1997)

## 4.2. Significance of the Study and Objectives

The main objective of this study was to develop and evaluate the applicability of digestion and laser ablation ICP-MS based methods as rapid screening tools for determining airborne trace elements in filters containing both contemporary African dust and local dust emissions.

The specific objectives of this work are the following:

1. Development and optimization of a LA-ICP-MS method for the analysis of African dust samples, including the study of potential sampling substrates.
2. Installation of a collection site for the periodical sampling of dust particulate in key points of interest in South Florida.
3. Development of a quantitative method for the assessment of toxic metals in filters.
4. Development and optimization of a digestion method for extraction of the available elemental content present in African dust filters and measurement on ICP-MS system.
5. Measurement of airborne particulate collected from a set of real samples, by the optimized LA-ICP-MS and digestion ICP-MS methods.
6. Evaluate the overall advantages and limitations of the proposed LA-ICP-MS and its feasibility against regular digestion ICP-MS methods for this particular application.
7. Interpret the elemental profiles to assess the potential influence of African dust inputs from storms.

### 4.3. Method Optimization for LA-ICP-MS of African Dust Filters

#### 4.3.1 Instrumentation and Measurement Parameters

ICP-MS analysis were conducted on a quadrupole ELAN DRC II (Perkin Elmer LAS, Shelton CT USA), used in the standard operation mode. A 213nm Nd-YAG laser (UP 213, New Wave, USA) was used for this work. Optimization of laser parameters included the study of different repetition rates (5Hz, 10Hz, 20Hz), ablation modes (line and raster), spot sizes (50, 100, 200 $\mu$ m), scan rates (from 5 to 20 $\mu$ m/s at 5 $\mu$ m/s increments) and energy level (from 20 to 60%, at 5% increments). Best ablation results, evaluated as the best precision and signal stability, were obtained using raster mode at 100 $\mu$ m spot size, 10 $\mu$ m/s scan rate, 35% energy and 10Hz. Helium was used as the carrier gas of the laser ablation particles.

The following elemental menu was used during the ICP-MS and LA-ICP-MS analysis:  $^9\text{Be}$ ,  $^{27}\text{Al}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{57}\text{Fe}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{75}\text{As}$ ,  $^{82}\text{Se}$ ,  $^{88}\text{Sr}$ ,  $^{107}\text{Ag}$ ,  $^{111}\text{Cd}$ ,  $^{117,118,120}\text{Sn}$ ,  $^{121,123}\text{Sb}$ ,  $^{137}\text{Ba}$ ,  $^{206,207,208}\text{Pb}$ .

A Scanning Electron Microscope Philips XL 30 with EDX detector (Philips, The Netherlands and EDAX, USA, respectively) was used for the imaging of the surface of the materials and to assess the elemental distribution (mapping) elements or standards deposited in the filters.

#### 4.3.2. Reagents and Standards

Glass micro fiber (PALL Life Sciences, USA) and Verapor ® polymeric filters (Versapor 450R, 0.45 $\mu$ m, PALL Life Sciences, USA) were studied as substrates at the development stage of the LA-ICP-MS method. Small pieces of the filters of 2 x 2 cm<sup>2</sup>

were used to determine the optimum parameters for the laser experiments.

For the calibration curves, single element solutions of lead, copper, barium, antimony, iron, nickel, vanadium and rhodium at  $1000\ \mu\text{g}\cdot\text{mL}^{-1}$  (Peak performance, CPI International, USA) were used to prepare the stock solutions. Individual stock solutions at 0, 50, 75, 100, 150 and  $300\ \mu\text{g mL}^{-1}$  were prepared for the experiment. A  $20\ \mu\text{L}$  spike of each of the stock solutions were added into the filter and dried overnight. Multi-element solutions of lead, barium, antimony, iron, nickel and vanadium were also prepared at 0, 50, 75, 100, 150 and  $300\ \mu\text{g mL}^{-1}$  and spiked into the filters as explained above. Rhodium was used as internal standard (ISTD) for all the experiments at a concentration level of  $75\ \mu\text{g mL}^{-1}$ . Nitric acid 0.8M was used to prepared the standards (Optima grade, Fisher Scientific, USA). NIST 2783 filter (Air Particulate on Filter Media, National Institute of Standards and Technology, USA) was evaluated as a control standard.

#### 4.3.3. Sample Preparation for LA-ICP-MS Analysis

A small piece of  $\sim 2\ \text{cm}$  by  $2\ \text{cm}$  was carefully cut from the sampling filter and fixed into a glass slide with tape. Samples were then stored in clean pill boxes. In order to minimize contamination, filters were cut with plastic scissors, manipulated with plastic tweezers under a clean hood (Air-clean systems, AC600, USA) and gloves were used at all times.

#### 4.3.4. Qualitative and Quantitative Strategies for Laser Ablation

All samples were acquired in transient mode (intensity vs. time) with a 25 second gas blank (helium gas through the ablation cell) followed by at least 120 seconds of

ablation of the corresponding sample and then a 30 second of blank to purge the ablation cell. Each sample was placed separately on the ablation cell to avoid any cross contamination. Data was acquired qualitatively and quantitatively.

All laser ablation data acquired was processed with GEOPRO software (1999, v 1.0, CETAC, USA). For the quantitative approach, the following calibration strategies were conducted:

#### 4.3.4.1. Drop Method using Addition of Single Element Spikes

Standard addition experiments were conducted on versapor filters. A 20 $\mu$ L spike of single-element standards were added to the filter at different concentration levels and let dry overnight. Rhodium was used as internal standard.

The “blank” control consisted of a spike of 20  $\mu$ L of a solution containing nitric acid 0.8M and the internal standard only.

#### 4.3.4.2. Drop Method using Addition of Multi- Element Spikes

The same method reported above was used but using multi-element standards composed of 3 or 6 elements respectively, plus rhodium as internal standard.

The calibration curve was composed of 5 data points ranging from 0-300  $\mu\text{g g}^{-1}$ .

#### 4.3.5. Method Optimization for Digestion ICP-MS of African Dust Filters

##### 4.3.5.1. Instrumentation and Measurement Parameters

Acid digestates of the filters were measured by ICP-MS using an ELAN DRCII (Perkin Elmer LAS, Shelton CT USA). The digestion method for filters was conducted on a hot block digestion system (Environmental Express, SC, USA).

#### 4.3.5.2. Reagents and Standards

All solutions were prepared using high purity water ( $\geq 18 \text{ M}\Omega\text{cm}^{-1}$ ) obtained with a Millipore direct Q VV3 system (Millipore, MA, USA). Trace elemental grade (optima grade) nitric acid and hydrogen peroxide (Fisher, Pittsburg, PA, USA) were used throughout the analysis. Two standard mixtures of elements in concentration of  $1000 \mu\text{g mL}^{-1}$  from AccuStandard (CT, USA) were used to prepare element calibration solutions for the following monitored isotopes:  $^9\text{Be}$ ,  $^{27}\text{Al}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{57}\text{Fe}$ ,  $^{60}\text{Ni}$ ,  $^{63}\text{Cu}$ ,  $^{66}\text{Zn}$ ,  $^{75}\text{As}$ ,  $^{82}\text{Se}$ ,  $^{88}\text{Sr}$ ,  $^{103}\text{Rh}$ ,  $^{107}\text{Ag}$ ,  $^{111}\text{Cd}$ ,  $^{117,118,120}\text{Sn}$ ,  $^{121,123}\text{Sb}$ ,  $^{137}\text{Ba}$ ,  $^{206,207,208}\text{Pb}$ . A standard solution of  $10 \mu\text{g mL}^{-1}$  for Rhodium from AccuStandard (CT, USA) was used as an internal standard at a measurement concentration of 50 ppb in the calibration curve.

#### 4.3.5.3. Sample Preparation for Digestion ICP-MS analysis

A five- point calibration curve ranging from 0 to 25 ppm was prepared to spike the filter. Approximately 0.25g of each filter was weighted and a  $50\mu\text{L}$  spike of each of the calibration solutions was added into the filter. The drop was dried overnight at room temperature and then the filter was digested. For the measurement of the samples, the exposed filters were spiked with  $50 \mu\text{L}$  of a solution containing the internal standard.

An aliquot of 5 mL (1+1) nitric acid trace metal grade was added to the vessel and cover with a watch glass and heated at  $95^\circ\text{C}$  for 15 minutes without boiling. Once the sample was cool, concentrated nitric acid (trace metal grade) was added and the solution was refluxed for 30 minutes. This procedure was repeated until no brown fumes are given off the sample. The sample was heated for an additional 1.5 hours avoiding boiling the sample or taking the sample to dryness. After this period of time, the sample was cooled

to room temperature. 2-5 mL of water (to lessen effervescence) and 0.5 mL of 30 % H<sub>2</sub>O<sub>2</sub> was added and the sample was placed in the hot block. The H<sub>2</sub>O<sub>2</sub> was continuously added until the sample color remained unchanged while heating for a total of 2 hours. Samples were then diluted to 50 mL with DDI water and a Filtermate® (Environmental Express, SC, USA) was used for filtration. After dilution, spiked levels ranged from 0 to 90 ppb.

Digestion blanks, laboratory blanks, and procedural blanks were also used to assess the introduction of contaminants during the digestion of the samples. In order to determine the ruggedness of the analytical method, fortified laboratory blanks (LBS) were analyzed with every set of samples analyzed. The LBS is made of filter spiked with a mixture of the elements of interest in the digestion vessel. The LBS is then processed through the whole analytical procedure. To assess precision, accuracy and recoveries, matrix spikes (MS) and duplicates (MSD) were analyzed with each batch of filter samples. MS consisted of the use of fortified filters, spiked with a mixture of the elements of interest.

External calibration curves ranging from 0 to 100 µg L<sup>-1</sup> was used to measure the digestates on the ICP-MS.

#### 4.3.6. Sample Collection

A super-high volume collector system (SHVC, Veridian Pacific Sierra Research ) provided by the United States Geological Survey (USGS) was used to collect the dust samples in a near shore location along the Port of Fort Lauderdale inlet located at 26°5'32.90N, 80°6'29.88W (see Figure 4.2). The sampler is housed in a stainless steel

shelter inside of a trailer. A PVC pipe of approximate 4 meters above the surface of the trailer is extended.

The sampler consists of a two stage centrifugal blower powered by a brushless, variable speed, maintenance free motor. The speed of the motor is controlled by a programmable logic controller (PLC) that accepts an input from a mass flow sensor mounted in the sample air flow stream. The PLC detects changes in the operator's pre-set flow rate due to changes in temperature, barometric pressure and pressure drop due to dust loading on filter media. It compensates for these changes by adjusting the motor speed to maintain the pre-set flow rate. Filters of 25x25 inches are manually loaded into the sampler. The versapor filters were cut from the 100 LF roll to fit the dimensions of the dust collection system. All filters were weighed to  $\pm 0.2500$  g before and after deployment to calculate the mass of dust collected.

In order to prevent contamination, filters were manipulated wearing gloves and regular cleaning and vacuuming of key components of the machine was conducted before installation of the new filter.

Prior to use, each filter was exposed to a light source and inspect for pinholes, particles and other imperfections. Filters were transported from and to the laboratory in large Ziploc bags. The sampler keeps a record of the volume of air sampled so that concentrations can be translated from ng/g of material to  $\text{ng/m}^3$  of air. Samples were collected at least on a monthly basis since September 2005 and for the entire year 2006. Table 4.1 shows the sampling dates of the filters collected at Port Everglades Station. Figure 4.3 shows a picture of the filter before and after collection of the dust, which reflects the loading of particulates by visual change of color.



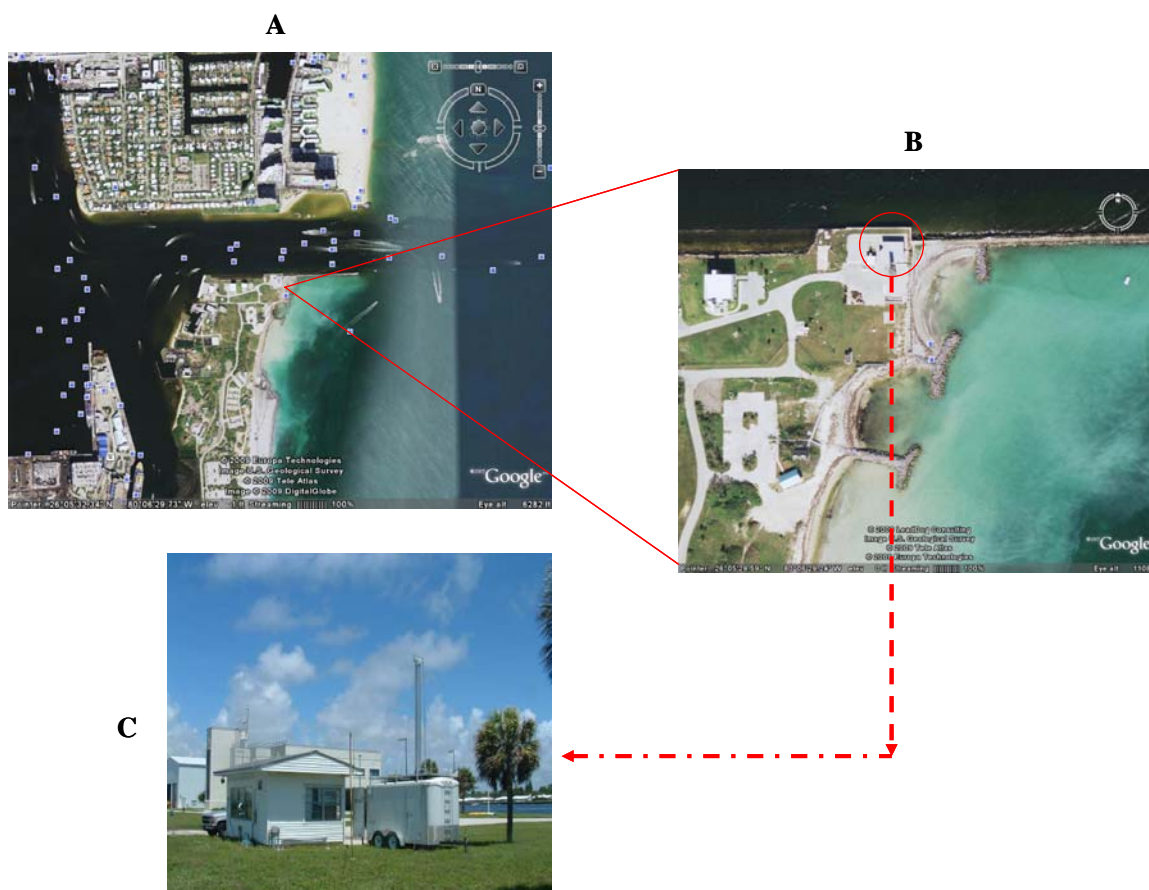
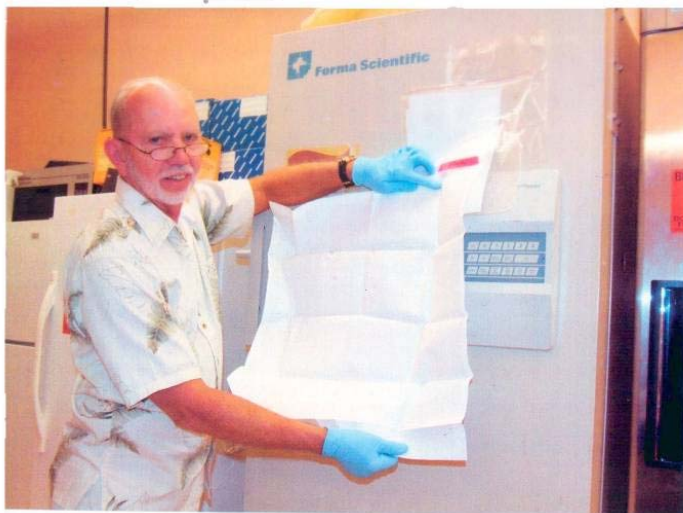


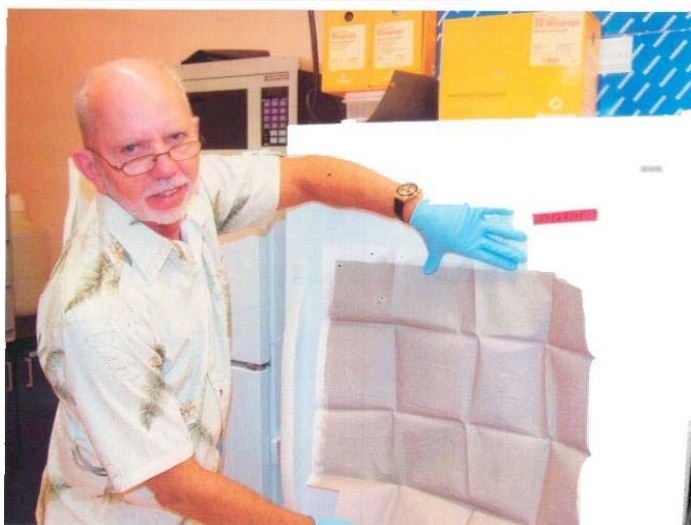
Figure 4.2. Description of sampling area for the collection of airborne particulate.  
 A) Port Everglades inlet; B) Location of the trailer at  $26^{\circ}5'32.90\text{N}$ ,  $80^{\circ}6'29.88\text{W}$ ;  
 C) Trailer with the sampler

Table 4.1 Sampling dates for African Dust collection on filters at Port Everglades Station during 2006

Sample ID	Starting Date	Ending Date	Total Volume of air filtered, m <sup>3</sup>	Dust Weight (g)
<b>Summer Season</b>				
5/7/2006	5/7/2006	5/25/2006	85,746	0.5091
5/25/2006	5/25/2006	6/8/2006	127,319	1.7392
6/8/2006	6/8/2006	6/20/2006	165,197	0.464
6/20/2006	6/20/2006	7/6/2006	189,344	0.1952
6/30/2006	6/30/2006	7/12/2006	205,971	0.017
7/12/2006	7/12/2006	7/23/2006	238,959	0.0716
7/23/2006	7/23/2006	8/5/2006	279,141	0.4438
8/5/2006	8/5/2006	8/13/2006	300,942	0.1258
8/13/2006	8/13/2006	8/25/2006	334,128	0.2232
8/25/2006	8/25/2006	10/10/2006	441,208	0.6417
10/11/2006	10/11/2006	10/24/2006	491,175	0.2221
<b>Winter and Spring Season</b>				
1/18/2006	1/18/2006	1/25/2006	328,713	0.265
1/25/2006	1/25/2006	2/6/2006	361,498	0.5565
2/6/2006	2/6/2006	2/10/2006	372,527	0.0483
2/10/2006	2/10/2006	2/15/2006	386,405	0.0717
2/15/2006	2/15/2006	2/22/2006	405,840	0.2513
2/22/2006	2/22/2006	3/1/2006	425,673	0.2641
3/1/2006	3/1/2006	3/8/2006	445,131	0.6408
3/8/2006	3/8/2006	3/16/2006	466,983	0.3688
3/16/2006	3/16/2006	3/30/2006	510,803	0.3711
3/30/2006	3/30/2006	4/17/2006	550,592	0.4619
4/17/2006	4/17/2006	4/24/2006	588,797	0.3671
4/24/2006	4/24/2006	5/1/2006	29,857	0.4336



**A**



**B**

Figure 4.3. Picture of the filter before (A) and after (B) collection of particulate

#### 4.4. Results and Discussion

Laser ablation ICP-MS is a versatile analytical technique that has been used in our research group as alternative mean of getting inorganic profiles in solid matrices including soils and sediments in a quick and straightforward way (Arroyo et al., 2009). Most of the inherent advantages of this technique such as ease of analysis, low sample consumption, reduced processing times; excellent precision and accuracy make it ideal for the analysis of particulate matter in filters. The advantages of using laser ablation for these types of matrices were extensively discussed in chapter 3.

##### 4.4.1. Method Development and Optimization

Laser ablation methods are matrix dependent because their analytical performance depends on the efficiency of removal of particles from the sample substrate, which is a consequence of the laser to sample interaction.

The development of a laser ablation method for airborne particulate is less straightforward than the analysis of bulk soils or sediments previously described. There are different aspects that should be considered for the development and optimization of a laser ablation method for particulate matter deposited in an inert surface, for example:

###### 4.4.1.1. Size of the Sample:

Both sample size and particulate size requirements should be appropriate for micro-sampling with laser ablation. In ambient air dust, particles size range from a few nanometer to hundreds of micrometers ( $\mu\text{m}$ ). In this study, the amount of dust typically collected in the sampling filter was in the order of few milligrams per filter (for a

monthly collection). From these filters, laser ablation is conducted on areas of few millimeters square and therefore the total amount of mass removed is fairly small. As an example, for a filter sample collected on 06/08/06, the total mass of dust collected for a period of 12 days (from 06/08/06 to 06/20/06) was 0.4640 g (See Table 4.1). The total volume of air filtered during that period was 165,197m<sup>3</sup>. The dusted area on that filter corresponds to 3118 cm<sup>2</sup> while the typical ablated area corresponds to 3.3 x 10<sup>4</sup> μm<sup>2</sup>. Assuming the dust was deposited homogeneously within the filter, the ablated area will correspond to a mass of dust of approximately 50 ng.

On the other hand, the particle size of the dust deposited on the filter is usually smaller than the “spot” size of the ablation mode in order to account for heterogeneity on the sample and signal stability on the laser ablation measurements.

#### 4.4.1.2. Manipulation of the Sample:

Minimal manipulation of the filters is also a key factor in the analysis of dust. In the case of soils and sediments, the nature and amount of sample allows for manipulation of the sample as a powder. Micro-milling produces very homogeneous pellets that facilitate the laser-sample interaction. Airborne particulates, however, get trapped between the substrate (filter) adding not only less flexibility to the sample manipulation but also complexity because now the filter becomes an integral part of the sample. For this reason, different types of filters were considered prior to the collection of the dust as part of the optimization of the method.

Since contamination of the filter before and after collection of the dust becomes critical for dust particulates. In order to avoid cross contamination, the filters were always

manipulated with gloves and stored in plastic bags. In order to transport the filters to the laboratory, they were bent inwards avoiding the direct touching of “dusted” areas.

#### 4.4.1.3. Sample Micro-Homogeneity

As with any other matrix, homogeneity at a micro-scale must be considered to assure representative sampling of the bulk elemental composition. Since the airborne particulate can not be homogenized as in the case of soils, larger areas must be sampled to overcome this limitation.

#### 4.4.1.4. Matrix Match Standards:

The availability of matrix match standards is of great utility for the development of laser ablation-based quantification strategies. As discussed before, there are reference standard materials available for soils and sediments that facilitate the quantification by LA-ICP-MS. On the other hand, there are fewer airborne particulate standards like NIST 1648 A and NIST 2783, and they are not designed for micro-sampling techniques such as laser ablation and/or for a large suite of elements at trace levels. More details about matching standards are discussed later in this chapter.

#### 4.4.1.5. Selection of the Filter Substrate

Two types of filter materials were evaluated, a) Glass micro fiber and b) Versapor® polymeric filter. The filters were evaluated in terms of background concentration for the elements of interest, proper interaction of the laser with the medium, depth penetration and reproducibility of results from laser ablation experiments.

In addition, the filters were also selected with the purpose of using them in the future for the analysis of organic materials, so resistance to organic solvents for extraction purposes was also considered.

Glass Micro Fibre filters are suitable for this analysis because they have low resistance to air flow, low affinity for moisture and high collection efficiency for particles of 0.3 microns or larger size (99%, as reported by the manufacturer).

The Versapor® membrane is an acrylic copolymer membrane cast on a non-woven nylon support. Advantages of this filter such as non-fiber shedding, low background of metals, durability and water resistance, all these characteristics plus cost were considered for the final selection of this filter as the sampling substrate.

Figure 4.4 shows SEM images of the morphology of both filters at different magnifications with dust deposits and with laser ablation tracks. The ablation lines on these imaged filters were conducted at high energy power (50%) and therefore they went through the filter layer. Dust particles are more easily observed on the polymeric filter than in the glass filter since the latter are visible trapped within the glass fibers.

Polymeric filters showed more stable transient signals than glass filters (see Figure 4.5), which may be a consequence of a more reproducible ablation pattern as shown in the SEM image of the surface of both filters. The transient signal is a consequence of the amount of material (dust and filter) transported to the ICP over time. The stability of the signal is therefore evaluated as the signal variation over time. The polymeric filter produced a “plateau” pattern during the ablation process while the glass filter produced a rather irregular signal with larger fluctuations over time. Based on previous experience with other matrices, signal stability is directly related to precision of

the measurements, thus an advantage for the polymeric filters.

Regardless of the unstable signal obtained by the glass fiber, they did provide the natural advantage of having a constant concentration of a major element such as silicon and therefore,  $^{29}\text{Si}$ , could be used as an internal standard to reduce variability. Nevertheless, in terms of reproducibility between samples, polymeric filters were equally viable but far less expensive.

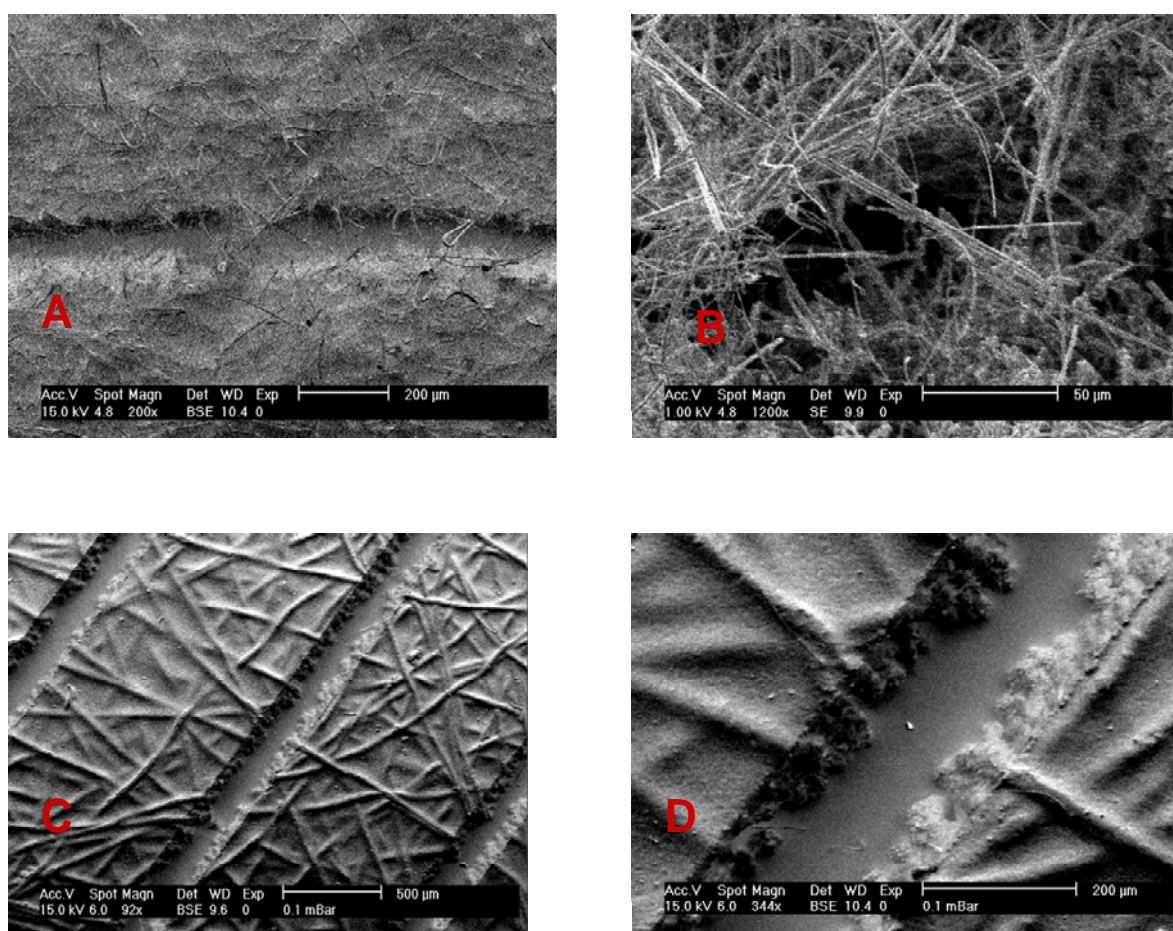


Figure 4.4. SEM images of the morphology of the surface of the filters and laser interaction with the glass type filters (A, B) and the polymer type filters (C, D). Images taken at 100X (left) and 1200X (right).



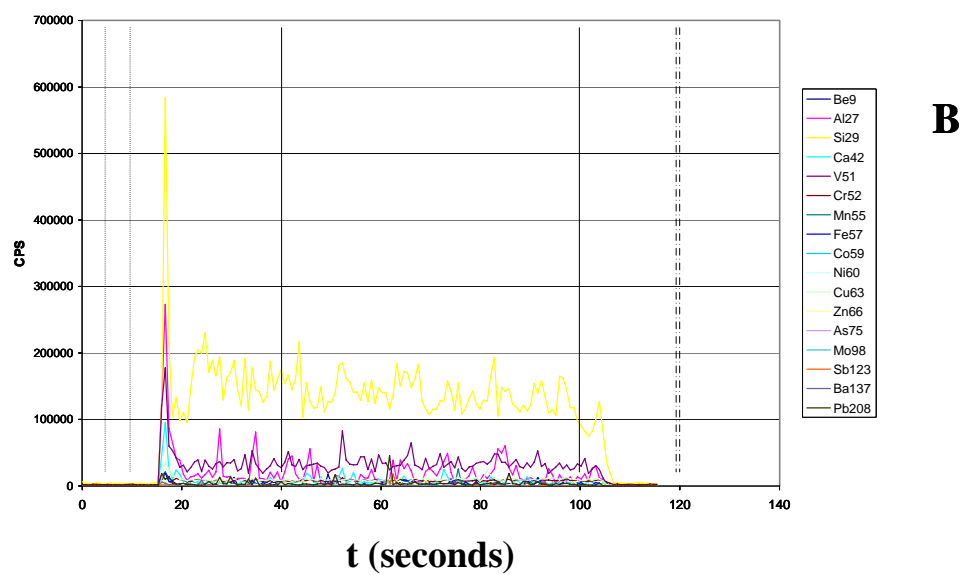
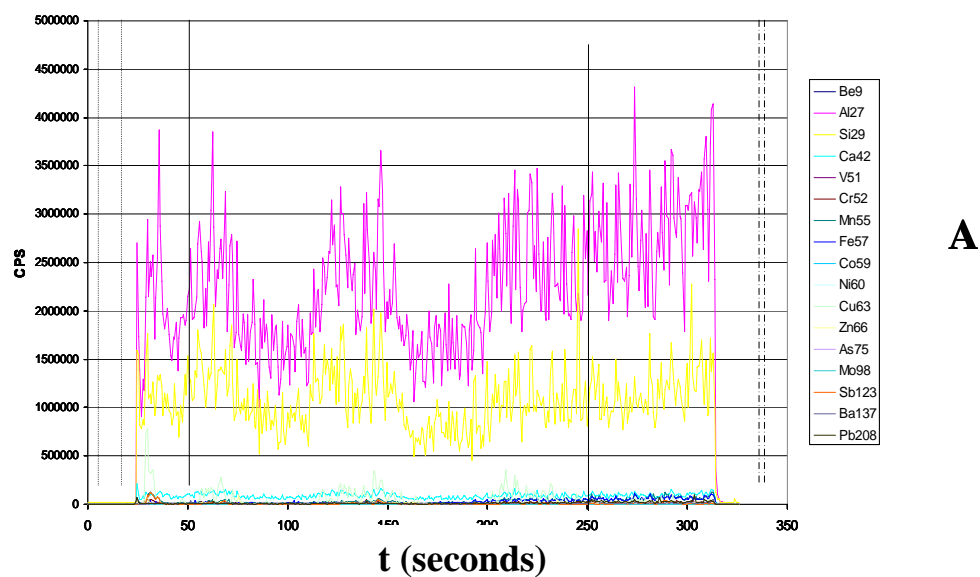


Figure 4.5. Laser ablation signal for an exposed on (A) glass fiber filter and (B) Versapor filter

#### 4.5. Optimization of Ablation Parameters

Two ablation modes: single line and raster (multiple lines in continuous mode) were studied. Spot mode was not even considered because the deposition of the airborne particulate is quite superficial leading not only to smaller sampling areas but also more interference from the filter as the laser penetrates into the substrate. The raster method was found to be the best approach for ablation of the Versapor filter. A total area of  $\sim 3.3 \times 10^{-4} \mu\text{m}^2$  was selected in order to speed up the experiment time and allow the possibility of replicates, without sacrificing the precision. The energy power of the laser was selected in order to optimize the amount of dust removed from the filter while minimizing the damage into the filter's surface. Figure 4.6 shows an SEM image of the laser interaction with the versapor filter at different energies. The optimum value of 35 % energy was able to penetrate the dust layers and the surface of the filter without going through the material completely. Figure 4.7 shows the surface damage at higher magnification as well as the morphology of particles deposited in the filter's surface.

Laser frequency was also studied at 5, 10 and 20 Hz. Ablation at 5 Hz did not produce sufficient removal of particles and therefore the ablation signal was poor and noisy. On the other hand, 20Hz created a more pronounced damage into the filter surface, thus rendering impractical. A frequency at 10 Hz provided the best results.

Scan rate was varied from 5 $\mu\text{m/s}$  to 20 $\mu\text{m/s}$  at 5 $\mu\text{m/s}$  intervals. Optimum signal and minimum damage of the filter was achieved at 15 $\mu\text{m/s}$ .

Spot sizes were measured at 50, 100 and 200 $\mu\text{m}$ , the best S/N results and signal reproducibility was achieved at a 100 $\mu\text{m}$  spot size. A summary of the optimized laser parameters is presented in Table 4.2.

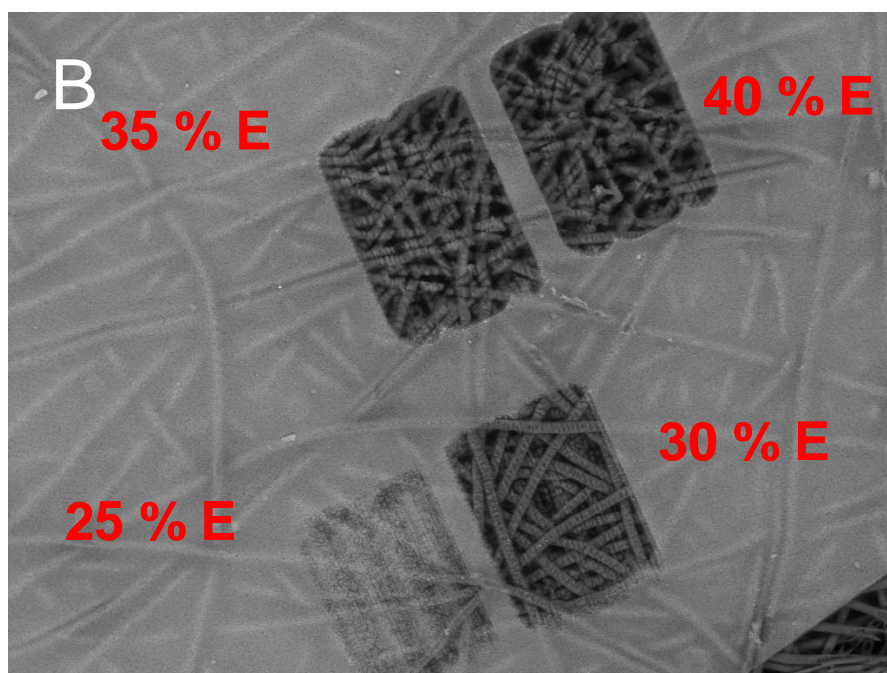
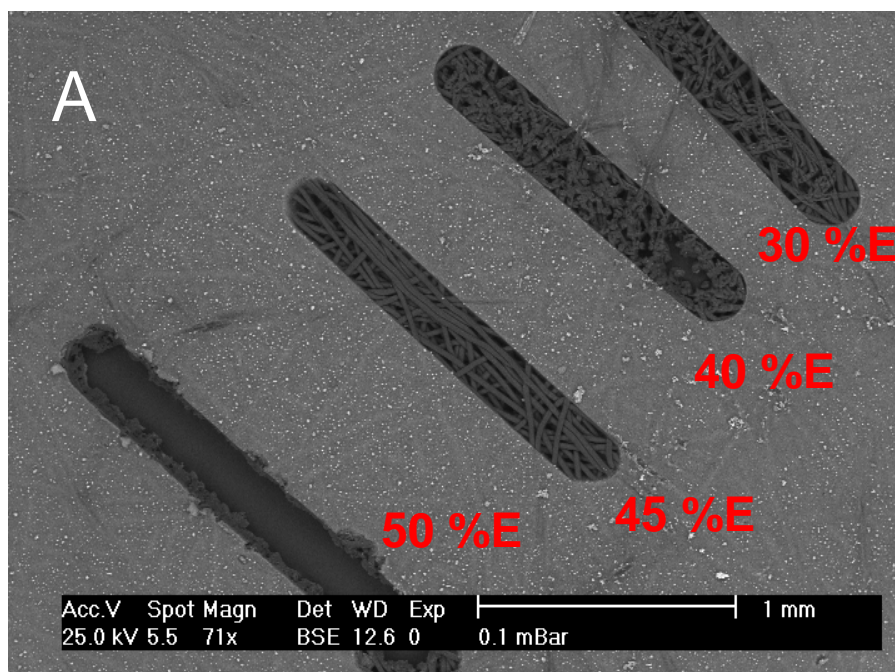


Figure 4.6. Laser energy optimization on Versapor Filter : a) using the single line mode at different energy levels and b) using the raster mode at different energy levels.

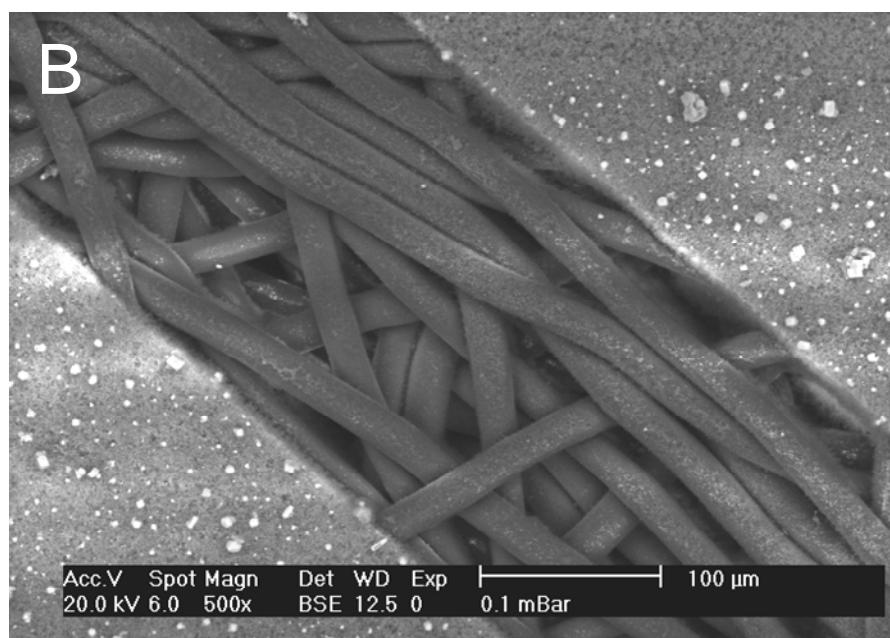
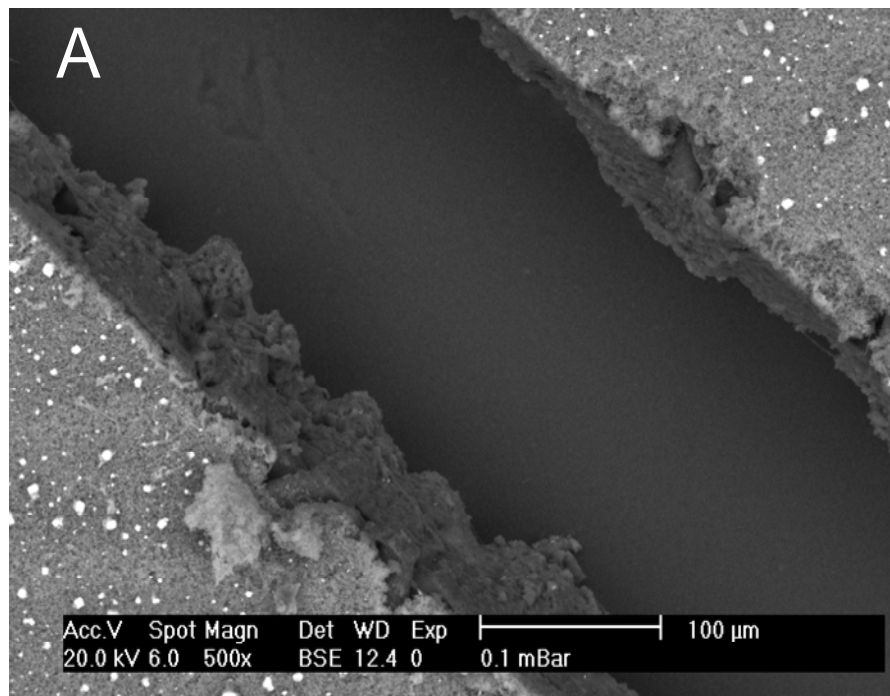


Figure 4.7. Detail of filter damage and particle deposition on Versapor filter. Ablation conducted at 50% (A) and 35% energy (B).

Table 4.2. Optimum New Wave 213 laser parameters for the ablation of Versapor Filters

Parameter	Value
Spot size	100 $\mu\text{m}$
Power	35% E ( $0.11 \text{ J/cm}^2$ )
Repetition rate	10Hz
Ar flow after the cell	$\sim 0.9 \text{ L/min}$
He flow through cell	$\sim 0.9 \text{ L/min}$
Time of ablation	90 s ( $\sim$ distance 1800 $\mu\text{m}$ lines)
Scan Rate	15 $\mu\text{m/s}$

#### 4.6. Mass removal during laser ablation

In order to estimate how much mass is removed during the ablation of the filter; a piezoelectric balance was connected to the laser ablation units working at the optimum conditions used for the collection of the ablation signal.

Table 4.3 shows the mass removed on six independent ablations on the Versapor filter containing deposited dust. The amount of material removed is in the order of  $\sim 300\text{ng}$ . Although maximum dust removal and minimum filter removal was optimized on the method, it is impossible to remove “only” dust particles. Therefore, only a percentage of this mass corresponds to airborne material removed during the ablation process. Because of that, further studies need to be conducted to determine whether or not these micro-amounts are representative of the overall filter.

Table 4.3. Mass of airborne particulate and filter removed during the ablation experiment.

<b>Replicate</b>	<b>Mass measured (mg/m<sup>3</sup>)</b>	<b>Total mass removed (ng)</b>
1	0.25	270
2	0.28	302
3	0.25	270
4	0.24	259
5	0.22	238
6	0.25	270
<b>mean</b>	<b>0.25</b>	<b>270</b>
Standard deviation	0.02	21
%RSD	8	8

#### 4.7. Analysis of real samples by LA-ICP-MS: Qualitative Approach

Qualitative evaluation of filters was conducted after optimization of the laser ablation method. Two pieces of ~2x2 cm were cut with plastic scissors from each of the original filter. One of the pieces was taken from the unexposed area of the filter and was measured as a “blank”. The other piece was removed from the area that has a visual deposit of dust. Each blank and sample pair was analyzed in triplicate (one piece of filter, three individual ablation rasters).

Ablations were conducted away from the edges of the piece to avoid any undesirable contamination from the scissors or from the sample manipulation.

Data reduction was conducted using the Geopro data handling software (CETAC Technologies, v 1.0 ). Each signal was integrated for 60 seconds. Background values of the blank were subtracted from the sample signal. Since the analysis conducted are only qualitative, the signal to noise ratio (S/N) was defined and calculated for each of the elements by the following equation:

$$S/N = (\text{intensity}_{\text{sample}} - \text{intensity}_{\text{blank}}) / \text{std dev}_{\text{blank}}$$

Only elements present at S/N ratios above 10 and with a relative standard deviation below 25% were considered as “present”. Elements with ratios between 10 and 20 were close the method limit of detection, while elements with an S/N above 20 were present at higher values. By assessing these presence/absence ratios we were able to establish the qualitative distribution of elements in the filters (Figure 4.8) during one year period and to narrow down the elemental menu of interest. This qualitative approach can be employed as a quick screening tool to monitor sudden inputs of target metals. The main advantage of the qualitative screening relies on the fast sample preparation, measurement and analysis of the filters. However, for this approach to be effective, further studies should be conducted to identify which are the key elements of interest that may be directly associated to African dust.

#### 4.8. Quantitative Determination

One of the main objectives of this project was to develop a quantitative approach that will allow for the determination of the elements by using either an external standard or the creation of “in filter” standardization. Standard reference materials NIST 2783 filter was tested during the method optimization as an alternative for quantification of

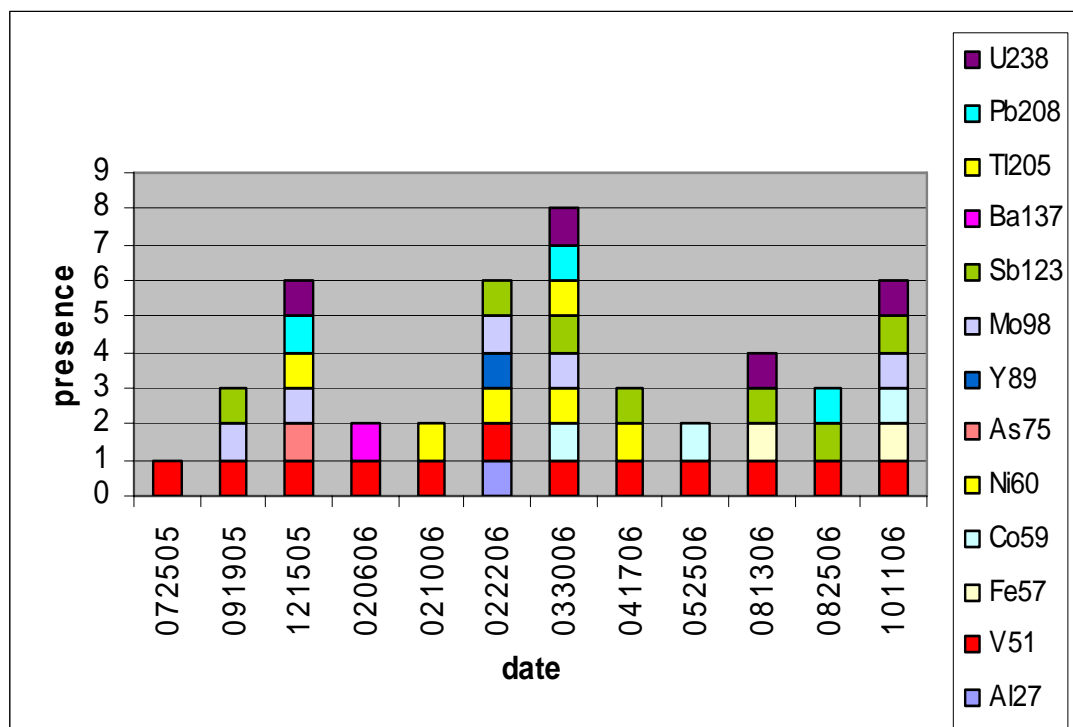


Figure 4.8. Distribution of elements by date, only elements showed on the graph were present on the samples. Measurements were conducted on Versapor filter, at optimum ablation conditions.

dust particulate, but it was not practical because the properties of the filter required ablation parameters very different from the optimum parameters found for the Versapor sampling filters. The laser produced partial melting of this standard even at low energy power, resulting impractical for LA-ICP-MS measurements.

Several quantitation strategies were developed to obtain quantitative information of the airborne trace elements on the versapor filters:

- Addition of Single-element and Multi-Element spikes using internal standard addition (Rhodium, Scandium) to the filter (Drop Method)
- Single point calibration (Drop Method)



- Multiple-point calibration (calibration Curve in Drop Method)
- Hot Block Digestion of the Filters (Acid Solution “No Laser Method”)

#### 4.9. Development of In House Standards (Drop)

The development of a matrix match standard was conducted by exploiting the hydrophobic characteristics of the Versapor Filter. Small pieces ( $2 \times 2 \text{ cm}^2$ ) were cut and affixed to a cover glass. A 20  $\mu\text{L}$  volume of a liquid spiked of either single element or a mixture of elements was added in different areas of the surface of the Versapor filter and the required drying time at atmospheric temperature and pressure was determined. The dry spot (smear) on the surface to the material should be representative of the deposition of the dust particles on the surface of the filter.

The estimation of the mass (ng) spiked to the filter was achieved by using the volume of the solution and the area of the drops. Figure 4.9 shows an SEM image of a typical dried drop deposited on the surface of the filter and the raster patterns performed with increasing energies of the laser. The image clearly shows a smooth distribution of the targeting element in most parts of the drop. This allowed enough space to obtain replicate values of this standard.

The total mass loading of the drop was estimated by multiplying the concentration of the analyte in the drop by the total volume of the drop. Since the drop is dried on the surface and due to the hydrophobic nature of the filter, it was assumed that all the mass is dried “on” the surface and does not goes “inside” the filter. Ablations are conducted only in a small portion of the dried drop ( $\sim 0.25\%$  of the area), therefore a ratio of the total area of the drop (assuming circular shape) and the total area ablated (calculated from the laser

ablation parameters) is used to estimate the total mass of each analyte ablated from spiked the filter. Microscopic images and SEM images were used to corroborate the estimated areas.

By using the same strategy a multi-element calibration curve was prepared for lead, antimony and barium. Figures 4.10 and 4.11 show the calibration curves for both single element and multi-element drop depositions. As could be seen by the figures, the calibration strategy for both single elements or for 3-element mixtures was very promising and reproducible. Nevertheless when the multi-element solution was increased from 3 to 6 elements or more, the drying of the drops became very irreproducible. This behavior was observed for different combinations of elemental mixtures and at different levels of fortification (10, 30, 50, 75, 100, 150, 250 and 300  $\mu\text{g mL}^{-1}$ ).

Figures 4.12 to 4.14; show pictures of the morphology of the drops spiked on filter, at different drying stages. It is clearly observed that the uniformity of the dried drop suffers significantly when more than 3 elements are added to the spiked solution. The morphology of the dried drop varies not only between replicates of equivalent concentration but also between spikes present at different concentration levels. The lack of uniformity on the drops was also associated to heterogeneity within the drop since different gradient concentrations were observed at different locations in the dried drop.

Different attempts to correct this phenomenon were conducted, for example adding surfactants and organic solvents, decreasing the concentration level and changing the drying conditions. However, no significant improving in the 6-element spike was achieved, limiting these in house standards to 3 element component which may not be practical for routine applications.

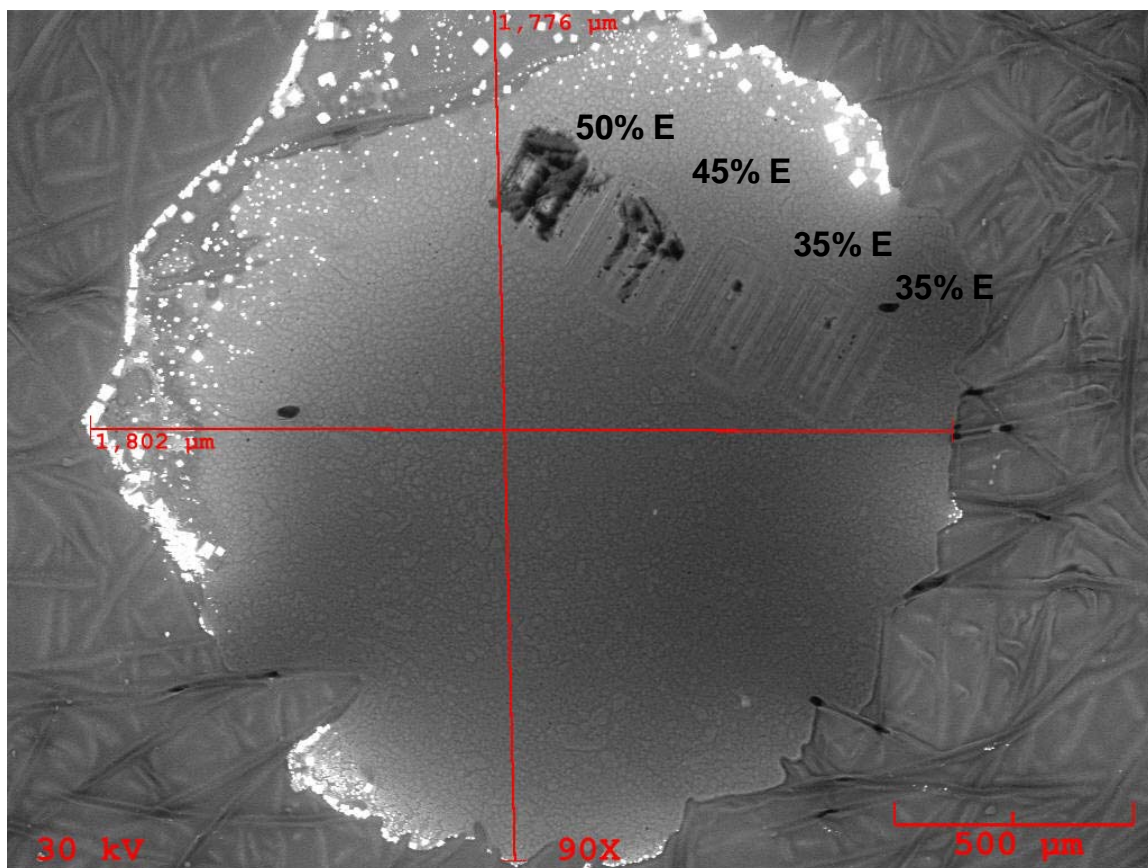


Figure 4.9. SEM image of the shape and morphology of a dry spiked- drop on the surface of the Versapor filter. Patterns for laser ablation rasters conducted at 50%, 45% and 35% energy are also imaged at the top-right side of the image.

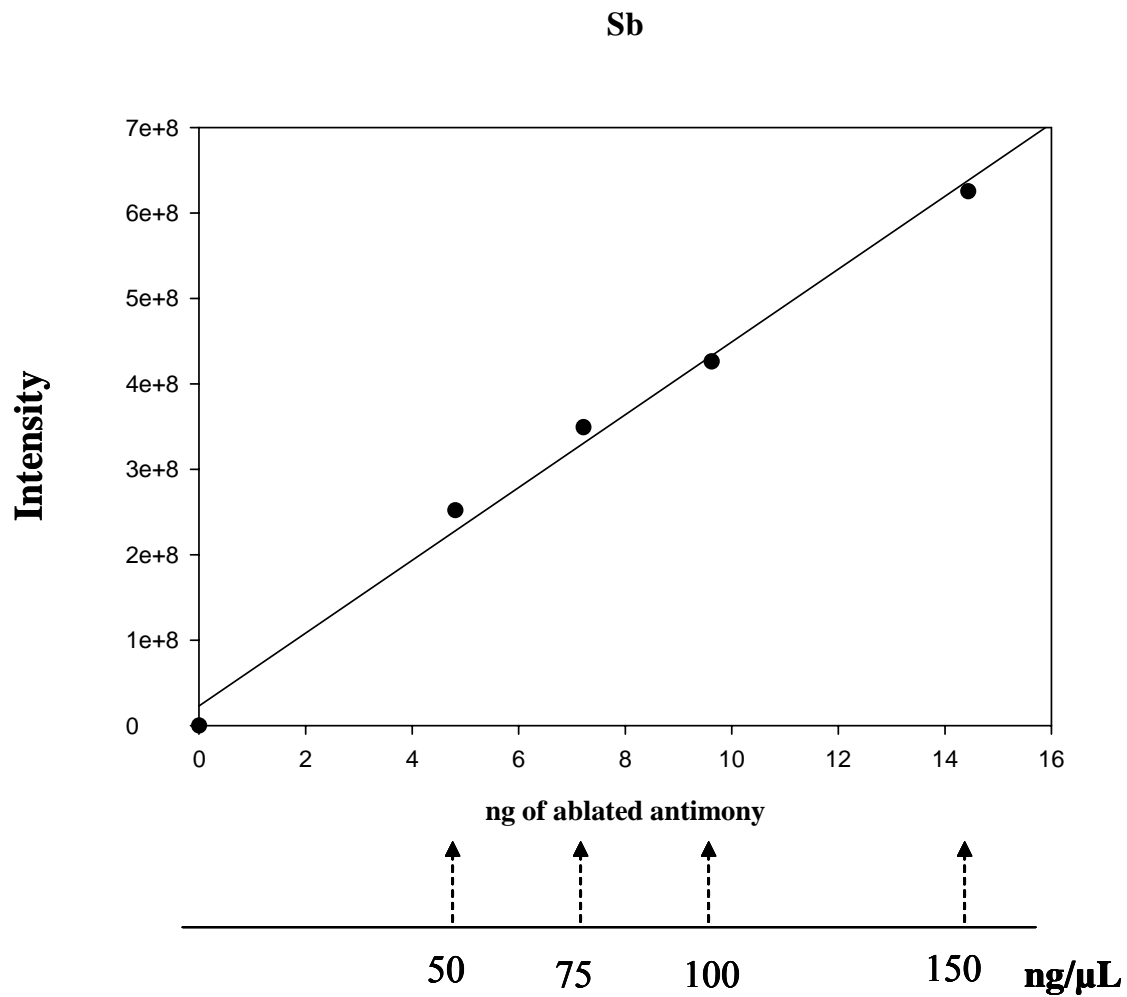


Figure 4.10. In-house filter calibration curve for antimony using the drop technique

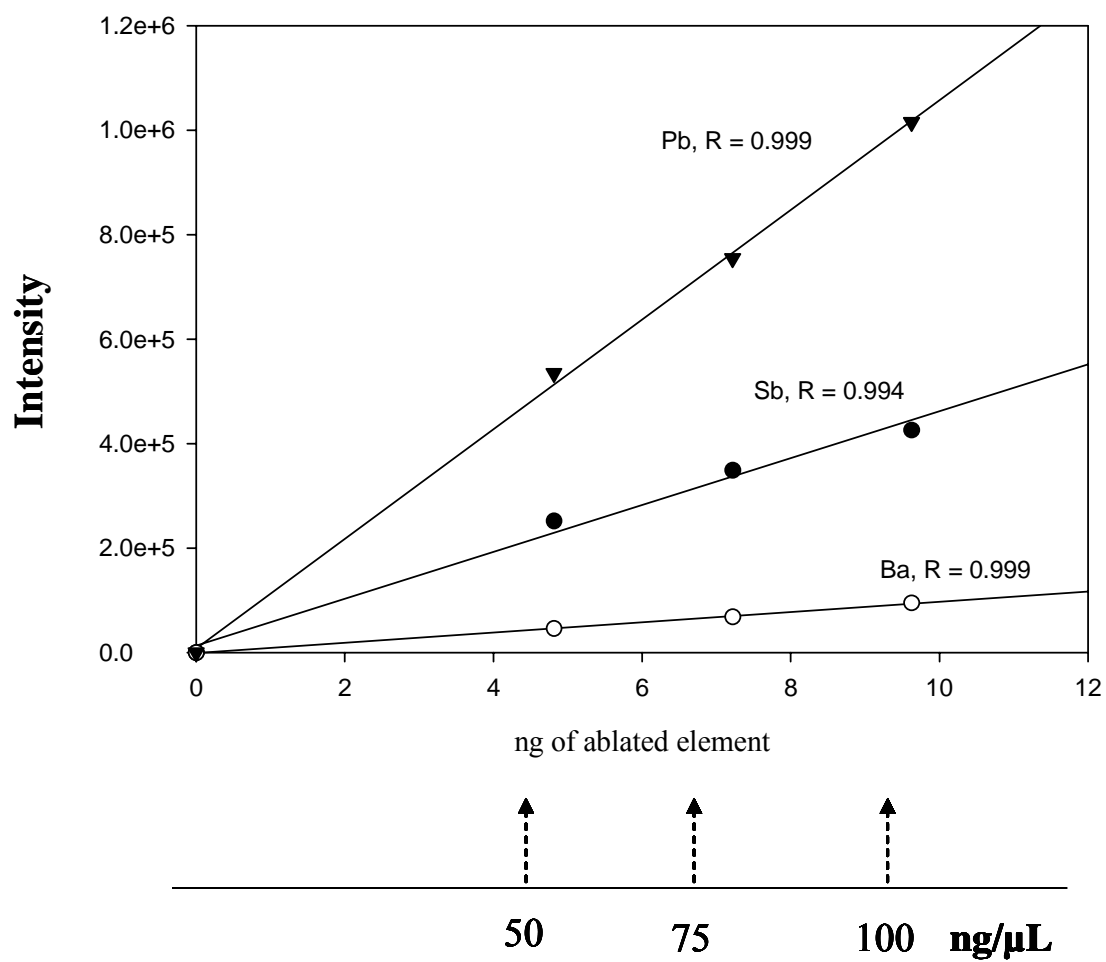


Figure 4.11. In-filter calibration curve for lead, antimony and barium using the drop technique

#### 4.10. Digestion of Filters by Hot Block Digestion (No Laser Method)

A digestion method was developed and optimized as an alternative to quantify the trace elements in the versapor filter. A higher volume than the one used in the drop method (50  $\mu\text{L}$  vs. 20  $\mu\text{L}$ ) was applied to small pieces of versapor filters, and air dried before the digestion process. The digestion protocol involves the use of  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  and common QA/QC samples for elemental analysis were used, such as filter blank (VB), laboratory blank spikes (LBS) and matrix spikes (MS). Nineteen different elements were included in the spike mixture and as shown in Table 3.4 an overall average recovery of 89% and 98% was obtained for the lowest and highest spike levels tested.

Table 4.4 present the values obtained for un-exposed digested versapor filters spiked with 19 elements at two different levels: 50 ppb for the laboratory blank spike (LBS) and 90 ppb for the quality control sample (QC). This quality control sample corresponds to a suite of elements coming from a different vendor and spiked on the surface of the versapor filter. Versapor blanks (VPB) represent concentration values at ng/ml measured for the digested blank filters without any spike.

The difference between the mean concentration of the LBS and the versapor filter blank values are presented as the “LBS mean – VPB” in the Table 3.4. Also the “QCmean – VPB”, represents the mean values obtained for the quality control sample after subtraction of the values from the versapor blank.

Table 4.4 indicates acceptable recovery results and precision were obtained for laboratory blank spikes and quality control samples spiked on Versapor filter with the exception of silver. Elements such as aluminum, copper and iron showed higher background levels in the versapor filter blanks (VPB) ranging from  $\sim 8 \mu\text{g L}^{-1}$  for iron up

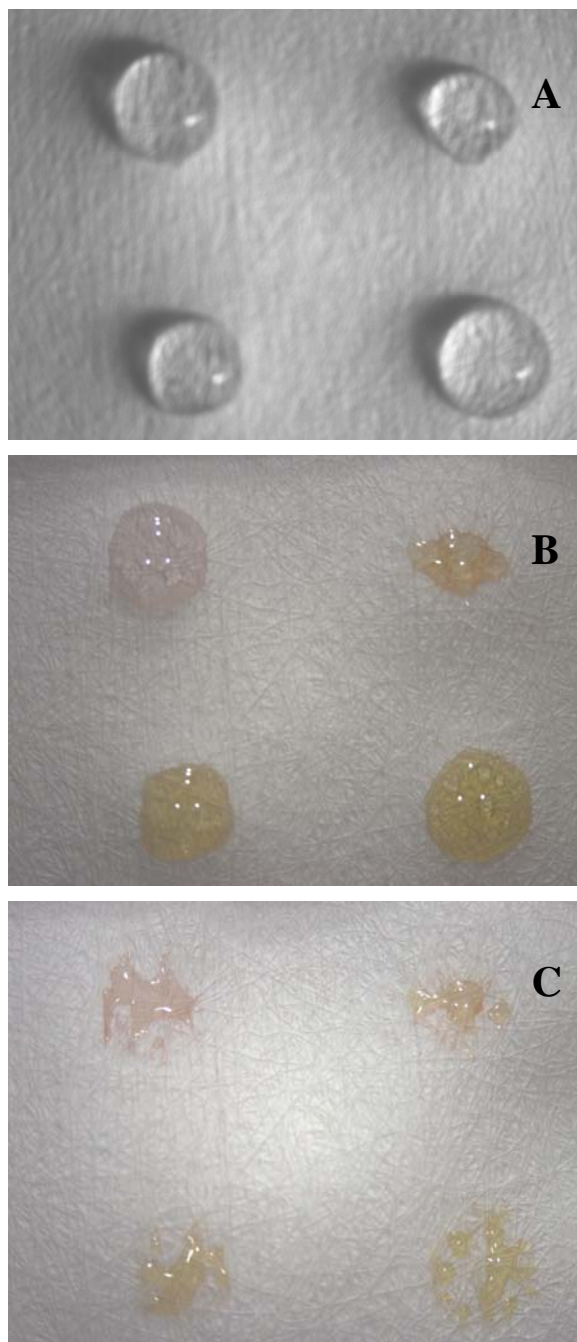


Figure 4.12. Pictures of the drying process of 20µL drops of a 6-multielement solution at A) 30 minutes, B) 4.5 hours and C) 7 hours after spike.

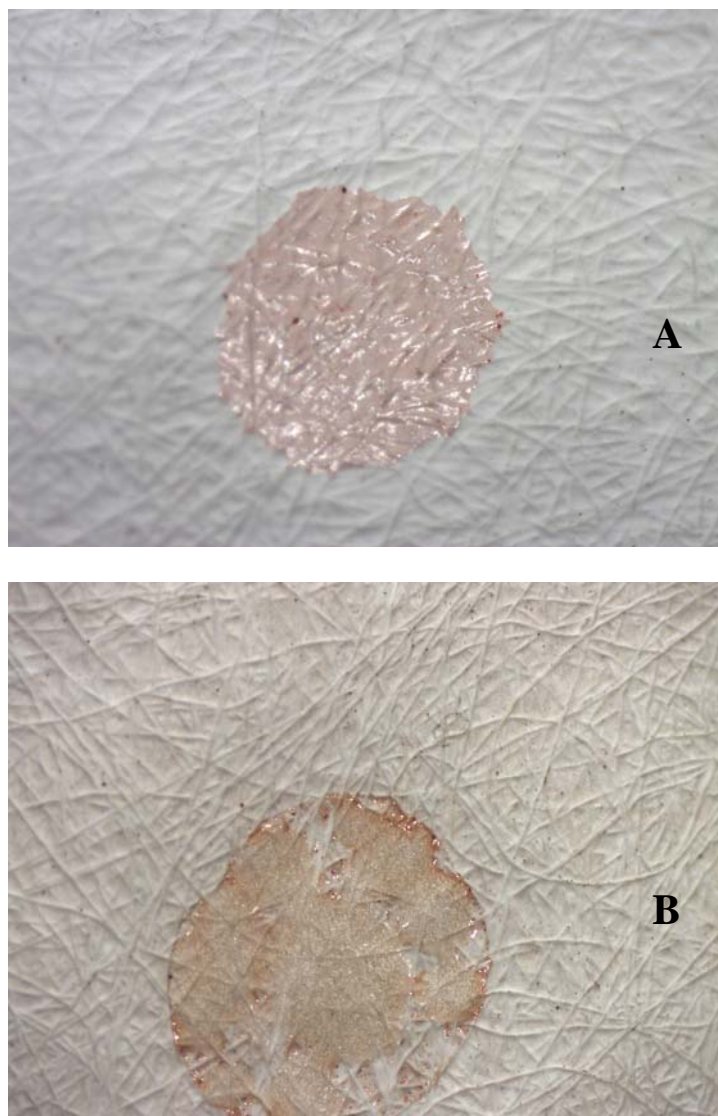


Figure 4.13. Microphotograph of a dried drop of  $20\mu\text{l}$  of a  $50\text{ ng}\mu\text{L}^{-1}$  solution containing  
A) three enriched elements and B) six enriched elements.



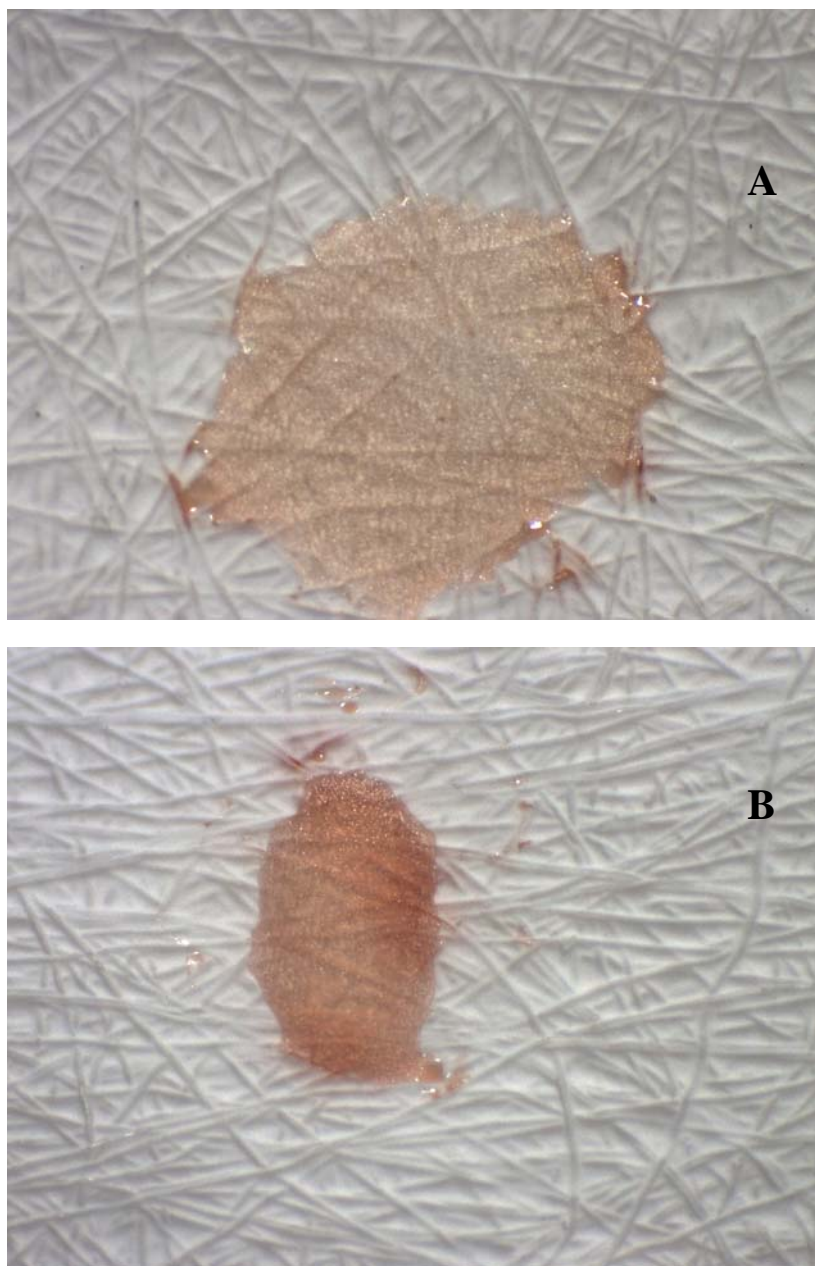


Figure 4.14. Microphotograph of dried drops for six-multi-element solution at A) 25 and B) 100  $\text{ng}\mu\text{L}^{-1}$

Table 4.4. Recovery efficiency for digested versapor filters spiked with 19 elements at 50  $\mu\text{gL}^{-1}$  (LBS) and 90  $\mu\text{gL}^{-1}$  (QC).

Analyte	LBS mean ( $\mu\text{g/L}$ )	%RSD	VP Blank ( $\mu\text{g/L}$ )	(LBS mean-VPB) ( $\mu\text{g/L}$ )	% recovery	QC mean ( $\mu\text{g/L}$ )	%RSD	VPB ( $\mu\text{g/L}$ )	QC mean-VPB ( $\mu\text{g/L}$ )	% recovery
Be	50	12	0.0	50	100	96	3.0	0.0	96	106
Al	62	14	12.3	50	100	81	3.2	12.3	69	76
V	54	13	0.0	54	107	85	2.8	0.0	85	95
Cr	54	13	2.1	52	104	83	2.7	2.1	81	89
Mn	54	13	0.2	54	108	88	3.1	0.2	88	97
Fe	60	15	7.7	52	104	97	3.7	7.7	89	99
Co	53	13	0.0	53	106	82	2.6	0.0	82	91
Ni	51	13	0.5	50	100	84	2.3	0.5	84	93
Cu	61	11	12.2	49	98	91	2.3	12.2	78	87
As	48	12	0.1	48	96	73	1.4	0.1	73	81
Se	45	12	0.6	45	90	70	0.9	0.6	69	77
Sr	50	13	0.2	50	100	84	2.4	0.2	84	93
Mo	51	13	0.2	51	102	80	2.1	0.2	80	89
Ag	6	27	0.0	6	13	7	18.7	0.0	7	8
Cd	46	13	0.0	46	91	70	1.5	0.0	70	78
Sn	45	13	0.1	45	91	73	2.3	0.1	73	81
Sb	36	14	0.0	36	71	56	3.6	0.0	56	62
Ba	51	13	0.1	50	101	121	2.7	0.1	121	134
Pb sum	49	13	0.2	49	98	69	0.3	0.2	68	76
overall					98					89

\* LBS: Laboratory Blank Spike; QC: Quality Control; VPB: Versapor Blank

to 12  $\mu\text{gL}^{-1}$  for aluminum and copper. These results show that the major contribution to the aluminum and iron contents in the filter blanks could be attributed to uncontrolled contamination at the laboratory (from reagents and laboratory environment).

Table 4.5 shows the concentrations detected on the reagent blanks, which consist in blanks from the acids and reagents used during the digestion procedure, and versapor blanks, which consist in unexposed areas cut from the versapor filter that has been used for sample collection and which has been digested. The copper content is mainly originated from contamination from the sampling device. This is expected since the “unexposed” areas of the filter correspond to the edges of the filters which do not collect

“dust” but are in closer contact to the metallic surface of the holder of the sampling device.

In spite of the values obtained from the reagent blank and the versapor filter blanks, the overall background levels of metals in the filter were considered appropriate for the collection of the particulate material and further analysis by ICP/MS. The efficiency in recoveries are evidence of the advantage of using Versapor filters which did

Table 4.5. Digestion results for digested reagent blanks (RB) and unexposed Versapor filter (VPB).

Element	Reagent blank, RB (acids, no filter)		Versapor filter blanks (VPB)	
	Mean ( $\mu\text{gL}^{-1}$ )	stdev	Mean ( $\mu\text{gL}^{-1}$ )	stdev
<b>Be</b>	nd		nd	nd
<b>Al</b>	8.93	4.54	12.29	4.04
<b>V</b>	nd		nd	0.01
<b>Cr</b>	0.32	0.03	2.09	0.37
<b>Mn</b>	0.10	0.04	0.15	0.01
<b>Fe</b>	6.85	2.31	7.73	1.19
<b>Co</b>	nd	0.00	nd	0.01
<b>Ni</b>	0.19	0.19	0.50	0.12
<b>Cu</b>	1.20	0.38	12.19	2.54
<b>As</b>	0.13	0.01	0.11	0.02
<b>Se</b>	0.06	0.01	0.65	0.17
<b>Sr</b>	0.08	0.03	0.20	0.06
<b>Mo</b>	0.18	0.02	0.22	0.07
<b>Ag</b>	nd	0.01	nd	0.00
<b>Cd</b>	nd	0.01	nd	0.01
<b>Sn</b>	0.15	0.15	0.12	0.08
<b>Sb</b>	nd	0.02	nd	0.03
<b>Ba</b>	0.22	0.12	0.14	0.02
<b>Pb sum</b>	0.04	0.02	0.24	0.37

not required complex total- total digestion methods or the use of HF as a common practice used in microwave extraction to remove the metals from similar matrices (Prospero et al., 2008).

#### 4.10.1. Analysis of Real samples by Acid Digestion

A total of 51 filter samples were collected for digestion analysis. This sample set corresponds to the 2006 collection. Using an analytical balance 0.2500 g of each filter was weighed and poured into the digestion cell. The concentrations determined by ICP-MS were then normalized to the dust weight and reported as micrograms of element per gram of dust. The total amount of the target analytes collected in the filter was then approximated by the ratio of the mass of the filter analyzed by acid digestion versus the total mass of the respective collected filter. The following formula was used to determine

$$\frac{(Metal\ Conc.(\mu g / L) * dilution(L) / mass\ of\ digested\ filter(g)) * whole\ filter\ weight(g)}{dust\ weight(g)}$$

these concentrations:

For example, to determine the value of vanadium for the filter collected on 01/25/2006, a concentration of 28  $\mu g/L$  was obtained in the ICP/MS tube. The dilution factor was 0.05 L which corresponds to the digestion cell volume, the weight of the digested filter was 0.2553 g. An assumption is made to consider that the concentration is homogeneous within the whole filter so, the weight of the whole filter is included in the formula, which in this case was 34.7415 g. Finally to obtain the value of the element concentration in the desired form, the micrograms of the element are divided by the dust

weight collected in the filter (0.5565g). This concentration is reported in the table for a value of 347  $\mu\text{g/g}$  of dust.

Table 4.6 shows the individual results for the concentrations detected during 2006. This table also includes the average Upper Crustal Abundances (UCA) as a way to relate these values with dust outbreaks and/or other sources of pollution. Summer average is reported for samples collected from June to August of 2006. These results shows a higher variability over time on the elemental concentrations as well as average values deviated from the UCA (positive and negative deviations), which could indicate multiple contributions to the elemental content on the air particulate other than “African dust” or dust from other sources. For example, lead was detected only in few of the sampling dates, mainly during the months of July and August and the concentrations are much higher than the UCA which may indicate anthropogenic pollution sources. Elements such as copper, vanadium and nickel were consistently higher than the UCA values in the majority of the collection dates which may be indicative of aerosols impacted by local and regional pollution sources. For example, in addition to the crustal abundance, vanadium is being associated to burning of fuels while nickel pollution is associated to smelters and incinerators (Prospero 2008).

As an example, the concentrations of iron and other elements are presented in Figures 4.15 to 4.20. Among the elements detected on the filter samples collected over the period of 2006, iron, copper, vanadium, aluminum and nickel were found at higher concentrations. It is interesting to note that spikes of iron and other elements were found at the summer months when larger amounts of African dust have been reported (Prospero and Lamb, 2003).

Table 4.6. Concentration of element on samples collected in Miami Area during 2006 and analyzed by ICP-MS.

Date	Element concentration (µg/g)								dust	volume	dust (ug/m <sup>3</sup> )
	V	Cr	Fe	Ni	Cu	Sr	Ba	Pb	weight (g)	(m <sup>3</sup> )	
011806	79	57	1578	62	1733	127	30	nd	0.2650	328713	0.806
012506	347	11	1108	97	787	76	88	nd	0.5565	361498	1.539
020606	443	682	18823	384	10065	445	409	nd	0.0483	372527	0.130
021006	1844	546	11542	577	6593	363	173	nd	0.0717	386405	0.186
021506	201	105	2197	80	1967	109	53	nd	0.2513	405840	0.619
022206	522	121	2389	170	1850	124	54	nd	0.2641	425673	0.620
030106	160	51	1750	62	962	66	38	nd	0.6408	445131	1.440
030806	35	52	1385	35	1293	159	21	nd	0.3688	466983	0.790
031606	343	85	2478	134	1342	156	60	nd	0.3711	510803	0.727
041706	155	91	2858	86	1311	137	44	nd	0.3671	588797	0.623
042406	169	72	3385	89	1134	125	nd	nd	0.4336	29857	14.523
050706	172	63	4329	85	878	255	58	3	0.5091	85746	5.937
052506	46	16	598	20	272	32	14	nd	1.7392	127319	13.660
060806	110	68	6635	59	991	228	55	nd	0.4640	165197	2.809
062006	79	308	5182	130	2554	251	59	nd	0.1952	189394	1.031
063006	378	1446	80465	1212	28214	1511	938	nd	0.0170	205971	0.083
072106	451	422	18801	284	6596	nd	nd	222	0.0716	238959	0.300
072306	61	82	8042	57	1073	117	70	43	0.4438	279141	1.590
080506	172	187	19553	159	4084	nd	nd	178	0.1258	300942	0.418
081306	212	122	8700	140	2276	nd	nd	119	0.2232	334128	0.668
082506	326	135	5592	158	792	241	110	4	0.6417	441208	1.454
101106	383	230	5521	197	11992	215	111	nd	0.2221	441175	0.503
<b>Average for 2006</b>	<b>300</b>	<b>225</b>	<b>9876</b>	<b>194</b>	<b>3656</b>	<b>251</b>	<b>134</b>	<b>95</b>			
stdev	373	324	16867	261	6250	322	220	93			
<b>Average Summer</b>	<b>224</b>	<b>346</b>	<b>19121</b>	<b>275</b>	<b>5822</b>	<b>469</b>	<b>247</b>	<b>113</b>			
stdev Summer	146	460	25433	385	9254	585	387	91			
<b>UCA *</b>	<b>106</b>	<b>74</b>	<b>41402</b>	<b>34</b>	<b>27</b>	<b>81</b>	<b>390</b>	<b>13</b>			

\*UCA: Upper Crustal Values, from Hu and Gao, 2008

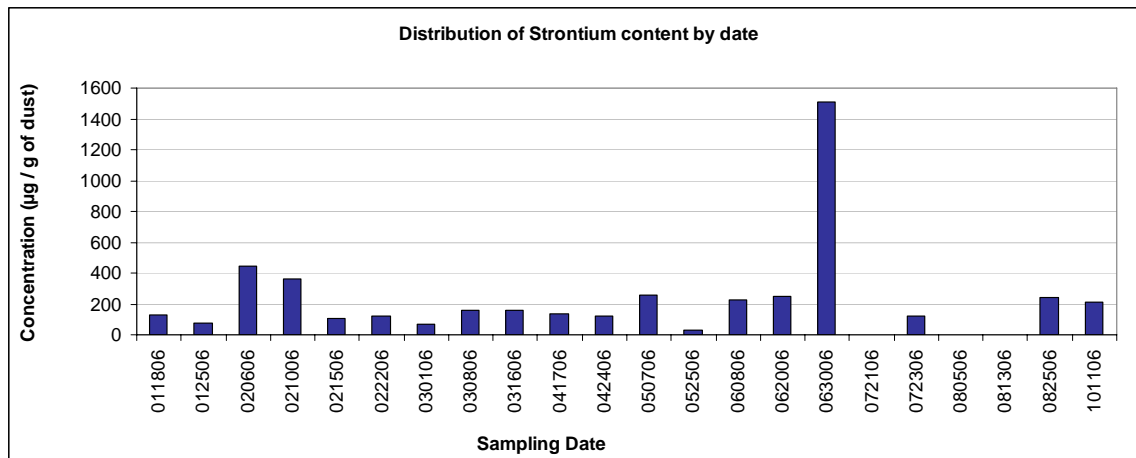


Figure 4.15. Iron concentrations related to dust concentration for 2006.

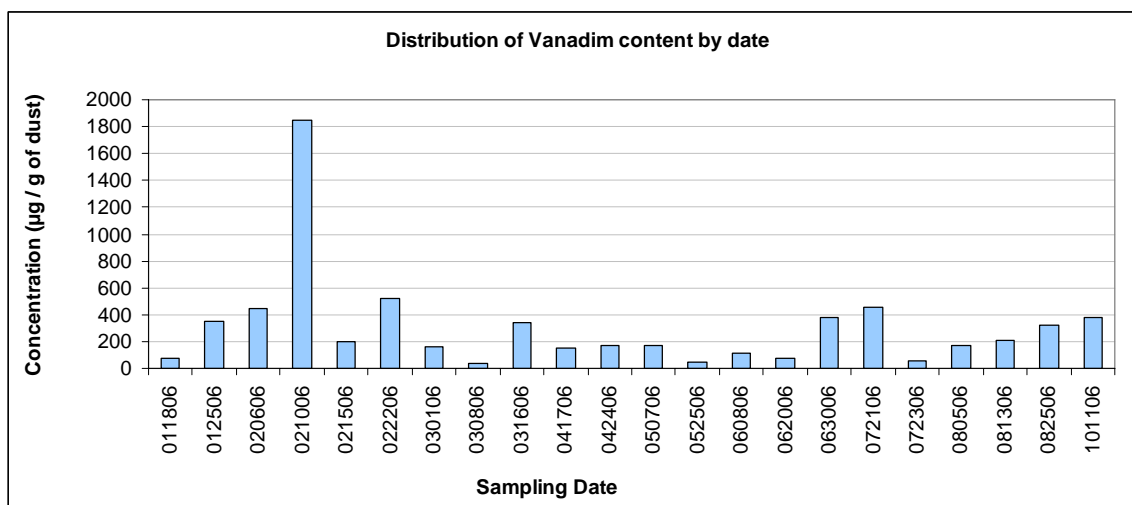


Figure 4.16 Strontium concentrations related to dust concentration for 2006.

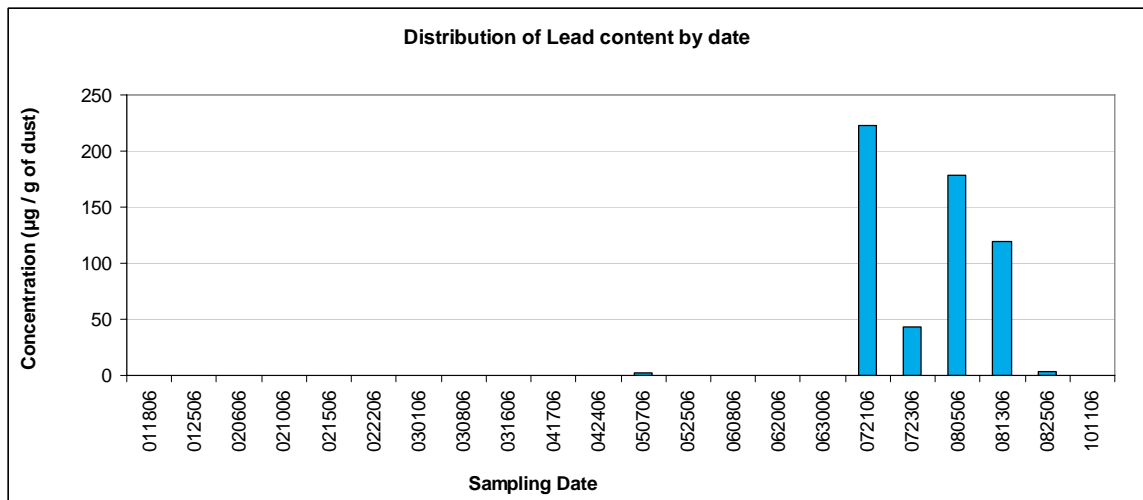


Figure 4.17. Vanadium concentrations related to dust concentration for 2006.

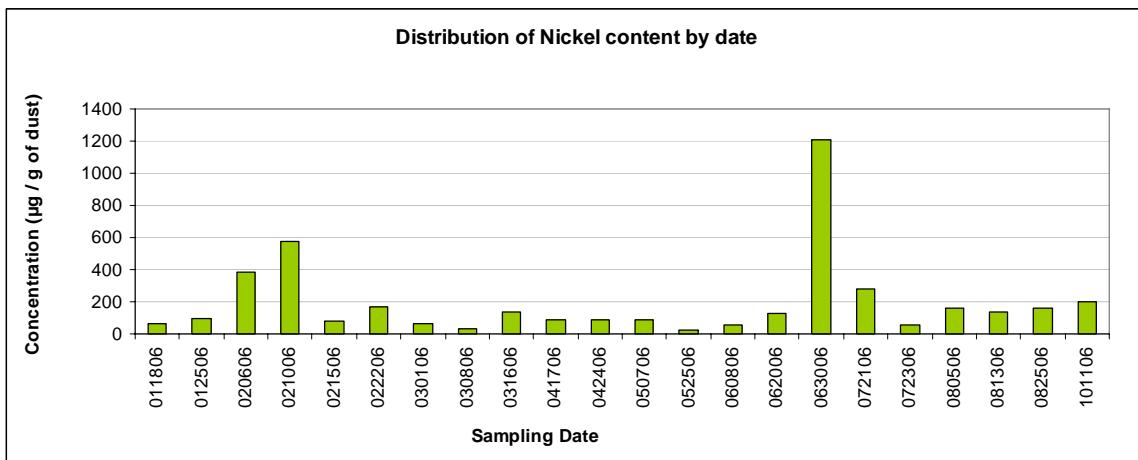


Figure 4.18. Lead concentrations related to dust concentration for 2006.



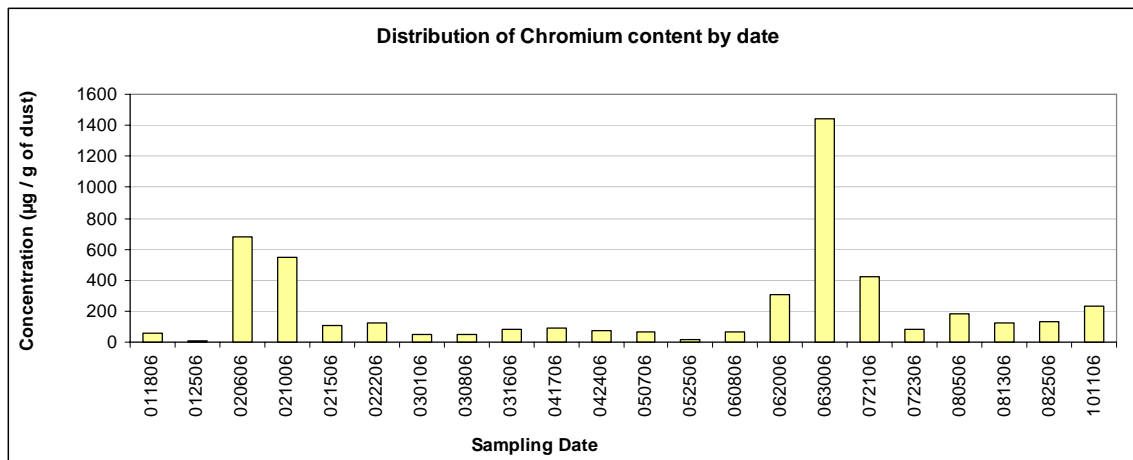


Figure 4.19. Nickel concentrations related to dust concentration for 2006.

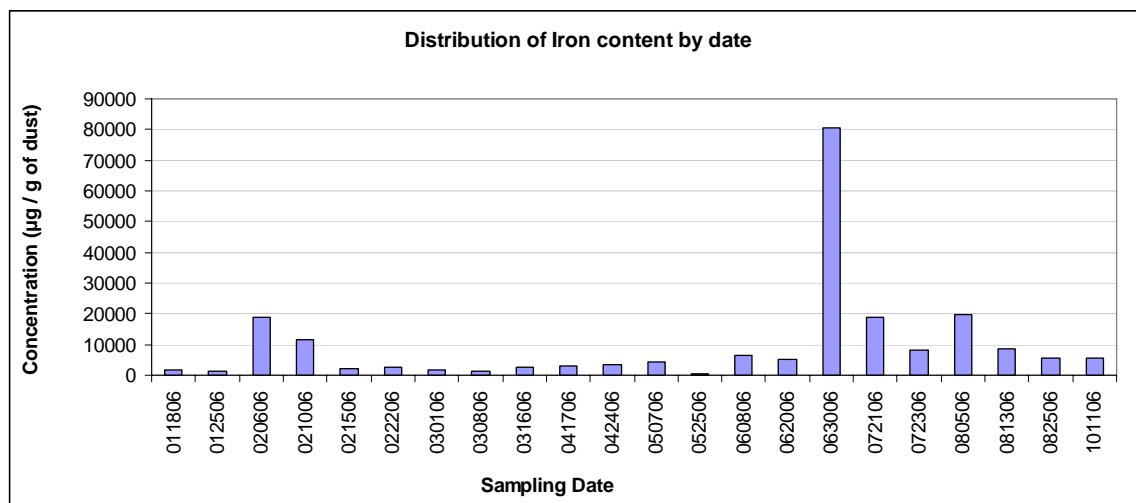


Figure 4.20. Chromium concentrations related to dust concentration for 2006.

#### 4.11. Data Normalization with Aluminum

As an additional attempt to understand how the elemental composition of dust may be linked to dust and/or anthropogenic sources, the concentration of the elements determined on the filter samples were normalized to the volume of sampled air and compared to aluminum. Aluminum was chosen as an indicator for mineral dust concentrations from crustal sources.

Figures 4.21 and 4.22 show the scatter plots for the elements versus aluminum. Each data point in the figure represents the average value of a specific sampling date. These elements can be separated in two main groups, according to their correlation to aluminum.

The first group, composed by iron, strontium and manganese, yields a positive correlation to aluminum ( $r^2 > 0.91$ ) which may indicate that these elements are not highly contaminated by non-crustal sources. Sample collected on June 30<sup>th</sup>, was removed from this correlation since it showed a higher level on all three elements (see Figures 4.15 and 4.16). It is important to indicate that manganese has much less data points since some of the samples were present at values below the detection limit.

The second group, represented on Figure 4.22 includes vanadium, chromium, copper and nickel, which does not exhibit a strong correlation to aluminum and also shows highly scattered data. These four elements also have the communality of being associated to pollution sources as discussed before.

Miami air masses are likely to be impacted by local and regional pollution sources, so these results are not unexpected. Moreover, Figure 4.22 shows that the correlation of these four elements to aluminum has a similar pattern.

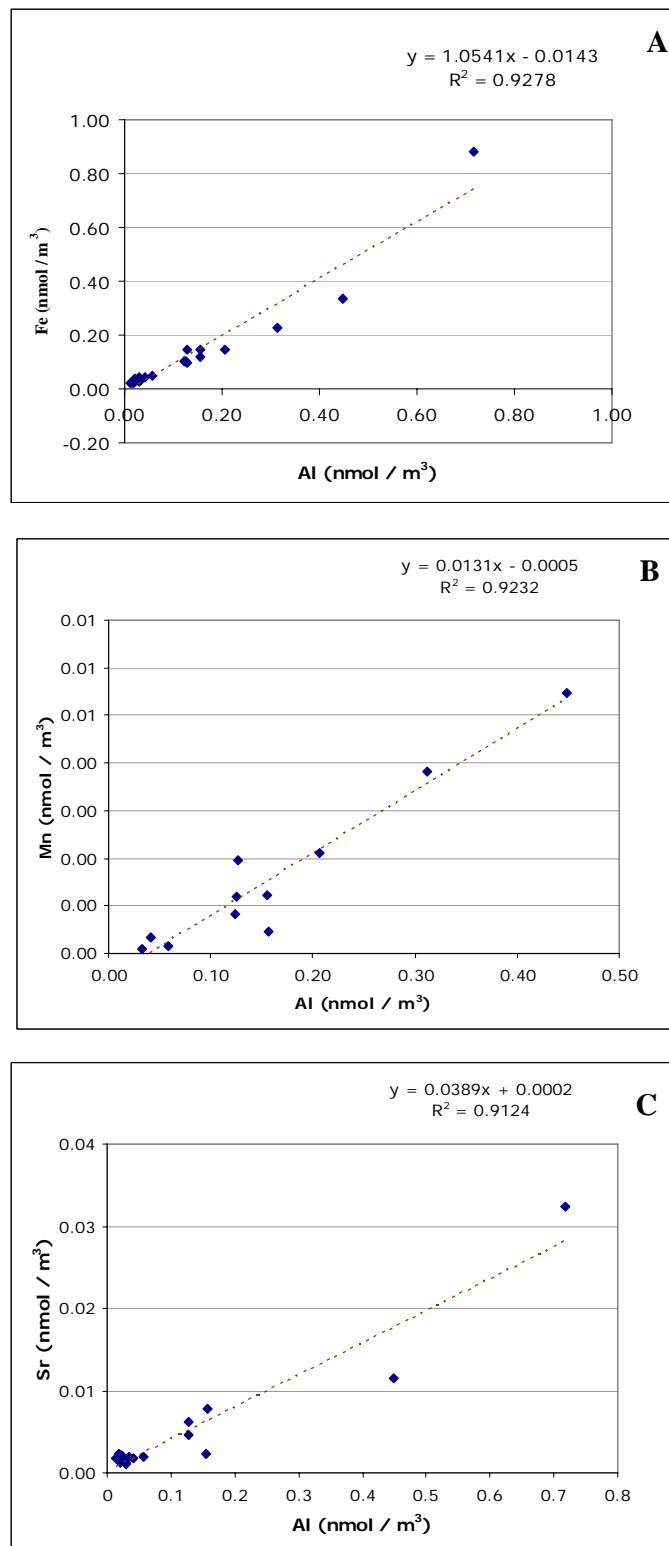


Figure 4.21. A) Correlation of iron/aluminum; B) correlation of manganese/aluminum and C) correlation strontium/aluminum. Abundances reported in nmol/m<sup>3</sup>.

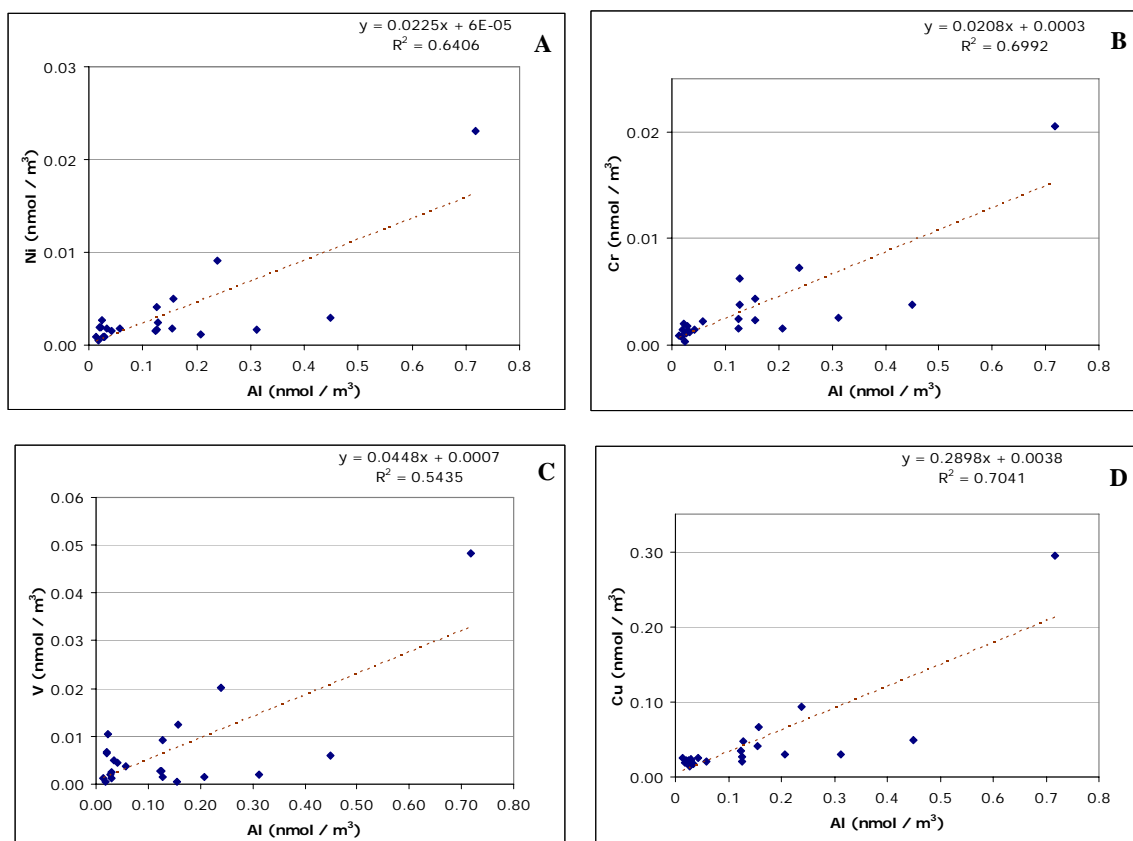


Figure 4.22. A) Correlation of nickel/aluminum; B) Correlation of chromium/aluminum C) Correlation of Vanadium/aluminum; D) Correlation copper/ aluminum. Abundances reported in nmol/m<sup>3</sup>.

For this reason, these elements were plotted versus each other to further determine if there is correlation to each other. Figure 4.23 shows an example of the positive correlation observed between elements of this four group to nickel, which may also corroborate this pollution-like abundances. In spite of these preliminary observations, the contribution of elemental profiles in air masses is very dynamic and complex and therefore, further studies need to be conducted in a) larger datasets, b) with extended element menu and c) in shorter ranges of collection times in order to better differentiate regional vs. transoceanic sources and to arrive to accurate conclusions.

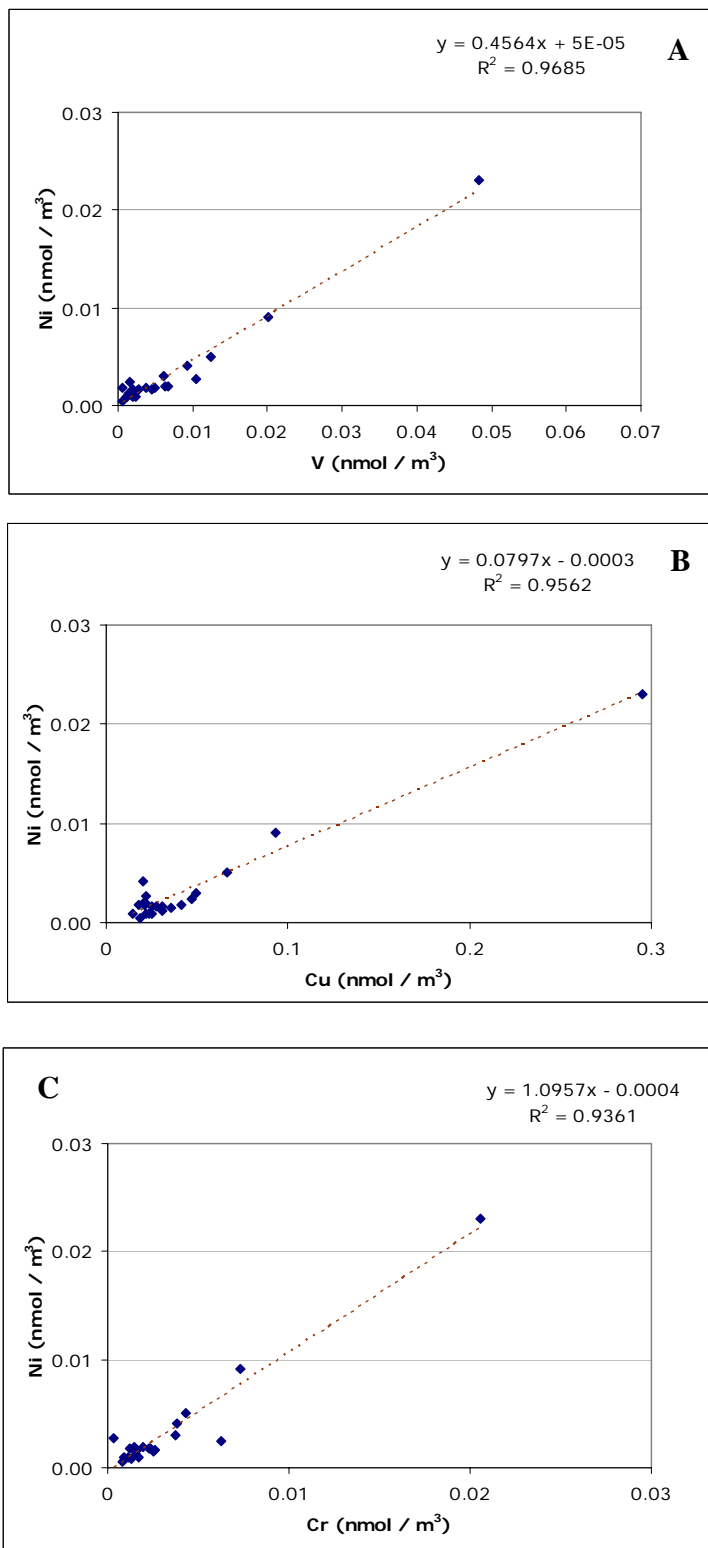


Figure 4.23. A) Correlation of nickel/vanadium; B) correlation of nickel/chromium and C) correlation of nickel/copper. Abundances reported in nmol/m<sup>3</sup>.

#### 4.12. Comparison of Digestion vs. Laser Ablation Data

The same samples were analyzed by LA-ICP-MS qualitatively and normalized to the dust weight, but elemental profiles for some of the elements were different than the ones obtained by digestion methods. As an example, Figure 4.24 shows the profile obtained by laser ablation which is similar to the one obtained by digestion ICP-MS (Figure 4.15), while vanadium profiles have different peak dates (see Figure 4.17 for digestion ICP-MS profile).

This may be attributed to the differences in absolute detection limits since many of the samples were not detected by LA-ICPMS. Also, the fact that LA-ICP-MS is removing just a small amount of material may not be homogeneous enough to provide a representative sample of the bulk filter. For instance, in digestion methods a mass of 0.25g was weighed per replicate. Using the same example presented on section 4.4.1.1, from the total dusted area of 3118cm<sup>2</sup> of that filter, an area of 1.5 x 1.5 cm was cut and weighed for digestion analysis, which corresponds to approximately  $3.3 \times 10^5$  ng of “dust”. In contrast, LA-ICP-MS would remove approximately 50ng of “dust” from the filter. This represents the sampling of about a 6000 times more dust material during the digestion protocol than during the laser method which gives a better opportunity to the digestion method to be more representative of the bulk elemental composition.

Additionally, since the quantitative method for laser ablation did not perform well for more than 3 elements at a time, it is difficult to do a thorough comparison between the laser ablation and the solution data. Alternatively, other quantitation strategies should be explored for LA-ICP-MS, such as deposition of in house solid standards or the use of a different filter to support liquid spikes, in order to allow this method to be practical and

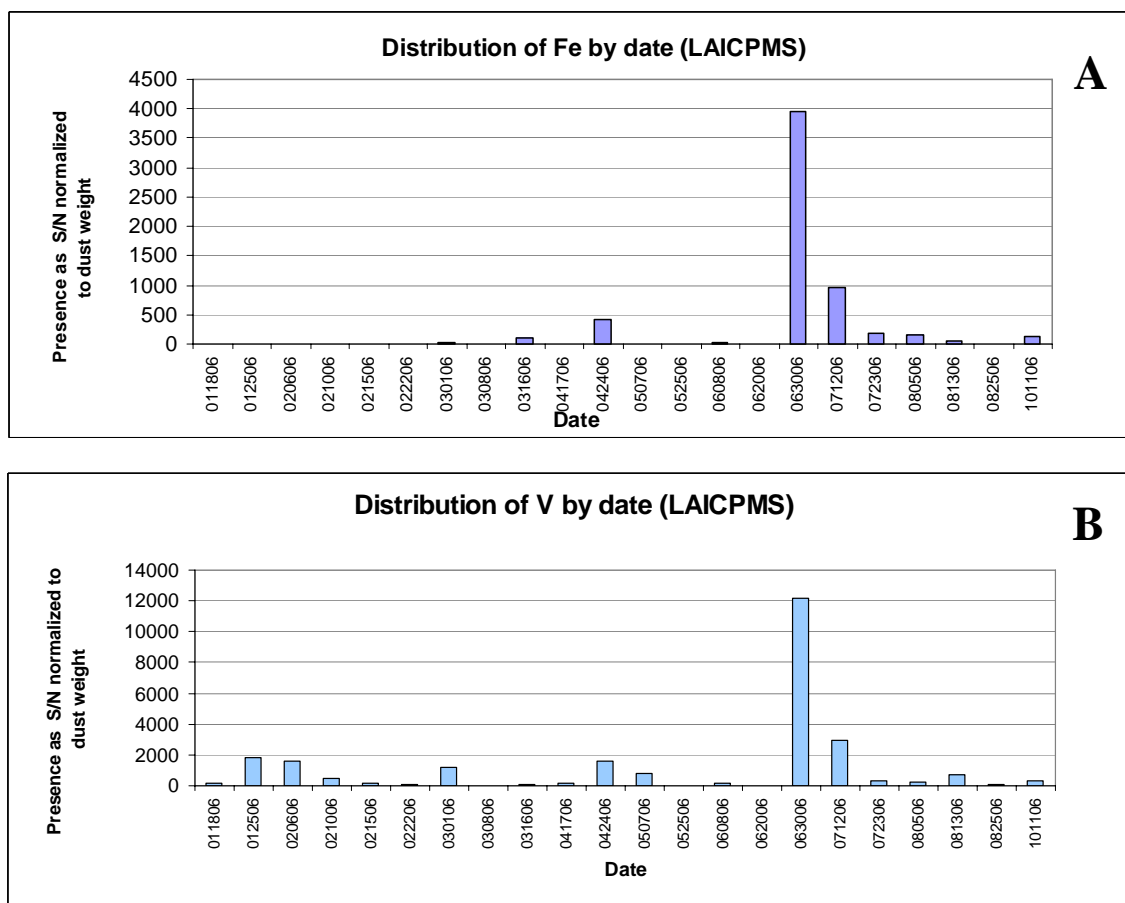


Figure 4.24. A) Iron and B) Vanadium distributions for Y 2006 dataset analyzed by LA-ICP-MS.

less time consuming than digestion protocols.

#### 4.13. Conclusions and Recommendations

The proposed LA-ICP-MS method allows a rapid qualitative screening of the elemental profile of dust particles deposited on filters and can be applied as a preliminary identification of potential sources of pollutants and dust particulates. External calibration curves showed good linearity for very low amounts of mass deposited but this approach

is limited for calibration curves made of 3-multielement subsets, which may not be practical for routine assessment.

Quantitative information could be obtained using laser ablation methods, but they will require adjustments on development of alternative quantitation strategies or a reduced elemental menu. For example, the addition of solid spiked standards prior to dust collection could be accomplished and could be equivalent of creating a “matrix match standard.”

Another alternative consist in changing the filter type to a non-hydrophobic surface so that liquid spikes can be better applied to generate in-house matrix standards. Larger ablation areas may be also necessary to improve the detection limits of low elements such as cadmium, silver or lead.

For generalized quantitative analysis, an acid digestion method may be more appropriate than LA-ICP-MS especially because a larger, more representative sample can be removed from the filter. Good recoveries (better than 88%) were obtained for the majority of the elements of interest using the simple digestion method. The polymeric filters have the added advantage of not requiring a total digestion method, thus simplifying the analytical protocol.



## CHAPTER 5

### 5.0. Conclusions

## 5.0 Conclusions

The main purpose of this study was to develop a set of new and robust routine analytical methods for the analysis of different environmental pollutants in complex matrices and to apply those methods to samples collected in protected areas in South Florida in support of the CARE project. Three analytical methods were developed for the quantification of both organic and inorganic species. The methods developed were validated and applied to different sampling stations located within protected areas in South Florida and the conclusive results are as follows:

### 5.1 Environmental Monitoring of Acidic Herbicides in Surface Waters by Electrospray Ionization Quadrupole Ion Trap Mass Spectrometry (LC-QIT/MS)

The complex nature and diverse physical-chemical characteristics, together with the high content of organic matter of the waters studied, made the isolation, concentration and fractionation of pollutants by SPE a difficult task.

The polymeric cartridges Oasis HLB exhibited a fair performance for elimination of the background interferences present in the water samples. The easy conditioning steps and the rapid setup, allowed for a straightforward pre-treatment of water samples by the autotrace system.

The chromatographic separation of the phenoxy acid herbicides was successfully accomplished with gradient elution program using methanol and acetic acid 1% in a C<sub>18</sub> column and by electrospray ionization in the negative mode (ESI<sup>-</sup>). This ionization technique with selected reaction monitoring, was effective for the detection and quantification of the target phenoxy herbicides.

Different parameters showed a great influence in the ionization process of the mass spectrometer. Focusing voltages, probe temperatures, and mobile phase composition to avoid ion suppression, were of primary importance as variables that control the detection, sensitivity and selectivity.

The analytical sensitivity of the phenoxy acid quantification method was evaluated through the use of internal calibration curves. The accuracy of the method was measured by running spiked replicates. The interday variation was tested for precision showed values below 30 % RSD. The MDL ranged from 1.9 to 4.5  $\mu\text{gL}^{-1}$  for the compounds tested.

The novelty of the proposed method for the screening analysis of phenoxy acid herbicides was to combine a polymeric SPE phase with LC/MS/MS for the simultaneous analysis of 12 acidic herbicides. Advantages over conventional extraction and detection methodologies include compatibility with aqueous matrices, minimization of solvents and simplification of the analysis for water samples with a high content of dissolved organic matter.

Nine compounds were efficiently extracted and analyzed using the proposed methodology. However, picloram, 2,4-DB and dinoseb were not recovered from the natural matrix).

Hyphenated chromatographic techniques, such as HPLC/ESI/MS in both full scan mode and SRM offer a good alternative for the screening and quantification of the analytes at screening concentrations in surface waters. This method required only 100 ml of sample to achieve limits of detection as low as 1.9  $\mu\text{gL}^{-1}$  for the suite of herbicides studied, which is at least 10 times below of the MCL requirements.

## 5.2. Laser Ablation ICP-MS of Soils and Sediments

A simple sample preparation and quantitation method by LA-ICP-MS was optimized and validated for soils and sediments, as a viable alternative for routine monitoring of contaminated sites and determination of background levels.

The novelty of the proposed method relies on the application of UV-laser ablation-ICP-MS for the simultaneous analysis of a large variety of elements of interest to the environmental sciences with limits of detection as low as 0.01 mg/kg. The micro-homogeneity and particle size studies showed that the milling methods and pellet formation provide efficient homogenization at the micro-scale to produce accurate chemical representation of the bulk sample. This sample preparation procedure improved not only the precision and accuracy of measurements, but also the cohesion of soils during pellet formation avoiding the use of binders that dilute the sample.

Despite the added steps, sample preparation times are more than adequate for routine applications. With a batch of three high speed mill systems, one technician can prepare ~72 samples/day. The LA-ICP-MS analysis can determine trace, minor and major elements simultaneously without requiring the use of multiple dilutions as often required in typical digestion methods. Laser ablation demonstrated to be an efficient sampling method for the elemental menu of interest (19 elements). The recoveries did not vary significantly by element or among soil type.

Spot size, grain size and the use of an appropriate internal standard were found to be the key factors, controlling the analytical performance.

Assessment of analytical parameters produced good accuracy and precision for the reported elements for certified soil and sediment standards. Additionally, the LA-ICP-

MS method performs very well in comparison to other ICP and AA digestion methods typically used by the soil community as established by the participation on the proficiency test exercises.

LA-ICP-MS also allows direct characterization of solids with minimum consumption of the sample (~ micrograms), which is critical in some of the forensic application where this method is intending to be used. Micromilling and pellet formation is also a valuable method for specimen preservation.

The robustness and applicability of the method was also tested for a large set of real sediment and soils with broad chemical and physical properties. From this second part of the study, the following conclusions can be drawn:

The proposed methodology can be applied for the rapid quantitative assessment of contaminated soils for major, minor and trace levels, including analytes such as lead, tin and antimony which are of particularly importance to determine anthropogenic sources and to establish remediation or clean up efforts.

When compared to traditional methods, Laser ablation can be considered an exhaustive sampling method that recovers significantly higher amounts of analytes than leachate or pseudo-total digestion protocols. Despite of the inherent differences between leachate digestions and exhaustive methods such as laser ablation, different statistical models such as non-parametric correlation tests, aluminum normalization and principal component analysis, demonstrated that there is good agreement between the informative value of the data acquired by both methods and therefore LA-ICP-MS can be also used to establish baseline concentrations and to establish enrichment levels.

The application of the LA-ICP-MS method to environmental forensics has several

advantages over the digestion methods discussed in this study, such as: providing reduced time of analysis and complexity, offering a single rapid alternative to conduct multi-elemental quantitative analysis of soils without requiring multiple digestion and independent measurement procedures.

### 5.3. Screening for Airborne Trace Elements by in situ Laser Ablation ICP-MS: Method Development and Preliminary Assessment

The proposed LA-ICP-MS method allows a rapid qualitative screening of the elemental profile of dust particles deposited on filters and can be applied as a preliminary identification of potential sources of pollutants and dust particulates.

External calibration curves showed good linearity for very low amounts of mass deposited but this approach is limited for calibration curves made of 3-multielement subsets, which may not be practical for routine assessment. Quantitative information could be obtained using laser ablation methods, but they will require adjustments on development of alternative quantitation strategies or a reduced elemental menu. For example, the addition of solid spiked standards prior to dust collection could be accomplished and could be equivalent of creating a “matrix match standard”. Another alternative consist in changing the filter type to a non-hydrophobic surface so that liquid spikes can be better applied to generate in-house matrix standards. Larger ablation areas may be also necessary to improve the detection limits of low elements such as cadmium, silver or lead.

For generalized quantitative analysis, an acid digestion method may be more appropriate than LA-ICP-MS especially because a larger, more representative sample can

be removed from the filter. Good recoveries (better than 88%) were obtained for the majority of the elements of interest using the simple digestion method. The polymeric filters have the added advantage of not requiring a total digestion method, thus simplifying the analytical protocol.

## CHAPTER 6

### 6.0. Future Work



## 6.0 Future Work

During the fractionation of the acidic herbicides, the possibility of performing a two-step elution in solid phase extraction by using an additional aliquot of dichloromethane should be considered in order to improve the recovery of the analytes. As an alternative, the use of newly available mixed beds cation exchanged plus reverse phase or mixed functionality may be applied in conjunction with the Oasis cartridges. The tandem cartridge combinations may also allow to process higher volumes of sample and therefore yield a significant improvement in the method detection limits. The presence of phenoxy acid metabolites like 2,4-dichlorophenol and 2,4,5-trichlorophenol are considered a priority for future method evaluation. Figures of merit for these compounds in terms of extraction, and detection in the LC/MS/MS system need to be further validated.

In order to have complete elemental baseline information in Florida, the proposed LA-ICP-MS method should be applied to a larger dataset including areas outside the studied protected areas similar the one conducted by Schropp.. By doing so, a complete spatial distribution of the metal content and their comparison against digestion protocols will be obtained at state level comparisons. A comprehensive geochemical study of the soils and sediments in other states should be designed by incorporating external laboratories as a two fold purpose: evaluate advantages and limitation of the LA-ICP-MS method for the elemental analysis of soils and sediments in other geographical regions, and at the same time develop a database that comprises the elemental profiles of these collected samples. Initially, government laboratories could be contacted for this purpose.

The development of appropriate sampling and analytical methods for the monitoring of atmospheric inputs in protected areas in South Florida was presented in this research. The study was useful to identify key factors for the practical analysis of dust particulates as well as the interpretation of the results but by no means definitive. Specific areas for further research: a) Laser ablation methods are promising but require the development of alternative in filter standards or the use of non-hydrophobic filters that will allow for spiking, use of inject devices; a possibility that needs to be explored. High limits of detection and in-homogeneity during sampling are inherent disadvantages of the LA approach. b) Digestion methods followed by ICP-MS measurements are a good alternative for the elemental analysis of dust. The developed method is efficient for the digestion of airborne particles in particular when collected on Versapor filters. c) In order to improve the interpretation capabilities, collections over shorter periods of time (at least weekly) is recommended for the summer months. The current elemental menu is dominated by pollution-like elements and therefore it will be useful to increase the target analyte list to other rare elements that may be better markers of African Dust emissions. The accurate determination of the total dust on the filter will greatly help on the interpretation of results. Since several elements were below detection limit when laser ablation or digestion methods were employed it is suggested to design smaller total areas of sampling as a mean to have a greater concentration factor on the filter. A larger dataset should be produced over an extended period of time to draw conclusions about the trends and elemental profile of the particles deposited on the filter and to differentiate anthropogenic local sources from African dust.

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