UNIVERSITY
OF TRENTO - Italy
DEPARTMENT OF INDUSTRIAL ENGINEERING

XXVI cycle

Doctoral School in Materials Science and Engineering

Micro-analytical methodologies for the characterization of airborne inorganic pollutants collected on unconventional substrates

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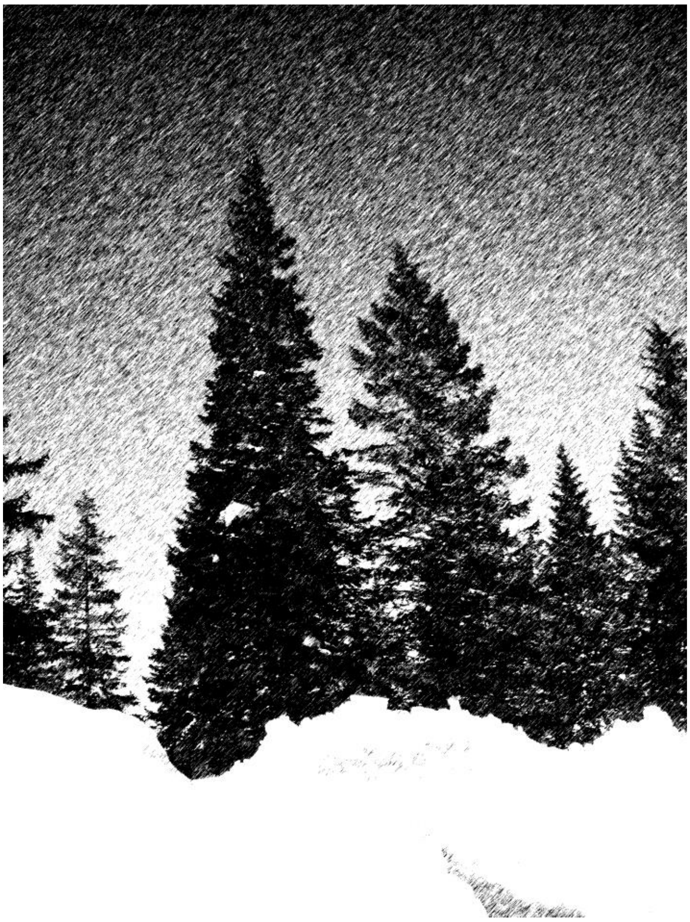

April 15, 2014

MICROANALYTICAL METHODOLOGIES FOR THE CHARACTERIZATION OF AIRBORNE INORGANIC POLLUTANTS COLLECTED ON UNCONVENTIONAL SUBSTRATES

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Department of Industrial Engineering

April 2014

University of Trento - Department of Industrial Engineering
Doctoral Thesis

Giulia Bertolotti - 2014
Published in Trento (Italy) – by University of Trento

To Kelly Tsuru Yeda
and my parents

Abstract/Summary

The present work regards the development of a methodology for the study of atmospheric particulate matter (PM) which is alternative to instrumental measurements. The methodology developed exploits the surfaces already present in the field as samplers of PM. In particular, conifer needles and building facades are employed to investigate different temporal ranges: conifer needles potentially retain particles circulating in the atmosphere from the recent past up to now, while building facades could retain particles from an older period up to know.

The field of application of the approach developed are the situations in which a wide territory must be monitored, eventually including remote locations, or information on past pollution scenario must be reconstructed in the absence of monitoring stations. For instance, the evaluation of the improved efficiency of off-gas abatement systems of industrial plants is a typical case of application. These pollution sources affect large areas and might have been active before regulation on air quality required constant monitoring of their emissions. Typically in such a case the methodology could assist in evaluating how large was in the past and it is nowadays the area of impact of the plant. In general, such an approach could be valuable whenever relying on instrumental measurements is cost and time consuming in terms of installing a large network of monitoring stations to study the dispersion of pollutants from a single or few sources.

To have a detailed description of the spatial distribution of pollutant particles, they are studied individually with subsequent higher magnification. Where no traces of a source are detected by scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (SEM-EDXS), the samples are analyzed with the higher resolution of transmission electron microscopy coupled with energy dispersive x-ray spectroscopy (TEM-EDXS) and selected area electron diffraction (SAED) in order to make sure that no smaller particles, able to travel farther from their source, are present at a certain site.

All data provided by electron microscopy analysis of particles colleted by conifer needles are placed in the context of elemental concentrations measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES), which is a bulk analytical technique. The same is not possible for the data on single particles present on building facades given the inorganic matrix of the substrate, especially in the case of metal oxide paints, which does not allow the bulk measurement.

Both the preparation of the samples for bulk analytical techniques and single particle analysis by electron microscopy were optimized.

For method development and evaluation, the analytical protocol was applied to estimate spatial and temporal trends of accumulation of inorganic pollutants that can be related with changes in the emissions of atmospheric pollutants by an electric arc furnace (EAF) steelmaking plant located in a test site. The benefits of combining the single particle and bulk analytical techniques emerged especially for the discrimination of the emissions from different sources.

Keywords

Particulate matter, air quality, conifer needles, passive sampling, scanning electron microscopy, transmission electron microscopy, inductively coupled plasma atomic emission spectroscopy, EAF emissions, electric arc furnace dust, fugitive emissions, biomonitoring.

Acknowledgments

The present study was carried out at the Department of Industrial Engineering of the University of Trento during the years 2011–2013.
I am grateful to my supervisor, Prof. Stefano Gialanella, for introducing me to the fascinating field of electron microscopy and for his support throughout this work. I wish also to express my gratitude to Prof. Marco Ragazzi and Dr. Elena C. Rada for having involved my thesis supervisor and me in air quality monitoring activities. I am indebted to them all also for their careful reading of my manuscript and for their constructive comments and corrections. My special thanks are extended to Ing. Alessandro Chisté and Ing. Marco Schiavon for all the fruitful discussions on sampling designs and the adventurous sampling campaigns undertaken together.

I would like to express my very great appreciation to Prof. Stefano Siboni for his statistical advices and Livio Zottele for discussing with me all about ICP-AES preparation and analysis.
FKV Company and in particular Dr. Sonia Fumagalli for the closed-vessel microwave-assisted digestion of few samples. These data were fundamental for method evaluation.
TEM training by Dr. Gloria Ischia and SEM training by Dr. Lorena Maines have been very helpful to carry out this work.
In addition, I wish to express my thanks to Prof. Fabrizia Fossati of the University of Parma (Italy), Dr. Fabiana Cristofolini and Dr. Elena Gottardini of the Research Centre of The Edmund Mach Foundation for addressing my botanical questions and to Dr. Paola Fermo of the University of Milan (Italy) for suggestions on application of ICP-AES in the environmental research field and on multivariate statistical analysis.
I am grateful to the students of the course MATBEC of spring 2013 who helped in performing the analysis on the samples from building facades collected in the framework of the case study.
Participating in the courses on different aspects of the relationship between environment and cultural heritage organized in 2010 and 2011 at the European Centre for Cultural Heritage in Ravello (Salerno, Italy) was inspirational. The list of the speakers in the courses would be quite long but I thank all of them.

A lot of people need to be acknowledged also if they did not directly contributed to this research project. They indeed contributed to keep my motivation high, improve myself as a human being and as a researcher and made my life in these past years very pleasant.
Dr. Gregory D. Smith of the Indianapolis Museum of Art who welcomed me in his laboratory during summer 2012. I owe him a lot of being able to work autonomously.
All the staff of the Indianapolis Museum of Art and Prof. Erich Uffelmann for teaching me how to be a good teacher.
Dr. Majlinda Lahaniatis for being a great model of a strong and kindhearted woman and scientist. Dr. Ing. Luca Lutterotti giving me the chance to learn about being a researcher every time he was in town.
My parents that often throughout my life believed in me more than I did myself.
Andrea Azzolini, without his encouragement and his determination in urging me to go on I would have quit during my first year.
Emanuele Zera who did not laugh at my weird practical laboratory questions.
Enrico Zappia for his daily patient support and for deciding to keep holding my hand with love in what will be after this thesis.

This work has been partially sponsored by the Mediterranean Scientific Organization of the Environmental Protection (MESAEP) in the framework of Dr. Emmanuel Lahaniatis Award.

Trento, March 15, 2014

Abbreviations and definitions

AAS = atomic absorption spectroscopy
APPA = Agenzia Provinaciale per la Protezione dell'Ambiente, Trento, Italy (Provincial Agency for Environment Protection)
APAT = Agenzia per la Protezione del'Ambiente e per i servizi Tecnici (Agency for Environment Protection and Technical Services)
ASTM = American Society for Testing and Materials
ATR-FTIR = attenuated total reflection Fourier transform infrared spectroscopy
BAT = best available technology
BSE = backscattered electrons
CCSEM = computer controlled scanning electron microscopy
CEN = Comité Européen de Normalisation (European Committee for Standardization)
DII = Department of Industrial Engineering, University of Trento, Italy (existing from 2013)
CPs = chlorinated paraffins
CRM = certified reference material
DCM = dichloromethane also known as methylene chloride (CH_2Cl_2)
DICAM = Department of Civil, Environmental and Mechanical Engineering, University of Trento, Italy (existing from 2013)
d.m. = dry matter (referred to the dry matter at 105°C)
DZ = deposition zone
EAF = electric arc furnace
EC = elemental carbon
EDXS = energy dispersive x-ray spectroscopy
EEA = European Environment Agency
EELS = electron energy loss spectrometry
EF = enrichment factor
EM = electron microscopy
EPA = Environmental Protection Agency of the United States of America (USA)
ESEM = environmental scanning electron microscopy
FAAS = flame atomic absorption spectroscopy
FE-SEM = field emission scanning electron microscopy
GFAAS = graphite furnace atomic absorption spectroscopy
HAADF-STEM = High-Angle Annular Dark Field – Scanning transmission electron microscopy
HVSEM = high vacuum scanning electron microscopy
IAEA = International Atomic Energy Agency
IARC = International Agency for Research in Cancer
IC = ion chromatography
ICP-AES = inductively coupled plasma atomic emission spectroscopy (same as ICP-OES)
ICP-OES = inductively coupled plasma optical emission spectroscopy (same as ICP-AES)
ISO = International Organization for Standardization
ISPRA = Istituto Superiore per la Protezione e la Ricerca Ambientale (Institute for Environmental Protection and Research)
LVSEM = low vacuum scanning electron microscopy
MCE = mixed cellulose esters
MESAEP = Mediterranean Scientific Association of Environmental Protection
μ-FTIR = micro Fourier transform infrared spectroscopy
MSDS = Material Safety Data Sheet
MSWI = municipal solid waste incinerator
MW = microwave
NIOSH = National Institute of Safety and Health of the United States of America (USA)
NMVOCs = non methane volatile organic compounds
OC = organic carbon
OM = optical microscopy
PAHs = polycyclic aromatic hydrocarbons
PC = polycarbonate
PCA = principal component analysis
PCBs = Polychlorinated biphenyls
PCDDs = polychlorinated dibenzo-p-dioxins
PCDFs = polychlorinated dibenzofurans
PGE = platinum group elements (Pt, Pd,Rh)
POPs = persistent organic pollutants
PM = particulate matter
PM$_{10}$ = particulate matter with an aerodynamic diameter lower than 10 μm (which includes also PM$_{2.5}$)
PM$_{2.5}$ = particulate matter with an aerodynamic diameter lower than 2.5 μm
PTFE = Teflon
SAED = selected area electron diffraction
SE = secondary electrons
SEM = scanning electron microscopy
SIMS = secondary ion mass spectroscopy
SMRE = Safety in Mines Research Establishment of the United Kingdom (UK)
SPA = single particle analysis
SPMDs = semi-permeable membrane devices (passive samplers)
SR-FTIR = synchrotron radiation Fourier transform infrared spectroscopy
SR-XRD = synchrotron radiation x-ray diffraction
STEM = scanning transmission electron microscopy
TEM = transmission electron microscopy
THF = tetrahydrofuran
ToF-SIMS = time-of-flight secondary ion mass spectroscopy
TSP = total suspended particulate matter
UF = ultrafine (referred to particles with diameter smaller than 0.1 μm)
UFPM = ultrafine particulate matter (referred to particles with diameter smaller than 0.1 μm)
VDI = Verein Deutscher Ingenieure
VOC = volatile organic compound
WDXS = wavelength dispersive x-ray spectroscopy
WHO = World Health Organization
XRD = x-ray diffraction
XRF = x-ray fluorescence

Contents

The references are provided at the end of each chapter (for appendixes at the end of the thesis) so that the reader can easily identify those relevant to the topic of his/her interest.

1 Introduction

> *"[..] prudence dictates that policy shifts should await the due process of science[...]"*
> Grahame TJ and Schlesinger RB, 2007[1]

Every local, national or international organization devoted to environment protection, public health protection or cultural heritage protection stresses the need for a better characterization of air pollutants, their properties and their effects. The knowledge provided by such activity is the basis for better monitoring, regulating and managing air quality and it is needed to improve the systems that reduce the emissions of pollutants or treat them.

The present thesis focuses on the characterization of the inorganic portion of particulate matter (PM) in the microscopic and below microscopic size range.

In chapter 2, a holistic view on the problem of inorganic air pollution, its effects and the possible ways to monitor it, is provided. From this, the specific objectives of the work will be defined in paragraph 2.3. This systemic view accounts for the materials science and industrial engineering perspective and includes environmental considerations and cultural heritage issues. It derives from the academic background of the Author, who holds a Bachelor and a Master in the field of natural sciences (chemistry, physics, geology, botany) applied to cultural heritage (historical buildings, monuments, objects in museum collections) conservation, and from the opportunity to develop this PhD research inside the Department of Industrial Engineering with a continuous collaboration with the Department of Civil, Environmental and Mechanical Engineering.

This thesis seeks to advance the field of PM characterization developing a strategy that allows the collection of air quality data without placing any instrumental monitoring station. This includes the obtainment of information on the past situation even if no instrument was placed at a selected site. This is thought to be useful when there is a need to monitor remote locations or to cover a wide area to establish, for example, the further distance to which a source exerts its influence on air quality. It may also be employed as a survey tool to select the sites where it is strategic to install instrumental monitoring stations. Far from suggesting a substitution of official monitoring techniques, such an approach is proposed for application in cases recognized as hard to investigate with conventional techniques, such as those of fugitive industrial emissions.

An innovative methodology will be developed in order to combine data obtained from bulk analytical techniques (in this work inductively coupled plasma atomic emission spectroscopy is employed, but atomic absorption spectroscopies, x-ray fluorescence or neutron activation analysis could be valuable alternatives) with those acquired by single particle analytical techniques. Among the single particle analytical techniques, in this work we refer to electron microscopy, both scanning and transmission, as they offer the unique opportunity to collect simultaneously morphological, chemical and crystallographic information on the sample. The inclusion of electron microscopy is in line with the need to understand better the exact property/ies of hazardous particles that determine their toxicity and with that of investigating the presence and risks posed by ultrafine and nanoparticles. These information support the process of air quality regulation/standard development which is going towards setting limits for smaller size fractions and it is evaluating whether it would be possible to regulate parameters other than the mass of certain size fractions of PM to better protect public health. Simultaneously, utilizing electron microscopy techniques allows the

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[1] Grahame, T. J. & Schlesinger, R. B. (2007). Health Effects of Airborne Particulate Matter: Do We Know Enough to Consider Regulating Specific Particle Types or Sources? Inhalation Toxicology 19, 457-481

development of specific analytical protocols that would be readily available in the case that new regulations would require the characterization of very small particles or provide information on the characteristics of single particles composing a hazardous sample. Electron microscopy is then both a tool for policy development and a research instrument[2] but it could be employed by environmental agencies in official monitoring activities to check if air quality complies with the limits established by regulations, if scientific data[3] would lead to a regulation shift.

Environmental samples are representative of a certain specific sampling period, thereby they cannot be collected again and have to be considered unique. Hence, a streamline analytical protocol will be designed: from a single sample both bulk and single particle data will be obtained. As the development of the analytical protocol is the core of the thesis, an entire chapter, chapter 3, is devoted to the matter.

The samples of PM will be obtained from two types of unconventional sampling substrates: conifer needles and building facades. The choice of collecting the samples from these surfaces, which are not purposely placed in the territory but have been and are there independently of any air quality monitoring network, is supported by the necessity in many remote locations to obtain data without moving instrumentations or more commonly to gather information on periods previous to the setting of instruments. Conifer trees are widely available especially in the Northern hemisphere and buildings covers complementary urbanized areas. Often the two type of samplers coexist giving information on two different time scales: conifer needles may have been exposed to the atmosphere for a period in the order of less than a dozen years; buildings, may have been constructed longer before and, if not restored, may have cumulatively collected contaminants even for centuries.

A case study focused on the characterization of the pollutants emitted by an electric arc furnace (EAF) steelmaking plant and their dispersion in the surrounding territory will be used for the development and partially the validation of the analytical protocol. The case study and the results obtained in that context are described in chapter 4.

The Author hopes that the advantageous collaboration experienced by our University and the local environmental agency, that seems to be reflected in many other case studies in the literature, will be a method of work to overcome the fact that the use of certain instrumentations, such as the electron microscopes, is expensive and not at disposal of every environmental monitoring agency.

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[2] The electron microscope is still a research technique because it cannot provide the measurements needed for official purposes and regulations do not require the amazing resolution of the instrument nor the variety of information it can provide. Current regulations establish a mass concentration limit for different size fraction of PM. Mass concentrations are provided by gravitational techniques and the size classes are defined by measuring the aerodynamic diameter that the electron microscope cannot measure.

[3] obtained from toxicological and epidemiological studies

2 Background

2.1 Particulate Matter Monitoring

> *"[…] today particulates may represent a greater threat to human health in urban areas than any other pollutant"*
> Beckett *et al.* (2000)

Since it was understood the importance of air quality for human health and the environment and the tight connection between the detection and removal of pollutants, there has been an increasing interest in obtaining information on pollutants at different levels:

- size: the concern about the smallest size fraction of PM has been recently growing as it seems that exposure to these particles causes adverse health effects and contributes to the development of respiratory and cardiovascular diseases;
- concentration: higher attention is given to the presence of pollutants even in minimum amounts using more sensitive and selective instruments or pre-treating the samples before the analysis in order to reduce the contribution of the matrix and increase the detectability of hazardous substances;
- chemistry: to understand whether toxicity is linked to a specific component and to assess if the pollutant is present in a bioavailable[4] form;
- temporal differences: the development of an environment protection-oriented mindset, which is favoring the establishment of regulations and policies, especially in the industrial and waste treatment sectors, highlighted the need to be able to make comparison with past air quality scenarios in order to evaluate the present situation in a more objective way;
- spatial differences: meteorological and geographical conditions play an important role in the spread of pollutants at different distances from the emitting sources, thus, it is currently believed that a large number of monitoring stations on the territory is required or that a reliable knowledge of the dispersion of pollutants has to be reached combining measurements and modeling.

Any study in the environmental field and in sanitary engineering is generally aimed at contributing to the reduction of the causes of adverse effects on the environment and especially on human health. Since air pollution caused more than seven million deaths in 2012 (WHO 2014) it is a field in which much has still to be done to improve the situation. The complexity of dealing with the problem is that the mixture of air pollutants is dynamic and in each territory many sources contribute to it.

This thesis would like to give a contribution in the field of monitoring and characterization of inorganic pollutants and in particular of PM.

PM has a recognized impact on human health as well as on ecosystems, climate, durability and appearance of materials. Therefore, it is a pollutant regulated in the majority of the countries and studied by a variety of discipines: atmospheric sciences, epidemiology, toxicology and exposure assessment disciplines. PM is the most important air pollutant in Asia while, thanks to regulations, its concentrations decreased in Europe and North America by the late 90s (WHO 2006).

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[4] Bioavailability is often related to solubility. Particles dissolving in aqueous solutions can be taken up by cells through ion channels and cleared relatively fast. On the contrary, insoluble particles are taken up by phagocytosis inducing intracellular signaling and are less easily cleared (Kochbach Bølling *et al.* 2009).

Suspended particulate matter is regulated[5] on the basis of its size which determines the ability of the particles to penetrate and reside in the breathing system and their transport dynamic. These aspects are important for health protection and for the assessment of the area of impact of different sources. Particles with an aerodynamic diameter larger than 10 microns are not able to penetrate the respiratory system, thus are not considered hazardous and not regulated. Particles below 10 microns of aerodynamic diameter (PM_{10}) are inhalable and called coarse particles. Particles below 2.5 microns of aerodynamic diameter ($PM_{2.5}$) are called fine particles. They are regulated through the indicator called $PM_{2.5}$ but also included in the coarse particle regulation, as the indicator PM_{10} used in most countries does not exclude this dimensional range. Although ultrafine (aerodynamic diameter smaller than 100 nm) and nano-sized particles are not separately regulated from fine particles from a policy perspective, they can be separately collected for scientific purposes. The limit thresholds in Europe and North America are expressed as mass concentrations in time for $PM_{2.5}$ and PM_{10}. The aerodynamic diameter is a conventional way to give a diameter for non-spherical particles considering their shape and density. It is used in air pollution control because it directly relates to the behavior of particles in a fluid such as air. $PM_{2.5}$ is made of fine particles with an atmospheric lifetime from days to weeks, the capability to be transported on a regional scale and the ease to move from outdoor to indoor environments (Bérubé *et al.* 2007; Watson *et al.* 2002; WHO 2006). Particles in this size range are most likely emitted by anthropogenic sources and in particular by combustion processes. PM_{10} instead is produced mainly by mechanical processes, such as construction and road dust resuspension (Beckett *et al.* 2000; Bérubé *et al.* 2007; WHO 2006), and have an atmospheric lifetime of 10-30 days (Kalavrouziotis and Koukoulakis 2009). Typical annual average concentrations of PM_{10} in Europe and North America are 20-70 $\mu g/m^3$ and 20-60 $\mu g/m^3$ respectively. PM travels shorter distances than gaseous pollutants, no matter the height at which it is emitted. A threshold below which PM does not pose a health risk cannot probably be identified, however for Western Europe and USA a concentration of 3-5 $\mu g/m^3$ of $PM_{2.5}$ is considered background (WHO 2006).

The World Health Organization (WHO) provides targets for PM air concentrations of different particle sizes that are aimed at increasing health protection (WHO 2006). These recommendations contribute in the processes by which states issue regulations setting emission limits for different sources and threshold concentrations to comply with in their territories. The Italian regulation for $PM_{2.5}$ will become effective starting from 2015 and sets the annual average limit to 25 $\mu g/m^3$. The Italian regulation for PM_{10} was issued in 2010 through the D.Lgs. 155 and sets an annual limit of 40 $\mu g/m^3$ and a daily limit of 50 $\mu g/m^3$. These limits should not be exceeded more than 35 times per year. However, it was observed that unless an annual average of about 30 $\mu g/m^3$ is recorded, it is not possible to comply with both thresholds. Since no causal relationship was observed between public health and annual PM_{10} concentration, the WHO does not provide targets for this standard and the USA revoked the limit threshold for it (ISPRA 2011). More details on the existing regulations and differences between the EU (including Italy) and the USA ones are provided in appendix A. In general, the trend in regulations is to set limits for smaller particles. In the beginning PM was regulated with no size separation as total suspended particulate (TSP), then the standard for PM_{10} was introduced and finally that of $PM_{2.5}$. Smaller particles including ultrafine particles, which are respirable, are still not regulated but research suggests they induce adverse health effects because of their large surface area per mass which causes increased biological activity (Bérubé *et al.* 2007; Kochbach Bølling *et al.* 2009; WHO 2006). If this will be confirmed regulations will include them in new standards especially considering that this size fraction dominates the number

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[5] Directive 2008/50/EC adopted in Italy through the D.Lgs. 13 agosto 2010, n.155

distribution of PM (Bernardoni 2009-2010; EPA 1987)[6]. Regulations may change not only concerning size. It was observed in epidemiological and toxicological studies that days with high PM mass concentrations and even days in which regulatory limits were exceeded, are not always associated with adverse health effects (Grahame and Schlesinger 2007). On the contrary, days with low PM mass concentrations may be associated with health consequences. Different hypothesis explaining this can be made, the most obvious being that it is not the mass but the presence of specific toxic components which is related to adverse health effects[7]. If this would be demonstrated, future air quality regulations may be based on chemical composition in addition to size-fractionated mass concentrations. The necessity to wait epidemiological and toxicological research results to update, revoke or issue regulations reflects also the necessity to respect the principle of environmental justice. For example, restricting the limits to urban areas without sufficient evidence that rural PM does not pose a risk for human health would not guarantee the protection of people living in those areas, thus violating the principle of environmental justice. Similarly, indicators different from the mass-based ones are not currently included in regulations: the evidence that certain chemical compositions are responsible for adverse effects is not sufficient, nor there is enough evidence against the absence of effects of other components (EPA 2006).

The data obtained from official monitoring of stationary emission sources, that is of monitoring activities aimed at checking compliance with regulations, cannot be directly related to exposure risks. Part of the pollutants, including part of PM, the so-called primary pollutants, are found in the same form at the source or in ambient air. However, others, called secondary pollutants, are produced in the atmosphere from transformations and interactions between primary ones. Many fine and ultrafine (UF) particles composing PM are secondary pollutants. Official monitoring data are gathered at the source. They can be combined with known pollutant emission rates (recorded in emission inventories) and meteorological information to obtain maps called dispersion or source-oriented models. These models can be used to predict which changes in air quality will be introduced if a new source will be added in a specific location. The limit of these models is given by the fact that they do not consider pollutant transformations and formation of secondary pollutants. Hence, a second kind of air quality models has been developed: they are called receptor-oriented models and are built on the basis of experimental data collected at receptors (living beings, water bodies, soil). This kind of models has the advantage of being able to identify eventual sources not listed in the local emission inventory. However, being built from experimental data, it cannot be used in a predictive way to evaluate the impact of a future source. Further details on receptor-oriented models and on the role of electron microscopy in providing information to build them are given in paragraph 2.2. It has to be highlighted that the two models are complementary, the first being predictive and the second explanatory.

To gather data on the territory in order to build receptor models or for official monitoring activities there is a tendency to use instruments that can provide the results in situ and that can be remotely controlled (Busetto *et al.* 2013; Namieśnik 2001). These instruments belong to the category called active samplers as they need a pump, thus power supply, to suck a sample of air and make the desired measurement. Often they are part of monitoring networks established by the agencies in charge of official monitoring. Since it is expensive to install and operate these stations – Ott (2007) reported that a single sampler costs between $10,000 and $40,000 which corresponds to about

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[6] This fraction is also increasing because of diesel combustion (diesel exhaust PM accounts for up to 80% of urban PM_{10}) and the spread of nanotechnologies producing many engineered nanomaterials (Bérubé *et al.* 2007). Especially, the latter source poses high occupational hazard.

[7] An alternative explanation is that when the mass concentration is high the toxic components are diluted. This hypothesis implies that current attempts in reducing mass concentrations are counterproductive (Grahame and Schlesinger 2007).

7,314-29,300 € considering that year exchange rate – there is a limited number of locations in which they are placed. Baraton and Merhari (2004) stated that even large cities cannot afford the costs of establishing a very dense network of air quality active monitoring stations. For instance, they report that only forty-five automatic stations are located in the 100-km-radius area of Paris (France) inhabited by eleven million people. The selection of the locations where these monitoring stations are placed is based on population density or on judgmental considerations regarding the possibility of exceedance of regulatory limits in certain sites. These selection criteria are not statistically based, thus the efficiency of the locations in representing people's exposure cannot be verified (Ott 2007). Moreover, these stations are insufficient to reflect the demonstrated different risks to which people may be exposed living in different zones of the same urban area (e.g. people living near a major road). This is of fundamental importance in epidemiology studies where the central monitoring approach has been substituted around 2002 by a personal exposure monitoring strategy (Grahame and Schlesinger 2007). Personal exposure monitoring takes into account that especially coarse particles, which tend to settle near their source, have a very large spatial variability, thus they could have spatially different effects, while central monitoring assumes that every person in an area is exposed to the same pollution load and is based on gathering data through the sparsely distributed official monitoring stations. Considering that "the expense of purchasing and operating a sufficient number of filter-based samplers to capture this variability is often impractical" (Ott *et al.* 2008a) but exposure assessment studies would benefit of having a larger number of monitoring stations, ground-based measurements based on the use of passive samplers are increasing. These samplers (Assael *et al.* 2010; Kot-Wasik *et al.* 2007; Namieśnik *et al.* 2005; Ouyang and Pawliszyn 2007; Seethapathy *et al.* 2008):

- are inexpensive compared to active devices;
- do not require power supply, as no suction pumps are needed;
- provide easily time weighted average concentration of pollutants based only upon exposure time without measuring sample volume;
- are small, thereby their transport between the sampling site and the laboratory is relatively cheap;
- are suitable for long-term collection of samples of pollutants;
- are easy to operate.

The measurement that can be obtained through the use of passive devices is a time-weighted average (TWA) of the concentration of pollutants over the sampling time. The limit of the detection for a certain pollutant depends on the sampling rate, the sampling time and on blank values of the unexposed sampler (Namieśnik *et al.* 2005).

The limitations to consider when using passive samplers are that (Assael *et al.* 2010; Kot-Wasik *et al.* 2007; Namieśnik *et al.* 2005; Seethapathy *et al.* 2008):

- they are unsuitable for monitoring short-term variations in pollutants concentration;
- they need to be carefully calibrated in order to convert the number of deposited particles into actual analyte concentration in the atmosphere. Therefore, a deep knowledge of the uptake kinetics of different compounds is required and often for calibration purposes passive devices need to be placed next to active instruments;
- they are difficult to be automated;
- they provide measurements with a high uncertainty due to their sensitivity to environmental parameters: temperature fluctuations, air movements, air humidity. The dependence from all these parameters made the US National Institute of Safety and Health (NIOSH) estimate an accuracy of ±25% and a bias of ±10% for the application of passive samplers in air quality studies.

Of course, depending on the situation any of these drawbacks can become an advantage. For instance, in the case where the concentration of a pollutant goes up and down in time, a very short-term measurement done by active sampling could bias the conclusions providing a very low or high value. On the contrary, an average, such as that recorded in the long-term by a passive sampler, may be more representative of the situation.

Some researchers included in the concept of passive sampling biomonitoring[8]. However, not all biomonitoring activities can be really considered passive collection of pollutants. Bioaccumulation techniques can be considered techniques employing the "passive" accumulation of pollutants by selected biological receptors (the most commonly used are mosses and lichens) (Nimis 1998). The organisms used for bioaccumulation studies must be tolerant to the substances to be monitored. Bioidication techniques estimate the changes in air quality from the study of the morphological and physiological changes in individual monitors or also from changes at the level of population or community of monitors. The organisms selected for bioindication studies must be spread in the territory, be sensitive to the pollutants to be investigated and have a long life cycle. Of the two described aspects of biomonitoring only the first, bioaccumulation, could be included in the concept of passive sampling. However, no confusion should be made with the fact that some Authors classify biomonitors as active or passive based on their presence in the territory (passive) or their positioning by the scientist who is conducting the study (active). Nimis (1998) already highlighted that this classification is not appropriate and in the context of this work both already existing biomonitors and biomonitors placed by an operator can be considered as passive samplers of pollutants as, at least for PM (object of investigation in this work), they do not exert any "sucking" action that can be assimilated to that of a pump of an active sampler. On the contrary, their sampling activity is related only to the fact that their surfaces are exposed to the atmosphere. The distinction among biomonitors already present at a site and those placed by a scientist in a place is however relevant for the kind of data that can be collected. If a biomonitor was already present at a site, the time of exposure includes periods previous to the beginning of the monitoring activity. Therefore, the use of these samplers present the advantage of offering wide spatial and temporal covering. Placing biomonitors at a site, instead, allows only the coverage of a wide territory but is no different than placing an instrument, thus in the context of this work present no advantages compared to instrumental monitoring.

The definition of passive sampler may include any surface exposed to the atmosphere, such as building facades.

Both passive and biomonitoring techniques, described above, can be employed to study the dispersion of pollutants emitted by point or linear sources[9]. Traffic is often studied in urban contexts where many monitoring stations are available to provide data, thereby passive sampling strategies might be more useful to monitor certain point sources located in remote regions (Pöykiö 2002).

The most difficult type of information to gather in air quality monitoring projects are those on the past air quality scenario (if no data where recorded in the period of interest) and those on fugitive emissions that are not released at a common point and often have an unpredictable emission dynamic. Two types of fugitive emissions exist: industrial and dust (Lillis and Young 1975). The first ones can be both gaseous and particulate. Both are those process or non-process related emissions escaping a factory without passing through the off-gas abatement system but through

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[8] Nimis (1998) defines biomonitoring as the analysis of components of ecosystems reactive to pollution for the estimation of deviations from normal situations.

[9] Linear sources are generally railways, highways or roads affected by high traffic (aggregated effects of all the single emission sources travelling on the line).

windows, doors, roof ventilators, etc. Although they are currently not clearly regulated[10], they should be carefully monitored to avoid that source operators release more fugitive dusts to meet the regulatory limits for the other emissions passing through the off-gas abatement system, i.e., those officially monitored. Fugitive dust is generally windblown or resuspended by moving vehicles and can be either of natural or anthropogenic origin but in any case in particulate form. It includes for instance dust storm and PM from unpaved roads. Fugitive dust is not regulated as control strategies are often too expensive or unreasonable (e.g. paving all roads, adding cohesive chemical compounds to soil, etc.).

In designing an air quality monitoring study the following steps are necessary:

1. identification of the problem and definition of the concentration ranges to be measured (trace levels are not always a need)
2. identification of the analytical method sensitive enough to provide the desired information and determination of the type of sample suitable for the investigation with such a technique
3. selection of the sampler able to provide the desired sample or adaptation of it or development of a new sampler tailored for the purpose.

Sometimes, as it is the case of this thesis or the needs of the case study described in chapter 4, steps 2 and 3 are inverted as the conditions require the use of a certain sampler and sample preparation and the analytical protocol must be adapted or completely developed to provide the required data from the available sample.

No matter the type of samplers and analytical technique selected, it must be considered that a sample is a very small “portion of the environment”, thus statistical consideration must be included both in the planning step (number of sites and sampling design) and during data interpretation.

Source apportionment is necessary in all monitoring studies. If the focus of the study is on one specific source, this has to be singled out from others. In other situations different sources must be distinguished in order to apportion to each of them its contribution to total PM concentration. Hence, the fingerprint or source signature of every source must be identified. This is a unique chemical (ratio of components, isotopes, chemical form in which an element is present, etc.) or physical (number or mass size distribution of the particles, shape of the particles, etc.) characteristic or combination of them. It is not easy identifying such unique features as many sources emit simultaneously pollutants in a dynamic way and their atmospheric transformations further complicate the picture. Measurements in different seasons are often undertaken to identify the source signatures. This is done because certain anthropogenic (e.g. those from heating systems) and natural (e.g. dust resuspension which is absent in winter in areas covered by snow) emissions are seasonal, while in general industrial emissions are not (Morawska and Zhang 2002). Alternatively, short sampling campaigns are conducted when a single source is dominating thanks to meteorological and/or to its specific emission dynamic (Watson *et al.* 2002). Jenq *et al.* (1992) determined source profiles for fugitive emissions of EAF steelmaking plants, cement plants and asphaltic concrete plants. Instead of source signatures in specific studies, if local sources are known, it is possible to utilize easily measurable markers or tracers for each of them (Morawska and Zhang 2002). For instance, barium is often used as a marker for brake wear emissions (Gietl *et al.* 2010). Generally, multivariate statistical analysis tools areused to discriminate different sources when many samples have been collected (Watson *et al.* 2002).

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[10] In the United States of America some attempts to regulate fugitive emissions have been done. Certain states issued nuisance regulations which deny the possibility for a source to exist if it causes harmful effects on any person. Other states have imposed the use of equipment to capture and control fugitive emissions. Others adopted visible emissions regulations and the less effective regulations released by other states were those indicating the necessity of “reasonable precautions to prevent fugitive emissions to become airborne”. The Environmental Protection Agency (EPA) considers a flexible regulatory approach the best strategy to successfully reduce the fugitive emissions of specific industrial plants that are very different from those released by other factories (Lillis and Young 1975).

The knowledge of specific industrial cycles and wear mechanisms is the basis for source apportionment. Therefore, materials science and industrial engineering may play a key role in air quality studies.

In the air quality field, industries are considered as stationary point sources of pollutants in the sense that a single plant is entirely contained in one 1x1 km^2 or smaller mesh of the grid dividing a territory in a typical emission inventory (WHO 2006).

Industries may emit pollutants because of their specific production process and products in use (i.e., toxic solvents) but also because of combustion activities that sustain the process and waste disposal procedures.

The aim of monitoring the emissions from a particular plant combined with the knowledge of the industrial process there undertaken and the emission abatement strategies there adopted may help in finding technological solutions that reduce the impact of these activities on the environment and human health.

Monitoring the emissions from a plant should include measuring pollutants concentrations at different sites around the factory as an industrial plant typically releases pollutants at different heights: chimneys disperse pollutants at high heights from the ground and at ground level there are, unfortunately, fugitive emissions. Pollutants that are emitted from a chimney influence a wider area because pollutants emitted at high heights are spread more in the atmosphere. On the contrary, ground-level emissions such as the fugitive ones determine higher local concentrations of pollutants (WHO 2006). The results of these different emissions are that industrial plants can affect areas of 20 km^2 (Machemer 2004). Unfortunately, only the measurements at the stacks are easily undertaken. These are used to produce emission inventories and air quality dispersion models. The measurement of fugitive emissions is much more complex as these emissions are not collected and released in a controlled manner (Sielicki *et al.* 2011). This difficulty is reflected in the design of the measurements for the case study described in chapter 4.

Industrial emissions are generally linked to high energy processes, such as combustions, hot processes (smelting, welding, laser cutting, etc.) and high velocity mechanical processes (grinding) (Elihn and Berg 2009). Combustion causes the release of smoke made of solid particles (0.05-1 μm). The type of particles depends on the fuel burnt (appendix B). When analyzing the contribution of a plant to combustion particles the emissions from other anthropogenic sources (diesel and gasoline vehicles, biomass burning, energy production) must be distinguished.

Hot processes can produce fumes containing solid particles (0.03-0.3 μm) formed by condensation of vapours through sublimation, distillation processes, calcification or other chemical reactions (f.e. Pb and Zn oxides are produced during oxidation and condensation of metals at high temperature).

High velocity mechanical processes (grinding) can cause the emission of dust (1 μm - 10 mm) such as sawdust coming from wood manufacturing or sand used during sandblasting.

The hazard caused by industrial air pollutants must include that posed to workers of the industry (occupational hazard) and that posed to the environment and the population living in the area affected by the plant.

Every industry emits characteristic pollutants and contributes in different amounts to the general air quality level. Metallurgic plants emits particles containing silicates and carbonates and sulphur gases. In particular, steelmaking plants are important emitters of PM, heavy metals and persistent organic pollutants (POPs). Ceramic industry emits heavy metals like Hf, U and Th. Cement production plants emit PM and SO_x, plants using solvents emit many non methane volatile organic compounds (NMVOCs), while chemical and petrochemical plants release organic sulphur compounds, heavy metals and hydrocarbons (Bernardoni 2009-2010; Romano *et al.* 2009). Appendix C list possible sources of metals including industrial plants. In 2007, the most important source of PM, heavy metals and POPs in Italy was the iron and steel industry (Romano *et al.* 2009).

The emissions from steelmaking plants using electric arc furnaces (EAFs) are described in appendix D and they are of interest for the case study.

2.1.1 Conifer needles as samplers of particulate matter

The reader should refer to the review by Bertolotti and Gialanella (2014). At the end of chapter 4, Table 4.4-1 compares the advantages and drawbacks of building facades and conifer needles as passive samplers of pollutants. Appendix E summarizes all studies in which conifer needles have been utilized specifying the analytical technique used, the pollutants analysed, the country in which the study took place and the general purpose of the investigation. From a quick look it emerges that atomic spectroscopies, among which also ICP-AES, have been extensively used. This reason and the fact that ICP-AES is multi-elemental and it has a very low detection limit, as a rule of thumb in the range of 1-10 ppb, leaded to select such a technique for bulk analysis in this work.

2.2 Electron Microscopy for particulate matter analysis

> *Will the electron-microscope techniques really "remain essentially research techniques" as predicted by Cartwright et al. in 1956?*[11]

Electron microscopes have already proven to be effective tools among the analytical techniques applied in the field of environmental and materials sciences. As they are still being improved, they are even more promising for the future. Already in the review published in 2000 on *Acta Materialia* by Newbury and Williams (2000), the electron microscopes are identified as the "materials characterization tool of the millennium" and many different applications in the field of materials science were presented. It can be stated that also in the field of environmental sciences the application of these instruments has been quite immediate. The first commercial transmission electron microscope appeared in 1936, but was spread after the Second World War, the first commercial Scanning Electron Microscope was available in 1965, the first study using these instruments to characterize PM was published, to our knowledge, in 1956 on the *Quarterly Journal of the Royal Meteorological Society* (Cartwright *et al.* 1956). The Authors of the article, from the Safety in Mines Research Establishment (SMRE) in Sheffield (UK), used the transmission electron microscope to complete the characterization of coal mine dust for what concerns spherical carbonaceous particles with diameters lower than 3 µm. Above this size they used an optical microscope. They used electron diffraction in the TEM to establish the amorphous or crystalline nature of the particles, while for chemical analysis no energy dispersive x-ray spectroscopy was available and their tests consisted in comparing the samples as such and after treatments (heating, exposure to hydrofluoric acid vapour, intensification of the electron beam) in order to determine the combustion temperature of the particles and the presence of quartz or sodium chloride (NaCl). Other early application of electron microscopy (EM) to study aerosols and fly-ash particles were also done in English-speaking countries (UK and Australia) and published in 1967 on the journal *Atmospheric Environment* (Heard and Wiffen 1967; Ramsden and Shibaoka 1967).

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[11] These authors in the first paper using TEM to study PM (Cartwright *et al.* 1956) were using projection counting to obtain surface and mass distributions of the particles sampled with a thermal precipitator on Formvar-coated substrates. In the discussion section of their article they wrote: "The electron-microscope techniques will probably remain essentially research techniques, even if the development of automatic evaluation of the records replaces the projection counting used at present".

Electron microscopes allow imaging with high resolution (SEM: 20-50 nm; TEM: 0.1-1 nm). In the field of atmospheric particle characterization this means the opportunity to conduct single particle analysis (SPA), sometimes called individual particle analysis (IPA) (Hunt and Johnson 2011), down to the nanoscale dimensional range. With an optical microscope it would be possible to observe only coarse particles larger than 0.3-0.7 μm, which correspond to the wavelength of light (Chow and Watson 1998), while with a TEM it is possible to study particles even smaller than 1 nm. This is very important considering that from the beginning of microscopy studies of PM it was observed that the number of particles below the limit of optical resolution is higher than that above it (Cartwright *et al.* 1956). More than just observe fine and UF particles it is possible to determine simultaneously their chemical composition as both scanning and transmission electron microscopes are generally coupled with energy dispersive X-ray spectroscopy (EDXS) systems or less commonly with wavelength dispersive X-ray spectroscopy (WDXS). Compared to x-ray fluorescence (XRF) or particle induced x-ray emission (PIXE), EDXS can detect light elements as most modern detectors are windowless and do not require working in low vacuum. Also maps showing the distribution of the elements in the particle under study can be drawn by the EDXS software. In TEM instruments, it is possible to obtain also crystallographic data through selected area electron diffraction (SAED). The opportunity to obtain morphological, compositional and crystallographic data in one go is not offered by any other technique of SPA. Nor micro-Raman spectroscopy (μ-RS), secondary ion mass spectroscopy (SIMS) or synchrotron based infrared spectroscopy (SR-FTIR) and diffraction (SR-XRD) can compete alone with electron microscopy regarding the completeness of characterization offered.

In environmental studies all these information may help to:

- understand the formation of particles, their transport, their reaction and deposition mechanisms, their environmental history, complementing bulk analytical techniques;
- identify the emission sources of certain pollutants in source apportionment studies;
- investigate soiling of building materials;
- understand the effects of PM and predict the potential health risks;
- predict the potential impact of PM on the environment (climate forcing, cloud formation, absorption and scattering of solar radiation);
- characterize $PM_{2.5}$ as required by the National Ambient Air Quality Standards (NAAQS) of the United States of America especially in case of exceedance of the limits (EPA 1998).

In particular, in source apportionment studies electron microscopy, especially the automated scanning electron microscope (CCSEM), plays a role in receptor modelling. Receptor models or receptor-oriented models are one of the possible mathematical models that can be used in air quality study and management to identify sources of pollutants and apportion the concentrations measured at a certain site to those sources. They are based on ambient measurements of the chemical and physical characteristics of the particles at the point of impact. The electron microscope:

- can be the principal analytical tool to obtain the information on the properties of the particles (composition, size and shape) collected at a receptor site on which the model is based;
- can be used to verify the data obtained by size-selective samplers;
- can be used to obtain information that validate source-oriented models;
- can be used to validate receptor models for source apportionment built on the basis of bulk analysis (EPA 1998).

Nonetheless the described enormous advantages, EM techniques were not applied until recent times for the analysis of airborne particles as much as other analytical methodologies mainly because of the difficulty of having a sample suitable for the examination with these techniques (Casuccio *et al.* 2004).

SEM specimens need to have an appropriate spacing among the particles, especially if they have to be observed in computer controlled mode. Particles have to be collected in a monolayer on a smooth conductive and thermally stable substrate (Sielicki *et al.* 2011; Willis *et al.* 2002). If the substrate is a polycarbonate filter a loading of the substrate of the order of 5-20 $\mu g/cm^2$ is recommended to minimize particle contact. By eye this loading is reached when the filter looks gray (Capannelli *et al.* 2011; Casuccio *et al.* 2004). Some particles may not have suitable conductivity for electron microscope conditions, thus coating the sample by sputtering with carbon, gold or gold/palladium could reduce the chance of volatilization of the particles and the consequent damage to the components of the instrument. This is very important when particles containing nitrogen, such as nitrates, are analyzed as nitrogen easily sublimate or when sulphur-containing particles are studied as they may be altered. The coating needs to have a thickness that does not hide textural details of the particles (Coz Diego 2008).

TEM specimens needs to be thin enough to be electron transparent (around 100 nm; Sielicki *et al.* 2011), disk shaped with a diameter of 3 mm, conducting and non-magnetic (Williams and Carter 2009). The methodology used in producing the TEM specimen might affect the analysis, thus it needs to be considered during the interpretation and discussion of the analytical results. The preparation technique is highly related to the previous step of sampling.

Figure 2.2.1 summarizes the techniques available to sample PM (EPA APTI 435). For each of them different sampling substrates are available and their suitability for electron microscopy will be described further in this paragraph.

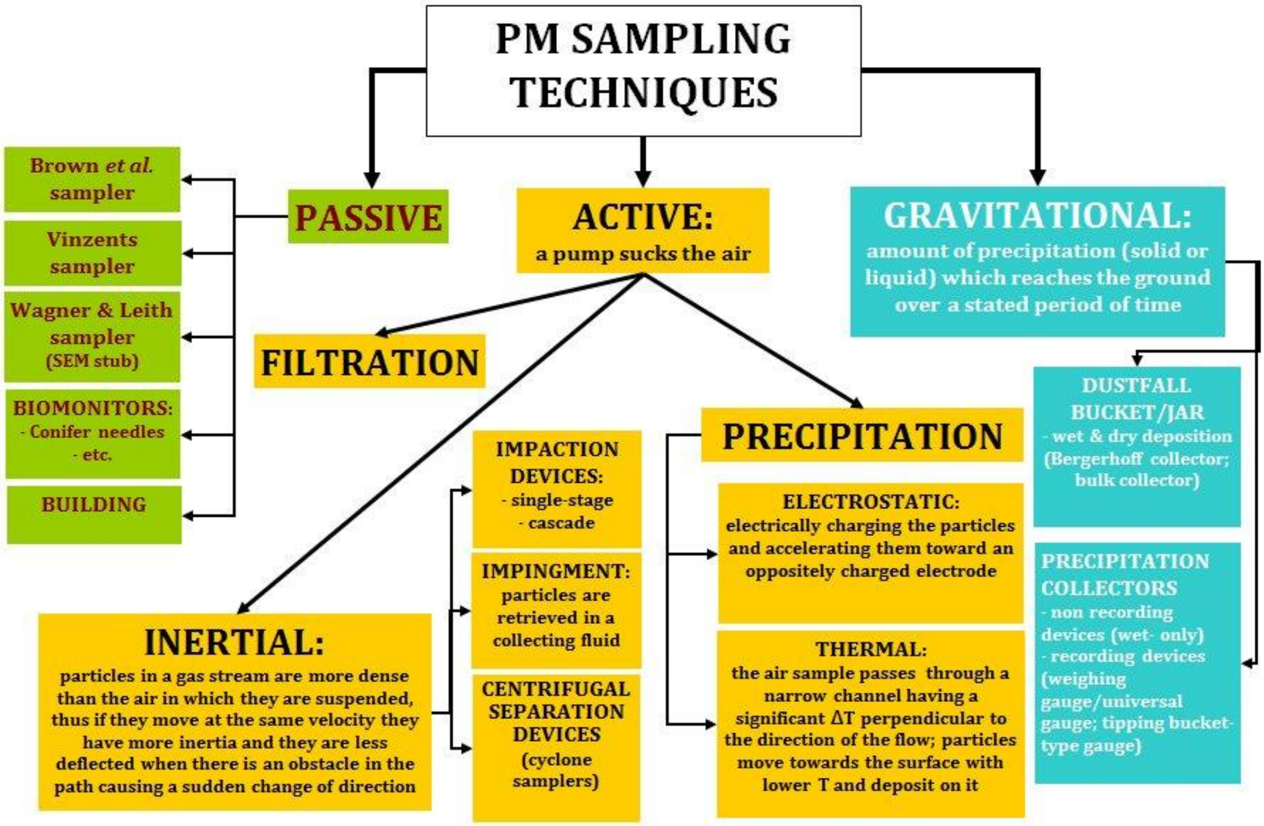

Figure 2.2.1 Techniques to sample particulate matter. In the graph under passive sampling also the two unconventional substrates used in this thesis are listed.

<u>Inertial devices</u> may use different collection matrix (glass microscope slides, beryllium disks, aluminium foils, silver foils, silicon wafers, TEM grids) as the physical and chemical properties of the substrate do not influence sampling given certain operating conditions of the sampler. These

collection surfaces are often coated with a thin film of oil or light grease, which enhances the retention of the particles. Substrates such as silver foil, aluminium foil, silicon wafer and beryllium disks have some drawbacks if used for EM analysis (Godoi *et al.* 2006; Laskin *et al.* 2006). The x-ray spectrum lines of silver overlap with other lines of interest in airborne particle analysis, thus silver foil is not suitable for EDXS analysis. Aluminum foil, due to its manufacturing process, present a line structure which makes particle recognition complicated. Moreover, the atomic number of aluminium is comparable with the atomic mass of calcium carbonate, a very common particle and building material, causing low contrast in backscattering electron imaging. For the same reason also the silicon wafer, that on the other hand has a very flat surface, is not a good substrate for electron microscopy coupled with EDXS. Beryllium is brittle, toxic, very expensive and reactive with sulphates, thereby it is not a good substrate for the analysis of atmospheric aerosols.

TEM grids are maybe the most interesting substrate for conducting single particle analysis. The direct use of a TEM grid for sampling avoids the introduction of artefacts that may happen during sampling preparation (Lorenzo *et al.* 2007). They are suitable both for SEM and TEM (Sielicki *et al.* 2011). They are made by a regular metallic mesh (copper, nickel or gold) coated with modified (by carbon or silica) Formvar (polyvinyl formal resin). The mesh with a signed middle centre favour the identification of the precise location of a certain particle. The same quadrant might be found again later allowing the analysis of the same particle with other techniques, such as time-of-flight secondary ion mass spectrometry (ToF-SIMS) (Godoi *et al.* 2006; Laskin *et al.* 2006). Dye *et al.* (1997) produced their own electron-beam transparent porous carbon film to be deposited over a copper mesh grid and compared its collection efficiency for PM_{10} in direct sampling through a cyclone sampler with that of other films that may be deposited on the grids: Formvar and holey carbon. It emerged that the self-produced porous film was the best compromise to have a good collection efficiency, also of the particles below 1 μm. The collection efficiency of the different types of TEM grids available should be evaluated for the size of interest of PM. Ouf *et al.* (2010) recommended sampling directly on TEM grids against filtration on PC membranes if TEM analysis has to be conducted. This technique was applied by Bang *et al.* (2004) who collected nano-sized PM on a TEM grid placed inside a portable thermal precipitator. Even if the thin film deposited on the mesh is composed of carbon it is possible to distinguish carbon-rich particles on it (Casuccio *et al.* 2004). Furthermore, TEM grids provide an almost flat background in the EDXS spectrum that makes it possible to clearly identify the peaks from the particles, as the low thickness of the film (of the order of 25 nm) makes this substrate transparent to a 20-keV electron beam reducing the scatter from the substrate. However, the material composing the grid (Cu, Ni or Au) may provide peaks in the spectrum due to the scattering of secondary electrons (Laskin *et al.* 2006; Sielicki *et al.* 2011; Willis et al. 2002). They almost do not contribute to the backscattered electron (BSE) signal ensuring good contrast in BSE imaging. However, with particles smaller than 1 μm the contribution of the grid to the EDX spectrum increases (Godoi *et al.* 2006; Laskin *et al.* 2006). Among the commercially available different types of TEM grids, the carbon-coated Formvar film supported on a standard Cu grid is the best compromise for microscopy as this film provides an optically flat background which guarantees the best conditions for imaging. The C-coating of the thin film improves the stability of the Formvar film to localized heating from the electron beam (Willis *et al.* 2002).

Filtration devices collect particles on filters that can be fibrous or membrane filters. Fibrous filters are made of woven material (individual fibers compressed into a cohesive mat), while membrane filters are made of continuous (plastic) material penetrated by microscopic pores (often obtained by etching). Fibrous filters capture particles at various depth and only the coarsest are retained on the surface. On the contrary, membrane filters are superficial ones, thus they are recommended for imaging techniques. For the same pore size fibrous filters are more resistant to the passage of air than membrane filters. The pore size of membrane filters is much more uniform and controlled than that of fibrous filters (Thompson and Walsh 1989).

Fibrous filters are available in different materials:

- glass fibres
- quartz fibres (borosilicates)
- cellulose fibres (chemical filters)
- mixed cellulose esters (MCE)
- Teflon (PTFE).

Quartz fibre filters are a subgroup of glass fibre filters characterized by resistance to high temperatures (they remain unchanged until 1000°C). They are obtained by fine glass fibres woven with or without an organic binder (maximum amount of binder: 5%) and compressed in a paper machine. They are indicated by European Directives for samplers of PM_{10} and $PM_{2.5}$. They are not hygroscopic, thus constitute a very good substrate for gravimetric analysis as their weight is stable with respect to humidity. However, they are very fragile. They are suitable to sample in corrosive atmosphere and they are recommended if sampling is aimed at determining organic and elemental carbon by ion chromatography (IC). They are appropriate for Raman analysis as they do not show Raman or fluorescent signals but the acquisition of data is not easy as they have a complex, not completely flat, surface morphology (Nelson *et al.* 2001). They are not suitable for X-ray fluorescence (XRF) and not recommended for EDXS as their content of Si, O and C (carbon may be present in the binder) influences the analysis (Casuccio *et al.* 2004; Willis *et al.* 2002). They have an efficiency higher than 99% for particles having aerodynamic diameter of at least 0.3 μm. They are generally too loaded to allow electron microscopy examination without transfer of the particles to another substrate (Capannelli *et al.* 2011).

Cellulose filters were designed for liquid-solid separation in chemistry. They are hygroscopic and not resistant to high temperatures. However, they have low metal content and they are suitable to determine trace metals in PM with different techniques. Whatman 41 is the most used commercial type.

Mixed Cellulose Esters (MCE) are dry gels made by inert cellulose nitrate or acetate. They melt at 70°C and they are fragile. They are recommended by EPA and NIOSH to sample asbestos fibres. For this purpose, two pore sizes are used: 0.45 μm and 0.8 μm. The first pore size is that recommended by EPA and NIOSH and it was proven by Vallero *et al.* (2009) to have higher retention efficiency for asbestos fibres of size equal or larger than 0.5 μm. If fibres longer than 5.0 μm need to be collected, no relevant difference in retention efficiency was observed among the two different porosities. The most common commercial type used is produced by the Millipore Corporation.

Teflon (PTFE) filters are indicated by European regulations for PM_{10} and $PM_{2.5}$ samplers. They are not hygroscopic, thus they are good substrates for gravimetric analysis (EPA 1999). They are also suitable for XRF and IC. EDXS is influenced by the presence of carbon and fluorine peaks (Willis *et al.* 2002). They are better than quartz substrate for electron microscopy but when used for PM_{10} sampling, as indicated by EU directives, they are generally too loaded (Capannelli *et al.* 2011). They have a collecting efficiency higher than 99% for particles with aerodynamic diameter of 0.01 μm (EPA 1999).

Fibrous filters are not suitable for microscopy as small particles are able to be trapped in the fibres throughout the depth of the filter and do not lie on the surface of the filters where only coarser particles are retained (Coz Diego 2008; Casuccio *et al.* 2004; Sielicki *et al.* 2011; Willis *et al.* 2002). The fact that the particles are at different depth increases the difficulty in focusing the sample for good quality imaging.

If fibrous filters have to be employed the particles deposited on them need to be transferred to a polycarbonate support to be directly observed by SEM. The process, however, can result in loss or transformation (morphology, chemistry) of particles and contamination, thus it can affect the representativeness of the sample (Casuccio *et al.* 2004; Willis *et al.* 2002).

Membrane filters are available in:

- nylon
- polycarbonate (PC).

Nylon substrates are particularly suitable to sample nitrates and other ions soluble in water. They are not hygroscopic, thus they can be used for gravimetric determinations. They have a collection efficiency of 99% for particles with aerodynamic diameter of 0.01 µm.

In nylon also woven net filters exist with larger meshes but those are less suitable for microscopy (compare Figure 2.2.2 and Figure 2.2.3).

Figure 2.2.2 Optical microscope image of a woven nylon net filter with 30 µm mesh.

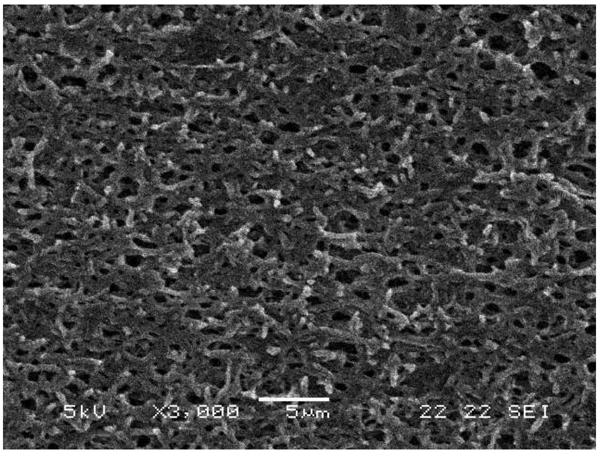

Figure 2.2.3 SEM micrograph of a nylon membrane with a 0.2 µm mesh.

Polycarbonate (PC) filters are superficial filters, ideal for SEM analysis because of their very smooth surface composed of light elements (H, C, O) on which the particles are easily distinguished, especially in backscattered electron (BSE) imaging mode, where the contrast with particles with high atomic number is high (Capannelli *et al.* 2011; Willis *et al.* 2002). The ideal load of the filter to distinguish the particles using a CCSEM is between 5 and 20 µm/cm^2. The surface of the membrane presents randomly distributed circular pores industrially obtained by ion irradiation followed by chemical etching. They are manufactured by Corning CoStar (formerly Nucleopore Corporation) and commercially available in different diameters ranging from 13 mm to 47 mm and pore sizes ranging from 0.01 µm to 20 µm (Casuccio *et al.* 2004; Willis *et al.* 2002). Capannelli *et al.* (2011) observed that those with 0.8 µm pore size have a collection efficiency of less than 50% for particles smaller than 200 nm, while those with 0.2 µm pore size are suitable also for nanoparticle collection with a better efficiency. These substrates are suitable also for Raman spectroscopy (Nelson *et al.* 2001). Particles tend to accumulate around the pores of the PC filter due to dipolar forces caused by gradients of the electric fields near the edges of the pores (Zamengo *et al.* 2009). The drawback of the use of these filters is that they are mainly composed of carbon, thus this substrate is not useful for carbon particles analysis (Coz Diego 2008; Sielicki *et al.* 2011; Willis *et al.* 2002). To reduce this problem, especially in the analysis of fine and ultrafine particles in which the volume of interaction where the x-rays are produced may be larger than the particle itself, the filter may be coated with palladium to attenuate the carbon contribution from the filter. The palladium peak appearing in the spectrum is an artefact and may also interfere with other peaks, such as the chlorine one. Moreover, palladium has a high atomic number and the bright background in backscattered electron imaging reduces the contrast with many particles (Casuccio *et al.* 2004). Lowering the accelerating voltage of the electron beam may help in reducing the contribution of the substrate to the EDXS signal (Godoi *et al.* 2006). Polycarbonate filters are recommended by EPA (1987) for the study of asbestos fibres.

Passive samplers for PM exist in three designs: the Brown *et al.* one, based on electrostatic force, the Vinzents one, based on the exposure of a transparent sticky surface and its analysis with a laser device, and the Wagner and Leith one which consists in a SEM stub with carbon tape adhesive exposed to the atmosphere (Seethapathy *et al.* 2008; Wagner and Leith 2001). The latter seems the best in the context of this work, as the collected sample is ready for analysis without further sample preparation. However, Prof. Peters' Research Group at the Occupational and Environmental Health Department of the University of Iowa (USA) identified two main limitations in this device: the necessity to know wind speed in order to estimate the load of particles of intermediate size by turbulent inertia and the need to protect the sampler from precipitations. That is why a PhD student of his research group developed a flat-plates shelter for the Wagner and Leith sampler. This shelter simultaneously protected the sampling surface from precipitations and reduced the wind-dependent turbulent inertial deposition (Ott 2007). He also modified the Wagner and Leith's sampler by adding a cover glass as collection substrate in order to be able to observe the sample and acquire pictures by light microscopy and later analyse these images with an image analysis software in order to obtain quantitative data (Ott *et al.* 2008b).

Precipitation devices (electrostatic precipitators, thermal precipitators, thermophoretic samplers, electrophoresis samplers) can utilize a variety of substrates: glass fibres, silicon wafer, silver foil, aluminium foil, beryllium disk and TEM grids. As early as in 1956, thermal precipitators were recognized as good samplers of PM to be studied by TEM because they favour the preservation of the state of aggregation of the particles. These Authors used Formvar-coated cover slips as substrates instead of glass slides and considered thermal precipitators more efficient than electrostatic precipitators and filtration devices with membranes (Cartwright *et al.* 1956). They also used heat-stable membranes of alumina to study the combustion temperature of the carbonaceous particles by counting the particles in the TEM micrographs after heating the sample at different subsequent temperatures.

The sampling surface of gravitational samplers is the cylindrical container itself: the bulk collector is made by a funnel and a bottle, the Bergerhoff collector is a simple bucket, while the wet-only collector is a bulk collector with a lid that avoids collection on dry days. This sampling system does not allow the obtainment of a material that can be directly observed by electron microscopy.

Similarly a directly observable sample is not obtained also when:

- fibrous filters are used to comply with official monitoring techniques;
- the sample is a powder scraped from a building facade or is soil;
- the sample is made by particles collected by brushing the leaves of trees;
- the sampler available for the study does not support an EM substrate.

In all these situations it is necessary to transfer the particles from an unsuitable substrate to a substrate suitable for electron microscopy.

From a fibrous filter it is possible to transfer the particles on a polycarbonate filter with an electric vibrating etching tool or with a chemical extraction (resuspension) followed by particle redeposition. The first option does not guarantee the transfer of all the particles, as it was observed that some became airborne (Khwaja *et al.* 2009). The second option requires two steps: in the first step the fibrous filter is immersed in a solvent (ethanol, acetone, hexane, distilled water) for dissolution of the particles, and the system is sonicated in order to obtain a homogeneous suspension; in the second step, the obtained suspension, eventually diluted, is percolated through a PC filter (liquid filtration) (Capannelli *et al.* 2011) or redeposited on the PC filter using a vacuum filtration apparatus (Willis *et al.* 2002). Capannelli *et al.* (2011) observed that the transfer is efficient from Teflon filters but not from quartz filters as during the extraction many quartz fibres are released and may settle together with the particles on the PC filter. If it is not necessary to conduct SEM analysis, particles deposited on a fibrous filter can be directly prepared for TEM investigation. In EPA's document (1987) a methodology to transfer PM sampled on MCE filters to TEM grids is reported. More recently, Vallero *et al.* (2009) wrote that for the analysis of asbestos fibres collected on MCE filters by TEM the filter is collapsed with an organic solvent, such as dimethylformamide (DMP), in a Jaffe washer, as described in ISO 10312:1995, and then its top layer is etched away with a low temperature plasma etcher.

Pollutants collected by gravitational samplers are generally dispersed in water or lie on the surface of the funnel as solid deposits. To study these samples by electron microscopy the easiest procedure is to transfer these particles on a PC filter percolating the solution through it or depositing the particles on it using a vacuum filtration apparatus. If SEM analysis is not needed, the solution can be deposited directly on a TEM grid.

Particles scraped with a scalpel from the facades of buildings and monuments can be analyzed directly by putting an aliquot of the powder on a double sided carbon tape on a SEM aluminium stub (direct preparation), by depositing the powder on a PC filter (redeposition) or by creating a solution concentrating the particles for SEM or for TEM analysis (resuspension). After having spread the particles on the tape it is recommended to gently blow air on it in order to blow out the particles that are not well adherent to the substrate (Bhattacharjee *et al.* 2011). The drawback of using a C-tape is its roughness of about 1000 nm which can sometimes make particle recognition challenging (Godoi *et al.* 2006). Deposition of the particles on a filter can be done by wet or dry preparation methodologies (Schiavon *et al.* 2004; Willis *et al.* 2002). Willis *et al.* (2002) describe two wet preparation procedures. Both methodologies present the risk of altering the chemistry, size or morphology of the particles or loosing particles during the dissolution in the solvent. For the dry preparation the same Authors (Willis *et al.* 2002) propose two systems, one using commercially available tools and another one using a laboratory built "puffer" which allows the reduction of the amount of sample needed to 0.1 mg. To analyze the dusts scraped from building facades by TEM it is possible to prepare a 0.1 wt% deionized water solution, then ultrasonicate it for 45 minutes and finally sprinkle it on a TEM grid and let it dry for 24 hours before examination (Bhattacharjee *et al.* 2011). If the samples are fragments of stone or plaster, they can be embedded in epoxy resin and polished in order to observe the cross section and study its internal structure. This preparation procedure is called polished mount.

Powders of airborne particles may also be collected by brushing the leaves of trees, as it was done by Lu *et al.* (2008). The researchers ultrasound dispersed the dust in alcohol and deposited it on a carbon coated Cu TEM grid.

After the analysis of the particles as they were sampled, more detailed analysis of specific classes of particles could be undertaken after acid attack of the sample. Powder samples can undergo hydrolysis by hydrochloric acid (HCl) 6M (Ausset *et al.* 1998; Del Monte *et al.* 2001) or 10 mol/L (Marinoni *et al.* 2003) in deionised water (Schiavon *et al.* 2004). With this acid attack sulphates and carbonates are dissolved and the insoluble particles are isolated and can be studied in detail.

Another type of transfer to be described here, is that of particles deposited on a substrate suitable for SEM analysis, such as a PC filter, to a TEM grid for further TEM investigation. This transfer may be operated in three ways:

- dissolution of the PC filter;
- dissolution of the particles;
- nanomanipulation inside an ESEM.

The PC filter can be dissolved with chloroform (trichloromethane: $CHCl_3$) in a Jaffe washer through the so-called “sandwich method” of indirect preparation of a TEM sample (Capannelli *et al.* 2011; Casuccio *et al.* 2004; Dye *et al.* 1997; Farrants *et al.* 1988; Ouf *et al.* 2010). During the dissolution of the filter volatile species, such as secondary organic aerosols, might be removed, thus for specific studies regarding those compounds it is better to sample directly on a TEM grid (Casuccio *et al.* 2004). The dissolution of the substrate might lead to alterations in the chemistry of the particles or in their morphology (Willis *et al.* 2002). An alternative procedure, also based on the use of a Jaffe washer is cutting the PC filter in halves and coat it with 10 nm C or 15 nm Ge, then punch out discs of 3 mm from the filter and place them on Cu meshed grids (200 mesh grids for C-coated filters; 500 mesh grids for Ge-coated filters) followed by the dissolution with 1-methyl-2-pyrroline of the PC filter in the Jaffe washer overnight (Kocbach *et al.* 2005). Kocbach *et al.* (2005) preferred C extraction replicas to Ge extraction replicas as the latter break easily during sample preparation and analysis. Alternatively to filter dissolution, as explained for the fibrous filters, the particles deposited on a PC membrane can be dissolved into isopropilic alcohol ($(CH_3)_2CHOH$) by submersing a 2 cm^2 section of the filter in the solvent in a test tube, sonicating it for 10 seconds and leaving it in the tube for 5 minutes (Ramirez Leal *et al.* 2007). An aliquot of the obtained suspension can then be placed on a TEM grid. A more complicated technique to transfer particles from the PC filter to a TEM grid is nano-manipulation (Godoi *et al.* 2006). The procedure is conducted inside the sample chamber of an ESEM in which the manipulators equipped with fine glass needles are placed and controlled from the exterior. The transfer inside the microscope guarantees that eventual changes introduced in the sample are observed while happening. Moreover, the exact location of a specific particle on the TEM grid can be recorded, as it is the operator who decides where to put the particle removed from the original substrate (Kaegi and Holzer 2003).

People researching in the field of electron microscopy applied to environmental particle characterization feel that sample preparation needs further research (EPA 1987; Sielicki *et al.* 2011), especially towards the development of a single methodology for SEM and TEM (Hassellöv *et al.* 2008).

The understanding of the reliability of EM techniques for the analysis of atmospheric inorganic particles (Ramirez Leal *et al.* 2007) and the technological advancements, such as the design of speciation samplers which allow the collection of airborne particles simultaneously on different filters (Casuccio *et al.* 2004), may point at a future where electron microscopy will be part of the standard analytical techniques for PM analysis (Sielicki *et al.* 2011). Moreover, as the attention is focusing on smaller sizes of PM, the most hazardous for human health (Bang and Murr 2002; Bhattacharjee *et al.* 2011; Nowack and Bucheli 2007), a more diffuse use of electron microscopy is likely.

Currently, European and Italian regulations[12] require the characterization of PM only from the point of view of its size with bulk mass analytical techniques (Capannelli *et al.* 2011). These analysis may also provide some chemical information but this is averaged over a population of particles which may be composed of individual units with different origin, dimensions, composition, morphology, crystal structure, thus different chemical and physical properties. Bulk analysis are not able to detect elements whose concentration is high but located only in a small number of particles (Yue *et al.* 2006). It has been highlighted that although the information collected for official air quality monitoring are useful, they are not sufficient to guarantee a thorough understanding of the pollution scenario: the toxicity of a particle can be related to one characteristic aspect (oxidation state, crystal structure, etc.) only that could not be identified through the analysis of a group of particles (bulk techniques) or expressed by the aerodynamic diameter and the mass obtained through the commonly used gravimetric analytical technique. It is then hoped that EM studies may contribute to

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[12] Directive 2008/50/EC adopted in Italy through the d.Lgs. 13 agosto 2010, n.155

broaden the knowledge on specific components of PM in relation to adverse health effects. This may lead in the future to precisely define types of particles of greatest concern to public health that have to be regulated by national or international institutions in charge of air quality management. Research on EM analytical protocols for PM is then to be considered as policy-relevant scientific research. In this context, an interesting application of SEM-EDXS and computer controlled SEM-EDXS (CCSEM-EDXS) in the environmental field is their combination with techniques of chemical extraction for the study of morphological and compositional changes of the particles after exposure to simulated body fluids (for example simulated gastric, intestinal and lung fluids) (Hunt and Johnson 2011).

The SEM allows the acquisition of 3D-like images of the surfaces of the particles. The SEM in airborne particles analysis is used in four modes: environmental or low vacuum (ESEM, LV-SEM), high vacuum (SEM or HV-SEM), computer controlled (CCSEM) and high resolution high vacuum, the latter generally provided by the use of a field emission source for the electron beam (FE-SEM). ESEM and LV-SEM allow the analysis of non-conducting samples without deposition of a metal coating on their surfaces (Kaegi and Holzer 2003). This is a great advantage as it guarantees to analyze the pollutants intact and to avoid that a metal coating not conforming to the morphology of the particles to be analyzed obstacles the observation of the true morphology of the particles (Bhattacharjee *et al.* 2011). ESEM also avoids the damage that can be caused by working under vacuum conditions, such as loss and alterations of the chemistry of volatile and unstable compounds, especially of particles containing water and of H_2SO_4, sulphates and organic compounds that tend to evaporate in the electron microscope (Justino *et al.* 2011; Sielicki *et al.* 2011). ESEM has been used to study the influence of relative humidity on the hydration reactivity of atmospheric particles and to follow the morphological and compositional transformation of particles due to reactions with nitric acid, that is the prevalent species in the atmosphere (Krueger *et al.* 2003). It has also been used to study fungal spores as particles contributing to respiratory diseases, as it allows the measurement of size and shape of the spores without previous dehydration (Reponen *et al.* 2001). Manual scanning electron microscopy allows the real time adaptation of the operating conditions to a specific sample and the easy identification of some fine features. However, it is a slow analytical method which allows the investigation of a limited number of particles, which is quite subjective in the selection of the particles to be studied and in their classification and which requires high expertise. The limitations of the manual mode brought to the development of the automated mode, the CCSEM, which was designed to fasten the analysis and guarantee better reproducibility (EPA 1998). CCSEM through a specific software allows automatic recognition and analysis of particles which can then be grouped into classes on the basis of their elemental composition determined by EDXS. The frequency/mass distributions for each particle class and for the total sample might be determined (Chow and Watson 1998; EPA 1998). It is similar in principle to the JetSCAN® automatic analysis system used in aeronautic to detect the degradation of oil washed components in aero gas turbine engines, which is one of the parameters monitored to evaluate if a certain component still meet the service requirements of performance and safety (Alcock and Shepherd 2005). Samples of oil metallic debris are collected by magnetic plugs after a certain number of flying hours and a CCSEM-EDXS system identifies the particles in the samples, acquires their EDXS spectrum and produces a database were the compositions are given against the number of flying hours. A diagnostic software compares the measured compositions with an internal database of alloys, thus an evaluation of the engine health is provided. One of the first applications of CCSEM in environmental science was the classification of fly-ash particles from European differently fuel-fired power stations in the framework of the project "Fly-ash and metals in Europe: Implications for human and environmental health" (FLAME) (Watt 1998). In this study the programme DIGISCAN was used to identify a particle after selection of a backscattered intensity threshold, acquire its EDXS spectrum and conduct some shape measurements in order to build a classification scheme for the different fly ash. A limit of computer controlled systems is that they have both a lower resolution (0.1 µm compared to the manual mode where nano-sized features can be generally distinguished) and a higher detection limit of EDXS (1-5 wt% compared to 0.1 wt% of the manual mode and to 0.01 of the WDXS) than the manual mode (EPA 1998; IAEA 2011). FE-SEM among the SEMs has the highest imaging resolution and allows the identification of particles down to about 0.02 µm (Willis *et al.* 2002).

In the field of conifer needles analysis, SEM is suitable to study the characteristics of the surface of the needles, such as morphology and structure, dimensional parameters, wax quality/degradation and structure, particles (dust, gypsum-crystallites) and microorganisms (bacteria, algae, fungal spores and hyphes, pollen grains, eggs, etc.) deposited on them and inside stomata (Di Guardo *et al.* 2003; Iwasaki and Tainosho 2003; Lehndorff *et al.* 2004; Lehndorff *et al.* 2006; Sartori 1998; Trimbacher and Weiss 1999). The observation of the morphology, and in particular the determination of the specific leaf area and the evaluation of surface roughness, have to be conducted on the same samples of needles on which contaminants are analysed considering that these parameters affect the amount of pollutants, especially of semivolatile organic pollutants, accumulated in the needles (Ratola *et al.* 2011).

TEM is the best instrument available to characterize UF particles. Since it can directly provide the crystal structure of the particle under investigation it is the technique of choice to analyse asbestos fibres (Chow and Watson 1998; Esquivel and Murr 2004; Vallero *et al.* 2009). High resolution TEM is also commonly used to study diesel soot as from its structure it is possible to determine its combustion history (Rainey *et al.* 1996).

TEM provides information averaged through the thickness of the specimen as the sample is viewed in transmission (Williams and Carter 2009). For example, the images acquired are 2D images of the 3D sample. TEM might be selected as a second step of the analytical protocol, to refine the source apportionment obtained by SEM and to investigate in depth specific particles of interest. Kaegi and Holzer (2003), for instance, identified by ESEM-EDXS five classes of particles and characterized one of these by TEM to identify the internal structure of the particles. At the end of this two-step analysis the Authors were able to describe soot aggregates over three scalar orders.

In the field of conifer needles analysis, TEM has been employed mainly to study the ultrastructure of conifer mesophyll cells (Viskari *et al.* 2000) and the alterations that happen as a consequence of pollution at the level of chloroplasts (Sartori 1998). These issues could not be studied by SEM whose resolution even under optimal conditions cannot go below 1 nm (Howard 2010). The resolution is one of the reason why a methodology to study particles deposited on conifer needles should be developed as, to our knowledge, this has never been done. The basis for the development of a TEM protocol is that particles superficially adsorbed in the epicuticular wax have, on average, a diameter smaller than 4 µm (Simmleit *et al.* 1989), thus they may include UF particles. However, it has to be kept in mind in this context that other Authors (Rossini Oliva and Raitio 2003) have observed that particles smaller than 1 µm are less easily removed by the surface of the needles than coarse particles, thus it could be hard to obtain this fraction for TEM analysis.

The elemental analysis of UF particles by EDXS coupled either with a TEM or FE-SEM is difficult as the volume from which the x-rays are produced, which depends on particle properties (conductivity, size, chemical composition), is of the order of 1 μm^3 that is much larger than an UF particle. This means that most of the signal would come from the substrate (Casuccio *et al.* 2004; Coz Diego 2008; Godoi *et al.* 2006; Laskin *et al.* 2006; Willis *et al.* 2002). Hence, it is recommended to characterize the signal coming from the blank substrate in order to be able to understand the fraction of signal coming from the particle.

For the study of nanoparticles, the High-Angle Annular Dark Field – Scanning TEM (HAADF-STEM) is recommended: its optical scheme allows the construction of an image based on the incoherent thermal scattering. This guarantees a contrast highly dependent on the atomic number, which is useful to identify the heavier nano-size particles present in a matrix (Utsunomiya and Ewing 2003). In a study by Salbu *et al.* (IAEA 2011) the possibility of studying crystalline phases by STEM-EDX was used to detect radioactive colloids of about 20 nm size and pseudo-colloids in the region of 100 nm.

Together with the many highlighted advantages, it has to be considered that the results obtained by EM come from a single particle or a part of it, which means that only a very small amount of a sample is analyzed (Utsunomiya and Ewing 2003). Therefore, it is necessary to use complementary bulk analysis to include the obtained data in a broader picture (Hassellöv *et al.* 2008; Jambers and Van Grieken 1996; Willis *et al.* 2002) as well as to process the results with statistical techniques. If these actions are not taken there is a no negligible risk to provide results, which are not representative of the sample (Dye *et al.* 1997). Moreover, measurements with other instrumentations are strictly necessary to obtain data on organic compounds as EDXS does not provide molecular information, which would allow the speciation of carbonaceous matter (Nelson *et al.* 2001; Sielicki *et al.* 2011). Also H cannot be measured by EDXS. This does not allow the study of the presence of hydroxides and obstacles the obtainment of information on particle hydration status (Gieré *et al.* 2012). To tackle these limitations related to the type of pollutants that can be investigated, it is possible to use hybrid instruments combining SEM with other techniques that provide molecular data. For example, SEM exists integrated with a micro-Raman spectroscopy system (Potgieter-Vermaak *et al.* 2010).

Another issue related to the use of EM in the study of PM is the impossibility to measure directly the aerodynamic diameter (diameter of a sphere of density 1 g/cm^3 with the same settling velocity of the particle under investigation under the gravitational force in static air in the same conditions of temperature, pressure and relative humidity). The aerodynamic diameter is used for health-related issues to estimate the respiratory exposure (UNI ISO 7708). This diameter better explains the behaviour of particles in fluids (air) and determines the ability of the particles to deposit by sedimentation in the lungs (Kochbach Bølling *et al.* 2009)[13]. The definition itself implies that any measure of the physical diameter of the particle cannot estimate the aerodynamic diameter. That is why the study by Reponen *et al.* (2001) aimed at determining a correlation among the physical and the aerodynamic diameters of fungal spores of six different species highlighted in conclusion the obvious necessity of knowing the density and shape – influenced also by the hydration state – of spores to correctly calculate the aerodynamic diameter. As it should have been expected, no clear trend in the relationship between physical and aerodynamic diameter was found. While microscopy is a direct method to measure a diameter, the aerodynamic diameter can be provided only through indirect measurement methods by using a cascade impactor or an aerodynamic particle sizer. The diameters that can be measured by processing micrographs acquired by EM are (Pabst and Gregorová 2007):

- the equivalent projected area diameter
- the Feret's diameter
- the Martin's diameter.

The projected area diameter (D_p) is the diameter equivalent to that of a sphere/circle with the same projected area as the particle of interest. Since it depends on the orientation of the particle, it is

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[13] The other important diameter for health purposes is the mobility equivalent diameter responsible for the ability of the particle to diffuse in the lungs. For spherical particles this is equal to the physical diameter (Kochbach Bølling *et al.* 2009).

generally used only when there is a preferential non-random orientation of the particles in the sample.

The Feret's diameter or caliper diameter is the normal distance between two parallel tangent planes touching the outline of the particle. The minimum and maximum Feret diameters are those perpendiculars that determine the minimum possible area of a rectangle enclosing the particle.

The Martin's diameter is the length of the chord dividing the projected particle area into two equal halves.

Any of these diameters reflect a specific 2D particle outline, thus it may be very different for anisotropic particle (disks, needles, fibers) depending on their orientation.

Formulas to estimate the aerodynamic diameter from the projected area diameter were provided by Ott *et al.* (2008b) and by Willis *et al.* (2002). To apply these formulas it is necessary to measure the projected area of the particle and its circularity through an image analysis software, such as ImageJ (Rasband 1997-2012), assign an aerodynamic shape factor and estimate the density of the particle from EDXS data (Ault *et al.* 2012), from the estimates of mass and volume of the particle or from theoretical considerations. In any case the value would be an estimate based on other estimates or assumptions, thus it would be affected by high uncertainty.

An additional problem related to the use of EM is that it cannot provide mass concentrations of selected size classes of particles comparable to those measured to comply with official air quality methods. It was found that mass concentration is greatly overestimated from EM data. This again because the total mass comes from the sum of the masses of single particles that are in turn estimated from estimates of size and chemistry (EDXS oxide measurements) affected by large errors especially for non-spherical particles (Wagner and Leith 2001).

A further issue is that of representativeness. Manual analysis has a low particle throughput and to guarantee an accurate sample representation many field of view should be examined. All the particles in each field of view should be analysed in order to reduce the potential for bias or operator subjectivity due to the fact that the eye is naturally attracted to larger features or distinctive morphologies. When possible CC-SEM-EDX reducing subjectivity and allowing larger number of particles to be analysed should be preferred. Deboudt *et al.* (2008) observed that in order to have representative results for the study of short term variation in PM by CC-SEM-EDX it is necessary to analyse more than 500 particles to obtain accurate size distribution and about 1000 particles for chemical properties. Meeting these requirements they were able to record the increase in the number of metal particles due to the dust plume of a steelworks.

A further limitation of electron microscopy is the fact that the electron beam impinging on the sample, while producing a broad range of signals, may damage the sample if the accelerating voltage used exceed the damage threshold of the particle under study (Nelson *et al.* 2001; Williams and Carter 2009). $(NH_4)_2SO_4$, NH_4NO_3 and biological particles (pollens, spores, etc.) are beam-sensitive particles. Finally, a significant obstacle in undertaking microscopic investigations is that the equipment is expensive (Jambers *et al.* 1995) as well as the hourly cost of the analysis which is around 50€/hour for SEM and 100€/hour for TEM.

As electron microscopes are not chosen by regulations on PM as standard instruments no reference methodologies, with the exception of asbestos characterization (ASTM D6480-05; ASTM D5755 – 09; ASTM D 5756-02; UNI EN ISO 1600-7; ISO 14966; ISO 10312; ISO 13794), have been defined to conduct the analysis. On the one hand, the absence of a standardized analytical procedure is negative as inter-laboratory comparisons of data might be difficult. On the other hand, the lack of a standard procedure allows the selection of the sampling surface and device with the best performance for a specific study, the comparison of different sampling systems and advanced research on the development of new sampling technologies aimed at providing materials analyzable by EM.

This study is in line with the approach proposed by Moroni *et al.* (2012): working to integrate bulk analysis (inductively coupled plasma atomic emission spectroscopy) with electron microscopy to obtain data analyzable by multivariate statistical tools to perform source apportionment.

2.3 Purpose and key objectives of this work

> *"The success of a sampling and analytical method depends on the compatibility or interfacing of these two parts. As technology progresses, improvements can be incorporated into either part of the method to improve performance, provided that the interface remains compatible."* (NIOSH 1995)

The scenarios presented in the previous subchapters lead to summarize the issues to be solved in PM monitoring as follows:

1. source apportionment of PM is getting challenging as a consequence of the multiple emission sources that create at each location a complex multipollutant scenario reflecting a history of transport and transformation in the atmosphere (Sieliki *et al.* 2011)
2. characterization of ultrafine (<0.1 µm) and nano (<0.01 µm) particles is fundamental:
    a. their concentration in the environment is increasing (Sieliki *et al.* 2011) due to vehicle emissions (Nel 2005), stationary emissions (Nowack and Bucheli 2007) and the proliferation of nanotechnologies (Bang *et al.* 2004)
    b. they persist in the atmosphere
    c. they are the most hazardous for human health (Bang and Murr 2002; Justino *et al.* 2011; Nel 2005; Zamengo *et al.* 2009)
3. new environmental policies and measures against the emission of pollutants must be evaluated with cost-effective strategies.

These issues require to gather information on PM at receptors and to use single particle analytical (SPA) techniques, such as electron microscopy (Sielicki *et al.* 2011). Both solutions pose special attention on sampling PM.

Sampling at receptors require the selection of techniques appropriate to monitor wide territories. Active sampling techniques do not meet this requirement as their need for power makes them expensive compared to passive samplers and limits the selection of the locations in which they can be placed. Moreover, the data that can be collected are limited to a relatively short time and no information can be gathered on periods preceding the installation of the instruments. Therefore, the present work will try to advance the field of passive sampling techniques for PM exploiting existing surfaces exposed to the atmosphere, such as building facades and vegetation.

Electron microscopy techniques, although applied in environmental analysis since long time (see paragraph 2.2), have always been limited by the necessity of depositing the sample on suitable substrates (Casuccio *et al.* 2004). Hence, the sampling systems exploited in this work will be selected and/or prepared for easy analysis by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

The idea of studying PM presence in a site at different time scales is further developed in this thesis combining the use of instruments to collect data on the present situation, biomonitors to assess changes in the recent past, and building facades to gather proofs of remote past contamination.

The strongest interest in ultrafine and nano PM is developed in this work through the optimization of analytical protocols for TEM. These protocols are focused on guaranteeing the representativeness of the data extracted (Dye *et al.* 1997), through techniques integrating the microscopic data into bulk measurements.

Innovative sampling technologies and electron microscopy will be combined in different multiscale streamline (optical→SEM→TEM) analytical protocols aimed at the successive refinement of source apportionment. For the ultrafine and nanoparticle size-range the focus of the design will be in developing efficient collection and sample preparation off-line procedures overcoming the monetary and time costs imposed by the dimensional range.

The stated goals are in line with the trends in the field of environmental pollutant analysis highlighted by Namieśnik (2001), which include the "search for methods applicable for determination of many analytes in the same sample during a single analytical process" and the "increase in the significance of bioanalysis and biomonitoring".

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3 Development of a streamline integrated analytical protocol including electron microscopy for the analysis of inorganic pollutants collected on conifer needles

> *"Seek to find the method of analysis that depends least on the skill of the operating chemist..."* Berzelius, 1814

This chapter deals with the development of a sample preparation and analysis procedure for conifer needles to meet my research objectives.

The protocol should allow from the collection of a certain amount of fresh needles the obtainment of their elemental concentration (distinguished in internal and from atmospheric deposition) by a bulk analytical technique and associate the presence of certain elements in exceedance with respect to "natural background levels" to certain types of airborne particles (morphology, chemical composition, possibly cristallography) identified by single particle analysis (SPA) (Bertolotti and Gialanella 2014).

As data from SPA have to be placed in the framework of bulk measurements, at first a method to estimate the total amount of metals due to atmospheric deposition by a bulk technique is developed.

The bulk analytical technique of choice is inductively coupled plasma atomic emission spectroscopy (ICP-AES). This technique was chosen as it guarantees very low detection limits (ppm or even ppb for certain elements) and is multielemental. Moreover, considering that plants contain a hardly dissolvable mineral fraction (Pöykiö and Perämäki 2003), it is a positive characteristic of the technique the fact that it can tolerate up to 1-2% of dissolved solid in the sample thanks to the high plasma temperatures (6000-10000 K) (Kalra *et al.* 1989; Lamble and Hill 1998). The sample preparation procedure and data interpretation guidelines for ICP-AES analysis of conifer needles are optimized considering literature sources of a variety of fields of study: analysis of foliage, commercial botanical products (dietary supplements of nutraceuticals, herbal products for medicine and cosmetics), soils, sediments and vegetables.

Once the bulk analytical methodology is developed, suitable sample preparation techniques and standard operating procedures are defined for the characterization of the individual particles by electron microscopy. Coarse and fine particles are characterized by SEM while ultrafine and nanoparticles by TEM. As in our institution a variable pressure scanning electron microscope (see technical details provided later) is available, it was decided to exploit the low-vacuum mode that simplifies the sample preparation step and reduces the chances of modifying the sample (see paragraph 2.2). The reasons behind the choice of using electron microscopy for the analysis of the particles are expressed in detail in paragraph 2.2, which described the application of the technique in the field of PM characterization. To summarize the objective, it can be stated that with these SPA techniques the contaminants identified with the bulk method can be associated to a specific morphology and particle size that in turn can provide indications for source apportionment. This is useful as it was already highlighted that from the elemental analysis of plants alone it is difficult to determine the source of heavy metals (Sawidis *et al.* 2011).

Environmental studies often require to analyse a huge number of samples, as many sites have to be investigated to characterize the air quality of an area. Hence, streamline analytical procedures, which allow the use of one sample for a whole set of characterizations "in cascade", have to be favoured. As the last step of each method development the steps of sample preparation and analysis by bulk and SPA techniques will be combined in a streamline protocol that allows the integration of the data collected by both kind of techniques. Indications on the time necessary to undertake our protocol will be given, although the availability of different sample preparation systems (e.g. a microwave-assisted digestion apparatus that can handle many sample simultaneously) or the use of different samples (e.g. other conifer species or biomonitors) could significantly change the time consumption.

Before describing the development of the protocol technical characteristics of the instruments used in this work are provided.
These information are followed by details on each step of method development:

1. identification of the most efficient washing agent to
    a. remove the epicuticular wax layer and the particles trapped in it in order to estimate the contribution of atmospheric deposition on the elemental concentrations measured by ICP-AES on the needles as they are collected
    b. not extract elements from the inside of the needles;
    c. deposit coarse particles homogeneously on a filter suitable (smooth and flat with no peaks overlapping those of atmospheric particles) for SEM-EDXS analysis (if this cannot be undertaken directly on the needles still guaranteeing representative results). The filter should have the right particle load to guarantee a good separation of the particles and, where such an instrument is available, CC-SEM-EDXS.
    d. deposit ultrafine and nanoparticles on TEM grids without damaging the film constituting the grids and guaranteeing a sufficient number of particles to obtain representative data on the morphology and composition of this size fraction
2. select the best sample preparation procedure for ICP-AES (drying, grinding, removal of the organic component and obtainment of a liquid sample)
3. select the best type of TEM grids (metal constituting the mesh, type of film) for the analysis of PM (with special attention to PM from industrial sources and vehicular traffic)
4. identify data treatment and interpretation strategies for each techniques with related quality evaluation of the data
5. determine the necessary amount of sample for each technique separately and identify the amount suitable for the streamline analysis
6. select the best storage conditions for each type of sample.

3.1 Technical characteristics of instruments used in this work

Open-vessel microwave-assisted digestion apparatus “Simultaneous Temperature Accelerated Reaction” **(STAR) SYSTEM 2** (CEM) allowing the automatic control of temperature and acid additions (up to 4 reagents). The dissolution vessels are made in Pyrex. The apparatus does not allow the automatic addition of hydrofluoric acid during the digestion which would guarantee the dissolution of silicates. Looking at the good side of not using HF, is the observation by Pöykiö *et al.* that its use would lead to not very reliable Cr determinations (Pöykiö *et al.* 2000).
Reagents used in our laboratory for the microwave-assisted digestion:

- hydrogen peroxide, mass fraction 30% by weight, Sigma Aldrich
- nitric acid, mass fraction 69.0-70.0%, ACS grade, J.T.Baker
- sulphuric acid, ACS grade
- deionized water.

Closed-vessel microwave-assisted digestion apparatus "**ETHOS One**" owned by FKV Company allowing the simultaneous digestion of multiple samples of the same type.

Microwave-assisted digestion apparatus "**Ultra WAVE**" owned by FKV Company allowing the simultaneous digestion of multiple samples, even different in matrix, in vessels with independent temperature and pressure control.

ICP-AES Spectro Ciros^{CCD}
Frequency 50 Hz
Power 6 kW
cooling argon-flow rate 12-20 l/min
auxiliary argon-flow rate 0.4-2 l/min
nebulizer argon-flow rate 0.1-1 l/min
The sample is introduced through a glass spray chamber with nebulizer, thus not suitable for HF containing samples. The spectrometer is simultaneous and the quantitation is done comparing the peak intensity for an element in the sample with that in the spectrum of external calibration standards. The standards used for calibration is the Multi IV Merck containing 40-80-200 ppb of many elements to which Ca, Mg, Na and K are added respectively in the following concentrations:
K 2-4-10ppm
Na 10-20-50 ppm
Mg 4-8-20 ppm
Ca 20-40-100 ppm.
The multielement solutions utilized in our laboratory are in pure HNO_3 1%.

Optical Microscope Wild Heerbrug with nine magnifications: 6.3X, 7X, 8X, 10X, 12.5X, 16X, 20X, 25X and 32X

Stereomicroscope Zeiss - Leica

Environmental Scanning Electron Microscope XL30 ESEM Philips with tungsten source. The maximum voltage is 30 KV. It can operate in high vacuum, low vacuum and "environmental" modes. It is equipped with detectors for secondary electrons and backscattered electrons and it is coupled with an EDAX Falcon microanalysis system that has the X-ray detector EDAX-C/U. As in particulate matter most elements are rarely as such, the quantification of their concentration will be expressed as oxides. This was especially done in consideration that the case study described in chapter 4 regards monitoring an electric arc furnace steelmaking plant whose emitted dust contain several oxide phases which form due to the highly reactive environment and the high temperatures (Baiutti *et al.* 2007). This choice result in attributing oxygen even to elements which are not in oxide form. The stechiometry of the oxide for each element was that suggested by the software managing the analysis (Genesis), however, certain elements may be present in reality in more than one oxide form or even only in a form not used for quantification.

Scanning electron microscope SEM JSM 5500 Jeol

Transmission Electron Microscope TEM/STEM Philips CM12 with tungsten source. It works at 120 KV and has a resolution of 3.0 Å. It is coupled with a Falcon multichannel EDXS system and a

EDAX SUTW sapphire detector (C/U). For the element quantification by EDXS the same considerations reported under ESEM are valid.

Flame atomic absorption spectroscopy system ContrAA 700 + AS 51s owned by FKV Company. For the determination of K concentration the burner was rotated of 90° and the line at 769.8974 nm was used for quantification.

3.2 Identification of the washing agent for detachment of the particles from the surface of the needles (including those entrapped in the epicuticular wax layer)

The selection of the washing agent and washing procedure is of fundamental importance as it affects the accuracy of the determination of the elemental concentrations. In our review (Bertolotti and Gialanella 2014) a critical summary of the washing agents used in the ***literature*** is provided. From it chloroform seemed the best option for sample preparation. However, it was decided to explore the possibility of a greener substitution considering also dichloromethane (DCM) (CH_2Cl_2) and a 1:1 solution (v/v) of DCM:cyclohexane. None in the literature tested DCM alone or the indicated solution. Iozza *et al.* (2009) used a 1:1 (v/v) solution of DCM and nHexane. In this work nHexane, a known neurotoxic (Patnaik 2007), was substituted with cyclohexane which has a higher threshold limit value-time-weighted average (TLW-TWA)[14] (300 ppm) than n-hexane (50 ppm) and it is also cheaper.

DCM was selected as solvent for washing the needles. Even if it is less efficient than chloroform in removing particles and wax, its suitability for electron microscopy sample preparation and its lower toxicity makes it better for this protocol. The selection criteria are here summarized:

- theoretical caluculations based on the comparison of the cohesion parameter of spruce epicuticular wax and the solubility parameter of the washing agents (Khayet and Fernández 2012) suggested that the 1:1(v/v) solution of DCM and cyclohexane and chloroform would be the most efficient in removing the wax (Table 3.2-1); however, the efficiency of the washing agents was also tested experimentally since the same total solubility parameter could be determined by different dispersion forces, polar forces and hydrogen bonding forces which lead to different dissolving behavior (Burke 1984)

Table 3.2-1 Solubility parameter difference ($\Delta\delta$) between the epicuticular wax and each washing agent calculated assuming a cohesion parameter for the wax of 18.6

water	*chloroform*	*DCM*	*isobutyl alcohol*	*toluene:THF 1:1 (v/v)*	*DCM:cyclohexane 1:1 (v/v)*
29.3	0.2	1.7	2.9	1.7	0.1

- gravimetric comparisons of the amount of material removed (Turunen *et al.* 1997; Beckett 2000; Teper 2009), within the limitations of being conducted on different susamples of needles that are by nature heterogeneous, suggested that DCM and chloroform are the most efficient solvents in removing materials from the surface of conifer needles;
- observations with an optical microscope by different users of our laboratory agreed in ranking chloroform as the best in cleaning the surface of the needles, although confirming

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[14] concentration in air to which workers can be exposed for 8-hour work period per day or 40-hour work period per week without adverse effect

the issue of needle heterogeneity (Table 3.2-2; Figure 3.2.1); the good efficiency of chloroform was also confirmed by SEM observations (Figure 3.2.2);

Table 3.2-2 Ranking after observation at OM of the needles based on the amount of wax observed. 1: higher amount of wax (solvent less efficient).

USER	NOT WASHED	isobutyl alcohol	toluene:THF	DCM	DCM:cyclohexane	chloroform
A	1	5	2	3	4	6
B	1	5/6	2	3/4	3/4	5/6
C	1	3/4/5	2	3/4/5	3/4/5	6
D	1	5	2	3/4	3/4	6
E	1	3/4	2	5/6	3/4	5/6

Figure 3.2.1 OM pictures of the surfaces of washed needles. Top: DCM Bottom: chloroform.

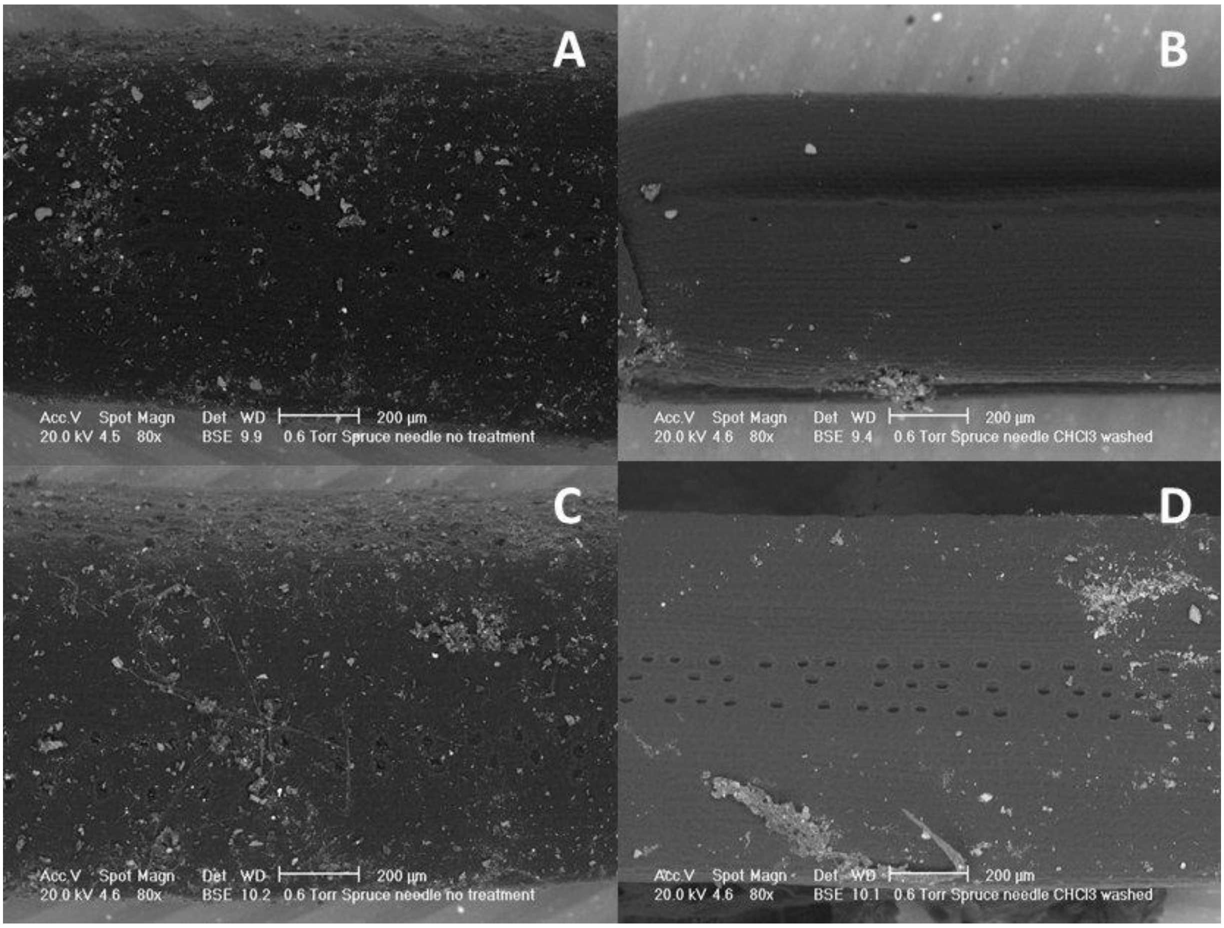

Figure 3.2.2 Comparison of the SEM micrographs acquired immediately near the base of needles unwashed (A) and washed with chloroform (B) and of central areas of needles needles unwashed (C) and washed with chloroform (D)

- chemical comparison by ICP-AES showed that soil/dust elements (Fe, Al, Si, Mn and Ti), constituting airborne PM everywhere, are removed from the needles more efficiently by chloroform (Figure 3.2.3) (Campbell and Plank 1992); it seems that certain elements leach from the interior of the needle after washing with chloroform (Figure 3.2.4), however this result was not confirmed analysing other samples; the 1:1(v/v) solution of DCM and cyclohexane is the least efficient, while DCM is the second most efficient after chloroform;

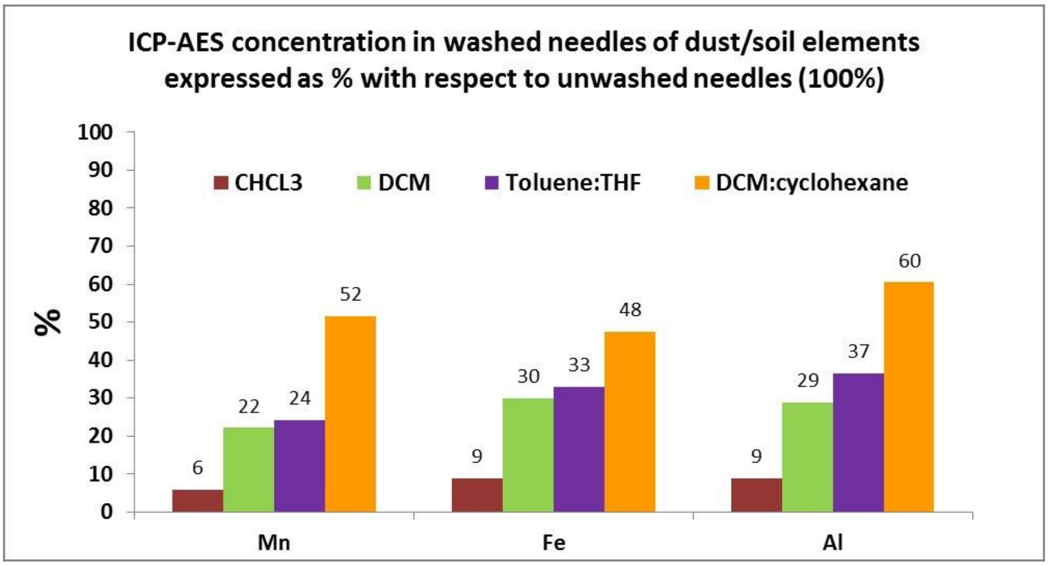

Figure 3.2.3 Relative concentrations of some of the elements (Mn, Fe, Al) characteristic of soil/dust after washing with different washing solutions. The elemental concentrations in unwashed needles are set to 100

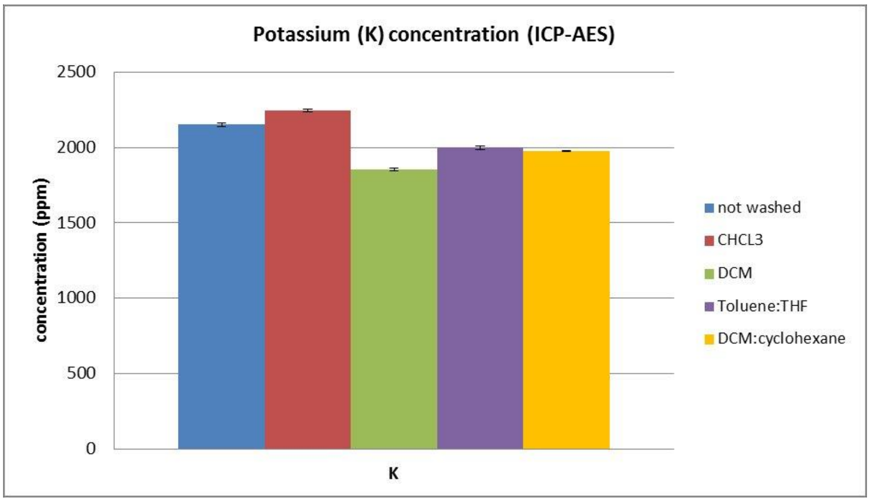

Figure 3.2.4 Evaluation of different washing solutions, chloroform (reddish brown), DCM (green), 1:1 (v/v) toluene:tetrahydrofuran (purple) and 1:1 (v/v) DCM:cyclohexane (yellow), in relation to the leaching of K from the interior of the needle during the washing procedure. K is mainly located intracellularly and leaches only if the cell membranes are damaged (Viksna *et al.* 1999)

- particle agglomeration on the membranes used as substrates for SEM-EDXS and on the TEM grids is less pronounced with DCM than with chloroform (compare Figure 3.2.5 and Figure 3.2.6);

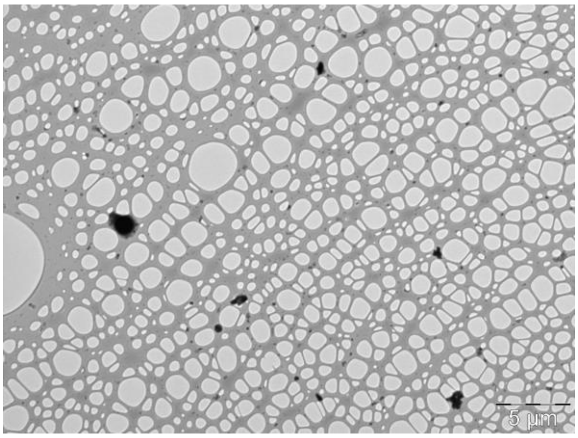

Figure 3.2.5 TEM micrograph of particles contained in the DCM residual solution after washing 1.5 g of spruce needles collected near an industrial area. The particles are not agglomerated in clusters and can be characterized individually both by EDXS and SAED techniques as well as imaged at higher resolution.

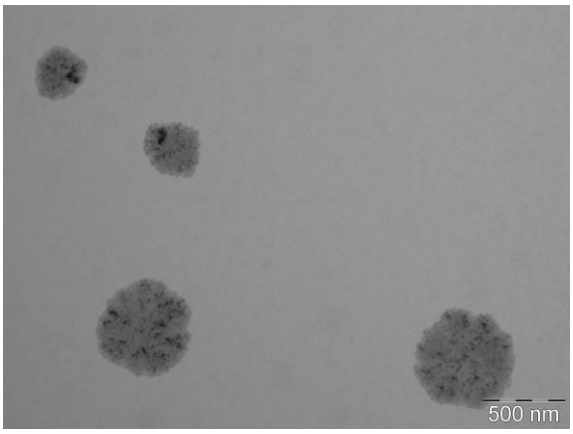

Figure 3.2.6 TEM micrograph of spheroidal clusters of particles contained in the chloroform residual solution after washing spruce needles collected near an industrial area. Particle agglomeration with chloroform was anticipated by Simmleit *et al.* (1989).

- filtering the DCM washing solution it is possible to obtain a good particle load of the membrane used as substrate for SEM-EDXS (Figure 3.2.7);

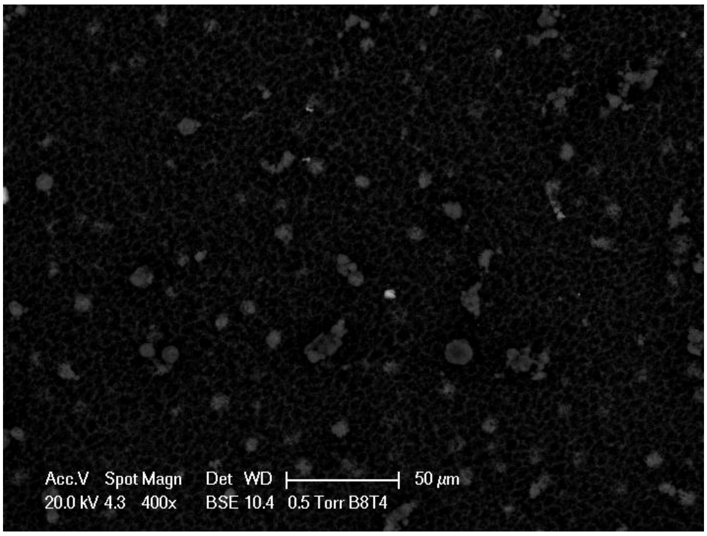

Figure 3.2.7 Micrograph of the particles deposited on a nylon 0.2 µm porosity membrane through filtration of a pre-filtered solution (size cut at 30 µm) of DCM in which about 1.6 g of needles collected at a polluted site had been washed.

- chloroform damages the film of the TEM grids more than DCM (compare Figure 3.2.8 and Figure 3.2.9);

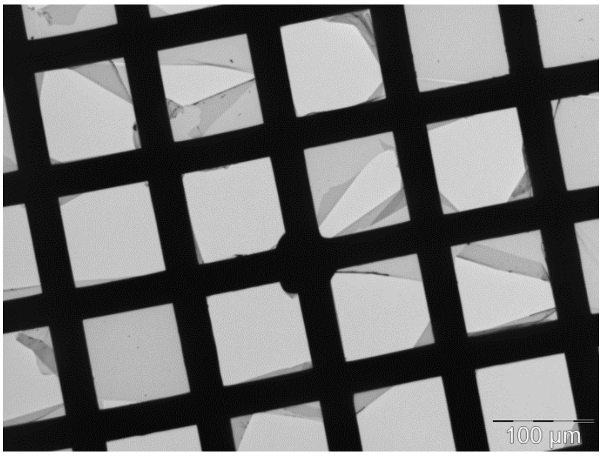

Figure 3.2.8 Center of an Au TEM grid after a drop of chloroform ($CHCl_3$) has been deposited. Although the film was already damaged before the application of the solvent, an increase in the size and number of holes in the film was observed.

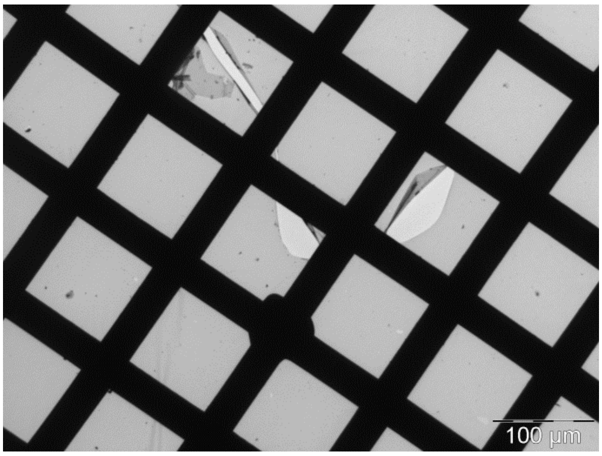

Figure 3.2.9 Center of an Au TEM grid after a drop of DCM has been deposited. The film was already damaged but the damage did not seem to increase significantly, at least in the central area of the grid, after the application of the DCM drop.

- DCM is less toxic than chloroform, as it has a threshold limit value (TLW) – time weighted average (TWA) of 50 ppm against the 10 ppm of chloroform.

3.3 Selection of the best sample preparation procedure for ICP-AES (drying, grinding, removal of the organic component and obtainment of a liquid sample)

The main drawback of ICP-AES is a cumbersome sample preparation which is required for the following reasons (Chan *et al.* 2011; Mackey *et al.* 2004; Pöykiö *et al.* 2000; Stefan *et al.* 2007; Vaisanen *et al.* 2008):

1. conifer needles are natural samples, thus they are highly heterogeneous. To obtain homogeneous size distribution and an adequate sample reproducibility, some procedures like milling, grinding and sieving are required; this phase of mechanical sample preparation favours efficient extraction of the metals in the successive step of sample digestion as it facilitates the interaction between sample components and the acid used in the digestion;
2. the organic component of the needles needs to be removed by wet or dry oxidation in order to determine the trace metal content;
3. the sample needs to be liquid to be analyzed by ICP spectroscopies.

To select a method for sample preparation these factors should be taken into account (Lamble and Hill 1998; Marguí *et al.* 2005; Rodushkin *et al.* 1999):

- level of recovery of the analyte of interest (metals) from the sample;
- reproducibility;
- levels of contamination that the method introduces, which in turn controls the achievable detection limit;
- amount of sample that can be handled (the quantity must be sufficient to guarantee the representativeness of the sample);
- time necessary to carry out the procedure, considering that in studies based on biomonitors the results have to be drawn on the basis of a large number of samples;
- reagent consumption, both for expenses evaluation and for environmental consideration;
- equipment and labour cost.

Drying is required in order to facilitate the obtainment of a powder to be transformed in a solution and to avoid biological transformations that may alter the sample. The literature presents a variety of drying procedures (Čeburnis and Stainnes 2000, Aboal *et al.* 2004, Adekola *et al.* 2002; Al-Alawi *et al.* 2007; Dmuchowski and Bytnerowicz 1995; Kord *et al.* 2010; Leśniewicz and Żyrnicki 2000; Rautio *et al.* 2010; Reimann *et al.* 2001; Rossini Oliva and Minogorance 2006; Suchara *et al.* 2011; Sun *et al.* 2010), thereby experimental tests on samples collected in the case study described in chapter 4 were conducted to establish the most suitable one. These tests showed that oven drying at 70°C for 48 hours allows the needles to reach constant weight.

Subsamples of powders obtained after drying the fresh needles at 70°C for 48 hours were used for the gravimetric determination of the total dry matter as the residue remaining after oven-drying the needle powder to 105°C. Since the dry weight of the needles increases over time (Muukkonen 2005) and washing procedures remove partially or totally the wax covering needle surface, the experiment was repeated for different types of samples from the case study in chapter 4 determined by the age of the needle and the washing treatment. Table 3.3-1 reports the measurements and also the factors that will be used to correct for water content the mass digested before ICP-AES analysis in compliance with the "*Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests – Part XII Sampling and Analysis of Needles and Leaves*" requiring the total concentration of elements in needles to be given by reference to 105°C-dried material (Rautio *et al.* 2010).

Table 3.3-1 Total dry matter and moisture after drying at 105°C for 16 hours spruce needle powders obtained from fresh needles dried at 70°C for 48 hours. The samples are part of the case study described in chapter 4.

SAMPLE TYPE		W1	W2	W3	$m_{dry105°C}$	TOTAL DRY MATTER	TOTAL MOISTURE	factor to correct digested mass for ICP-AES
		container	m_{fresh}	container + $m_{dry105°C}$				
age cohort	washed	g	g	g	g	%	%	
2008	**no**	2.5887	2.0022	4.5200	1.9313	96.5	3.5	0.9646
2012	**yes**	2.5865	2.1013	4.5903	2.0038	95.4	4.6	0.9536
2008	**yes**	2.6338	2.1383	4.6858	2.0520	96.0	4.0	0.9596
2008	**yes**	2.6187	1.3486	3.9045	1.2858	95.3	4.7	0.9534
2012	**no**	2.5850	2.0178	4.5345	1.9495	96.6	3.4	0.9662
2012	**no**	2.5856	2.0411	4.5518	1.9662	96.3	3.7	0.9633

The standard deviation for dry matter of unwashed samples is 0.1 which is acceptable (National), while that for washed samples is 0.4. The standard deviation for all the samples is of 0.6.

The results on the unwashed powder (average moisture 3.5%) parallel the moisture content of the powder sold as standard of pine needles (SRM 1575a) by NIST which is of about 3%. The washed needles are richer in moisture (4.4% on average) probably since washing removes the hydrophobic epicuticular wax. As the treatment (washed vs unwashed) influences more the total dry matter (standard deviation 0.6) than the age (standard deviation 0.1), two separate factors will be calculated to correct the mass measurements during sample preparation for ICP-AES from the average of the factors of samples belonging to the same treatment group. The factor for washed samples will be 0.9556 ± 0.0007 and that for unwashed samples will be 0.9647 ± 0.0008.

Regarding **homogenization**, two different mechanical preparation have been tested: manual grinding in an agate mortar and use of a coffee grinder. Our comparative test showed that the use of an agate mortar to obtain a powder is not recommended: grinding requires a long time, leaves a lot fibrous particles and causes the sample to absorb significant amount of moisture. The tested coffee grinder turned out to be rather effective in producing a quite homogenous powder easily and rapidly. It was decided to avoid sieving the sample in order to reduce the risk of contamination.

The **removal of the organic component and the obtainment of a liquid sample** can be accomplished in two steps (combustion and dissolution) or one go through a digestion procedure: an oxidation process supported by heat that ensures the decomposition of the organic matter present in the sample (wet decomposition). Combustion methods can be conducted in open systems (dry ashing, low temperature ashing) or in closed systems (flask combustion). Together with traditional wet decomposition methods (open system/hot plate digestion or closed system/bomb decomposition), they are characterized by a high risk of contamination and of loss of volatile elements. Moreover, they are time-consuming and require a skilled and careful operator. Therefore, in this work it was decided to employ a wet decomposition method in which the energy for the dissolution reactions is provided by microwaves. The use of a microwave-assisted digestion procedure presents many advantages (Agazzi and Pirola 2000; Borkowska-Burnecka 2000; Lamble and Hill 1998; Marguí *et al.* 2005; Pöykiö and Perämäki 2003; Stoeppler 1997) :

- the automatic control of temperature and addition of reagents ensure both an intra-laboratory and an inter-laboratory reproducible process;
- the consumption of reagents is reduced;
- the skills of the operator affect less the final result of the digestion procedure;
- the health risk for the analyst is reduced as he/her does not handle solvents and the vapors produced during the digestion process are treated by a vapor containment system;
- the contamination is reduced;
- the loss of volatile elements is reduced;
- the digestion time is reduced by a factor of 2-5 because the microwaves penetrate the containers heating directly the solution inside.

Two types of systems are available for the microwave-assisted sample preparation: the open-vessel system and the closed-vessel system. In our laboratory only the first one is available, thus it was necessary to develop an analytical protocol that, within the limitations of this kind of apparatus[15], could reach the maximum possible destruction of organic matter (matrix decomposition) and dissolution of the metals. The difficulties in tailoring a suitable digestion program to prepare conifer needles samples have already been pointed out in other studies (Marguí *et al.* 2005; Ohlsson 2012; Sucharová and Suchara 2006):

- conifer needles, as all plant materials, are heterogeneous, thus it is difficult to create a procedure that is valid for all their possible chemical compositions;
- the high silicon content of both the needle and of atmospheric PM (which varies also from sample to sample), does not allow the complete solubilization of metals. Primary silicates, silicic acid and silica colloids or gels can sequestrate target analytes such as aluminum, iron, chromium, titanium, nickel, vanadium, copper and manganese. It seems that an additional hydrofluoric acid or NaF attack after a more common digestion program with nitric, sulfuric acids and hydrogen peroxide, or even better the addition of tetrafluoroboric acid (HBF_4) guarantees to achieve the total decomposition of the sample as silicon is removed in the form of tetrafluoride.

Method development begun by trying on one of the samples collected for the case study described in chapter 4 the digestion program suggested in the manual of the instrument (CEM) for the NIST

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[15] Open-cavity systems are nowadays commercialized and used only when it is necessary to mineralize large amounts of samples (up to 15 g) or very reactive materials that with a close-vessel system could not be digested as they would generate large amount of gases. In these cases it is accepted to compromise with the not obtainment of a complete dissolution, due to the necessity to use a high-boiling point acid and risk of loss of volatile elements and contamination.

standard “pine needles 1575”. Since sample and reagent consumptions were quite high in this program, it was tested whether half of the indicated amounts were sufficient. 500 mg of sample seemed a reasonable amount since 100-200 mg are to be considered minimal amounts to guarantee sample homogeneity (Rautio *et al.* 2010) and a sample mass higher than 500 mg results in dark solutions after microwave-assisted digestion (Mingorance 2002). The entire digestion has been carefully watched and suggestions given in the manual (CEM 1996) were followed to improve the program. After a series of modifications and different attempts, we designed a program (Table 3.3-2) which allowed the obtainement of a clear digestate with no residues, besides some white precipitates (Figure 3.3.1). The white precipitates are likely sulfates and silicates and were removed by vacuum filtration with a Whatman cellulose nitrate filter with 0.3 μm porosity. Sulfates are due to the use of H_2SO_4 while silicates are present due to the lack of use of hydrofluoric acid (HF). The hypothesis was confirmed by LV-SEM-EDXS analysis of two filters (one was used to filter the certified reference material sample NIST 1575a) (Figure 3.3.2). EDXS analysis (an example of EDX spectrum obtained is given in Figure 3.3.3) of the particles deposited on the filters revealed that the elements present were mainly O, Si and S. Sometimes also Ca, K, Mg, Na and Al. It can be inferred that since certain elements are linked to the not-dissolved silicates, they remain trapped in their structure and do not pass to the solution (among the listed elements this is to be expected especially for Al). Alternatively, some elements could co-precipitate with the relatively insoluble $CaSO_4$ (Jones *et al.* 1991). This analysis offered also the opportunity to verify that where no particles are deposited, there is no Na, a typical contaminant due to the use of filters (Kalra *et al.* 1989).

Table 3.3-2 Microwave-assisted digestion program applied to a vessel containing 500 mg of dried needles powder, a small amount of deionized water and 1.25 ml of sulphuric acid.

STAGE	RAMP TIME	TEMP °C	TIME AT PARAMETER	REAGENT	ALIQUOT SIZE (mL)
0	0	amb	0	6 mL nitric	0
1	6 min	100	0	none	none
2	2 min	130	1 min	1 mL nitric	0.5 mL
3	2 min	180	5 min	5 mL nitric	0.5 mL
4	0	130	10 min	10 mL peroxide	0.5 mL

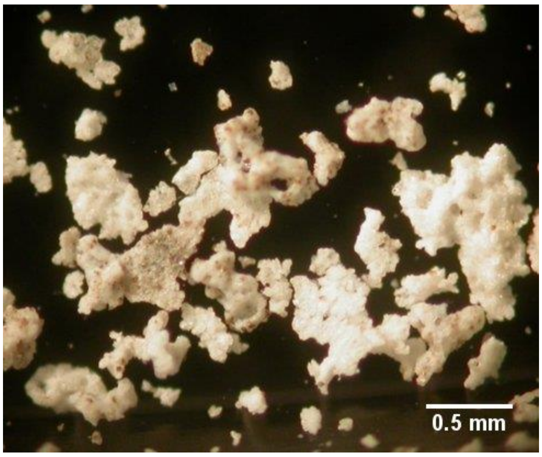

Figure 3.3.1 OM picture of the white precipitates present in the digestate. The precipitates are likely calcium and barium sulphates and silicates.

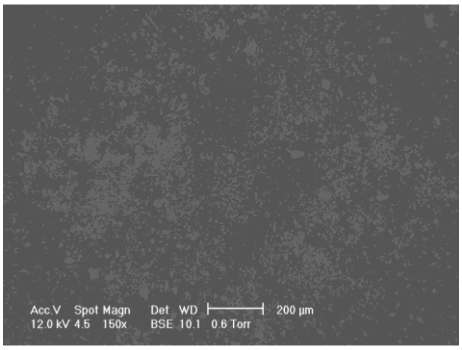

Figure 3.3.2 SEM micrograph of the surface of the filter with silicate and sulfate particles.

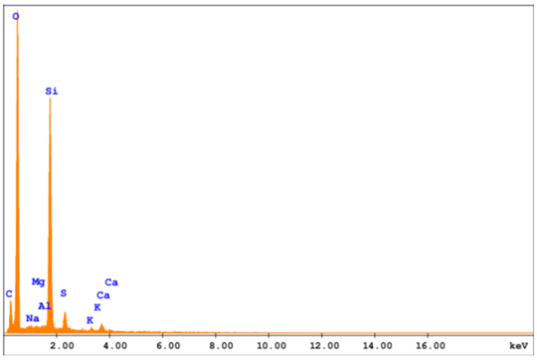

Figure 3.3.3 EDXS typical spectrum of the particles of silicates found on the filter. In some of them a higher content of sulfur and calcium was present.

The program designed (Table 3.3-3) was used to digest the certified reference material (CRM) NIST 1575a (Pine needles) and a series of samples. When the evaluation of the quality of our data based on the CRM was accomplished, as it will be described later, even with the digestion apparatus used, Al was recovered in an acceptable amount. This was quite unexpected as nitric acid does not form easily soluble nitrates with Al mainly as a result of surface oxide formation; moreover, Al can be easily sequestrated by Si compounds and the literature suggests low recovery without use of hydrofluoric acid (Stefan *et al.* 2007) and only HNO_3 and H_2O_2 (Pöykiö *et al.*, 2000). Based on this observation it was hypothesized that there was room for improvements in the developed digestion program. A factorial experiment was designed to understand the factors most influencing the digestion results and determine what could eventually be improved. The factors listed in Table 3.3-3 were selected and given two alternative values on the basis of the digestion program suggested for pine needles in the manual of the microwave-assisted digestion apparatus (CEM), literature-based considerations and careful exam of the suggestion by EPA given in method 3052 (EPA 1996).

Table 3.3-3 Variables and their values that will be used for the Youden Ruggedness Test (Dux 1986) to identify which variables have a stronger impact on the result of ICP-AES measurements in order to improve the analytical protocol within the limitation of the use of an open-vessel digestion system.

m: manual of our instrument
l: from examination of literature
EPA3025: amount of hydrogen peroxide in catalytic quantities (0.5-2 ml) the highest range value was picked as they were using HF, thus possibly dissolving better the organic matter

VARIABLE	VARIABLE ID	STAGE	LOW VALUE (-)	HIGH VALUE (+)
MW T (°C)	T1	1	100	130 (m)
HEAT TIME (min)	t1	1	3 (m)	6
MW max T (°C)	T3	3	180 (l)	250 (m)
SAMPLE MASS (g)	M	0	0.250 (l)	1 (m)
TOT VOLUME OF HNO_3 (mL)	N	0 to 3	23	24 (m)
VOLUME OF H_2SO_4 (mL)	S	0	0	2.5 (m)
VOLUME OF H_2O_2 (mL)	H	4	2 (EPA3052)	20 (m)

The seven selected factors were combined following Table 3.3-4 in what is called a Youden Ruggedness Test (Dux 1986) which requires a total of eight analysis.

Table 3.3-4 Combination of variables in each of the eight analysis required to perform the Youden Ruggedness Test (Dux 1986) and identify what factors influence most our results.

Analysis	T1	t1	T3	M	N	S	H
1	130	6	250	1	24	2.5	20
2	130	6	180	1	23	0	2
3	130	3	250	0.250	24	0	2
4	130	3	180	0.250	23	2.5	20
5	100	6	250	0.250	23	2.5	2
6	100	6	180	0.250	24	0	20
7	100	3	250	1	23	0	20
8	100	3	180	1	24	2.5	2

The effect for one variable (f.e. T1) of going from the low value to the high value (from 100 to 130°C) is evaluated calculating the difference between the sum of the concentration of the element of interest in 1,2,3 and 4 divided by four and the sum of the concentration of the same element in 5,6,7 and 8 divided by 4. The variables that influence most the concentration of a certain element will have a difference considerably larger than the others, thus are those that must be controlled in the analytical method.

As the CRM had certified concentrations the test was performed on this sample in order to see if the recovery of the elements was being improved.

Table 3.3-5 Summary of the results of the Youden Ruggedness Test. For each element the factors influencing more the measured concentration are in bold. The column "closest" identify the sample number that allowed a concentration determination closest to the certified one. The column "contaminated sample" lists the samples that contain a too high amount of the element suggesting that some contamination was introduced.

	amount H_2O_2	amount H_2SO_4	sample mass	ramp time 1	T1	T2 & T4	T3	closest	contaminated sample	certified
Fe	40	42/57	55	46	38	47	45	2 (30)	7	46
Mn	15	**78/114**	**167**	49	66	88	37	**2 (371)**	none	488
Zn	14	13/14	25	18	15	15	12	7 (19)	8	38
Al	0	**69/100**	**170**	50	75	75	34	2 (388)	none	580
Cu	1	0	1	0	0	0	0	**8 (2.6)**	none	2.8
Na	22	7/10	42	12	2	2	10	**7 (58)**	2 & 8	63
K	48	**352/582**	**1251**	**263**	**433**	**485**	**139**	2 (2582)	none	4170
Mg	**145**	**43/129**	**549**	60	24	53	94	**2 (846)**	none	1060
Ca	**311**	**139/341**	**1178**	**215**	**133**	**215**	**207**	2 (1855)	none	2500
Cr	0	0	0	0	0	0	0	4 (0.3)	none	0.4
Co	0	0/1	2	2	2	0	0	6 (<IDL)	all except 6	0.061
Ni	0	0	1	0	0	0	0	2 & 8 (1)	none	1.47
Pb	< IDL									0.067
Cd										0.233

From the Youden Ruggedness test (Table 3.3-5) it emerges that the recovery and quantification of Cu, Cr, Co and Ni cannot be improved by changing any of the parameters considered in the test. For the other elements it seems that many factors at the same time play a significant role in determining the final concentration measurement, thus none in particular could be identified to improve our current sample preparation procedure. All samples except one were contaminated with Co. This suggested a possible contamination of many of our flasks or of the ICP-AES system with this element.

From this factorial experiment it was understood that the method could not have been improved more.

Other methods developed for STAR 2 were tested. The “mild digest” one which does not use sulfuric acid or temperatures above 120°C, should have reduced the precipitation of Ba and Ca sulfates and determined a lower reagent blank signal. The attempt was completely unsuccessful as the obtained digestate was yellow and full of undissolved solids. This is probably due to the scarce oxidation efficiency of nitric acid at these low temperatures. The program for soil analysis was also tested, as it was noticed in the literature that some researchers use digestion protocols developed for this sample matrix. With this program, reaching maximum 90°C, too much material was obtained. The failure of these programs is in agreement with some Authors who consider necessary a minimum temperature of 140°C to digest organic samples.

It was then decided to utilize the digestion program previously developed (Table 3.3-2).

3.4 Selection of the best type of TEM grids for the analysis of PM (with special attention to PM from industrial sources and vehicular traffic)

“*The analytical community is presented with a new frontier of environmental investigation that can only commence with the development of innovative approaches to the quantitative measurement of man-made nanomaterials in the environment*” Alan G. Howard 2010

Experimental tests leaded to recommend the use of holey carbon film with gold mesh grids. Such a substrate guarantees high contrast with the particles for imaging and low contribution of signal to the EDX spectrum (gold peaks do not interfere with elements typical of airborne particles). Furthermore, it allows the characterization of particles containing copper, which are characteristic of many pollution sources (appendix C), and present less contaminant particles on its surface than grids with a continuous carbon-based film. On continuos film grids carbonaceous, siliceous and calcareous particles in a variety of morphologies were identified in high amounts (an example is given in Figure 3.4.1). Their number was significantly reduced with the holey carbon film. However, even there, few particles which could be taken for anthropogenic airborne particles were identified (an example is given in Figure 3.4.2).

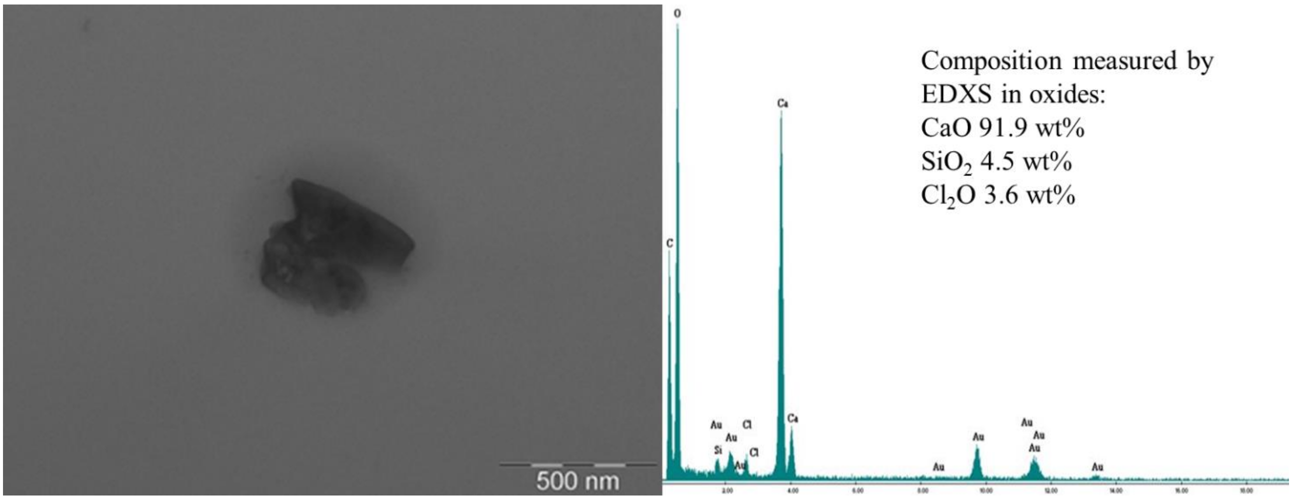

Figure 3.4.1 Example of a typical contaminant present on new Au grids with continuous C-film. Chlorine concentration reported as Cl_2O is an artifact of the EDXS quantification method used.

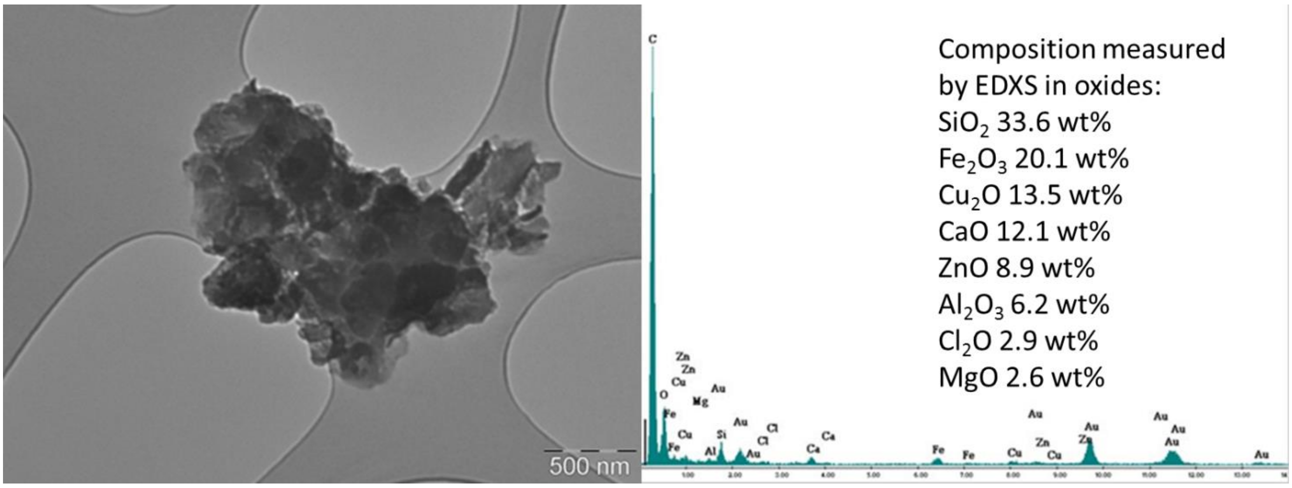

Figure 3.4.2 Micrograph and relative EDX spectrum of a contaminant particle detected on TEM grids with gold mesh and holey carbon film. Chlorine concentration reported as oxide is an artifact of the EDXS quantification method used.

3.5 Development of a sample preparation procedure for SEM-EDXS

At first needles were analysed directly mounted on a stub with double-sided carbon tape (Figure 3.5.1). Direct needle observation is found in the literature (Iwasaki and Tainosho 2003; Karhu and Huttunen 1986; Lehndorff *et al.* 2006; Ratola *et al.* 2011; Trimbacher and Weiss 1999; Urbat *et al.* 2004; Viskari 2000; Viskari *et al.* 2000) with the only difference that all the Authors used high vacuum conditions while this work explored the opportunity of using low vacuum conditions (0.6 Torr) to avoid sample coating. In preliminary tests it was observed that no collapse of the biological structure of spruce needles occurred if they were fresh, thus this work eliminated the usual drying step in sample preparation reducing contamination risks.

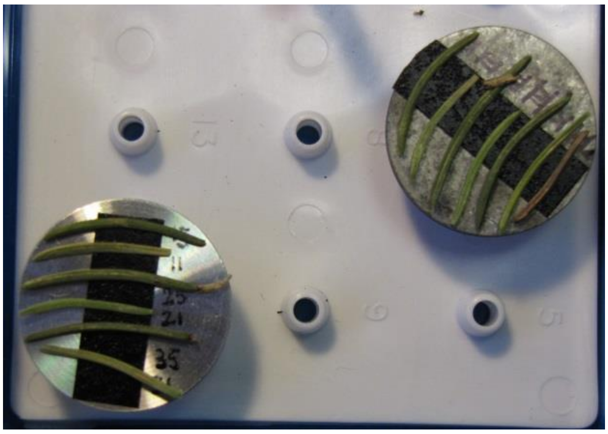

Figure 3.5.1 Spruce needles mounted with double-sided carbon tape on Al SEM stubs (diameter: 2 cm)

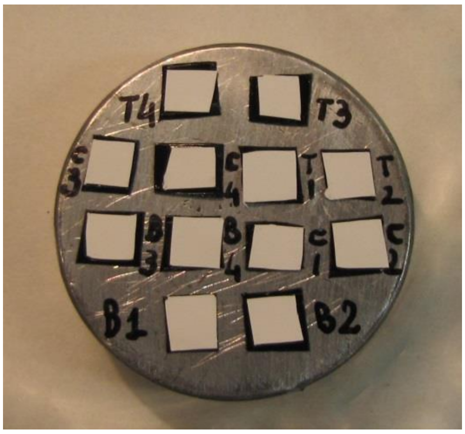

Figure 3.5.2 Nylon membrane squares (5 x 5 mm^2) mounted with double-sided carbon tape on a Al-Cu alloy disk.

From these preliminary experiments aimed at obtaining quantitative information from the scientific analysis of the micrographs acquired by SEM, it was concluded that observing the particles directly on the needle was not the way to go. Firstly, the needle is not flat and secondly the presence of the epicuticular wax around the particles made it difficult for the image analysis software to distinguish the contours of the particles and provide realistic information on their size and number (a piece of wax could embed more than one particle becoming a single one in the counting process leading to underestimate the number of particles present in the field of view[16]. Finally, especially with a manual system, such as the one available in our laboratory, the analysis of particles on the needle leads to results whose representativeness is questionable: for time consumption reasons, the researcher ends up analysing particles deposited on one or very few needles per type to be able to analyse the large number of samples characteristic of a large monitoring project.

These considerations suggested that the particles needed to be re-deposited on a better substrate: a filter. It was discussed in paragraph 2.2 that polycarbonate membranes are the best for SEM, but that similar suitability is expected from nylon ones. The latter can resist the solvents used for washing the needles, thus allowing the integration of the deposition of the particles for SEM analysis in the same protocol of ICP-AES analysis, consuming the same sample. The other advantage of depositing the particles on filters is that potentially they are available for other non-destructive (optical microscopy for the study of the coarse fraction, x-ray fluorescence, instrumental neutron activation analysis) or destructive analysis (water extraction for the determination of soluble ions – nitrates and sulphates – by UV or visible spectroscopy) that could complement the characterization and eventually solve new questions during data interpretation. They can also give information on the organic component of PM through, for instance, extraction in an organic solvent followed by IR or UV spectroscopy.

Since coarse particles and possible coarse wax agglomerates are not interesting for SEM, it was decided to introduce a double filtration with a cut at 30 μm and a cut at 0.2 μm. To be observed by SEM and provide representative results the filters should have a suitable loading. Willis *et al.* (2002) gave indications about ideal loading for CC-SEM: 5-20 $\mu g/cm^2$, in any case maximum 30 $\mu g/cm^2$. However, it has to be taken into account that Willis *et al.* (2002) recommend loading for filters capturing particles in active samplers. Hence, the weight they measured is due to particles only. Here, instead, the weight includes also the epicuticular wax removed from the surface.

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[16] Particle analysis improves notably also on the needle surface using the plugin Trainable Weka Segmentation of Fiji (Schindelin *et al.* 2012), a distribution of the software ImageJ tailored for application in the life sciences.

Using filters and membranes with 47 mm diameter (area of 17.35 cm^2) to filter 50 ml of DCM in which about 1.5 g of needles collected at a polluted site were washed a loading of 79 $\mu g/cm^2$ was obtained on the 0.2 μm membrane. An area of this filter was observed by LVSEM showing that the particles are well separated and that in BSE mode it is easy to distinguish those rich in high-Z elements (brighter) and the numerous carbonaceous ones (Figure 3.2.7).

Placing the entire filter in the SEM chamber is not always convenient, thus our approach was to observe their whole surface at the optical microscope and select areas with different particle concentrations. A mask to cut squares of the filters with 5 mm side was prepared on an acetate sheet. The squares were cut with a clean steel scalpel following the procedure described in Willis *et al.* (2002) for polycarbonate membranes. The square sections were mounted with double-sided carbon tape on a SEM stub (Figure 3.5.2).

3.6 *Identification of data treatment and interpretation strategies for each techniques with related quality evaluation of the data*

ICP-AES sample preparation

The first step in the assessment of the efficiency of the sample preparation procedure developed was verifying that the grinder was not releasing contaminants. The elemental composition of a grinded sample of dried cellulose powder[17] and another one not grinded at different levels of usage of the grinder (30 and 55 samples grinded before the test respectively) were compared. The analytical technique employed for this comparison was ICP-AES as it is sensitive to ppb concentrations, thus it guarantees the detection of even small amounts of contaminants. No contamination was detected.

The evaluation of the sample digestion protocol based on the use of the open-vessel system available in the Department, was done submitting to the FKV Company the certified reference material (CRM) 1575a Pine Needles and a series of samples for sample preparation in closed-vessel systems, known to be better[18] than open-vessel digestion. The sample set was comparatively prepared also with our open-cavity system. The FKV Company used two different closed-vessel apparatus, described at the beginning of this chapter, and for each of them a specific digestion program (Table 3.6-1 and Table 3.6-2). The two sets of samples (prepared with our open-cavity system and prepared by FKV in closed-vessel systems) were both analyzed with our ICP-AES system in the same conditions.

Table 3.6-1 Microwave-assisted digestion program applied to a vessel containing 500 mg of dried needles powder, 4 ml of HNO_3 and 1 ml H_2O_2 in the closed-vessel system UltraWAVE Milestone.

STEP	TIME (min)	POWER (W)	TEMPERATURE (°C)
1	22	1500	250
2	10	1500	250

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[17] Cellulose fibers (medium) sold from Sigma as high purity cellulose powder for partition chromatography or in metabolic pathway and carbohydrate applications and to study biofuel and biorefinery applications.

[18] Reduced risk of contamination and loss of analytes, lower amount of reagent consumed, higher temperatures reached and shorter time employed.

Table 3.6-2 Microwave-assisted digestion program applied to a vessel containing 500 mg of dried needles powder, 7 ml of HNO_3 and 1 ml H_2O_2 in the closed-vessel system EthosONE Milestone.

STEP	TIME (min)	POWER (W)	TEMPERATURE (°C)
1	15	1500	200
2	10	1500	200

The comparison of the experimentally obtained concentrations and those provided in the certificate of the CRM was done to check if with 95% confidence the measurements met the 25% accuracy criterion (AC) (NIOSH 1995): the result must be within ±25% of the true concentration with a probability of 0.95 for an individual observation. A way to apply the 25% AC is to calculate the t-value from the experimental measurements on the standard using the following formula:

$$|t| = \frac{|\bar{x} - certified|}{st.dev}\sqrt{N}$$

where $\bar{x}$ is the average of the N concentration measurements for an element of the CRM in the laboratory following the desired sample preparation procedure, *certified* is the concentrations indicated for that element on the certificate accompanying the CRM and *st.dev* is the 25% of the concentration given in the certificate as that would be the maximum spread from the reference value accepted for method validation. If this experimental t is less than the t-value provided by a t-table for 95% confidence level and infinite degrees of freedom (a certified reference material is assumed to have been measured infinite times to provide the reference concentration), then the difference in concentration is not significant and the method (sample preparation + analysis) can be considered accurate[19]. The best measurements are those obtained on the samples digested in closed-vessel systems (UW ICP-AES and ET ICP-AES) (Table 3.6-3). With these systems 10 elements out of 15 and 9 elements out of 15 were respectively within the 25%AC and 4/5 of them were also directly in the interval provided on the certificate. Both samples were contaminated with Na and Co (ET also with Fe) and contained less K and Cd than the amount given on the certificate. Na is the main constituent of glassware and could derive also from filter paper (the filter paper used by FKV staff was not analysed for contamination).

Discrepancy with the concentrations provided by NIST even after UW and ET digestions could have been anticipated considering that at the NIST they used a combination of acids (nitric acid, hydrofluoric acid, hydrochloric acid, perchloric acid and aqua regia) which allows the complete dissolution of the sample. Being limited by our digestion apparatus, the total dissolution of the sample cannot be accomplished (also the NIST uses a closed-vessel apparatus for microwave digestion).

It can also be concluded from this part of the study that the use of HF is not necessary for the dissolution of the metals contained in conifer needles: the elements typically sequestered by Si compounds (Al, Cr, Ni, Cu, Mn), were all within the 25% AC when the closed-vessel digestion apparatus was used, with the exception of Fe in the sample digested by ET.

As the concentrations measured on NIST 1575a were closer to those on the associated certificate when using the two close-vessel systems it was necessary to estimate the deviation between the concentration measured with our ICP-AES in the same operating conditions when the sample is

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[19] Similarly, it is also possible to determine the confidence interval for each concentration measured as

$$\bar{x} \pm \frac{t \times st.dev}{\sqrt{N}}$$

where t is the t-value taken from a t-table if not specified for 95% confidence, st.dev is the standard deviation of N measurements of the same sample of CRM, and verify if it is included or not in the confidence interval built around the certified concentration subtracting or summing its 25%. The difference between $\bar{x}$ and certified is often called bias.

prepared with our open vessel system (indicated as CEM) and with the close-vessel system. The following graphs (Figure 3.6.1) show for each element emission line the amount recovered with open vessel digestion (CEM) (each bar of the histogram is a sample) with respect to closed-vessel digestion (UW stands for UltraWAVE apparatus, each point of the line is a sample) considered as the real concentration after the good accuracy obtained on the CRM with this sample preparation procedure.

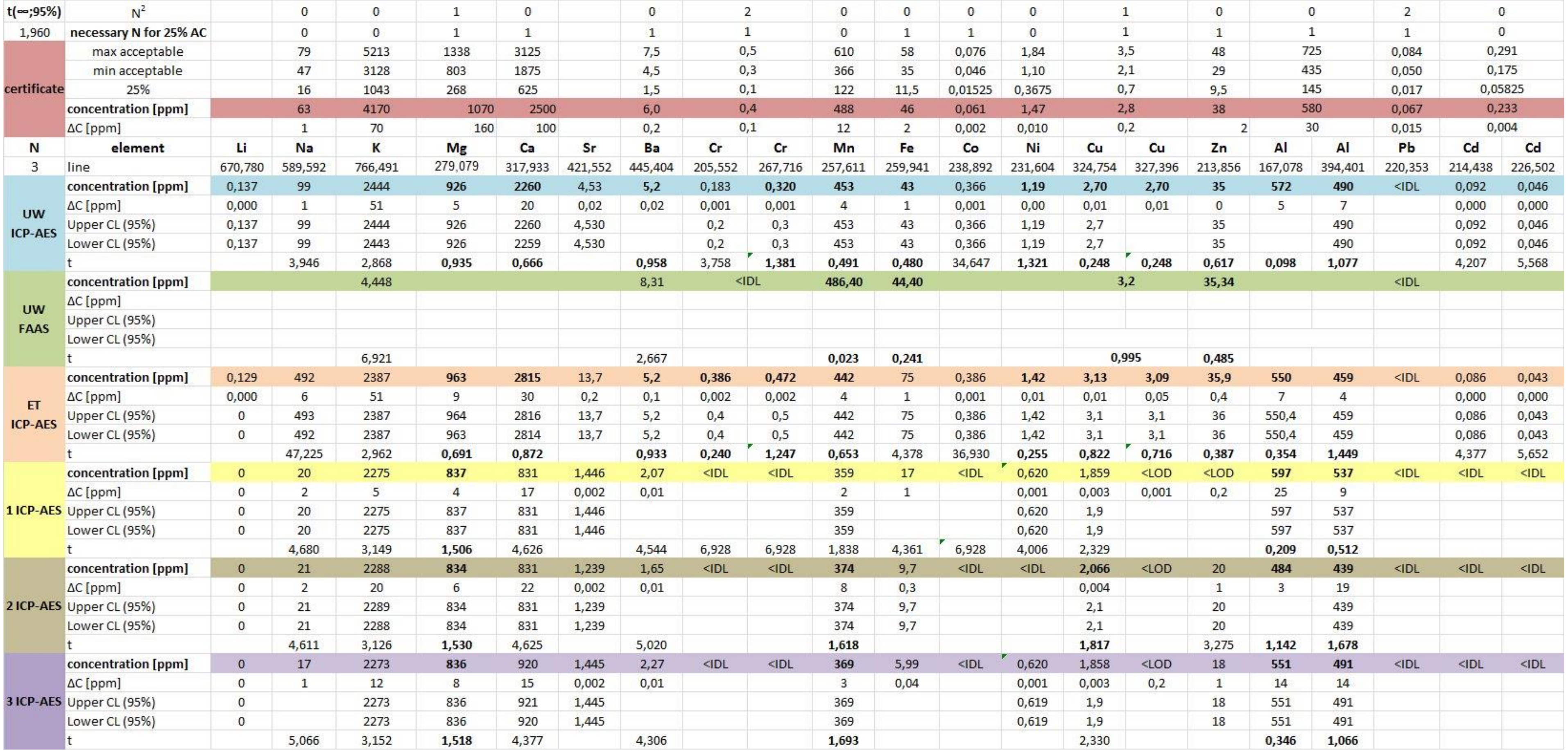
It can be observed that by digesting the samples with our system we almost always underestimate element concentrations. This is due to different phenomena:

- volatilization
- precipitation of insoluble sulfates due to the use of H_2SO_4 (Jones *et al.*1991; Twyman 2005): Ca, Ba, Sr, Pb.

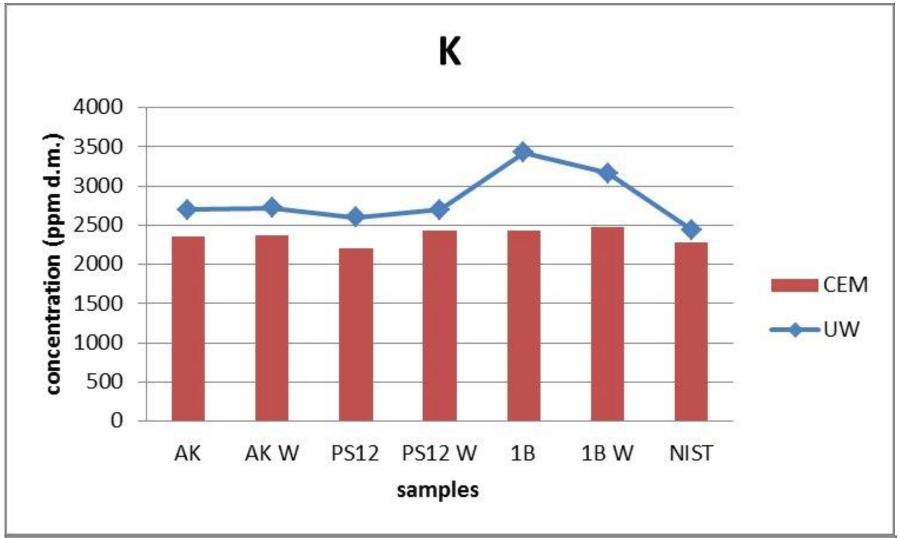
The histograms for Li, Mn, Ni and Cd highlight that the underestimation is particularly significant when it leads to exclude the presence of a certain element in a sample in which instead there is a low amount. For two samples (1B and 1BW) sometimes element concentrations are overestimated (for Mg this happens with samples PS12 and PS12W). This may be due to contamination which is more likely when an open-vessel apparatus is used. The absence of Zn in samples 1B and 1BW digested with the open-vessel system is due to a very high reagent blank Zn content for that set of measurements with consequent high limit of detection.

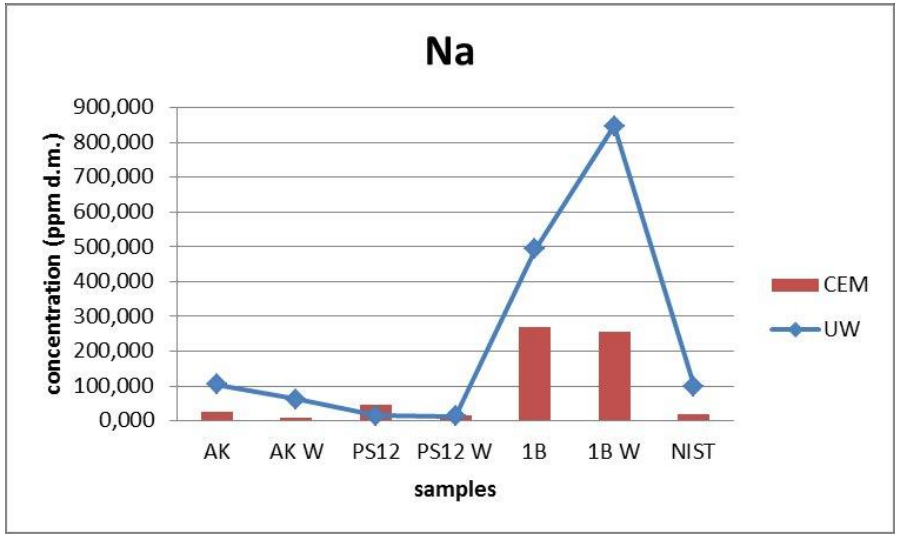
The results here described highlight that preparing the sample with an open-vessel digestion apparatus, even if the digestion program is optimized for the sample to be dissolved, leads to results that can be used only in a relative way, that is for intra-laboratory comparison. They cannot definitely be taken as valid for comparisons with other laboratories or the literature, assuming published data have the required analytical quality. To further investigate the quality of the sample preparation technique, combustion in air with an external heat source (Bunsen burner) of needle dry powder in a platinum crucible followed by dissolution of the ashes in either hydrochloric (ashHCl in Table 3.6-4) or nitric (ashHNO_3 in Table 3.6-4) acid was tested. This technique is definitely not recommended in the literature due to the high risk of sample volatilization and contamination (Jones *et al.* 1991; Stoeppler 1997). This undesirable sample preparation was performed on the CRM (NIST 1575a) and, depending on the acid used to dissolve the ashes, led to have 2 or 3 element concentrations within the values provided on the certificate considering the 25% accuracy criterion. Since with our open-vessel system 3 elements were on average recovered with the same statistical confidence, the scarce quality of our digestion method was confirmed.

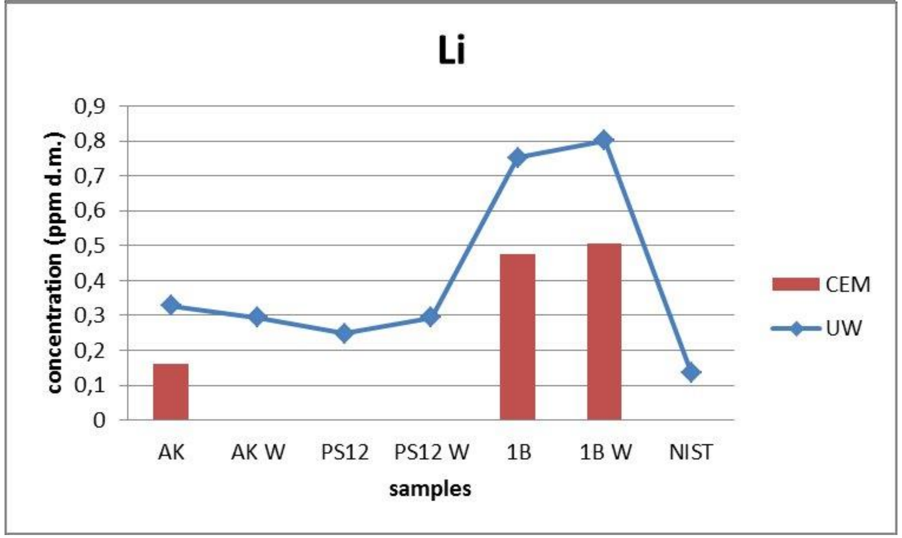
Table 3.6-3 ICP-AES elemental concentrations (ppm) of the NIST CRM 1575a (Pine needles) after open-vessel microwave-assisted digestion of the sample with a CEM system using both nitric and sulphuric acid (1 ICP-AES, 2 ICP-AES and 3 ICP-AES) and after closed-vessel microwave assisted digestion with two different Milestone system (UW: UltraWAVE, ET: EthosONE) with nitric acid only. The concentrations are compared with those provided on the NIST certificate. The concentrations that with a 95% confidence meet the 25% accuracy criterion are in bold characters. The uncertainties were calculated through error propagation. The UW ICP-AES and ET ICP-AES uncertainty do not include the uncertainty on the volume measurement. The t- value for 95% confidence and infinite DF is 1.960 and concentrations are accurate if the experimental t-value given in the table is lower than this one. DL = instrument detection limit, LOD = limit of detection

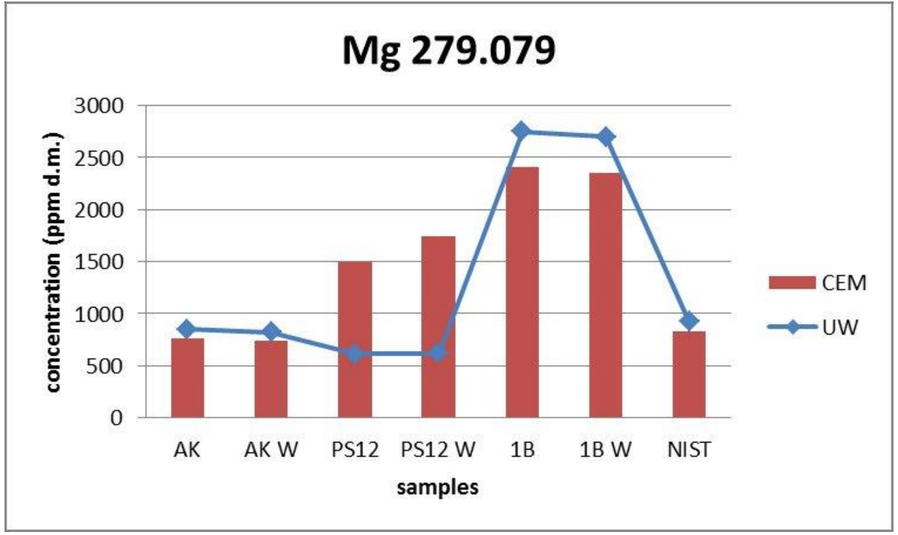


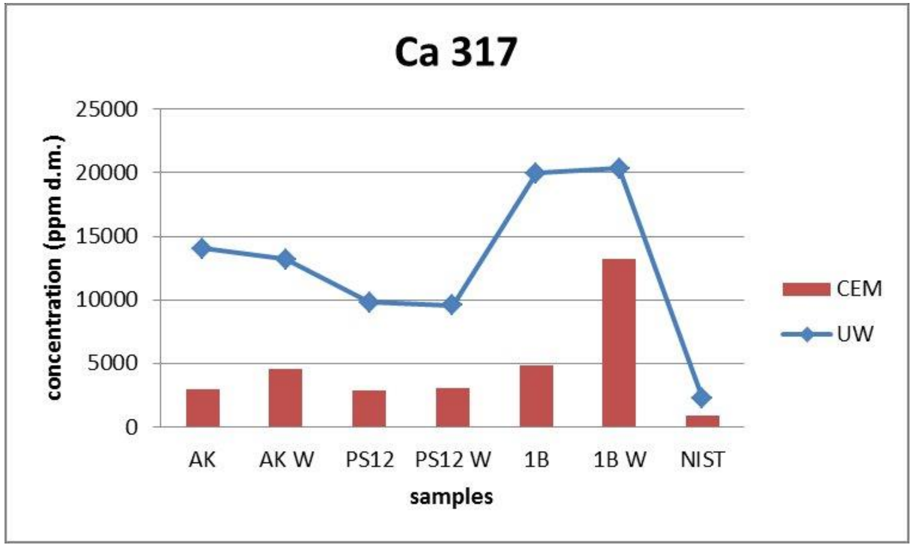
t(∞;95%)	N^2		0	0	1	0		0	2		0	0	0	0	1		0	0		2	0	
1,960	**necessary N for 25% AC**		0	0	1	1		1	1		0	1	1	0	1		1	1		1	0	
certificate	max acceptable		79	5213	1338	3125		7,5	0,5		610	58	0,076	1,84	3,5		48	725		0,084	0,291	
	min acceptable		47	3128	803	1875		4,5	0,3		366	35	0,046	1,10	2,1		29	435		0,050	0,175	
	25%		16	1043	268	625		1,5	0,1		122	11,5	0,01525	0,3675	0,7		9,5	145		0,017	0,05825	
	concentration [ppm]		63	4170	1070	2500		6,0	0,4		488	46	0,061	1,47	2,8		38	580		0,067	0,233	
	ΔC [ppm]		1	70	160	100		0,2	0,1		12	2	0,002	0,010	0,2		2	30		0,015	0,004	
N	**element**	**Li**	**Na**	**K**	**Mg**	**Ca**	**Sr**	**Ba**	**Cr**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Cu**	**Zn**	**Al**	**Al**	**Pb**	**Cd**	**Cd**
3	line	670,780	589,592	766,491	279,079	317,933	421,552	445,404	205,552	267,716	257,611	259,941	238,892	231,604	324,754	327,396	213,856	167,078	394,401	220,353	214,438	226,502
UW ICP-AES	**concentration [ppm]**	0,137	99	2444	**926**	**2260**	4,53	**5,2**	0,183	**0,320**	**453**	**43**	0,366	**1,19**	**2,70**	**2,70**	**35**	**572**	**490**	<IDL	0,092	0,046
	ΔC [ppm]	0,000	1	51	5	20	0,02	0,02	0,001	0,001	4	1	0,001	0,00	0,01	0,01	0	5	7		0,000	0,000
	Upper CL (95%)	0,137	99	2444	926	2260	4,530		0,2	0,3	453	43	0,366	1,19	2,7		35		490		0,092	0,046
	Lower CL (95%)	0,137	99	2443	926	2259	4,530		0,2	0,3	453	43	0,366	1,19	2,7		35		490		0,092	0,046
	t		3,946	2,868	**0,935**	**0,666**		**0,958**	3,758	**1,381**	**0,491**	**0,480**	34,647	**1,321**	**0,248**	**0,248**	**0,617**	**0,098**	**1,077**		4,207	5,568
UW FAAS	**concentration [ppm]**			4,448				8,31	<IDL		**486,40**	**44,40**			3,2		**35,34**			<IDL		
	ΔC [ppm]																					
	Upper CL (95%)																					
	Lower CL (95%)																					
	t			6,921				2,667			**0,023**	**0,241**			**0,995**		**0,485**					
ET ICP-AES	**concentration [ppm]**	0,129	492	2387	**963**	**2815**	13,7	**5,2**	**0,386**	**0,472**	**442**	75	0,386	**1,42**	**3,13**	**3,09**	**35,9**	**550**	**459**	<IDL	0,086	0,043
	ΔC [ppm]	0,000	6	51	9	30	0,2	0,1	0,002	0,002	4	1	0,001	0,01	0,01	0,05	0,4	7	4		0,000	0,000
	Upper CL (95%)	0	493	2387	964	2816	13,7	5,2	0,4	0,5	442	75	0,386	1,42	3,1	3,1	36	550,4	459		0,086	0,043
	Lower CL (95%)	0	492	2387	963	2814	13,7	5,2	0,4	0,5	442	75	0,386	1,42	3,1	3,1	36	550,4	459		0,086	0,043
	t		47,225	2,962	**0,691**	**0,872**		**0,933**	**0,240**	**1,247**	**0,653**	4,378	36,930	**0,255**	**0,822**	**0,716**	**0,387**	**0,354**	**1,449**		4,377	5,652
1 ICP-AES	**concentration [ppm]**	0	20	2275	**837**	831	1,446	2,07	<IDL	<IDL	359	17	<IDL	0,620	1,859	<LOD	<LOD	**597**	**537**	<IDL	<IDL	<IDL
	ΔC [ppm]	0	2	5	4	17	0,002	0,01			2	1		0,001	0,003	0,001	0,2	25	9			
	Upper CL (95%)	0	20	2275	837	831	1,446				359			0,620	1,9			597	537			
	Lower CL (95%)	0	20	2275	837	831	1,446				359			0,620	1,9			597	537			
	t		4,680	3,149	**1,506**	4,626		4,544	6,928	6,928	1,838	4,361	6,928	4,006	2,329			**0,209**	**0,512**			
2 ICP-AES	**concentration [ppm]**	0	21	2288	**834**	831	1,239	1,65	<IDL	<IDL	**374**	9,7	<IDL	<IDL	**2,066**	<LOD	20	**484**	**439**	<IDL	<IDL	<IDL
	ΔC [ppm]	0	2	20	6	22	0,002	0,01			8	0,3			0,004		1	3	19			
	Upper CL (95%)	0	21	2289	834	831	1,239				374	9,7			2,1		20		439			
	Lower CL (95%)	0	21	2288	834	831	1,239				374	9,7			2,1		20		439			
	t		4,611	3,126	**1,530**	4,625		5,020			**1,618**				**1,817**		3,275	**1,142**	**1,678**			
3 ICP-AES	**concentration [ppm]**	0	17	2273	**836**	920	1,445	2,27	<IDL	<IDL	**369**	5,99	<IDL	0,620	1,858	<LOD	18	**551**	**491**	<IDL	<IDL	<IDL
	ΔC [ppm]	0	1	12	8	15	0,002	0,01			3	0,04		0,001	0,003	0,2	1	14	14			
	Upper CL (95%)	0		2273	836	921	1,445				369			0,619	1,9		18	551	491			
	Lower CL (95%)	0		2273	836	920	1,445				369			0,619	1,9		18	551	491			
	t		5,066	3,152	**1,518**	4,377		4,306			**1,693**				2,330			**0,346**	**1,066**			

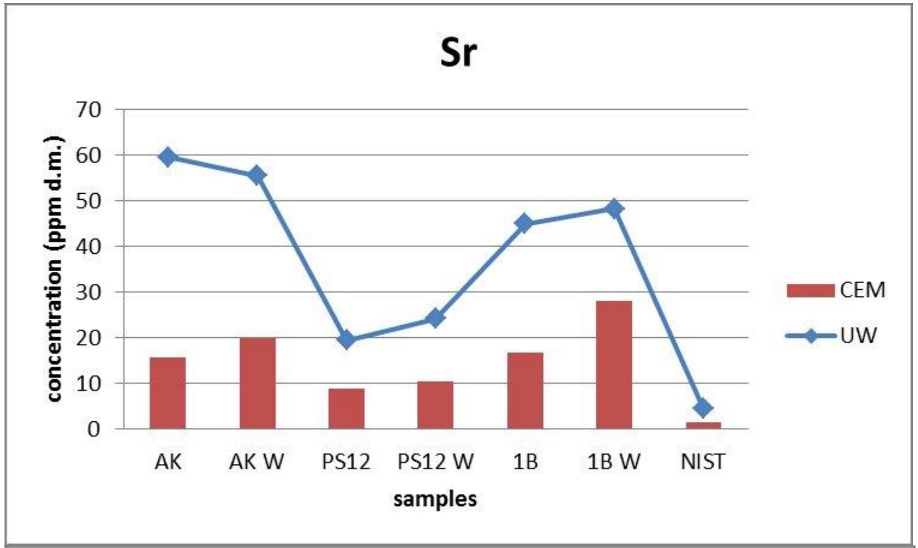

K
concentration (ppm d.m.)
4000
3500
3000
2500
2000
1500
1000
500
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

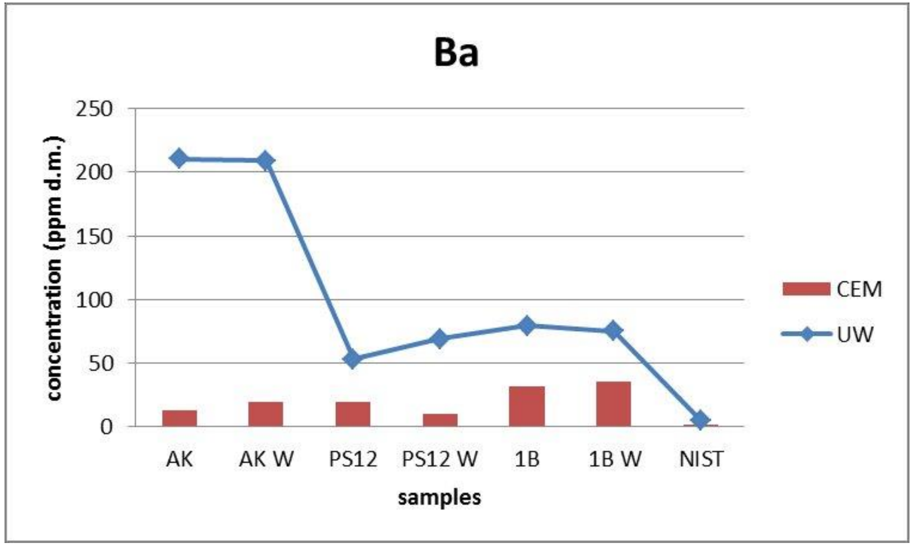

Na
concentration (ppm d.m.)
900,000
800,000
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0,000
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

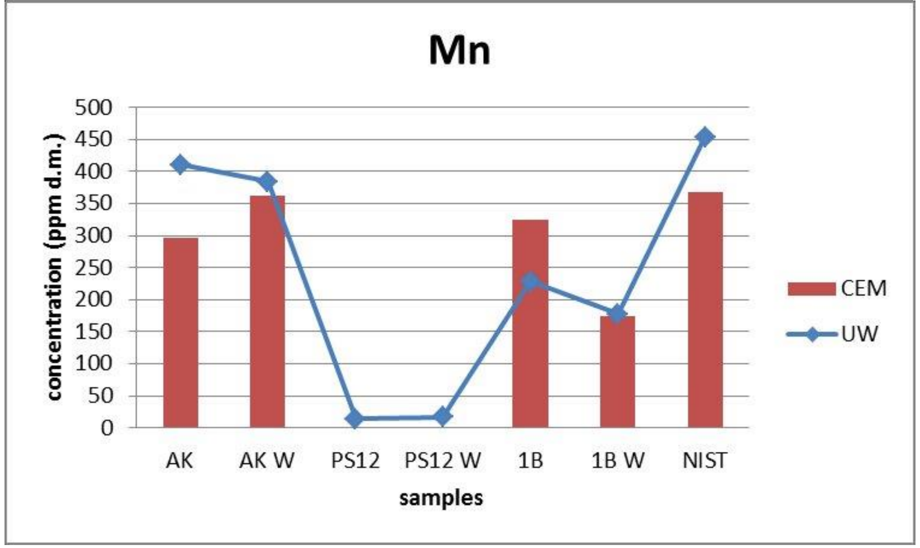

Li
concentration (ppm d.m.)
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

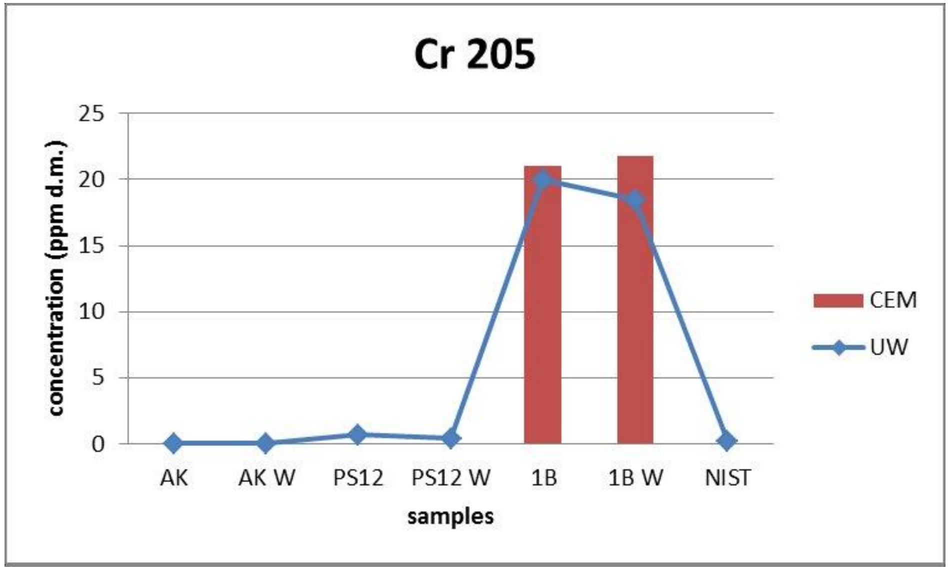

Mg 279.079
concentration (ppm d.m.)
3000
2500
2000
1500
1000
500
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

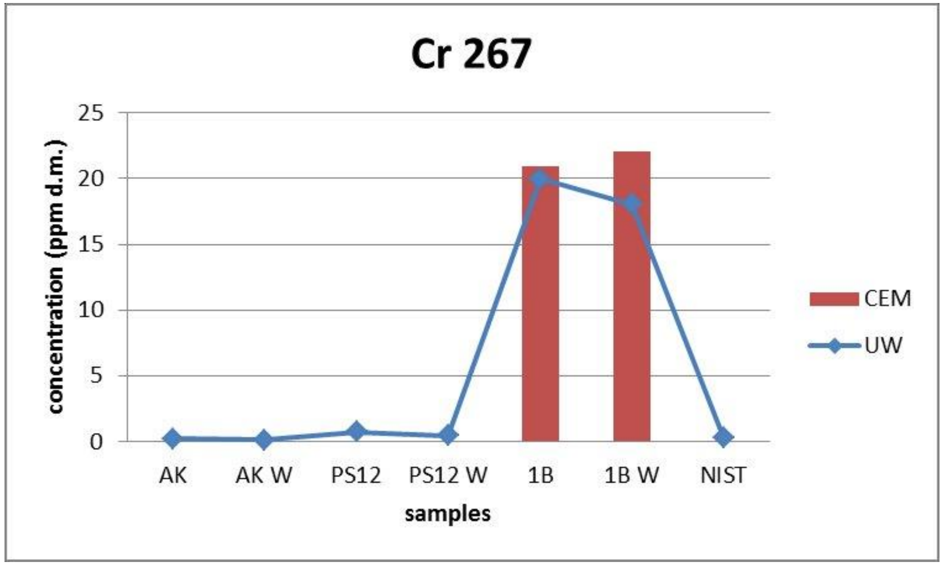

Ca 317
concentration (ppm d.m.)
25000
20000
15000
10000
5000
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

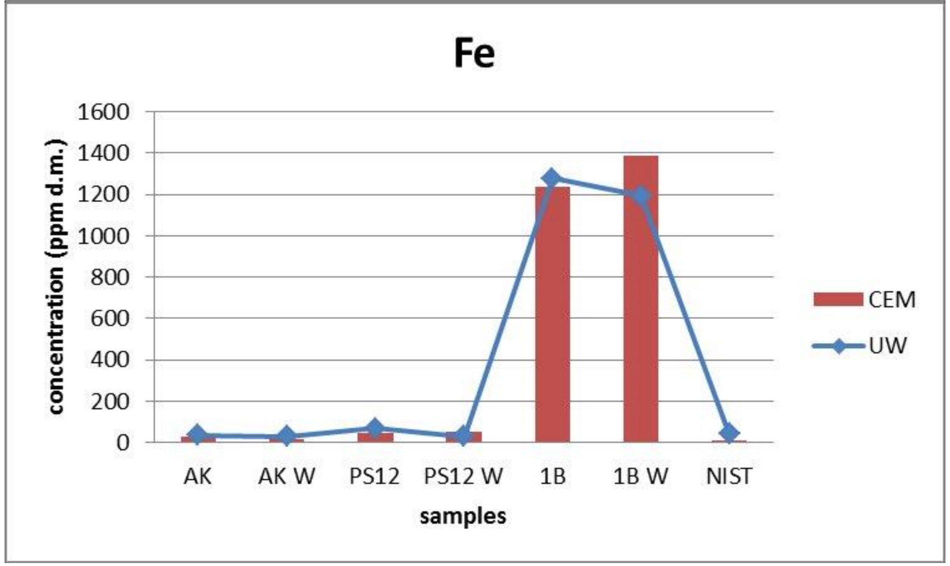

Sr
concentration (ppm d.m.)
70
60
50
40
30
20
10
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

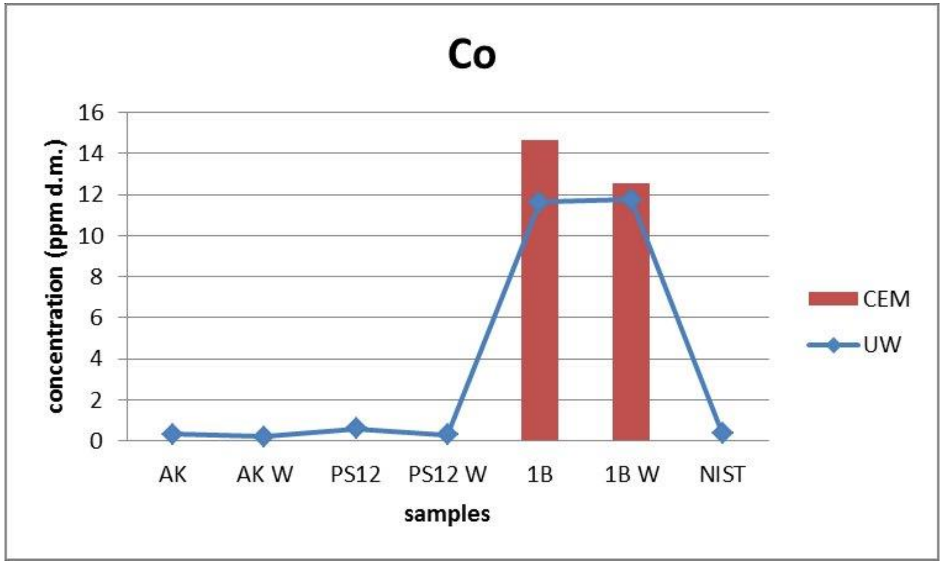

Ba
concentration (ppm d.m.)
250
200
150
100
50
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

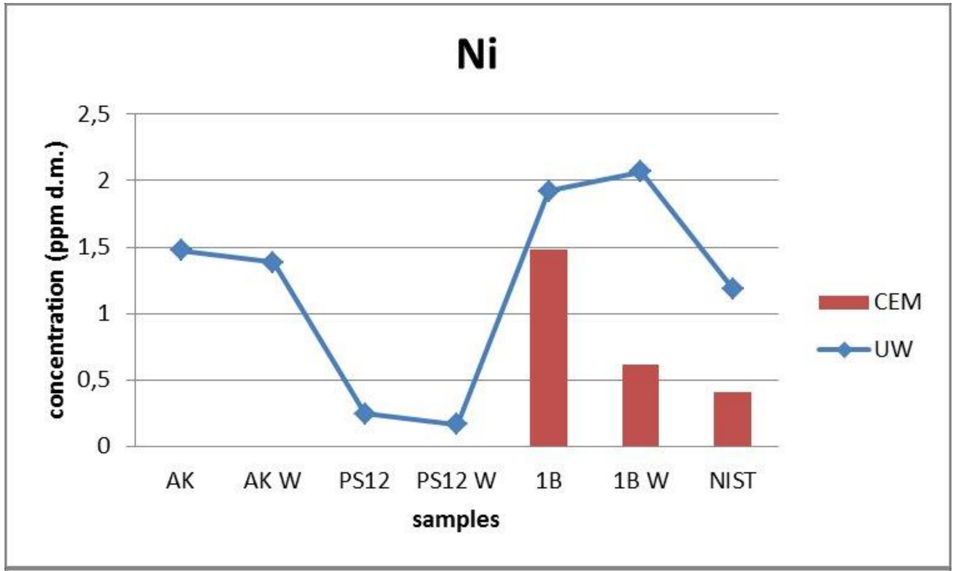

Mn
concentration (ppm d.m.)
500
450
400
350
300
250
200
150
100
50
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

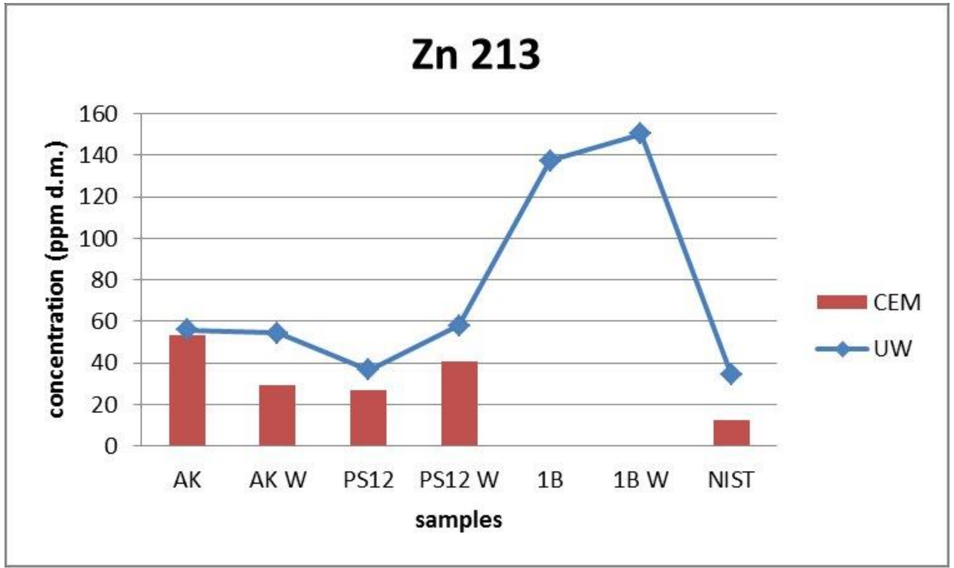

Cr 205
concentration (ppm d.m.)
25
20
15
10
5
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

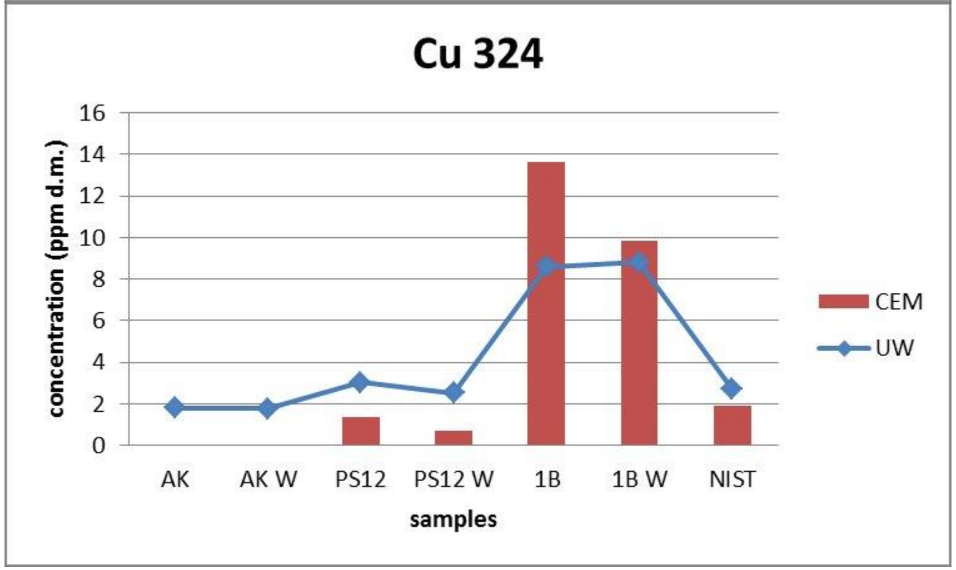

Cr 267
concentration (ppm d.m.)
25
20
15
10
5
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

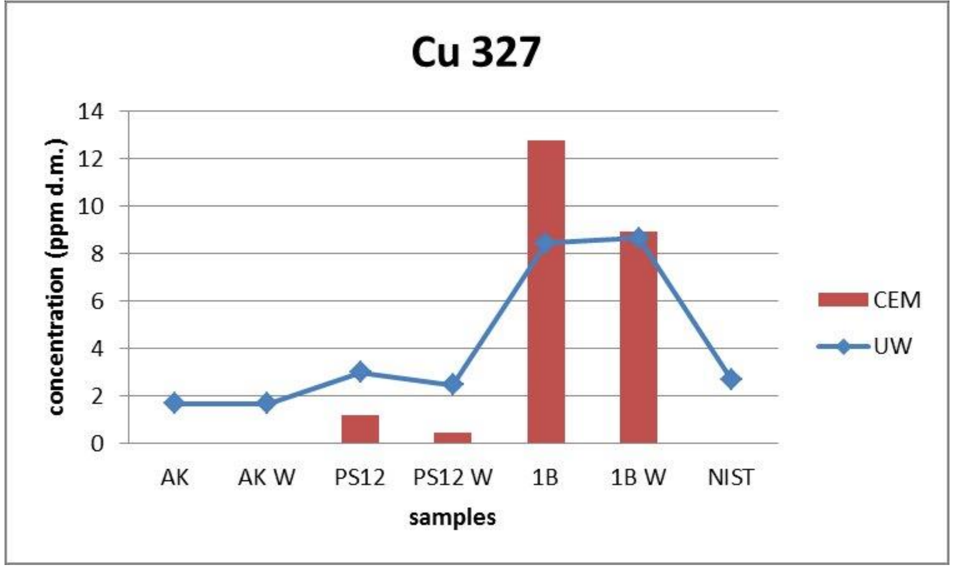

Fe
concentration (ppm d.m.)
1600
1400
1200
1000
800
600
400
200
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

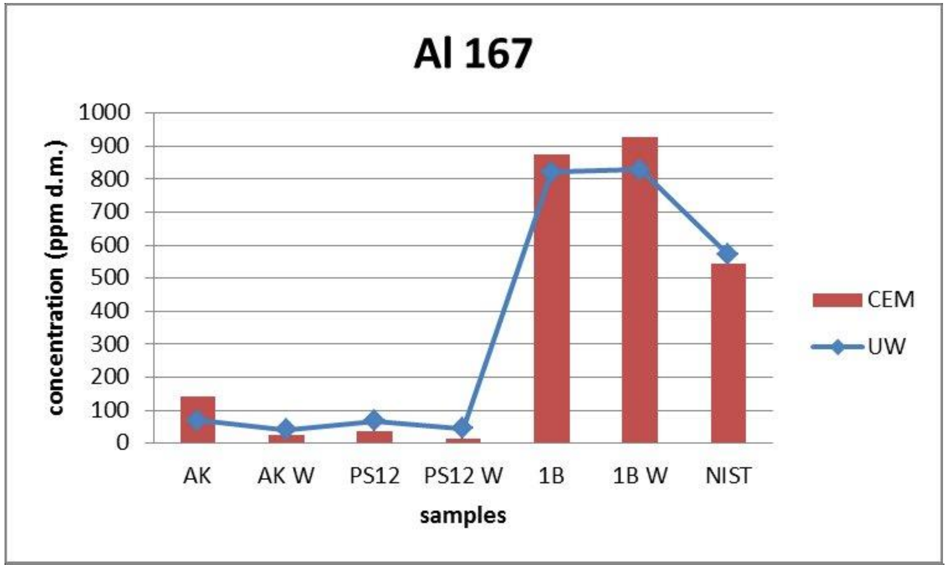

Co
concentration (ppm d.m.)
16
14
12
10
8
6
4
2
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

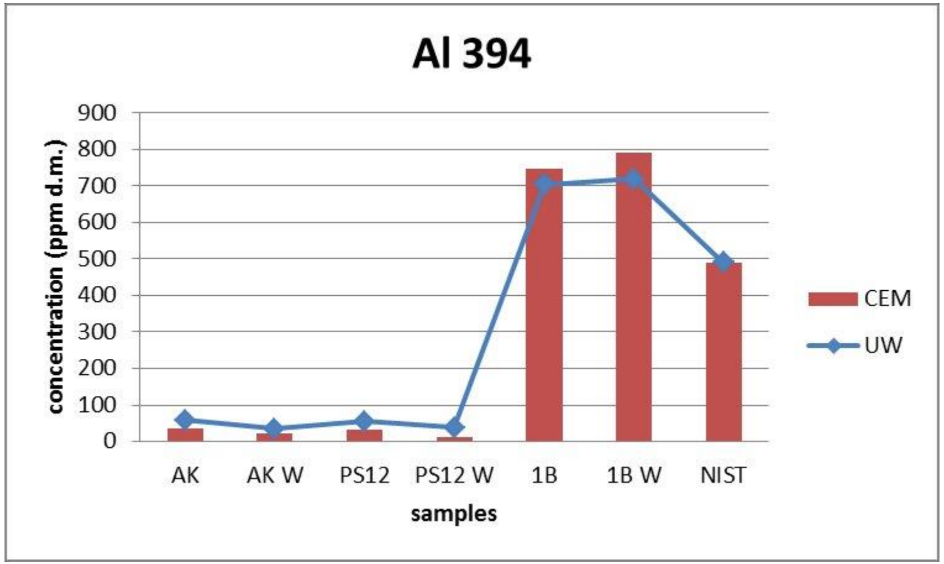

Ni
concentration (ppm d.m.)
2,5
2
1,5
1
0,5
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

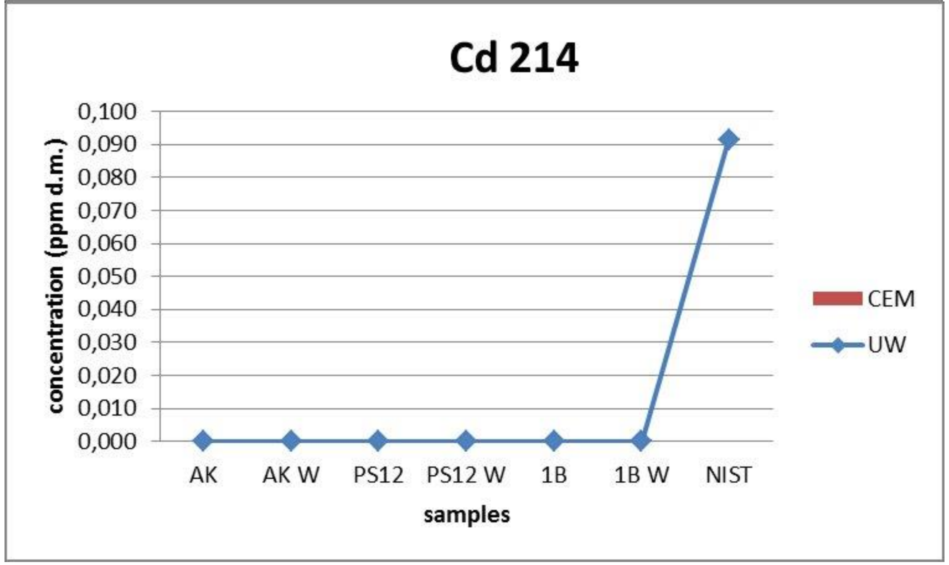

Zn 213
concentration (ppm d.m.)
160
140
120
100
80
60
40
20
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

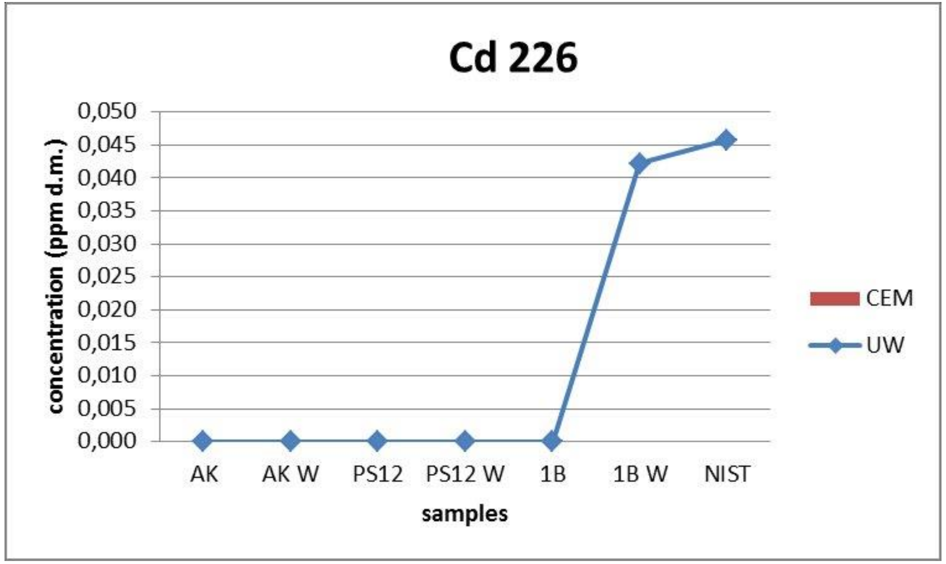

Cu 324
concentration (ppm d.m.)
16
14
12
10
8
6
4
2
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW


Cu 327
concentration (ppm d.m.)
14
12
10
8
6
4
2
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW


Al 167
concentration (ppm d.m.)
1000
900
800
700
600
500
400
300
200
100
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW


Al 394
concentration (ppm d.m.)
900
800
700
600
500
400
300
200
100
0
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW


Cd 214
concentration (ppm d.m.)
0,100
0,090
0,080
0,070
0,060
0,050
0,040
0,030
0,020
0,010
0,000
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW


Cd 226
concentration (ppm d.m.)
0,050
0,045
0,040
0,035
0,030
0,025
0,020
0,015
0,010
0,005
0,000
AK
AK W
PS12
PS12 W
1B
1B W
NIST
samples
CEM
UW

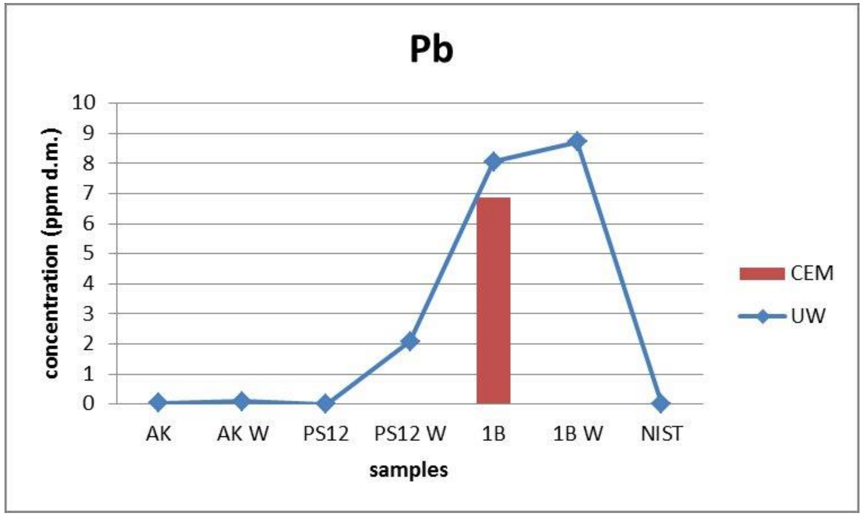


Figure 3.6.1 Graphs of the concentrations measured by ICP-AES after digestion in our open-vessel apparatus (CEM) and the UltraWave closed-vessel apparatus owned by FKV Company (UW).

Table 3.6-4 ICP-AES elemental concentrations (ppm) of the NIST CRM 1575a (Pine needles) measured after open-vessel microwave-assisted digestion of the sample with a CEM system and using both nitric and sulphuric acid (average of 3 samples) and after dry ashing in a Pt crucible and dilution in nitric (ashHNO_3) or hydrochloric acid (ashHCl) compared with the concentrations provided by the NIST certificate. Bold: concentrations that meet the 25% accuracy criterion with a 95% confidence. The uncertainties were calculated through error propagation. The t- value for 95% confidence and infinite DF is 1.960 and concentrations are accurate if the experimental t-value given in the table is lower than this one. IDL = instrument detection limit, LOD = limit of detection

t(∞;95%)	N^2		0	0	1	0		0	2		0	0	0	0	1		0	0		2	0	
1,960	**necessary N for 25% AC**		0	0	1	1		1	1		0	1	1	0	1		1	1		1	0	
certificate	max acceptable		79	5213	1338	3125		7,5	0,5		610	58	0,076	1,84	3,5		48	725		0,084	0,291	
	min acceptable		47	3128	803	1875		4,5	0,3		366	35	0,046	1,10	2,1		29	435		0,050	0,175	
	25%		16	1043	268	625		1,5	0,1		122	11,5	0,01525	0,3675	0,7		9,5	145		0,017	0,05825	
	concentration [ppm]		63	4170	1070	2500		6,0	0,4		488	46	0,061	1,47	2,8		38	580		0,067	0,233	
	ΔC [ppm]		1	70	160	100		0,2	0,1		12	2	0,002	0,010	0,2		2	30		0,015	0,004	
N	**element**	**Li**	**Na**	**K**	**Mg**	**Ca**	**Sr**	**Ba**	**Cr**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Cu**	**Zn**	**Al**	**Al**	**Pb**	**Cd**	**Cd**
3	line	670,780	589,592	766,491	279,079	317,933	421,552	445,404	205,552	267,716	257,611	259,941	238,892	231,604	324,754	327,396	213,856	167,078	394,401	220,353	214,438	226,502
average of 3 CEM	**concentration [ppm]**	0,000	19	2279	**836**	861	1,377	1,996	<IDL	<IDL	**367**	10,9	<IDL	0,413	1,927	<LOD	19	**544**	**489**	<IDL	<IDL	<IDL
	ΔC [ppm]	0	1	12	6	18	0,002041	0,005653			4	0,3		0,001	0,003	0,104	1	14	14			
	Upper CL (95%)	0	20,7593	2278,720867	835,6149872	860,9614	1,37675	#DIV/0!			367,093	9,710609		0,619482	1,927483		18,79307	574,2624	489,136			
	Lower CL (95%)	0	20,75434	2278,507231	835,5007169	860,6699	1,37675	#DIV/0!			367,093	9,7056407		0,619482	1,927483		18,79307	574,2475	489,136			
	t		4,786	3,142	**1,518**	4,543		4,623			**1,717**	5,285		4,980	2,159		3,502	**0,427**	**1,085**			
ashHCl ICP-AES	**concentration [ppm]**	0,12	101	2761		**2251**	3,964	**4,8**			>IDL	153	70,878	0,721	**2,6**	**2,5**	24	>IDL	>IDL	<IDL	1,201	<IDL
	ΔC [ppm]	0,004	3,572	175,535		425,551	0,139	0,168				5,36	2,479	0,025	0,092	0,088	0,844				0,042	
	Upper CL (95%)	0,12	101	2761		2251						153	70,878	0,721	2,6	2,5	24				1,201	
	Lower CL (95%)	0,12	101	2761		2251						153	70,878	0,721	2,6	2,5	24				1,201	
	t		4,143	2,342		**0,691**		**1,380**	6,928			16,051	8043,190	3,530	**0,388**	**0,685**	2,526				28,783	
ashHNO_3 ICP-AES	**concentration [ppm]**	0	100	2428		**1930**	3,243	4,1			>IDL	74	43,235	**1,76**	4,6	4,6	14	>IDL	>IDL	0,540	<IDL	<IDL
	ΔC [ppm]		3,988	128,039		429,207	0,127	0,159				2,922	1,700	0,069	0,181	0,181	0,563			0,021		
	Upper CL (95%)		100	2428		1931						74	43,235	1,76	4,6	4,6	14			0,540		
	Lower CL (95%)		100	2428		1930						74	43,235	1,76	4,6	4,6	14			0,540		
	t		4,037	2,894		**1,579**		2,248				4,264	4903,578	**1,348**	4,439	4,439	4,317			48,911		

ICP-AES measurement

The evaluation of the quality of the data obtained on conifer needles through ICP-AES has to be divided into 3 parts:

- evaluation of the quality of the single datum;
- evaluation of the quality of the analytical method;
- evaluation of the quality of the analytical system.

The determination of the quality of the single datum includes running calibration standard each time before the measurements to determine the instrument detection limits (IDL). These are the minimum and maximum concentrations that the instrument can measure for each element in the clean matrix for the selected wavelength. When a sample is analyzed only the measurements whose concentrations are inside the range defined by the IDLs can be considered. The maximum corresponds to the limit of linearity and it is the upper limit of quantitation as the calibration curve after becomes nonlinear. Another aspect of the quality of the single datum is the fact that the analytes are dissolved in a solvent matrix that may contain small amounts of the same elements of interest. It is then necessary to prepare a reagent blank sample that is the background for sample digestion (perform a digestion with no samples but only the reagents) and then analyze the result after performing the same sample preparation steps that samples undergo to calculate the limit of detection (LOD). The LOD is the smallest concentration of an element in a sample that you can distinguish from the blank at a 99% confidence. For the elements in the range defined by the IDLs you then know with 99% confidence that instrumental signal higher than the LOD is due to the analyte. The LOD can be calculated as three standard deviation above the mean blank signal (3-σ criterion) (Mitra 2003; Thomsen *et al.* 2003). If the concentration for an element measured by the instrument is above the LOD you can state that the element is present in the sample. However, to report its concentration the measured value needs to be above another concentration called limit of quantification (LOQ), higher than the LOD, which is 10 standard deviations above the mean blank signal (Dean 2005). Introducing the LOQ avoids to have the chance of false negatives that is not guaranteed by the LOD which tells only that the element is present with a certain probability. The other two information that must be provided in order to evaluate the quality of a single concentration measured by ICP-AES are the accuracy and the precision of the measurement. The accuracy can be determined measuring a certified reference material (CRM). The National Institute of Standards and Technology (NIST) is an agency of the U.S. Department of Commerce which developed many environmental standard reference materials® (SRMs) among which also natural matrix, such as the standard 1575a Pine Needles. This was released in 2002 to replace the original SRM 1575 issued in 1976 and currently out of stock (Zeisler *et al.* 2006). This standard is a dried and sterilized powder made of needles of loblolly trees (*Pinus taeda*) collected in North Carolina, USA, that can be used for trace elements analysis (Mackey *et al.* 2004). The standard is accompanied by a document in which certain element concentrations are certified, because they have been determined by many independent analytical techniques, others are given as reference, because they were measured by one technique only, and others are provided as information because there was insufficient data regarding the uncertainty of the measurements. The Institute for Reference Materials and Measurements (IRMM), part of the European Commission Joint Research Centre, released in the past a certified material for trace elements analysis of spruce needles, called BCR 101. This material was a mixed sample of fifty Norway spruce trees (Wyttenbach *et al.* 1993) but it is unfortunately not present in the 2012 catalogue of the products sold by the Institute. The use of reference materials, although useful to check the reproducibility of the analytical procedure and to allow inter-laboratory comparisons, has not always been reported as satisfactory in the environmental field. As observed by Ernst (Ernst 1995), “nature will never produce a reference plant with regard to the concentration of chemical elements" and some Researchers (Wyttenbach *et al.* 1993) reported that the standards SRM 1575 and BCR 101 had an atypical content of ten elements (Al, As, Br, Cr, Fe, La, Mo, Na, Sb, Sc, Th), higher than many samples of Norway spruce needles measured by neutron activation analysis (NAA) in their research. The Authors of the paper attributed this discrepancy to the presence of aerosols deposited on the surface of the needles of the reference materials. The suitability of NIST 1575a for each study should be evaluated as it is generally better to use a CRM with concentrations similar to those that need to be measured. Part XVI of the "Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests" suggests the use of other agricultural CRM with concentrations in the desired range if the specific CRM is not suitable (König *et al.* 2010). When comparing the measured concentrations of the CRM and the values given in the accompanying document ideally the measured values should lie within the uncertainty limits of the certified elemental concentrations (Castro *et al.* 2009; Lamble and Hill 1998; Rodushkin *et al.* 1999). Generally, however, the previously described 25% accuracy criterion (AC) is aceepted (NIOSH 1995).

Once a laboratory routinely analyze this kind of material the CRM or a set of self-prepared needle standards should be used at the beginning and at the end of the analysis sequence to verify respectively the calibration and the eventual shifts in the analytical procedure happened during the analyses of the real samples (Jones *et al.* 1991).

In this work the NIST standard 1575a (Pine needles) previously described was used as CRM. The results of the comparative measurements on differently prepared samples were reported in Table 3.6-3. Most of the results have already been discussed and suggested that the digestion technique reduced the accuracy of the process and allowed the recovery within the 25% AC of only three elements: Mg, Mn and Al.

The use of a reference sample might also help in selecting the best lines in the emission spectrum to avoid interferences and in evaluating the recovery for each element by spiking the CRM with aqueous solutions of known concentrations of certain elements before digestion (Kalra *et al.* 1989). Looking at the results of the comparison (Figure 3.6.1) it seems that for Cr and Al the concentration difference due to the open-vessel digestion procedure was the same no matter the line used for quantification, thereby an average of the values obtained by the two lines can be used.

The precision of the result is given by the relative standard deviation (RSD) which is the ratio between the standard deviation of the three concentration measurements (SD) on the sample and the mean concentration ($\bar{x}$), both automatically provided by the ICP-AES software:

$$RSD = 100 * \frac{SD}{\bar{x}}$$

An alternative measure of precision, leading to the same value, was provided by Robarge *et al.* (1989) calculating the percentage coefficient of variation (%CV) of duplicate measurements as follows:

$$\%CV = 200 \times \frac{I}{\sqrt{2}}$$

where I is an industrial statistic calculated as

$$I = \frac{|A - B|}{A + B}$$

where A and B are the concentrations of the duplicate measurements.

Three samples of NIST were separately prepared and analyzed. Table 3.6-5 reports the RSD calculated for each element. The precision was good only for Li, K, Mg and Ni.

Table 3.6-5 RSD calculated as 100*(SD/average conc between 3 samples) of the ICP-AES concentrations measured on three samples of the CRM NIST 1575a. n.m = not measurable because all measurements <IDL or LOD

element	**Li**	**Na**	**K**	**Mg**	**Ca**	**Sr**	**Ba**	**Cr**	**Mn**
RSD	0.0	11.5	0.4	0.2	6.0	8.6	15.8	n.m.	2.1
element	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Al**	**Pb**	**Cd**	
RSD	51.5	n.m.	0.02	6.2	14.0	10.2	n.m.	n.m.	

However, the standard deviation of the elemental concentration measurements in the CRM may not be a true measurement of precision if the standard material contains very different concentrations than the samples. Therefore, at least two aliquots of the same sample must be processed separately and analyzed under the exact same conditions. Ideally different operators should prepare and analyze the sample with the whole procedure to estimate the reproducibility of the method. If the number of samples is very large replicate measurements could be done on 5% of the samples randomly selected (König *et al.* 2010).

A sample of 2008 needles collected at site F of the case study described in chapter 4 was prepared twice to evaluate precision (Table 3.6-6). The measurements were even less precise than those obtained on the NIST sample, with reasonable precision only for Sr and Fe.

Table 3.6-6 RSD calculated as 100*(SD/average conc between 2 samples) of the ICP-AES concentrations measured on two aliquots of 2008 needles collected at site F prepared and analyzed separately (case study chapter 4). n.m = not measurable because both measurements <IDL or LOD

element	**Li**	**Na**	**K**	**Mg**	**Ca**	**Sr**	**Ba**	**Cr**	**Mn**	**Fe**
RSD	n.m.	n.m.	12.2	6.0	9.6	0.7	23.9	n.m.	24.6	0.9
element	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Al**	**Pb**	**Bi**	**Ag**	**Cd**
RSD	n.m.	n.m.	24.0	n.m.	n.m.	67.1	n.m.	n.m.	n.m.	n.m.

Element concentration is calculated as

$$C[ppm\ d.m.] = \frac{\left(\left(conc_{ICP-AES} \times \frac{V_{flask}}{V_{pipette}}\right) \times V\right) - Blank}{m_{digested}\left(1 - \frac{m_{fresh} - m_{dry105°C}}{m_{fresh}}\right)}$$

where $conc_{ICP-AES}$ is the average of 3 measurements in mg/l provided by the ICP-AES software for a certain element that it is above the instrument detection limit (IDL); $V_{pipette}$ is the ml of the original sample solution which is transferred to a V_{flask} ml volumetric flask for dilution; V is the final extraction volume in liters; *blank* is the mg of that element in the reagent blank sample; $m_{digested}$ is the mass digested, generally between 250 and 750 mg and in our case of about 500 mg, m_{fresh} is the weight of the fresh needles used for the determination of dry weight at 105°C and $m_{dry105°C}$ is the weight of the same needles after oven drying at 105°C for 16 hours. Therefore, to estimate the overall uncertainty on the measurement of the concentration of an element in the sample the uncertainties on the following measurements must be determined:

- mass of the sample
- volume (0.1 l) of the solution
- volume of the flasks used for dilutions
- volume of the pipette used for transferring an amount of the original solution for dilution
- mass of the element in the blank.

The mass of the sample is measured with a digital balance, thus its uncertainty (Δm) comes from a rectangular probability distribution: there is a range in which the same mass will appear on the display. The standard deviation on the mass is given by 0.29*resolution of the balance (ISO 2008). Since the smallest significant digit on our analytical balance is 0.0001 g the standard deviation of the measurement of the mass of the sample that will be digested is 0.000029 g. The uncertainty of the volume (ΔV) measurement was calculated gravimetrically determining ten times the mass of deionized water (density approximately of 1 g/ml) placed in the flask used to collect the result of the filtration of the digested sample filled up to the 100 ml mark (Ohlsson 2012) (Table 3.6-7). This measurement revealed that the volume really reached is slightly less than the desired. The average of the 10 measurements (99.71 ml) will be used in the calculations of element concentrations as V instead of 100 ml in order to avoid the introduction of a systematic error, while the standard deviation (0.05 ml) on these measurements will be used as ΔV. Since in certain cases the solution must be diluted to measure the concentrations of elements present in amounts higher that the maximum instrument detection limit, the uncertainty on the volumes measured filling other one-mark flasks (50 ml, 25 ml and 10 ml) was calculated with the same method (Table 3.6-7). The uncertainty of the pipette used to transfer 1 ml of the original solution to the new flask for dilution was obtained from the manufacturer: 0.005 ml.

Table 3.6-7 Gravimetric determination of the volumes measured by filling with deionized water the flask up to the mark.

N	**100 ml flask**	**50 ml flask**	**25 ml flask**	**10 ml flask**
	mass of water (g) = volume (ml)	**mass of water (g) = volume (ml)**	**mass of water (g) = volume (ml)**	**mass of water (g) = volume (ml)**
1	99.62	49.74	24.88	9.92
2	99.73	49.74	25.15	9.97
3	99.69	49.81	24.89	9.95
4	99.74	49.79	24.89	9.92
5	99.69	49.78	24.93	9.92
6	99.68	49.78	24.88	10.05
7	99.79	49.80	24.89	9.96
8	99.78	49.77	24.88	9.93
9	99.68	49.91	24.88	9.97
10	99.70	49.81	24.89	9.95
AVERAGE	99.71	49.79	24.92	9.95
ST.DEV.	0.05	0.05	0.08	0.04

If we call total element content (TEC) the product of the concentration given by ICP, the inverse of the dilution factor and the final volume it can be calculated an uncertainity on it as:

$$\Delta TEC\ [mg] = TEC * \left(\frac{SDconc_{ICP-AES}}{conc_{ICP-AES}} + \frac{\Delta V_{flask}}{V_{flask}} + \frac{\Delta V_{pipette}}{V_{pipette}} + \frac{\Delta V}{V}\right).$$

If no dilution was done before ICP-AES the equation is reduced to:

$$\Delta TEC\ [mg] = TEC * \left(\frac{SDconc_{ICP-AES}}{conc_{ICP-AES}} + \frac{\Delta V}{V}\right).$$

The other uncertainty playing a role in the determination of the uncertainty on the concentration of an element in the sample is the uncertainty on the measurement of the concentration of that element in the reagent blank sample which was also obtained by an ICP-AES measurement without sample (thus mass) in it. This uncertainty can be estimated using the same volume uncertainty measured before through this calculation:

$$\Delta Blank_{mass} = Blank_{mass} * \left(\frac{SDBlank_{ICP-AES}}{Blank_{ICP-AES}} + \frac{\Delta V}{V} \right)$$

where $SDBlank_{ICP\text{-}AES}$ and $Blank_{ICP\text{-}AES}$ are respectively the standard deviation and the concentration of the element provided after three measurement of the blank sample by the ICP-AES software and the blank mass is obtained multiplying the $\mathrm{Blank}_{\mathrm{ICP\text{-}AES}}$ for the volume of the solution (0.1 l).

If we call the whole numerator of the concentration calculus blank corrected element content (BC) it can be calculated an uncertainity on it as the sum of the uncertainty on the TEC and the uncertainty on the blank:

$$\Delta BC[mg] = \Delta TEC + \Delta Blank_{mass} .$$

The uncertainty on the mass corrected for its water content (denominator of the concentration calculation) is given by:

$$\Delta m[kg] = m_{digested}\left(\frac{\Delta m_{digested}}{m_{digested}} + \frac{\Delta factor}{factor}\right)$$

where *factor* is the factor calculated from the measurement of the dry mass at 105°C (for chapter 4 case study it was given in Table 3.3-1).

The uncertainty of the concentration of each element in ppm dry matter (d.m.) is then given by:

$$\Delta C[ppm\ d.m.] = C\left(\frac{\Delta BC}{BC} + \frac{\Delta m}{m}\right)$$

The **quality of the analytical method** regards not only the measurement step but also the previous sample preparation procedure. Moreover, the measurements of precision previously reported are not completely independent being done on different aliquots of a single sample. The total method precision should include both the analytical error and the sampling error.

Two samples of needles of the same age cohort (2012) were collected the same day from two different trees at the same site (site B of the case study described in chapter 4), kept separately, prepared separately and analyzed on the same day. Comparing Table 3.6-6 with Table 3.6-8 it can be seen that including the sampling step reduces the precision of the measurement for all elements except Ca, Cr, Ni, Al, Pb, Bi, Ag and Cd. This suggests that to reduce intersample variance due to the heterogeneity of the needles and improve the quality of the analytical method, it would be better to collect a mixed sample in the field or produce later in the laboratory a composite sample by combining aliquots of dried powders from samples taken from different trees.

Table 3.6-8 RSD calculated as 100*(SD/average conc between 2 samples) of the ICP-AES concentrations measured on two samples of 2012 needles collected at site B prepared and analyzed separately (case study chapter 4). n.m = not measurable because both measurements <IDL or LOD

element	**Li**	**Na**	**K**	**Mg**	**Ca**	**Sr**	**Ba**	**Cr**	**Mn**	**Fe**
RSD	0.2	18.7	61.4	6.9	1.6	51.1	120.7	n.m.	82.6	96.9
element	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Al**	**Pb**	**Bi**	**Ag**	**Cd**
RSD	35.2	n.m.	56.7	40.9	70.7	30.1	n.m.	n.m.	n.m.	n.m.

The **quality of the analytical system** can be evaluated by comparison of the results on a set of samples with an independent analytical method. For the comparison one of the two methods must be assumed as able to provide the true concentration. A plot can be built where the results from one analytical technique are graphed versus those obtained with the other for the same sample.

In this study, flame atomic absorption spectroscopy (FAAS) measurements were conducted by FKV Company on samples digested with the UltraWave system not for all elements, as the technique is not multielemental. The analysis was focused on the metals of interest for the case study described in chapter 4 and on K which is useful to evaluate leaching of internal elements due to the washing procedure. Some of the elements (Cr and Pb) with FAAS are below the instrumental detection limit (IDL) of the system which is higher than that of ICP-AES. FAAS allowed the measurement within the 25% AC of 4 elements of the 15 present on the certificate of the CRM (Mg, Ca, Co, Ni, Al and Cd not measured with this technique). Three of them were also in the confidence interval provided on the certificate (see row UW FAAS in Table 3.6-3). K concentration measured by FAAS was, however, totally out of the expected ranges for spruce as, no matter the sample, K amount was always between 4.3 and 4.7 ppm which is not realistic (König *et al.* 2010). As K determination was done after rotating the burner and using a secondary K line for quantification the different operating conditions might have biased the result. Figure 3.6.2 shows the plots of the ICP-AES concentration versus that measured by FAAS and highlights how the correspondence is quite good (the red line is the plot of the line with slope 1 and 0 intercept and the equation plotted on the graph is that of the least square line calculated from the experimental points).

SEM-EDXS data collection and analysis

"*[…] microscopy can be dangerous at times as the operator has to select which particles to study and may be led to the most interesting feature, rather than the typical.*" Alan G. Howard 2010

Our preliminary studies suggested that analysing the particles removed from many needles by washing and deposited on nylon membranes by filtration guaranteed more representative results than analysing particles directly on the surface of the needles.

Once the areas of the filter to be observed have been selected as explained in the part of this chapter dealing with SEM sample preparation, the number of fields of view for each of the areas must be established. Beckett *et al.* (2000) randomly selected five fields of view at 5000X magnification by moving the microscope stage in a series of random movements through its x and y axes. Since we have a manual SEM system we can only perform semi-automated particle counting and sizing by acquiring SEM micrographs (with selected criteria) and later processing them with a scientific image analysis software. We use ImageJ (Rasband 1997-2011) or its distribution for applications in the life sciences called Fiji (Schindelin *et al.* 2012).

This part of the analytical protocol was developed adapting the methods described in Zamengo *et al.* (2009) and Millipore Corporation (1998) to our situation and it is reported in paragraph 3.7.

The defined analytical strategy was tested on a sample coming from a polluted site (site B of the case study described in chapter 4) and on a sample coming from a suburban/rural area which was expected to be less polluted (site O of the case study described in chapter 4). Both samples consisted of 2008 needles which have been exposed to the atmosphere for about five years and a month. They were washed in DCM and the residual solution containing the particles removed and the wax was filtered previously with a 30 µm nylon filter and the result with a 0.2 µm nylon membrane. The general aspect of the 30 µm filter is the one shown in Figure 3.6.3, where agglomerates of particles (Figure 3.6.4) likely in wax are shown on the nylon mesh. The general aspect of the 0.2 µm memebrane is shown in Figure 3.6.5 where not very bright subspherical particles are present everywhere. EDXS revealed that these particles are carbonaceous, thus they might be wax clots.

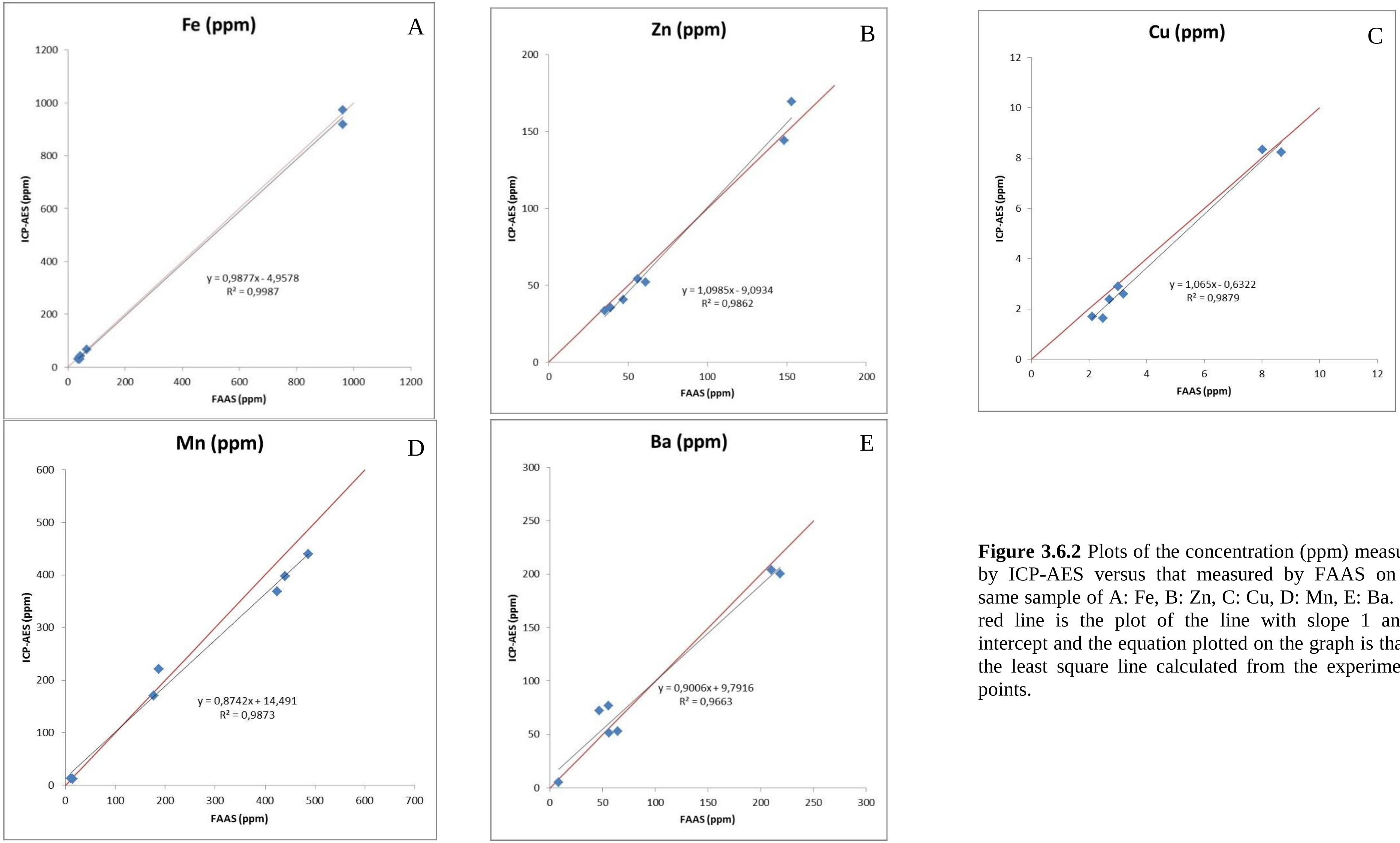


Figure 3.6.2 Plots of the concentration (ppm) measured by ICP-AES versus that measured by FAAS on the same sample of A: Fe, B: Zn, C: Cu, D: Mn, E: Ba. The red line is the plot of the line with slope 1 and 0 intercept and the equation plotted on the graph is that of the least square line calculated from the experimental points.

At the polluted site about 30 particles were classified as bright in BSE mode on both filters. However, on the 30 µm porosity filter most of them were of anthropogenic origin while on the 0.2 µm porosity membrane the majority was natural particles. The porosity of the filter did not seem to affect the analysis result as in both cases particles from below a micron up to ten microns were analyzed. The same particle classes with few exception were identified as characteristic of the site.

The structure of the 30 µm porosity filter gave some issues in the study of particle morphology by image analysis softwares as some particles partially ended up under the mesh (Figure 3.6.6). At the low-pollution site, the 0.2 µm porosity membrane was quite empty of bright particles. On the 30 µm porosity filter there is slightly more particles. In any case very few particles were analyzed: 3 on the 0.2 µm membrane and 5 on the 30 µm filter. Zincite monocrystals were identified on both, thus they could be considered a typical anthropogenic particle at the site (if this would be confirmed analyzing other DZs). Compromising with the fact that few particles may end up partially below the mesh (Figure 3.6.6), it seems then better to observe the 30 µm filter. It is here highlighted that this filter is slightly more difficult to be cut as it is attracted to the acetate mask and tends to break not sharply when cut with a scalpel. It is also suggested to analyze the particles through Fiji (Schindelin *et al.* 2012) exploiting the *Trainable Weka Segmentation* plugin which successfully can help in distinguishing a bright particle from the surrounding wax in an agglomerate.

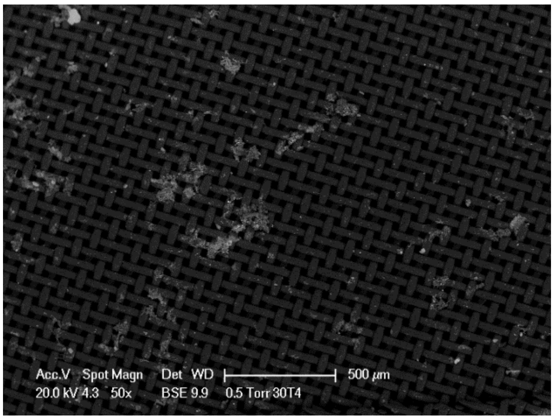


Figure 3.6.3 General aspect of 30 µm porosity nylon filters with agglomerates of particles (detail given in Figure 3.6.4).

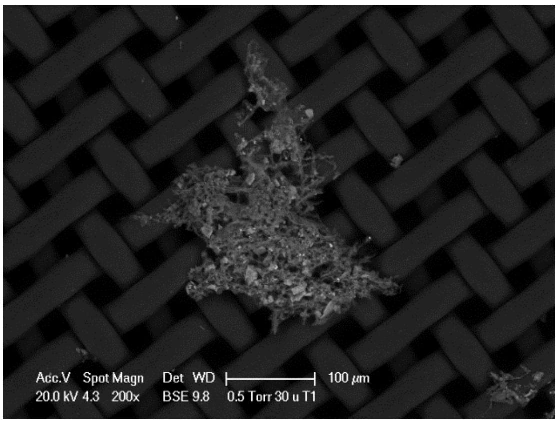


Figure 3.6.4 Typical particle agglomerate on the 30 µm porosity nylon filters.

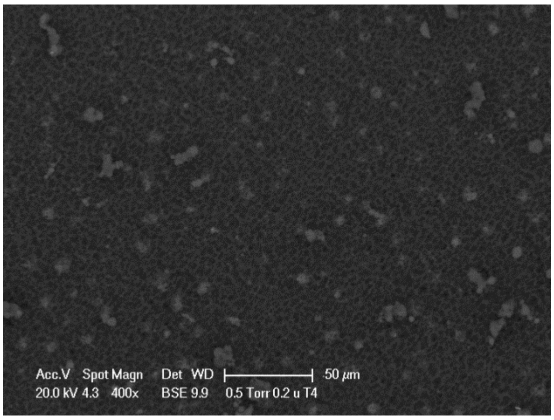


Figure 3.6.5 General aspect of 0.2 µm porosity nylon membranes with many non-bright subspherical particles, that might be wax clots.

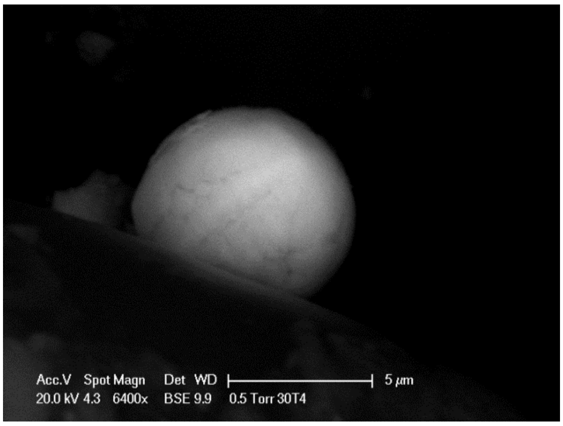


Figure 3.6.6 Micrograph of a ferrosphere (90.1 wt% Fe_2O_3) of about 7.7 µm Feret diameter which contains also, 3.8 wt% Al_2O_3, 2.8 wt% SiO_2, 1.6 wt% MnO, 0.9 wt% SO_3, 0.8 wt% Cl_2O, 0.7 wt% CaO. Chlorine concentration expressed as oxide is an artifact of the EDXS quantification method used.

TEM sample preparation, data acquisition and analysis

Many aspects regarding the selection of the substrate and sample preparation have already been discussed. An attempt was made to make the method more quantitative to have a drop of sample as large as the grid with known volume. Unfortunately, below 80 µl the drop hardly detached from the tip of the pipette and at this volume size the drop spread on an area already larger than the grid. Therefore, it does not help reducing the size of the drop below a certain amount. To guarantee the detachment of the drop it was decided to take up 100 µl of the sample in the pipette. Other attempts to further improve the method are here presented. Not all of them are part of the final proposed protocol (paragraph 3.7) as their development was driven by specific needs of the case study described in chapter 4.

Magnetic separation was introduced to maximize the time spent in the analysis of the particles of interest. This was possible as the case study was aimed at characterizing particles emitted from a steelmaking plant which are expected to be rich in Fe, an element magnetic even in the nanoscale. Although not for TEM, a similar strategy was used by Magiera *et al.* (2011) that separated dust samples suspended in isopropanol while these were in an ultrasonic bath. The use of the ultrasonic bath should promote the separation of individual grains and avoid their agglomeration.

In this work about 1.5 g of fresh 2008 needles were washed in some of the solvents previously tested.

A magnet was passed outside the becker all around the solution of the washing solvent (THF:toluene; $CHCL_3$; DCM; DCM:cyclohexane) containing the epicuticular wax and the particles (Figure 3.6.7). At naked eye, the separation with the hand magnet did not seem to be effective with the THF:toluene solution. On the contrary in chloroform, DCM e DCM:cyclohexane particles following the magnet were observed. Keeping the magnet outside the bottom of the becker the solution was poured in another becker so that only magnetic particles would remain in the original becker attracted by the magnet (Figure 3.6.8). Some solvent was poured on the magnetic particles. After stirring, an aliquot of the solution was picked up with a pipette and placed in a closed glass vial for TEM sample preparation.

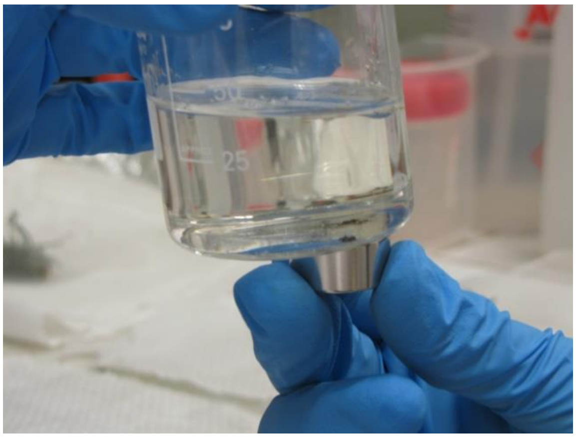


Figure 3.6.7 The magnet passed around the becker attracts the magnetic particles removed from the needles contained in the DCM solution.

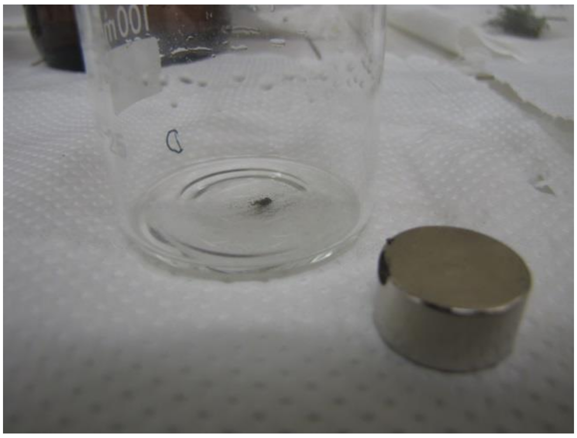


Figure 3.6.8 The becker after magnetic separation containing on the bottom only the magnetic fraction of the particles washed by DCM from the surface of the needles. On the right the magnet used for separation.

Chloroform, DCM and DCM:cyclohexane samples, in which the movement of particles following the magnet was observed, were selected for testing the efficiency of the magnetic and dimensional separation. As observed in other tests, chloroform favoured the agglomeration of the particles much more than the other two solutions (Figure 3.6.9). An ultrasound treatment was not efficient in dispersing the agglomerates observed.

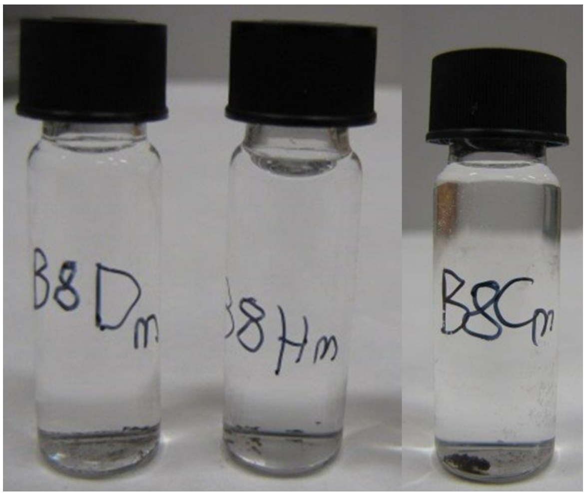


Figure 3.6.9 Subsamples of the residues of the washing solutions after magnetic separation. B8Dm: sample B2008 washed in DCM (sample on the left); B8Hm: sample B2008 washed in DCM:cyclohexane (1:1 v/v) (sample in the middle); B8Cm: sample B2008 washed in chloroform after 3 minutes ca of ultrasound treatment (sample on the right).

The samples were filtered without vacuum with nylon membranes with 0.2 µm porosity. The membranes can be observed in Figure 3.6.10. The filtration was efficient in removing coarse particles as the solutions looked clear after this process.

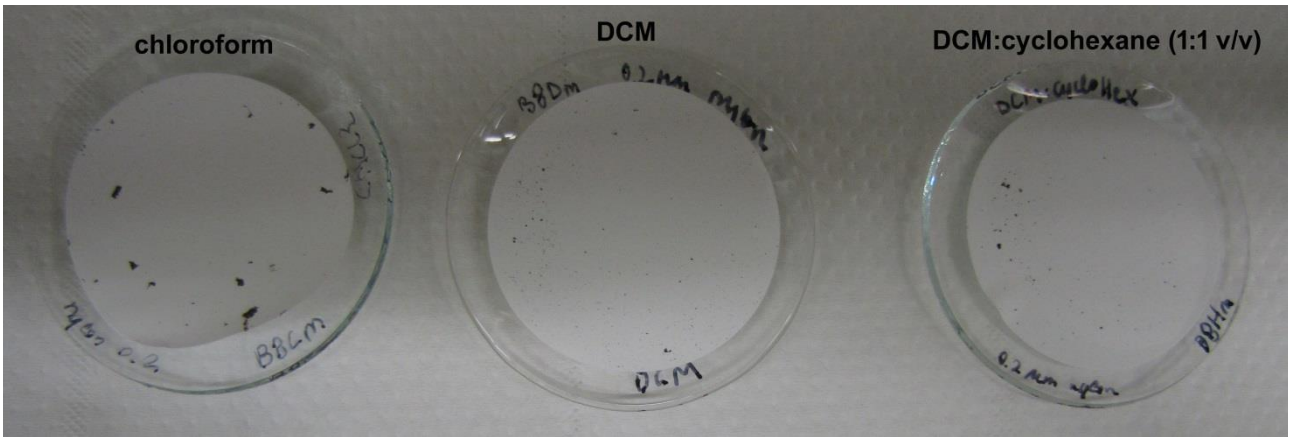
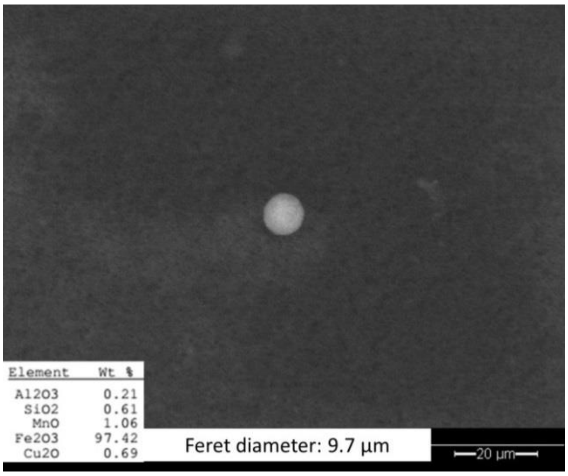


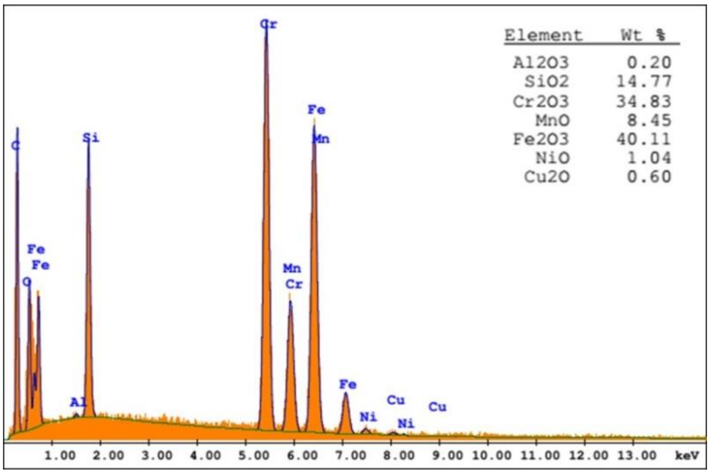
Figure 3.6.10 Nylon membranes (0.2 µm porosity) after filtration of the washing solutions compared on the sample B2008.

The obtained filtered solution were placed again in the ultrasound bath for 3 minutes, then a drop was placed on a gold 3 mm TEM grid with 200 mesh and carbon film. The sample was observed by TEM. Many particles rich in carbon were present in the DCM sample but none with Fe or magnetic composition. Most particles were small but there were some above the filter cut-off size of 0.2 µm. In the chloroform sample, more particles were observable, although only one of those characterized contained Fe and Ni. All the other particles were carbonaceous, or Ca-rich. The filters were examined with LVSEM-EDXS. The nylon membranes relative to the DCM solution had on its surface many particles that could be apportioned to the steelworks. These particles were spheres containing Fe as main element and also other metals characteristic of the steelmaking process (Mn, Zn, Cr, Cu) (see Figure 3.6.11 as an example) or coarse particles with irregular shapes containing Fe, Cr, Mn and Cu (see Figure 3.6.12 as an example). Even particles looking less bright contained little amount of Fe, thus it cannot be excluded that the magnetic separation was efficient.



Element	Wt %
Al_2O_3	0.21
SiO_2	0.61
MnO	1.06
Fe_2O_3	97.42
Cu_2O	0.69

Figure 3.6.11 SEM micrograph of a spherical Fe-rich particle and relative composition in oxides obtained by EDXS.



Element	Wt %
Al_2O_3	0.20
SiO_2	14.77
Cr_2O_3	34.83
MnO	8.45
Fe_2O_3	40.11
NiO	1.04
Cu_2O	0.60

Figure 3.6.12 EDX spectrum and composition in oxides of a particle very bright in BSE imaging mode that had irregular shape and Feret diameter of 26.6 µm.

In other experiments, where no magnetic separation was employed, particles that could be apportioned to the steelmaking plant were detected by TEM on samples from the same site. Therefore, it can be hypothesized that when magnetic separation is introduced, the particles tend to agglomerate and maybe they precipitate at the bottom of the glass vial. This would be supported by Iobstraibizer *et al.* (2014) who observed at the stereomicroscope that separated magnetic spherules tend to attract each other forming chain segments.

3.7 The final streamline analytical protocol: integration of the previous phases

The analytical protocol developed is shown in Figure 3.7.1. Each step is described in detail in the following text.

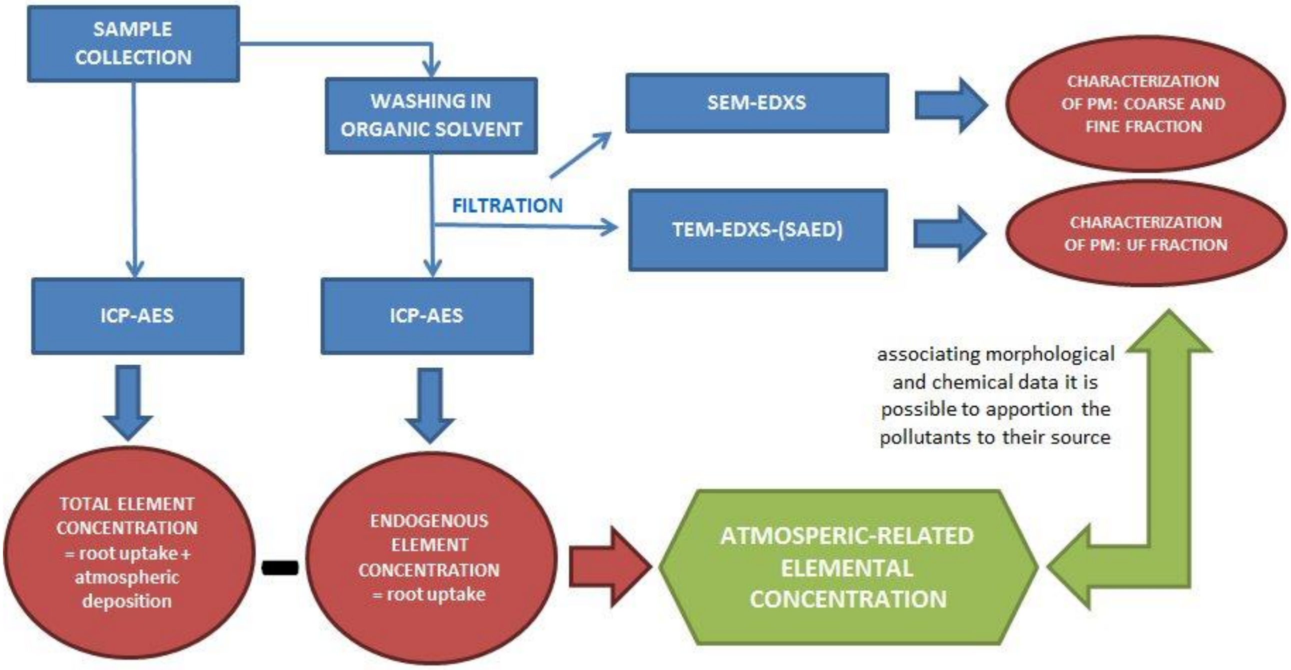


Figure 3.7.1 Scheme of the analytical protocol for the analysis of particulate matter collected by conifer needles.

A. SAMPLING

1. **Scope**: collecting conifer needles at different sites representative of different exposure periods to the atmosphere in sufficient amount for statistical representativeness and complete characterization of the deposited particulate matter.
2. **Basic principle**: Conifers allow the association of the pollutants with a time period of exposure as different age classes of needles can be easily distinguished and sampled (Figure 3.7.2).

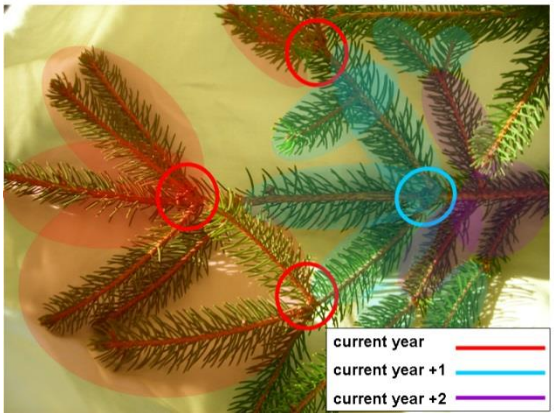


Figure 3.7.2 Every year a new set of needles is produced. This set is clearly distinguishable from previous ones because it is separated from them by a node. Counting back the nodes from the exterior to the interior of a branch it is possible to know when a certain set of needles was produced and sample different age classes. As they were produced in different years they have been exposed for different periods

3. **Apparatus and reagents**: Maps and notebook to record sampling location and observations, pruners, labeled plastic bags, equipment to clean the pruners

4. **Safety precautions**: Care using cutting tools (pruners): lock pruners when not in use, do not twist pruners while cutting

5. **Procedure:**
   a. Select the sites
      i. for gradient studies, which are aimed at evaluating the effect of point (power stations, factories, incinerators, etc.) or linear (highways, etc.) sources of pollution, the needles are sampled at increasing distance from the source. In these studies it is important to select the sampling sites on the basis of dispersion models and in a way that they have similar wind exposure. This will ensure to have the same contribution from other pollution sources located at the upcoming wind direction and to detect only effects depending on distance and direction with respect to the source. The distances are chosen in view of the expected variability of the concentration of the pollutants and, as concentration is generally higher in proximity of its source, more samples will be collected there.
      ii. a pristine or background location for comparison with the sites under investigation must be non-contaminated and with similar microclimatic conditions (VDI 2004; VDI 2007; Viskari 2000)

   b. Collect 6 g of fresh needles for each sample (needles of different age are different samples even if collected at the same site), corresponding to about 6 twigs, from different trees at a site by cutting the twigs with clean pruners
   c. Place the twigs in labeled plastic bags and close them

6. **Remarks**:
   a. Sampling should be statistically designed. Two statistical approaches may be employed. The first one is the classical random sampling design, stratified or not, based on the selection of sites in a territory with random numbers which are used to identify the spatial coordinates of the sites to be sampled. An example of this design is given in Turunen *et al.* (1997). They employed a factorial design with randomized complete blocks which was possible as they had to sample in a forest. Another example of selection of sampling sites using a random number table was used within each sampling transect by Robarge *et al.* (1989). In most cases it is possible only to employ a geostatistical design which is based on the description of the variables to be measured on the territory, assumed as a random field, and the introduction of judgement based on existing knowledge of the context in the selection of the sites. Although, the first approach may guarantee to avoid bias due to inappropriate considerations in the selection of the sampling sites, geostatistical sampling seems the most appropriate statistical approach to conifer needle-based air quality monitoring studies. The possibility of relying on previous knowledge, expected spatial variations in the concentrations of the monitored pollutants or information from dispersion models could reduce the number of samples required to obtain meaningful results (thus the costs and time expenses of the study) as well as to avoid the problem of going to the randomly selected site and find no conifers or no accessible trees, as, for instance, when they are located on private properties. The book edited by Keith (1988) provides more details on the two sampling designs and especially on the geostatistical approach.
   b. Samples should be collected in autumn or winter as the internal elemental concentrations are more stable in current year needles because their growth is complete (Jones *et al.* 1991) and also the epicuticular wax is fully developed (Prügel *et al.* 1994).
   c. Collect the same day needles from all needed age cohort and sites. A different sampling campaign means different exposure time and may produce bias in the results.
   d. The number of twigs to be collected given in 5b has to be taken as reference only. The Author is aware that twigs of older needles cohorts have less needles attached, thus more of them would be necessary to have about 6 g of fresh material. Moreover, these indications were developed for spruce and different amounts might be necessary for other species.
   e. To determine the dried mass at 105°C additional needles are required. Hence, a subset of samples must be collected in larger amount (12 g of fresh needles are suggested, within the limitations described in 6d, to use about 2 g of dry powder for the dry matter determination).
   f. It is not advisable to sample after intense rain because it is more likely that particles were washed away from the surfaces of the needles

7. **Quality assurance**:
   a. Wear a new pair of gloves to collect each sample

   b. Clean the pruners after the collection of each sample

8. **Comments**
   a. The amount of fresh needles to be sampled was calculated as twice the amount of dry powder (duplicate analysis) which has to be digested for ICP-AES (500 mg). Since each sample has to be divided in two subsamples (one to be washed for the internal element determination) it is necessary to have four times the amount to be digested of dry powder in corresponding fresh needles. It was observed that 1.5 g of fresh spruce needles give a little bit more than the amount of dry powder necessary for one digestion.
   b. Likely, more representative results are obtained if the same amount of needle dry powder from different trees present at a site, kept and processed separately from collection, are mixed in the laboratory (composite sample) instead of mixing in the field needles from different trees present at a site

B. DECONTAMINATION AND WAX REMOVAL

1. **Scope**: prepare subsamples for the determination of the endogenous element concentrations due to element uptake via roots and obtain the solution from which electron microscopy samples can be prepared

2. **Basic principle**: the measurement of the element concentrations coming from pollutant sources and wind-blown soil dust cannot be undertaken directly. Subtracting the endogenous element concentration measured on a washed subsample of needles from the total element concentration measured on an unwashed subsample of needles allows the estimation of the atmospheric contribution of interest.

3. **Apparatus and reagents**: 2 beckers, commercial glass fiber net with at most a 1 mm^2 mesh, DCM, water

4. **Safety precautions**:
   a. DCM is hazardous for the operator, thus the washing procedure must be undertaken under a vented fume hood and the washed needles and wet nets must be left there to dry

5. **Procedure**:
   a. The needles are detached from the twigs.
   b. About 1.5 g of needles are placed in a piece of net
   c. Put about 70 ml of water in a 100 ml becker and 50 ml of DCM in another one
   d. Dip three times the net in DCM for about 15 seconds and rinse each time in the water
   e. Prepare samples for SEM and TEM

6. **Remarks**:
   a. only deionized or ultrapure water must be employed to rinse the samples.

7. **Quality assurance**:
    a. Detach the needles from the twigs wearing a separate pair of gloves for each sample (you may keep the sample-dedicated pair of gloves for subsequent sample preparation operations)

8. **Comments**
    a. 1.5 g allow the obtainment of only the amount of powder necessary to prepare one sample. We recommend to wash at least 3 g either repeating the procedure twice or adapting the method by selecting appropriate glassware (a 100 ml beker does not allow proper washing of 3 g of needles)
    b. It is necessary to prepare more aliquots of a selected number of samples as part will be used for the determination of the dry matter at 105°C
    c. 1.5 g allows to have a sufficient number of particles deposited on a TEM grid for analysis at a polluted site. If lower amounts are washed an evaporation step of an aliquot of the residual washing solution must be introduced to have a sufficient number of particles for TEM-EDXS-SAED analysis.

C. DRYING (ALL SAMPLES)

1. **Scope**: remove all surface and combined water to facilitate the obtainment of a powder and guarantee sample stability/preservation

2. **Basic principle**: a temperature below 80°C allows the removal of all combined water but avoids thermal decomposition and reduction in needle dry weight (Campbell and Plank 1992; Jones *et al.* 1991; Stefan *et al.* 2007)

3. **Apparatus and reagents**: aluminum containers (e.g. cooking containers), labels, aluminum foil, oven

4. **Safety precautions**:
    a. oven surfaces and containers removed from it are hot and may pose burn hazard

5. **Procedure**:
    a. Label the aluminum containers to be used
    b. Place each sample (either twigs of unwashed needles or needles washed) in its aluminum container
    c. Cover the containers with aluminum foil with holes
    d. Place the containers in the oven at 70°C for 48 hours in air at ambient pressure

6. **Remarks**:
    a. Drying should be conducted as soon as possible after sampling or the washing step.
    b. Covering the containers is recommended where ovens are used for multiple purposes (increased contamination risk) and if the air flow in the oven is strong triggering container upset. If no contamination risk exist it would be better to dry the powders with open containers.

7. **Quality assurance**:
    - **a.** Handle each sample with its own dedicated pair of gloves

D. PARTICLE-SIZE REDUCTION

1. **Scope**: make the sample homogenous and favour the interaction of sample components with the digestion reagents used in phase F

2. **Basic principle**: conifer needles are fibrous samples, thus a grinder guarantees particle-size reduction in short time avoiding excessive moisture absorption

3. **Apparatus and reagents**: coffee grinder, spoon-spatula, plastic air-tight containers, brush, sorbent paper, deionized water, compressed air, gloves, dried cellulose powder

4. **Safety precautions**:
    - a. always unplug the grinder when not in use and before cleaning, do not place the grinder in water or other liquid, do not operate the grinder if the cord or the plug are damaged, all other general precautions typical of electrical appliances

5. **Procedure**:
    - a. Place a sample in the coffee grinder and grind it until a fine homogeneous powder is obtained
    - b. Remove the powder from the grinder with the spatula and place it in a labeled plastic-air tight container

6. **Remarks**:
    - a. Make sure no peduncles or twig pieces are attached to the needles before grinding
    - b. Coffee grinders are not for laboratory use, thus they cannot be cleaned with organic solvents
    - c. Grinders often cannot be used continuously. Read the instructions from the manufacturer

7. **Quality assurance**:
    - a. The grinder must be carefully cleaned with a clean brush, compressed air and humid sorbent paper after each sample is ground
    - b. Gloves must be worn while there is a risk to contaminate the needles or the powder
    - c. The grinder must be checked for contamination after a certain number of samples have been grinded by comparing the elemental concentration of a sample of dried cellulose powder with that of a sample of the same material grinded before analysis

8. **Comments**
    - a. Store the dry powder samples in a moisture-free environment in sealed containers until digestion and analysis

E. DRYING (SUBSAMPLE FOR DRY MATTER DETERMINATION)

1. **Scope**: determination of the dry matter of each category of conifer needle samples

2. **Basic principle**: oven drying removes moisture and total dry matter can be determined gravimetrically comparing sample weight before and after oven drying.

3. **Apparatus and reagents**: aluminum containers (e.g. cooking containers), labels, aluminum foil, oven, electronic balance (accurate to 0.0001 g)

4. **Safety precautions**:
   a. oven surfaces and containers removed from it are hot and may pose burn hazard

5. **Procedure**:
   a. Label the aluminum containers to be used
   b. Place one empty aluminum container for a selected number of samples for each sample category (washed and unwashed) and other three empty containers in the oven at 105°C for at least 2 hours (leave the oven on)
   c. Warm the balance by sequentially placing the 3 empty containers on the balance for about 20 seconds each
   d. Remove each container from the oven and weigh it rapidly to nearest 0.1 mg (W1)
   e. Allow the containers to cool
   f. Weight about 2 g of dried needle powder in each container to nearest 0.1 mg (W2 or m_{fresh})
   g. Shake the container so that the powder is uniformely distributed in them and cover them with a piece of aluminum foil with holes
   h. Place the sample containers and the three empty containers for 16 hours (overnight) in the oven at 105°C
   i. Warm the balance as in c and weigh the containers after removal of the aluminum foil cover to nearest 0.1 mg (W3)

6. **Remarks**:
   a. Covering the containers is recommended where ovens are used for multiple purposes (increased contamination risk) and if the air flow in the oven is strong triggering container upset or powder volatilization. If no contamination risk exist it would be better to dry the powders with open containers.

7. **Data analysis and interpretation**:
   a. Calculate the dry mass ($m_{dry105°C}$):
   $$m_{dry105°C} = W3 - W1$$
   b. Calculate the factor to correct the mass that will be digested for ICP-AES analysis separately for washed and unwashed samples:
   $$factor = 1 - \frac{m_{fresh} - m_{dry105°C}}{m_{fresh}}$$

8. **Quality assurance**:
   a. The factor to express ICP-AES result should be calculated on many samples including all the variables of the study (needle age, different pollution levels, washed and unwashed needles

9. **Comments**
    a. Our experimental measurements on spruce needles showed that the highest variability in dry weight was not related to the age cohort but to the washing treatment that is why in 7.b it is required to calculate a different factor for each treatment.

F. ORGANIC MATTER DESTRUCTION – MICROWAVE ASSISTED DIGESTION

1. **Scope**: remove the organic component of the needles and obtain a liquid sample suitable for ICP-AES.

2. **Basic principle**: microwave radiation is used as a source of heat to support the oxidation process that the sample undergoes after addition of nitric acid and hydrogen peroxide. The reaction leads to the formation of soluble nitrates (Gonzalez *et al.* 2009)

3. **Apparatus and reagents**: electronic balance (ccurate to 0.0001 g), microwave-assisted digestion apparatus, digestion vessels, concentrated nitric acid (69.0-70.0%), hydrogen peroxide (30%), high-purity water, vacuum filtration apparatus, Whatman membrane (3.0 µm porosity), funnel, 100 ml volumetric flask

4. **Safety precautions**:
    a. Nitric acid fumes are toxic, the digestion apparatus should direct the fumes to a hood
    b. Use laboratory-grade microwave equipment

5. **Procedure**:
    a. Follow the whole procedure once skipping step b to obtain the reagent blank
    b. Weigh about 500 mg of dried needle powder and transfer it quantitatively to a digestion vessel
    c. Pour the minimum amount of water necessary to remove the sample from the walls of the vessel
    d. Add the digestion reagents
    e. Swirl gently the vessel so that all the sample comes into contact with the reagents
    f. Install the safety pressure relief valve in compliance with manufacturer’s instructions
    g. Make sure the external surfaces of the digestion vessel are dry
    h. Place the vessel in the digestion apparatus, and follow manufacturer’s instructions
    i. Start the digestion program
    j. Remove the vessels from the digestion apparatus and allow the solution to cool down
    k. Vacuum filter the digestate with a Whatman membrane with 3.0 µm porosity and quantitatively transfer the digestate to a 100 ml volumetric flask with a funnel. Complete transfer of the sample can be achieved rinsing twice the digestion vessel, filtration apparatus and the funnel with small amounts of water
    l. add 1% of nitric acid and make up to the mark with high-purity water

6. **Remarks**:
    a. All the reagents used for the microwave-assisted digestion of the sample must be of analytical grade and only ultrapure water must be employed to previously dilute the sample and after the digestion to reach the volume of solution useful for ICP-AES determination.
    b. Silicates, silic acid and silica colloids or gels are not destroyed, thus the concentration of analytes possibly sequestrated by these structures (Al, Fe, Cr, Ti, Ni, V, Cu and Mn) might be underestimated.

7. **Quality assurance**:
    a. All the operations must be standardized including vessel and funnel rinsing for quantitative sample transfer and filtration.

8. **Comments:**
    a. the Author worked with an open-vessel microwave apparatus under temperature control. As it was confirmed the better performance of closed-vessel systems, these should be used and a suitable protocol should be defined according to manufacturer's instruction. Although generic, the steps here listed are then to be verified before application.
    b. From our experimental studies it seemed not necessary to employ hydrofluoric acid to destroy silicate structures. However, a comparative study could be undertaken to check whether this statement is true with the specific digestion apparatus and program used.

G. DETERMINATION OF ELEMENT CONCENTRATIONS BY ICP-AES

1. **Scope**: ICP-AES analysis is aimed at the chemical characterization of the PM deposited on the surface of conifer needles.

2. **Basic principle**: ICP-AES provides multi-element analysis by exciting sample elements with a high-temperature argon plasma so that optical radiation is emitted at element-specific spectral lines.

3. **Apparatus and reagents**: ICP-AES system, standard solutions, certified reference material.

4. **Procedure**:
    a. Ignite the plasma and follow manufacturer's recommendation for torch alignment and instrument initialization
    b. Allow the instrument to become thermally stable[20]

---

[20] This time is perfect to prepare the spreadsheets for calculation of element concentration and uncertainties and for determination of the accuracy of the measurement by comparison with the certified reference material. Having them ready before the analysis allows the immediate evaluation of the reagent blank and of the accuracy of the measurement avoiding to waste the samples in case there is a contamination problem or reduced analytical performance.

c. Select the elements and wavelengths of interest (see 5.c) preparing a suitable analytical method
d. Perform calibration using the standard solutions
e. Analyze the reagent blank. If it seems contaminated, the problem must be solved before continuing with the analysis. In case the contamination is likely due to the flask containing the blank or to the digestion step, prepare a new reagent blank and analyze this.
f. Analyze the certified reference material (NIST 1575a). If the concentrations do not comply with the 25% accuracy criterion, try to identify the problem before continuing with the analysis of the samples.
g. Analyze the samples
h. Dilute the samples whose concentrations exceed the maximum instrument detection limit and reanalyze them.

5. **Remarks**:
   a. boron and silicon are not measured if quartz vessel are used for acid digestion. In any case, the measurement of these elements might be biased by the interaction with the quartz of the plasma torch;
   b. nitrogen is not measured if nitric acid is used for sample digestion;
   c. if the calibration of the instrument is good for the lines listed in Table 3.7-1, these are recommended for the analysis of conifer needles. If the calibration is better for other lines refer to those for the calculation of element concentration.

6. **Data analysis and interpretation**:
   a. Calculate the limit of detection (LOD) as the mean reagent blank concentration plus 3 times its standard deviation and the limit of quantification (LOQ) as the mean reagent blank concentration plus 10 times its standard deviation
   b. Calculate element concentrations with reference to 105°C dry sample mass with the following formula:

$$C[ppm] = \frac{\left((conc_{ICP-AES}) \times V\right) - Blank}{m_{digested}(1 - \frac{m_{fresh} - m_{dry105°C}}{m_{fresh}})}$$

and of the diluted samples with the following formula

$$C[ppm\ d.m.] = \frac{\left(\left(conc_{ICP-AES} \times \frac{V_{flask}}{V_{pipette}}\right) \times V\right) - Blank}{m_{digested}(1 - \frac{m_{fresh} - m_{dry105°C}}{m_{fresh}})}$$

where $conc_{ICP-AES}$ is the average of 3 measurements in mg/l provided by the ICP-AES software for a certain element that it is above the instrument detection limit (IDL); V is the final extraction volume in liters (0.1 l); $V_{pipette}$ is the ml of the original sample solution transferred to a V_{flask} ml volumetric flask for dilution; *blank* is the average concentration of that element in the reagent blank sample (see explanation of this sample in quality of the single datum) in mg/l; $m_{digested}$ is the mass digested, m_{fresh} is the weight of the fresh needles used for the determination of dry weight at 105°C and $m_{dry105°C}$ is the weight of the same needles after oven drying at 105°C for hours.

c. Subtract the concentration of each element measured in the washed subsample from that measured in the unwashed sample to obtain the atmospheric concentration and calculate its uncertainty through error propagation (sum of the uncertainties)
d. Verify the validity of the selected background location by comparing its total element concentrations in current year needles and needles from one year go with the concentrations published in forest health studies for the same species and age cohort (e.g. Jones *et al.* 1991; König *et al.* 2010). If the location is suitable calculate a maximum pollution threshold as the upper limit of the atmospheric concentration at the background location plus three times its standard deviation.
e. Check which samples have atmospheric concentrations above the threshold calculated in 6d
f. Calculate enrichment factors with respect to the background location as the ratio of the concentration at the site and the concentration at the background for both total and atmospheric concentration
g. If soil data are available calculate the enrichment factors with respect to soil concentrations as the ratio of the concentration in the unwashed needles and the concentration in the soil
h. If the number of samples is three times the number of the elements of interest, in any case never less than the same number, perform principal component analysis on the data set and identify whether there are correlations among elements suggesting a common source in the study area

Table 3.7-1 ICP-AES lines recommended for conifer needles (EPA method IO-3.4; Kalra 1998; Rautio *et al.* 2010; Stefan *et al.* 2007)

ELEMENT	ICP-AES analytical wavelengths (nm)
P	213.618
Ca	317.933 or 318.127 (ICP-AES is a very sensitive Ca determination method so it is better to use a lower sensitively wavelength). At 317.933 only comparatively high concentration of Cr and Fe interfere
Mg	285.213 (ICP-AES is a very sensitive Mg determination method, so it is better to use a lower sensitively wavelength for the determination) At 285.213 only very high concentration of Cr and Fe interfere
K	766.491 (the detection limit is better but there is a Mg line nearby poorly resolved) or 769.867
Na	588.995 the wing of nearby Ar line causes the background to slope or 589.592 there is overlap with a Fe line
Zn	213.857 best detection limit but structured background due to NO band emission. Also Ni and Cu in higher concentration can interfere 206.19
Mn	257.610 or 260.568
Fe	238.204
Cu	327.395 or 324.754 (at this structured background due to OH band emission) At 224.700 background due to NO band emission and wing overlap with Fe and Pb
Pb	220.253 ICP-AES sometimes too high detection limits
Al	167.081 interfere with Fe and P 308.22 396.152 background cause by Ca (background correction recommended)
Cd	214.439 226.50
Ba	493.41
Ce	446.02
Co	228.62 238.9
Cr	357.87 283.6
La	379.48
Li	670.78
Ni	231.60
Pd	363.47
Pt	265.95
Rh	343.49
Sb	206.84
V	292.40
Zr	339.20

7. **Quality assurance**:
    a. Evaluate accuracy checking whether the concentrations measured on the certified reference material (NIST 1575a) comply with the 25% accuracy criterion[21]
    b. Evaluate precision calculating the relative standard deviation (RSD) of measurements conducted on replicate samples
    c. Evaluate the quality of the analytical method measuring the concentration of selected samples with an independent technique such as atomic absorption spectroscopy (AAS), instrumental neutron activation analysis (INAA), inductively coupled plasma atomic mass spectrometry (ICP-MS) or x-ray fluorescence (XRF).

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[21] Each concentration must be within ±25% of the certified concentration with a probability of 0.95 for an individual observation.

8. **Comments**
   a. ICP-AES analysis alone provides a very rough source apportionment (natural vs anthropogenic elements) and is not even able to assess whether ambiguous elements such as Fe are of natural or anthropogenic origin
   b. In the literature many use EF with respect to element concentrations in the upper crust. Refer to the paper by Reimann and De Caritat (2000) to understand why this approach is not recommended
   c. Evaluate: the precision of only the measurement looking at the RSD provided by the instrument; of the sample preparation and the measurement together analyzing aliquots of the same sample independently prepared; of sampling, sample preparation and analysis measuring the concentration on samples of the same type (site and age of the needle) kept separately from collection and independently prepared and analyzed
   d. Store the solutions in plastic bottles with screw cap
   e. If the concentrations of certain elements exceed expected values for the species possible sources of contamination must be considered, such as glassware, reagents, filter paper and dust from the laboratory atmosphere (König *et al.* 2010; Mitra 2003; Stoeppler 1997; Twyman 2005).

H. SINGLE PARTICLE ANALYSIS BY SEM-EDXS

1. **Scope**: SEM-EDXS is aimed at source apportionment based on the morphological and chemical characterization of PM deposited on the surface of conifer needles.

2. **Basic principle**: particles rich in heavy metals look bright in BSE, thus they are easily identified and characterized for their morphology. The EDX microprobe can be employed to discriminate particles of different origins based on their chemical composition.

3. **Apparatus and reagents**: filtration apparatus (flask and Büchner funnel), nylon filter (30 µm porosity), plastic Petri capsules, optical microscope, glass slide, scalpel, mask cut from an acetate foil long as the diameter of the filter with holes of 5x5 mm^2 cut along one radius (the internal square hole must be in the centre of the filter), marker, aluminum stubs or cylinders/disks, double-sided carbon tape, either a high vacuum system or a variable pressure scanning electron microscope, EDXS system coupled with the microscope. If a high vacuum microscope has to be used a metal coater and the metal for coating are necessary.

4. **Safety precautions**:
   a. DCM is irritant and permeates in case of skin contact, thus gloves must be worn. It is also potentially carcinogenic and hazardous if inhaled, thus filtration must be conducted under a vented hood and filters must be left dry under the same safety device.

5. **Procedure**:
    a. Filter the residual washing solution through the 30 µm filter and place the filter to dry on a glass Petri dish under the hood
    b. When the filters are dry place them back in a labelled plastic Petri dish
    c. Observe the filters at the optical microscope
    d. Cut filter squares with different particle density
    e. Attach the filter squares (DZ) on a stub with the carbon tape
    f. Observe the samples in backscattered electron mode with high contrast to brightness ratio so that particle contours are well defined (the same ratio should be maintained while collecting different micrographs)
    g. Acquire 9 micrographs and relative EDX spectra in whole field mode starting from the top left corner of the square and moving with a relative stage coordinate system of 1 mm on the x or y direction to acquire the new image following the scheme in Figure 3.7.3; record in which field of view there are bright particles

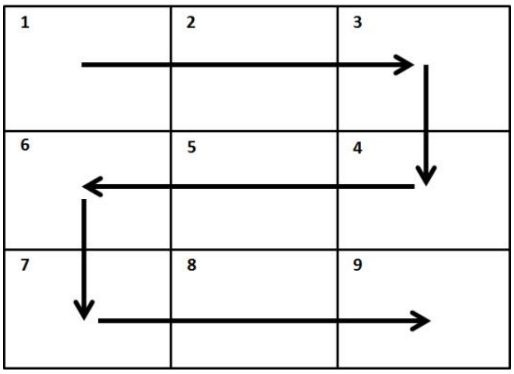

Figure 3.7.3 Scheme of SEM micrograph acquisition on each DZ

    h. Acquire the EDX spectrum in spot mode and micrographs at higher resolution of the bright particles previously observed in each field

6. **Remarks**:
    a. As soon as the filters are dried they must be placed in their plastic Petri dish as dried they tend to fly in the vented hood
    b. Each micrograph should be acquired twice one with and one without the databar: the first micrograph is used to calibrate the images based on the microscope scalebar, while the latter one will be used for thresholding within scientific image analysis (the white databar is taken otherwise as maximum brightness)

7. **Data analysis and interpretation**:
    a. Process the micrographs with a scientific image analysis software[22]
    b. Estimate the aerodynamic diameter ($D_{\mathrm{aerodyanamic}}$) of the particles containing heavy metals from the projected area diameter ($D_{\mathrm{projected\ area}}$) (Ott *et al.* 2008b):

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[22] Among the open access softwares we recommend here Fiji (Schindelin *et al.* 2012) over the original ImageJ as the "Trainable Weka Segmentation" plugin allows the definition of pixel of interest and not to train the software in performing image segmentation by selecting only the particles with the desirable brightness.

$$D_{aerodynamic} = D_{projected\ area} \times \frac{1}{S_V} \times \sqrt{\frac{\rho_{particle}}{S_D \times \rho_0}}$$

$$= D_{projected\ area} \times circularity_{ImageJ} \times \sqrt{\frac{\rho_{particle}}{S_D \times \rho_0}}$$

where the area of the particle, or *projected area*, is obtained through an image analysis software, S_V is the volumetric shape factor and S_D is the aerodynamic shape factor. Since S_V is defined as 1 divided by the circularity, $1/S_V$ is exactly the circularity provided by the ImageJ software (Rasband 1997-2012). This is calculated as:

$$circularity_{ImageJ} = 4\pi \frac{area}{perimeter^2}$$

Hence, S_V is equal to 1 if the particle is spherical. S_D is sometimes assigned a value of 1.4 that was proposed by Davies in 1979 for a dust made of quartz, sand, talc, anthracite coal and bituminous coal. The density of the particle ($\rho_{particle}$) is sometimes assumed to be equal to 2 g/cm^3 considering an average density between crustal materials and anthropogenic material (Wagner and Macher 2003 reported in Ott *et al.* 2008b). Alternatively, it could be calculated from a weighted average of the densities of the oxides composing the particle, based on the amounts measured by EDXS in spot mode (Ault *et al.* 2012).

8. **Quality assurance**:
   a. Avoid filter contamination by moving them with plastic tweezers

9. **Comments**
   a. By recording 9 micrographs per DZ and cutting 4 DZ per filter, 36 fields of view can be acquired, meeting the suggestion by Millipore corporation (1998) (min 26 fields at a given magnification). Zamengo *et al.* (2009) acquired more micrographs (48 micrographs for each filter – 12 micrograhs/DZ).

I. SINGLE PARTICLE ANALYSIS BY TEM-EDXS

1. **Scope**: TEM-EDXS-SAED is aimed at the morphological, chemical and crystallographic characterization of ultrafine PM deposited on the surface of conifer needles.

2. **Basic principle**: the high resolution of the TEM can be used to characterize ultrafine and nanoparticles. The EDX microprobe can be employed to discriminate particles of different origins based on their chemical composition, while SAED technique can be employed to acquire crystallographic data

3. **Apparatus and reagents**: ultrasound bath, cellulose filter, plastic Petri dish, pipette, Au-mesh holey carbon TEM grids

4. **Safety precautions**:

   a. DCM is irritant and permeates in case of skin contact, thus gloves must be worn. It is also potentially carcinogenic and hazardous if inhaled, thus sample preparation and eventual solvent evaporation must be conducted under a vented hood.

5. **Procedure**:
   a. Place the glass vial containing an aliquot of the washing solution obtained in B5 in the ultrasound bath for a few minutes
   b. Place a TEM grid on a filter in a labeled plastic Petri dish
   c. Place a drop (100 µl) of the solution on the grid
   d. Turn on the microscope
   e. Place the grid in the sample holder and insert the sample holder in the column
   f. Collect micrographs, EDX spectra and SAED patterns of a statistically significant number of particles

6. **Remarks**:
   a. If the aliquot of the washing solution came from washing less than 1.5 g of needles it is recommended before placing the vial in the ultrasound bath to evaporate part of the solvent so that particles are concentrated. In preliminary experiments it was shown that this step is necessary even for samples coming from polluted sites if 250 mg only are washed
   b. Check for contamination of the film at least an empty grid for each set to be used
   c. Use a low-background sample holder in order to avoid the presence of Zn peaks in the EDX spectrum not due to the sample

7. **Data analysis and interpretation**:
   a. Process the diffraction patterns with a software (e.g. *Process Diffraction* - Lábár 2012)

8. **Comments**
   a. In not polluted sites even particle concentration through evaporation does not allow the obtainment of a sufficient number of particles on the grid to characterize a site. However, if the same sample preparation procedure (amount of sample, type and amount of solvent, volume of the drop deposited on the grid) was undertaken for different sites a situation like this leads to conclude that there is comparatively less PM (of an unspecified type) at the location.

J. INTEGRATED DATA INTERPRETATION

1. **Scope**: source apportionment combining single particle and bulk chemical data

2. **Basic principle**: single particle analysis can associate a “face” to each element that from ICP-AES analysis is likely of atmospheric provenance

3. **Procedure**:
   a. Classify the particles analyzed by EM at each site

    b. Apportion the classes of the particles to a source through comparison with particle atlas and other literature sources
    c. Apportion the elements from bulk analysis to a source by observing in which particle class they are found by EM

4. **Data analysis and interpretation**:
    a. Compare which particle classes, thus sources, affect each site
    b. Verify, when possible, what inferred through comparison with data obtained through conventional monitoring techniques

5. **Statistics**:
    a. If the number of samples is sufficiently large, employ multivariate statistical tools to correlate bulk and single particle data

6. **Comments**
    a. The whole analytical protocol is quite time-consuming. Sampling time depends on the number of samples to be collected and the distances among the different sites. The sample preparation and analytical steps are affected by instrument availability. Interpretation time is of course related to the expertise of the operator and the availability of databases for data comparison

3.8 Future developments

Among the possible improvements to the method in 3.7, it is here suggested to:

- study the variance of element concentrations in order to calculate the minimum number of samples to be collected at each site for each age cohort which guarantees statistical reliability. This could be done by sampling from different trees in a sampling area a sufficient number of samples to build a relative frequency histogram describing the distribution of each element of interest and allowing the calculation of the minimum number of samples per year per site based on that distribution;
- improve the washing procedure:
    - test with DCM whether the “tube-technique” described in Viksna *et al.* (1999) is better than the immersion procedure used in this work;
    - optimize the current immersion procedure by establishing the number of immersions guaranteeing the best particle removal and no leaching of elements from the inside of the needles;
- evaluate the reliability of the subtraction strategy used in this work to estimate atmospheric element concentrations by co-located soil sampling and verification of the existence of a correlation between soil concentration and the concentration of the washed needles (endogenous). The article by Tsikritzis *et al.* (2002) is a reference to start developing such a strategy;
- determine the method detection limit (MDL) of ICP-AES measurements by calculating three times the standard deviation of the element concentrations obtained from 7 reagent blanks spiked with the LOD amount (measured previously on another blank). This allows to account for the noise in the measurement due to a tiny amount of the analyte;
- improve the quality control of ICP-AES analysis by checking sampling instruments for contamination. For instance, Reimann and De Caritat (2000) analyzed polyethylene gloves

and plastic bags used for sampling, while Keith (1988) suggested to analyze a sample of water (field blank) used to rinse the sampling tool after the usual intersample cleaning procedure;

- analyze composite samples made in laboratory by mixing equal aliquots of different samples collected at one site to check whether such a procedure increases the quality of the measurement (higher precision and representativeness of the site);
- evaluate the reliability of ICP-AES measurements by analyzing the same samples in random order on different days;
- explore the opportunity to utilize x-ray fluorescence (XRF) in place of ICP-AES to obtain multi-elemental bulk data on conifer needle samples. The use of XRF for the analysis of dry powdered plant materials dates back to 1977 (Hutton and Norrish 1977; Li and Fan 1995; Mudroch 1977; Norrish and Hutton 1977). XRF has the advantage with respect to ICP-AES that the analysis can be conducted directly on solid samples, thus the sample preparation step is reduced as well as the possibility of contamination. The cost, both in terms of time and of reagents used, is low for this technique. The timing involved in the analytical procedure is very important in biomonitoring-based studies, as to investigate the spatial distribution of certain pollutants it is necessary to analyse a large number of samples. Another advantage is the possibility to quantify P, S, Cl and Br, whose concentrations are difficult to measure with other techniques. On the other hand, XRF is not able to achieve the low detection limits of ICP-AES and in certain configurations has limited sensitivity for pollutants like cadmium and lead. In view of the important developments, particularly in the detection systems, that the technique is having the papers by Li and Fan (1995) and Marguí *et al.* (2005 and 2009) could be used as a basis to further XRF application in pollution monitoring through conifer needle analysis;
- develop a filtration methodology guaranteeing homogeneous distribution of the particles on the nylon membrane in order to be able to extend the SEM-EDXS results obtained on a subset of deposition zones to the whole filter surface and make quantitiative comparisons among different sites. Depending on the type of homogeneity obtained (radial or not) alternative strategies are proposed in the literature (Figure 3.8.1). The acetate mask we prepared allows the cutting of squares of the filters with 5 mm side in the same position on a 47 mm diameter membrane and in case of a homogeneous radial distribution (Figure 3.8.1 - a) it should guarantee inter-sample reproducibility of the cutting procedure, thus the possibility of comparing different samples (needle age, site)

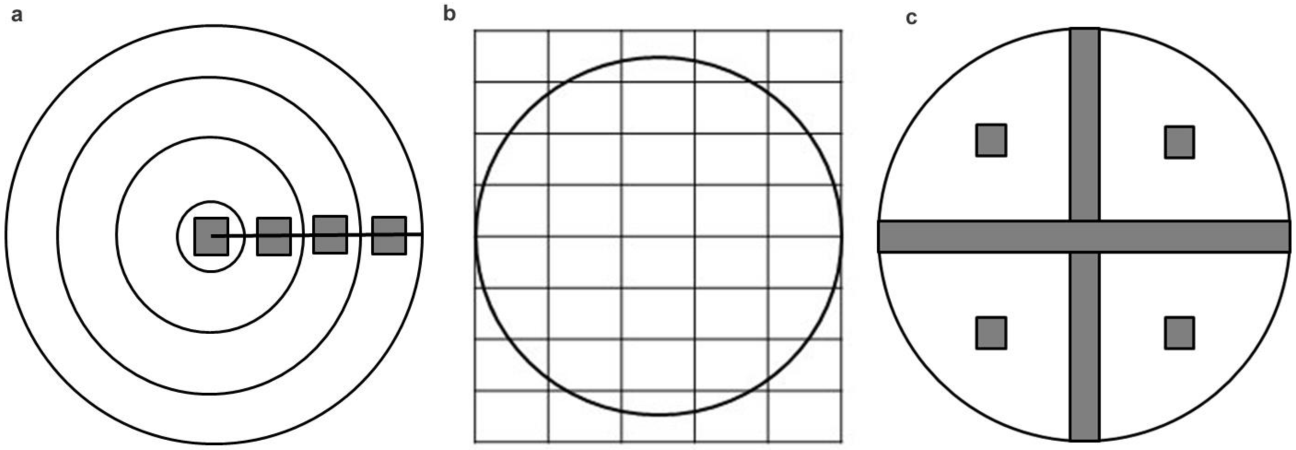

Figure 3.8.1 Different strategies of selections of areas of filters for analysis by SEM. a: If there is a homogeneous radial distribution, the filter/membrane is virtually divided into circular concentric deposition zones (DZs) by dividing in

equal parts the radius of the filter (Zamengo *et al.* 2009) b and c: if particles are homogeneously spread on the surface, random numbers can be used to select the square section of the filter defined by superimposition of a numbered acetate grid (b: Beckett *et al.* 2000) or at least twenty-six fields are analysed on perpendicular directions (left to right and top to bottom) and four additional fields randomly selected in the four quadrants created by the previously used perpendicular directions are analysed (c: Millipore Corporation 1998)

- refer element and particle concentrations to the surface of the needles analyzed to better characterize this sampler (capture efficiency) or develop a quantitative approach to compare different sites. In order to do so biometrical data on the needles are needed. We began to investigate possible strategies to obtain biometrical data on the needles acquiring SEM micrographs at low magnification (80X) which covered the whole needle surface. We analyzed the micrographs with ImageJ (Rasband 1997-2011) and calculated the total area (TA) and the fraction of needle surface where PM deposits, called total deposition area (TDA) (Zamengo *et al.* 2009). The total area of the needle (TA) measured in the preliminary study was of 21.98 mm^2, while the TDA obtained was of 0.95 mm^2. The deposition factor (DF) was calculated as the ratio between the TDA and the TA: 0.04. These numbers refer only to one side of the needle and should be extended to the whole sampling surface in order to obtain a needle deposition factor useful for comparison with other sampling surfaces. Pullman (2009) describes a method to estimate needle area from the empirical determination of a relationship between dry weight and surface area. In a preliminary study we tried to employ it but, as Figure 3.8.2 shows, it was not possible to identify a clear relationship (R^2 of 0.38).

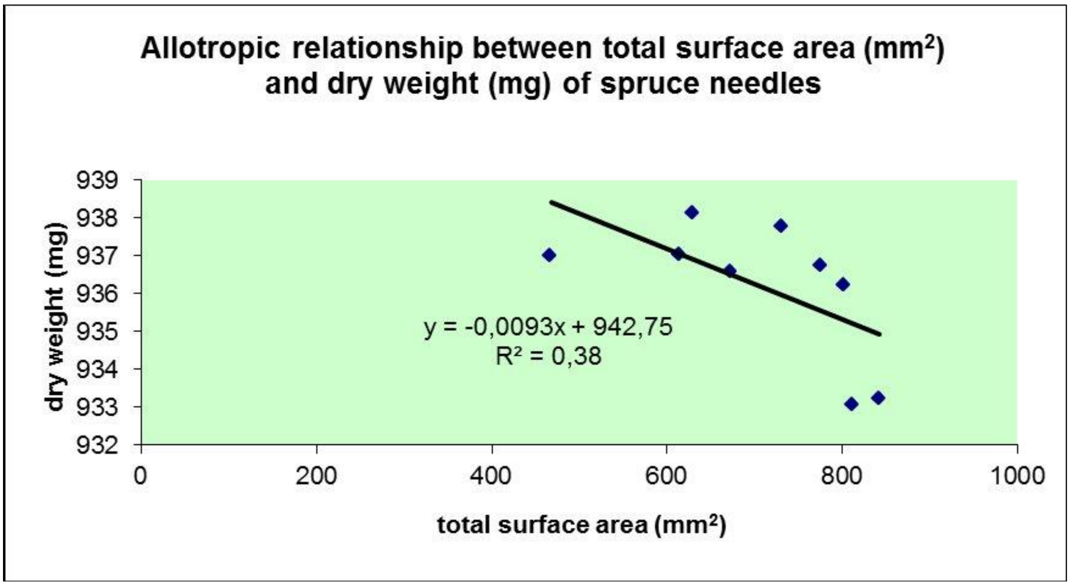

Figure 3.8.2 Experimental data to find a relationship between needle surface area and dry weight allowing the estimation of needle area without measuring it. The experimental procedure is described in Pullman (2009)

It is here recommended to use as a basis to further these studies the article by Flower-Ellis and Olsson (1993) who already criticized the tested approach as dry weight is tree-, season- and age- dependent and as using the projected area to estimate the TA may be biased by a wrong selection of the conversion factor. These Authors proposed a new method to calculate area and volume of Scots pine needles from their length. Their approach cannot be directly extended to other conifer species as their needles have a completely different section profile and different characteristics but could be a model for studies with other species

- Invest on multivariate statistical analysis techniques, such as PCA and cluster analysis, to integrate bulk and SPA results. In preliminary attempts, it was observed that as PCA evaluates sample variances it can be performed on the total element concentrations. Even if the washing treatment would still be necessary to prepare the samples for EM, by developing multivariate analytical strategies a higher number of samples could be analyzed by ICP-AES, as each sample does not necessarily need to be divided into two subsamples. This would increase the reliability of the obtained data.

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4 Case study: Source apportionment near a steelworks: analysis of PM collected on special passive samplers (district of buildings and spruce needles) in the industrial Alpine town of Borgo Valsugana (Trento, Italy)

> *"Both fugitive emissions (industrial) and fugitive dust emissions […] are difficult to quantify […] Both problems will generally require the use of non-conventional air pollution control measures."*
>
> Lillis and Young (1975)

4.1 Purpose of the study

This study was conducted as part of the integrated environmental characterization of the area of Borgo Valsugana (Trento, Italy). The site was intensely monitored to evaluate the environmental incidence of the local steelworks, with special attention to the possible exposure of the population to contaminants (www.appa.provincia.tn.it/news/pagina199.html). The steelworks is located in an industrial and commercial area at the North periphery of Borgo Valsugana (Figure 4.1.1) which has about 6850 inhabitants and it is located in the floor of a valley at an altitude of less than 400 m a.s.l..

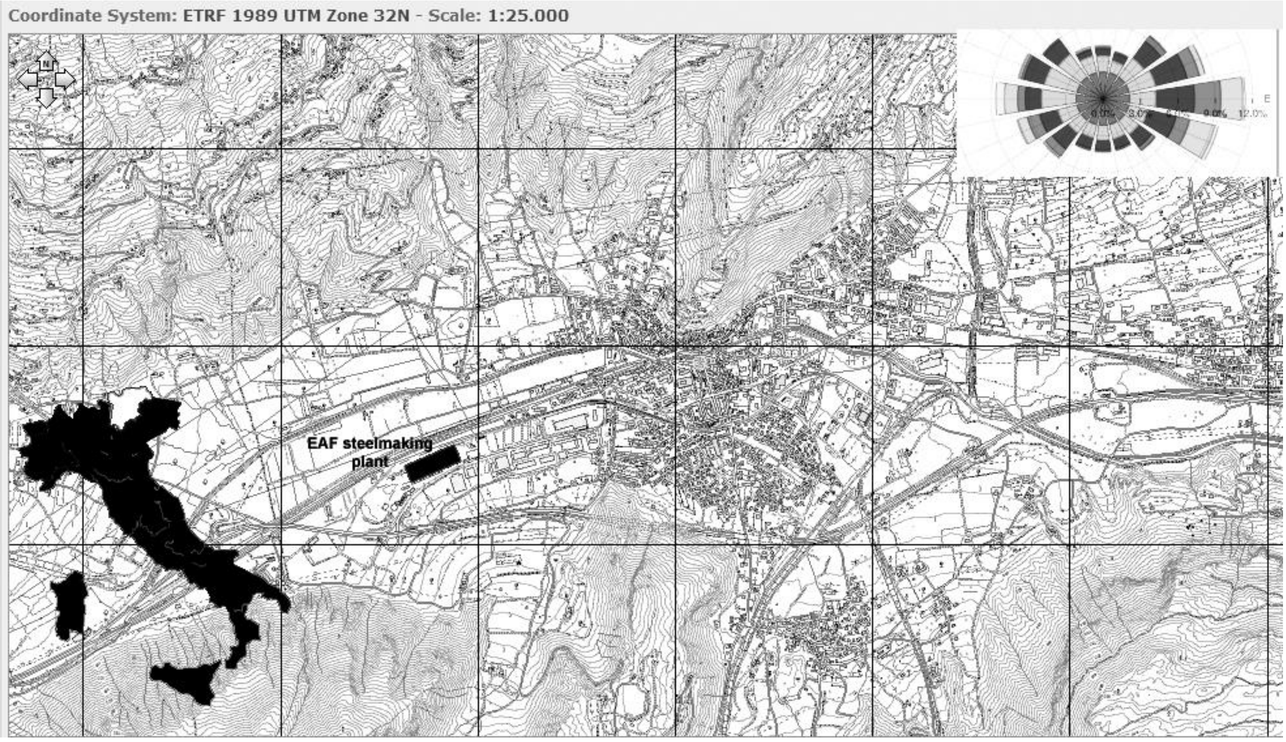

Figure 4.1.1 Map of the study area (1:25000) with location of the point emission source monitored: EAF steelmaking plant (edited from Carta Tecnica Provinciale). On the left location in Italy of the broader Trentino region, in which the site under investigation is located. On the right wind rose relative to the years 2008-2010 draw from data collected by the meteorological station better describing the meteorological conditions near the plant. The main wind direction is East-West, similarly to the main orientation of the valley.

The emissions of steelmaking plants depend on the technology used, on the raw materials employed, on the production cycle and on the off-gas abatement system installed. Because of the variability of these parameters the conclusions obtained in one context cannot be extended to the whole industrial sector and specific studies on the impact of single facilities are required.

The plant under investigation utilizes the electric arc furnace (EAF) technology to produce steel using ferrous scraps and cast iron (sheet metal, scrap from industrial demolition, scrap from structural work demolition, sheared heavy scrap, turnings) as raw materials. In particular, the following iron alloys are used: Fe-Si-Mn, Fe-Mn, Fe-Si and Fe-Cr. The plant begun its activity in 1979. In 1995-1996 the plant switched to a night time (10 pm to 6 am) and weekend production, while from 2011 numerous shut down have followed a judicial attachment due to exceedance of regulatory limits (2009) and a change in the ownership of the plant. Many improvements in the off-gas abatement system were undertaken in the years and the purpose of this study is to contribute to the evaluation of their efficiency. In other words, this study wants to support the reconstruction of the dispersion of pollutants emitted by the steelworks in order to assess whether inorganic pollutant concentrations were higher in the past and/or were affecting a larger area. The evaluation of the improvements does not regard process emissions alone, as their eventual reduction does not exclude risks for environment and human health due to the fugitive emissions which are much more difficult to quantify. Since the plant has been operating for many years and in the beginning the dust abated were discharged in a through near the plant, the soils surrounding it may be contaminated and wind blowing and trucks leaving the plant may cause the spread of the toxic components (WHO 2006). Moreover, the shop presents many openings which favour the dispersion of secondary and fugitive emissions to the outdoor environment. Therefore, besides the evaluation of the impact of stack emissions on local air quality, it is important to estimate the contribution of resuspension of metal-containing soils and wind blowing of raw materials from exposed stockpiles, unloading and stocking of raw materials, EAF and ladle/refining furnace slags and dust collected from the abatement system, demolition of refractory materials of the ladles which is accomplished outdoor.

Particular attention will be given to lead and cadmium listed as priority metals in the Italian emission inventory and to all possible tracers of the steel production process with an EAF furnace (iron, manganese, zinc, chromium, nickel) (see appendix C and D).

The study is also aimed at the collection of data from documentary sources and other proxies that can support the interpretation of the analytical results and the reconstruction of the past pollution scenario.

The data presented in this chapter were collected from various sources by the Department of Civil, Environmental and Mechanical Engineering and by the Author herself in local libraries and online databases, such as that of the local environmental Agency (www.appa.provincia.tn.it/news/pagina199.html). Other sources of information are listed in the references at the end of the chapter.

4.2 Study design

> *"[…] that which cannot be reliably sampled is seldom*
> *worth the care and expense of the analyses."*
> Michael J. Barcelona (Keith 1988)

The study design must allow the collection of information highlighting the temporal and spatial variation in the deposition of pollutants in the territory resulting from the improvements in the off-gas abatement system. This means that for the success of the study it is necessary to know the plant, in particular its characteristic emission of inorganic pollutants, the concentration and the dipersion of pollutants at present time and the concentration and the dispersion of pollutants in the past, before the implementation of the improvements in the off-gas abatement system.

To study the emissions of the plant and their current dispersion active samplers are the best instrument for sample collection. They are able to monitor short-term variations in pollutant concentrations, thereby to isolate the steelmaking emissions from those of other sources: it is possible to collect samples and compare what happens when the plant is not operating – day or periods of shut down – compared to when it is operating – night and weekends. This is very important as by working at night there is an increased possibility to cause adverse health effects on the population as the wind blows towards the urban area.

To study how large is the area of impact of the plant it is necessary to cover a wide territory as industrial activities may affect areas of 20 km^2 (Machemer 2004). This could be very expensive with instruments requiring power, such as active samplers. Moreover, the steelworks is located in the floor of a valley and monitoring its effect means reaching also mountaineous area. If the present situation was the only concern of the work, instrumental passive samplers of particulate matter might have been a good sampling strategy. However, to evaluate the efficiency of the improvements in the off-gas abatement system it was necessary to collect information on the past pollution scenario when no monitoring stations were installed as no monitoring activity was undergoing. This led to decide to use two types of “unconventional” samplers of PM. The suitability of conifer needles to collect PM and the analytical protocols for their use were already discussed in previous chapters and in the articles by Bertolotti and Gialanella (2014) and Bertolotti *et al.* (2014). These samplers allow the obtainment of information on recent past contamination as generally no needles older than a decade are available depending on the species used. In the case of this project, however, many improvements in the off-gas abatement system were undertaken in remote past and it would not be possible to study their eventual effect with conifer needles. Hence, it was decided to try to exploit building facades to extend the temporal range of investigation (Figure 4.2.1).

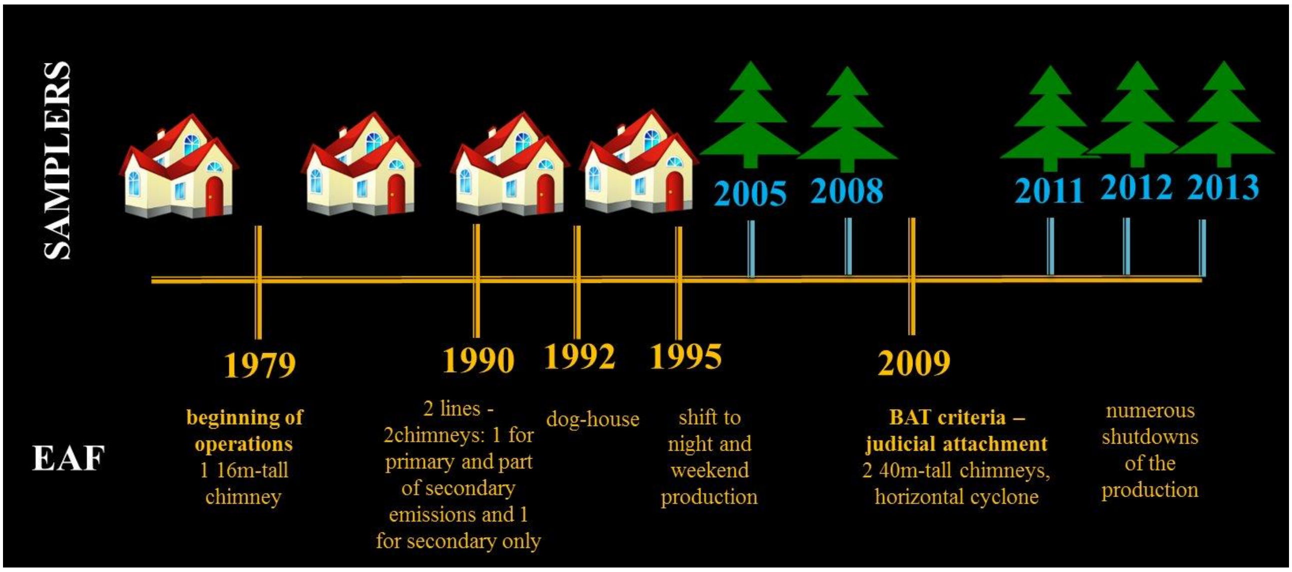

Figure 4.2.1 The study of the dispersion of pollutants at different time scales requires different “unconventional” passive samplers.

The use of building facades and monuments as passive samplers of pollutants was suggested already by Alves and Sanjurio-Sánchez (2011), Rampazzi *et al.* (2011) and Monna *et al.* (2008). Previously, many studies proved the correspondence between atmospheric pollutants and pollutants deposited on exposed surfaces (Derbez and Lefèvre 1996; Lefèvre 1995; Montana *et al.* 2008; Nord *et al.* 1994; Orecchio 2010). Compared to conifer needles the use of buildings hardly allows the association of the deposits to a specific time interval, since it is rare to find a stratigraphy with known chronology (e.g. paint coatings). An attempt in facing this issue and restrict the time period to which materials deposited on the buildings refer is that of sampling in the same location conifer needles so that at least there is a chance to verify that what is found on the building was not present in recent deposition on the needles.

The sampling program was designed in order to fulfill the minimum requirements suggested by the American Chemical Society Committee on Environmental Improvement which include:

- a statistical design selected on the basis of the goal of the project
- instructions for sample collection, labeling, storage and transport to the laboratory.

In the design of the sampling strategy a series of considerations directed the Author to rely on geostatistical sampling principles rather than on classical random sampling. First, sampling buildings and conifers requires to have them in the site identified for sampling. If the sites were selected on the basis of random numbers it could have often be the case of not having what had to be sampled in the location, or having it in not accessible places such as private properties (Keith 1988). Moreover, since source-oriented models were available for many pollutants, there were already suggestions on likely spatial differences due to the different impact of the source on the territory.

Information on the uncertainty introduced by sampling were given in chapter 3.

The map in Figure 4.2.2 shows the sampling points for both spruce needles (black) and buildings (red). More details on site selection and standard operating procedure are given in the following subparagraphs.

The site named PS for the spruce needles and SC for the buildings is considered as a local background location from dispersion models and organic micro-pollutant characterization (www.appa.provincia.tn.it/news/pagina199.html). Having a local background site is of paramount importance as this is exposed to similar microclimatic conditions. Locations such as those selected by Iobstraibizer *et al.* (2014) in other valleys or at different altitudes were avoided considering the specificity of meteorological phenomena in mountainous valleys (Giovannini *et al.* 2011; Gohm *et al.* 2009; Schnitzhofer *et al.* 2009).

Besides the gradient study approach, that is a comparative study among the different sites on the territory with respect to the influence of the EAF plant on the air quality, an attempt was made to provide an evaluation of each location itself by comparison with the background site and with the literature.

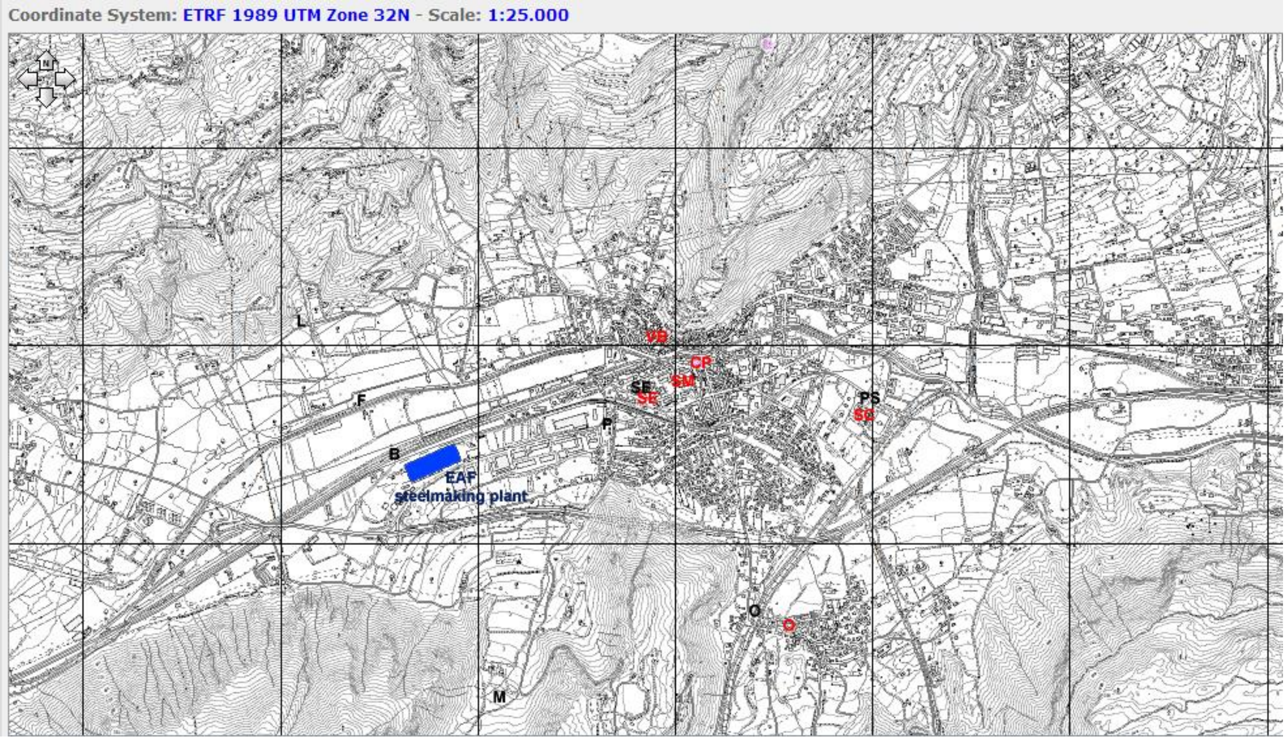

Figure 4.2.2 Map of the study area (1:25000) with location of the point emission source monitored: EAF steelmaking plant in blue, sites where spruce needles were sampled in black (L, F, B, M, P, SE, O, PS) and sites were samples from building were collected in red (SE, SM, CP, VB, SC, O) (edited from Carta Tecnica Provinciale).

4.2.1 Buildings

Sampling

For the purpose of the study it would have been better sampling private buildings, on average more ancient and less maintained than public ones. However, involving private owners to have the authorization to sample from their buildings would have increased the level of social alert on the topic of air pollution which has been for years a sensitive one and had triggered controversial feelings towards the presence of the steelworks. From the one hand, the steelworks is part of the industrial activities constituting the backbone of the economy of the town since the XIX century and especially from the 1960s (Fontana 2005). On the other hand, the steelworks has always been seen as a threat to air quality and many inhabitants of Borgo Valsugana associate it with unpleasant odours and fumes.

Hence, it was decided to use only public buildings as sampling points nonetheless not all interesting locations with respect to the point source and to the pollution dispersion models were considered. That is why using conifer needles might be in similar situations a preferable strategy, although it does not provide information on periods as old as on those potentially provided by the use of building facades. A sample (VB) was collected from a private building in the historical centre of Borgo Valsugana. The sample was already detached and available on the soil without requiring any authorization for its collection. It is hoped that if the methodology developed in this study results useful for the purpose, more private buildings will be sampled. Some of them and their accessories, such as stone portals, were described already by Fabris (2009) as blackened by street smog.

The buildings sampled in this study are listed in Table 4.2-1 together with information on the latest intervention on the facades. Within the described constraint of avoiding private structures, the buildings were selected in order to be representative of different periods of exposure, different locations in the urban district (urban, suburban, background) and different visible decay patterns.

Before sampling the conditions of all the facades of the buildings were recorded in a written report and by photographic documentation. Photographs were often acquired with metric scale and colorimetric reference bar. Even though the buildings sampled are not of cultural heritage concern, this stage of visual assessment was conducted according to the standard reference EN16096:2012 "Conservation of cultural property – Condition survey and report of built cultural heritage" and included recording information about local environmental conditions and use of adjacent buildings. This standard was issued in 2012 by the technical committee 346 of the European Committee for Standardization (CEN) that is concerned with the conservation of cultural heritage. However, since this work is not finalized to the conservation of the buildings but to the obtainment of information on the air quality to which they are exposed, no risk assessment and recommendations were produced.

The recommendations given in the standard reference EN16085:2012 "*Conservation of Cultural property – Methodology for sampling from materials of cultural property – General rules*" were used as guidelines for practical operations in the field.

From all the buildings the smallest necessary samples were collected exploiting, when possible, the morphology of decay, such as already detached fragments. A clean scalpel and tweezers were used to collect the samples. All samples were collected without the aid of any ladder, thus sampling height was never higher than 2 m above the ground. Whenever possible, samples were collected from both vertical and horizontal surfaces including as many variables as possible: surfaces exposed to rainwash and sheltered, different heights from the ground (different contribution of dust resuspension), different roughness of the surface and material, different architectonic elements, different orientations and exposure to sun and wind. A sampling data sheet was filled for every building reporting the number of samples taken, the location of the sampling point, the type of decay observed. Each sample was placed in a separate sealed labelled plastic bag.

Analysis

Source apportionment of metals and particles detected on building samples on the basis of bulk analysis is even harder than with conifer needles. The integration time of the pollutants to the surface is not known and even measuring isotopic ratios gives only rough indication on the anthropogenic versus natural source of certain elements (Monna *et al.* 2008). Moreover, most of the buildings sampled were painted which likely means that metals are also part of the substrate.

Hence, it was decided that only SEM-EDXS would have been employed to characterize PM deposited on the surface of the facades of buildings.

Powder samples were observed after being spread on a double-sided carbon tape attached on a SEM stub. Fragment samples were cut into two parts. One was used for the observation of the surface, while the other was embedded in epoxy resin for the study of the cross section.

Table 4.2-1 Characteristics of the sampling sites were powder and fragments of building facades were collected for the analysis of past contamination.

Location, distance (m) and direction with respect to the steelworks	Historical and current use of the building	Conservation state	Area type, use of adjacent buildings and possible pollution sources	Latest intervention on the facades	Sample ID
Town: Via IV Novembre, 1360 m E-NE	Elementary school “Vittorino da Feltre”	optimum	Urban area nearby a kindergarden, railway station, residential buildings, intense vehicular traffic on the road in front of the south side of the building	1994	SE
Town: Via Spagolla, 1490 m E-NE	Middle school “Ora e Veglia”	optimum	Urban area nearby a kindergarden and residential buildings. Vehicular traffic is a possible source as car can both pass and park on the street.	1992	SM
Town: Piazza De Gasperi 19, 1530 m E-NE	Previously “Fascio” building, currently police station and city hall	good	Urban area (main square) residential buildings, public restrooms	1977	CP
Town: Via Gozzer, 2370 m E	Sport centre	optimum	Suburban, rural	1992 (also year of construction)	SC
inhabited centre at the outskirts of the town: Frazione Olle, Via Molinari, 2100 m SE	Previously elementary school	good	Residential Residential buildings	1950	O
Historical Centre 1500m E-NE	Private house	mediocre	Residential buildings	unknown	VB

4.2.2 Spruce needles

Sampling

The species of conifer selected for this study is *Picea abies* (K.) or *Picea excelsa* (L.), also known as Norway spruce. This species comprises trees that may live 400-500 years characterized by needles with rhomboidal section with two or three stomatal lines on all sides inserted in cushions that remain in the branch after the fall of the needles. In Italy this species is diffuse in all the Alps, except the Maritime ones, and in two locations on the Appenine (Passo del Cerreto and Alpi delle Potenze). The maximum altitude limit on the Alps is of 2000-2200 m and the minimum depends on rainfall pattern and might varies from 300 m (in the areas with more rain) to 1000 m (in the arid areas) (Gellini 1985). Spruce is also a very important species in the subalpine zone (Günthardt-Goerg 1986). In Borgo Valsugana and Trentino Region in general these conifers are present in woodlands together with European beech (1.6% and other species (0.2%): Picea abies (62%), silver fir (12%), larch (17.5%), Scots pine (5.4%), Swiss pine (1.5%) (I.T.C. e G. "G. Gozzer" Borgo Valsugana, a. s. 95/96. c. 3A. 1995/1996). The species has a large genetic variability that determines differences in the composition of the epicuticular wax from one geographical location to another (Günthardt-Goerg 1986). Since Mn is one of the elements characteristic of the emissions from steelworks, the use of spruce is also recommended as this species is tolerant to this element and probably compartimentalize it within the needle (Wyttenbach *et al.* 1995).

A preliminary sampling campaign was undertaken to evaluate sampling design, verify the suitability of sample unit sizes for the analysis to be conducted, establish the analytical protocol as described in chapter 3 of this thesis. This sampling campaign included needles of different ages from the site closest to the plant (site B, Table 4.2-2) which was expected to have higher concentrations of particles and airborne metals (Kemppainen *et al.* 2003; Pöykiö *et al.* 2010; Rautio and Huttunen 2003). From these experiments it emerged that for each sample was sufficient to collect 3 twigs to have more than 1.5 g of fresh needles. Then, the sampling experiments were successively refined towards the obtainment of data for this case study.

It is generally recommended to sample conifer needles in autumn or winter to reduce the differences in element content due to the growth of current year needles (Jones *et al.* 1991). In the present studies some sampling campaigns were conducted in spring and early summer and the effects of seasonal changes in element concentrations were avoided by sampling the different age classes at one specific date as suggested by Wyttenbach *et al.* (1995).

The samples were collected along a transect perpendicular to the main wind direction, which corresponds to the main axis of the valley (East-West) (www.appa.provincia.tn.it/news/pagina199.html) (Figure 4.2.2). The transect consisted of five sampling sites at increasing distances from the EAF plant whose characteristics are described in Table 4.2-2. At each site, from one or more trees, needles were collected at relative different position on the branches of the same tree in order to have samples of different ages: 2005, 2008, 2011, 2012 and 2013 needles (Table 4.2-2). These sites were selected on the basis of dispersion models in order to represent different levels of pollution and include both high and low concentrations areas (VDI 2004). In addition, needles were sampled from trees in location corresponding to the building sampling campaign (samples O and PS) in order to be able to make comparisons and infer temporal data on both recent and less recent periods.

All the samples were collected at a maximum height of 2 m above the ground. When possible a sample was made of twigs collected from branches at different orientations on the tree. The twigs were cut wearing a different pair of gloves for each sample and using pruners cleaned between each sample with alcohol and deionized water. Every sample was kept in a closed labeled PET bag from the sampling site to the laboratory.

Our laboratory was also provided with a sample of spruce needles (AK) collected within the Denali National Park, Alaska, USA which was hypothesized to be relatively not contaminated[23].

Analysis

For the analytical protocol used refer to chapter 3. Unless differently stated, the needles were washed with a 1:1 (v/v) solution of Tol:THF. As a better calibration for quantification of Zn concentration by ICP-AES was obtained with the 213.856 nm line, rather than with the 206.191 nm, the first was selected for element quantification.

The percentage of crustal and of anthropogenic elements was calculated with respect to the total atmospheric ppm measured (sum of the concentrations of all the elements that provide a positive subtraction not washed-washed concentration). Two types of enrichment factors were computed:

- the enrichment factor with respect to the background location PS (EF_B):

$$\boldsymbol{EF_B = \frac{element\ concentration\ at\ the\ site}{element\ concentration\ at\ the\ background\ location}}$$

- the enrichment factor with respect to the soil (EF_S):

$$\boldsymbol{EF_S = \frac{total\ element\ concentration\ in\ the\ needles}{element\ concentration\ in\ the\ soil}}$$

While the first calculation can be undertaken both referring to the total concentration (not washed needles) and to the atmospheric concentration (not washed needles minus washed needles) given that the type of concentration used is consistent between the numerator and the denominator, EF_S can be calculated only using the total concentration data as it is an expression of what cannot be explained by soil uptake. Unfortunately, there were no available data regarding the concentration of Al, Li, Sr and Ba in the soil (www.appa.provincia.tn.it/news/pagina199.html), thus for these elements it was not possible to undertake the calculation. To state whether a site is polluted with this approach, the criteria by Mingorance *et al.* (2007) was used to evaluate the calculated EF_Bs: an EF larger than 2 indicates pollution. The results of the EF_S calculations were interpreted following the classification by Feng *et al.* (2009).

In few cases, high-vacuum SEM-EDXS was employed after coating the spruce needles with carbon.

TEM grids were prepared by placing a 100 µl drop of residual washing solution of 1.5 g needles on an Au mesh holey C TEM grid.

Electron diffraction patterns obtained by SAED were processed using the Process Diffraction software (Lábár 2012).

General analytical strategy

Table 4.2-3 summarizes the general analytical strategy. Reasons for many of the described steps derive from the analysis of the literature and the evaluation of the state of the art reported in chapter 2 of this thesis. Many of the listed operations were described in detail in chapter 3 of this thesis.

The aerodynamic diameter of a particle is estimated from electron microscopy data with the formula provided by Ott *et al.* (2008) reported in paragraph 3.7. The densities of the particles employed in the calculations were estimated from the composition measured by EDXS.

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[23] The vegetation of the Denali National Park was classified among the Western National Parks of the USA as having the third lowest concentrations of semi-volatile organic compounds (SOCs), nutrients, metals and mercury (Landers *et al.* 2008).

Table 4.2-2 Characteristics of the sampling sites were spruce needles of different age cohorts (2005, 2008, 2011, 2012) were collected for the study of recent contamination.

Sampling site ID	Direction with respect to the steelworks	Approximate distance from the steelworks (m)	Number of individual trees sampled	Context	Other evident sources of pollution	Needle age cohort
B	NW	120	1	Along the street and the cycling path that lead to Borgo passing on the side of the steelworks. On the other side of the street with respect to the steelworks.	traffic	2005 2008 2011 2012 2013
F	NW	500	1	along a little river in a rural area	agriculture	2008 2012
L	NW	1000	3	on a non-busy street in a rural area	traffic, domestic, agriculture	2008 2012
P	E	1000	5	trees on a street (road-side trees) leading to the industrial area where cars are required to slow down or stop.	traffic, industry cutting and storing aluminium and producing doors, windows and panels in aluminium, domestic heating	2008 2012
M	S-SE	1100	5	in a forestry area with few houses	none	2008 2012
O	SE	1910	1	Along a cycling path on the side of a torrent nearby a residential area at the outskirts of the town	domestic heating	2008 2012 2013
PS	E	2370		Suburban-rural area: near the sport centre of the town including an outdoor track and field stadium, an indoor gym and an indoor pool; near few houses and the fireman station	It is considered a background location for the EAF plant as no organic contaminants characteristic of steelmaking facilities from scraps were identified in previous monitoring activities	2008 2012 2013

Table 4.2-3 General analytical plan.

EXPERIMENT HYPOTHESIS	**Experimental hypothesis**	The concentration of particles emitted by the steelworks found on spruce needles and building facades is dependent on exposure time and on sampling location
	How can the hypothesis be tested	Identifying the particles emitted by the steelworks on the needles and on building facades and compare the amounts present on needles of different ages collected in different places
WHAT INFORMATION/DATA ARE NEEDED	**Resolution, type of data**	Resolution sufficient to identify particles of aerodynamic diameter < 10 µm and possibility to quantify the amount of particles of this size which is emitted from the steelworks. To single out particles emitted by the steelmaking plant it is necessary to use a SPA technique
CONTROLS DESIGN	**What controls are needed**	A bulk measurement (ICP-AES) of the concentration of metals in and on the needles to “validate” the measurements obtained by SPA
ANALYSIS PLAN	**How can the information be obtained**	1)identify the particles of interest in the BSE image (particles emitted by the EAF plant are rich in high-Z elements, thus look brighter in the micrograph) 2)acquire the EDX spectrum of these particles 3)measure other data on the particles, such as diameter and circularity, to fully characterize the emissions of the plant
	How will the statistical test work	Statistical technique to compare the data measured on different needles (e.g. PCA) and for the bulk technique to test whether there is an association with the detection of certain metals and the presence of certain particle classes
EQUIPMENT CHOICE AND SETUP	**What type of equipment is needed**	SEM-EDXS, TEM-EDXS, ImageJ software, Process Diffraction software oven, grinder, MW-assisted digestion apparatus, ICP-AES
	Process optimization: imaging equipment and sample preparation	For what concern SEM it is necessary to define reproducible strategies to select the fields of view to be analyzed and operating conditions; for ICP it is necessary to optimize sample preparation and quality of the measurements.
DATA ACQUISITION		Acquisition of BSE micrographs and EDX spectra Measurement of metal concentrations due to atmospheric deposition
IMAGE PROCESSING		Optimization of contrast and background, spatial calibration of the image, thresholding and particle analysis
MEASUREMENTS		Problem to be solved: avoid artifacts (particles not really existing) due to the substrate (the needle, the wax, the filter)
STATISTICAL ANALYSIS	**How to draw conclusions**	Comparing the obtained data

4.3 Results of the study

4.3.1 Buildings

The scope of this paragraph is summarizing the results of the study of the pollutants detected on the samples obtained from building facades.

The size of the particles analyzed apportioned to the EAF plant seems to be related with distance from the plant but not with the years of exposure of the buildings to the atmosphere. The finest particles (Feret diameter below 1 µm) apportioned to the EAF plant are present closer to the site (elementary school in Borgo, 1360 m away from the plant) and then their number increases with the distance from 1530 m away (city hall/police station in Borgo) up to the background location (sport centre in Borgo) (Figure 4.3.1). A spatial dependence of the size of EAF spherical particles was observed also by Iobstraibizer *et al.* (2014) in samples of PM collected by mosses. The Authors observed sub-micron spheres farther from the plant (up to 15 km from the plant), while close by they detected spheres of hundreds of microns. In the present study, the coarsest particles (Feret diameter above 10 µm) apportioned to the EAF plant are present only close to the source (elementary school in Borgo, 1360 m away from the plant) and at 1500 m away (historical centre, Borgo). The presence of coarse particles in the historical centre could be linked to the years of exposure of this building. Since it is private, we are not aware of the last intervention on the facades but from a visual inspection it seems likely that the surfaces have not been touched for a long time. It can be hypothesized that the presence of coarse particles witnesses a higher impact of the plant on the territory in past times.

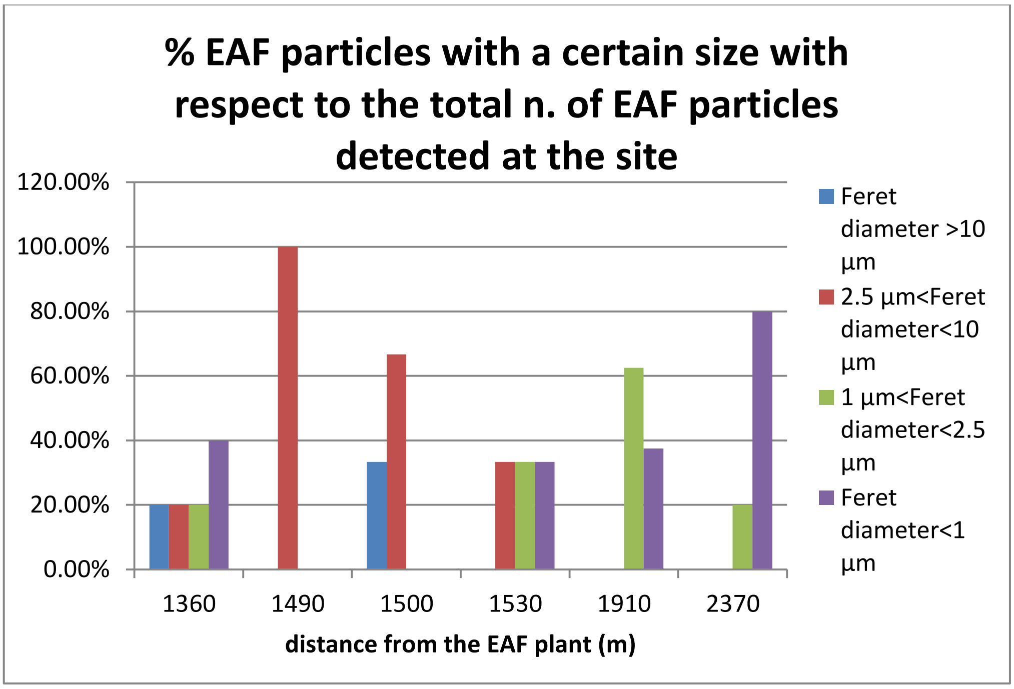

Figure 4.3.1 Percentage of particles apportioned to the EAF steelmaking plant of a certain size with respect to the total number of particles apportioned to the EAF plant at the same site. The sites are identified by their distance from the plant: 1360 m (elementary school Borgo), 1490 m (middle school Borgo), 1500 m (historical centre of Borgo Valsugana), 1530 m (city hall/police station Borgo), 1910 m (Olle), 2370 m (sport centre Borgo).

The strategy of acquiring whole field EDX spectra in areas of the plaster substrate where there were no particles showed that when a paint was present, the paint was titanium based and not zinc based. Hence, if Zn was detected in an EDX spectrum it was for sure located in the particle.

Spherical particles characteristic of EAF steelmaking plant emissions (Appendix D) were identified in higher number in fragment samples from site O (Figure 4.3.2): they contained ZnO, MnO, in addition to Fe_2O_3 (49-66 wt%). Of these oxides only Fe_2O_3 was measured by EDXS also in the substrate (yellow paint) but in much lower amounts (2.8-3.3 wt%); a further finding supporting the apportionment of the spherical particles in Olle to the EAF plant is the low amount (2-3 wt%, maximum 7 wt%) of SiO_2 and Al_2O_3 that are instead dominant in combustion spheres from other sources (appendix B).

These spheres were also detected on top of the gypsum layer in which the surface of the sample VB was altered (Figure 4.3.3).

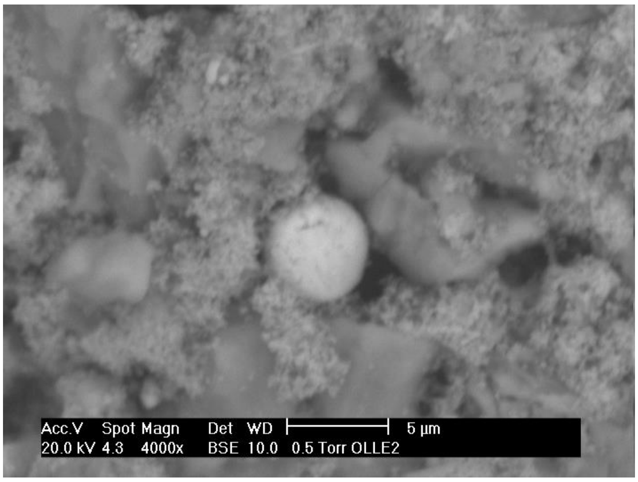

Figure 4.3.2 SEM micrograph showing a coarse spherical particle (5.6 µm) with dendritic surface texture collected on a sample from site O. The composition of the sphere measured by EDXS in spot mode is 65.8 wt% Fe_2O_3, 13.6 wt% TiO_2, 11.6 wt% CaO, 2.7 wt% SiO_2, 2.2 wt% Al_2O_3 and other elements below 1 wt%. CaO, TiO_2, SiO_2, Al_2O_3, MgO, SO_3, K_2O, Na_2O could come from the substrate whose composition was measured by EDXS in whole field mode.

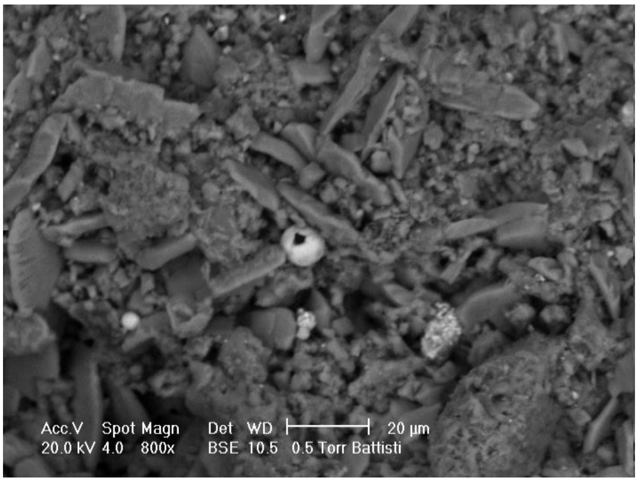

Figure 4.3.3 SEM micrograph of a spherical particle (11.0 µm ca Feret diameter) on top of the gypsum layer present on the sample at site VB. The particle is apportioned to the EAF emissions as it contains 50.7 wt% Fe_2O_3 and 14.9 wt% ZnO.

A special kind of spherical particle that could be apportioned to the steelmaking plant was detected on the sample collected at site SM. The sphere had a titaniferous composition and was apportioned to the EAF plant considering that titanium oxide is the most common modern pigment and that many scraps charged in the furnace are painted (Begonha and Sequeira Braga 1996).

Even the background location, site SC, seemed not totally unaffected by the steelmaking plant. Fine particles (0.6-2.1 µm Feret diameter) which can be apportioned to the EAF emissions were identified on a sample coming from the façade oriented towards the centre of the town and the steelmaking facility. The apportionment is based on the fact that these particles are spherical and contain ZnO (2.0-7.7 wt%) together with a certain amount of Fe_2O_3 (7.9-18.0 wt%), SiO_2 (31.8-73.6 wt%) and Al_2O_3 (4.2-8.6 wt%) (appendix D). The silicoaluminate composition is characteristic of fly ash from many sources (appendix B) but we believe that the presence of ZnO and in some particles also of a very low content of MnO and Cr_2O_3 are diagnostic features (see appendix D). Moreover, Iobstraibizer *et al.* (2014) analysed dust collected inside the plant under investigation and detected in the less magnetic fraction spherules with high Si, Ca and Al contents. The retention of these small particles in the substrate may have been favoured by the roughness and porosity of this piece of plaster (Figure 4.3.4), compared to the others analysed. The fine dimension of the particles detected at site SC (most of the particles were below 1 µm in Feret diameter) is compatible with transport to this location at 2370 m from the steelmaking plant. On the same fragment a coarse ferrosphere was detected. The sphere does not contain any element supporting an origin from the EAF plant. Moreover, being coarse (8.2 µm is the estimated aerodynamic diameter) it is not expected to have sufficient atmospheric lifetime to reach this site if emitted from the steelmaking plant. The presence of another industrial combustion source closer to the area could be hypothesized, however further investigations on the emission and transport dynamic of EAF combustion particles is required as a very coarse (31.7 µm Feret diameter) ferrosphere was detected also at site O, which is 1910 m far from the steelmaking plant.

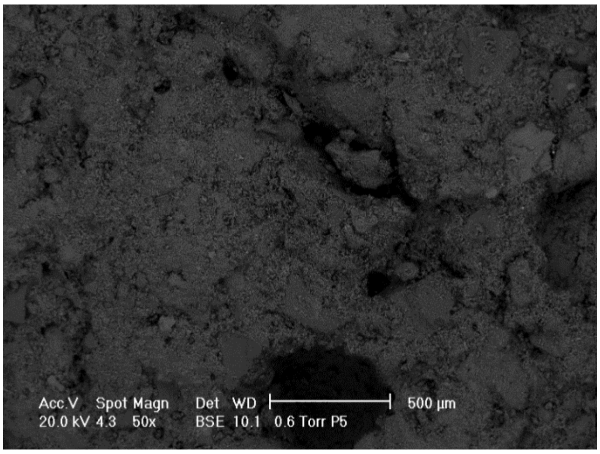

Figure 4.3.4 SEM micrograph showing the general aspect of a fragment sample collected from the South façade (back side) of the Sport Centre main building (gym) (site SC). The fragment painted in yellow has a rough, porous texture.

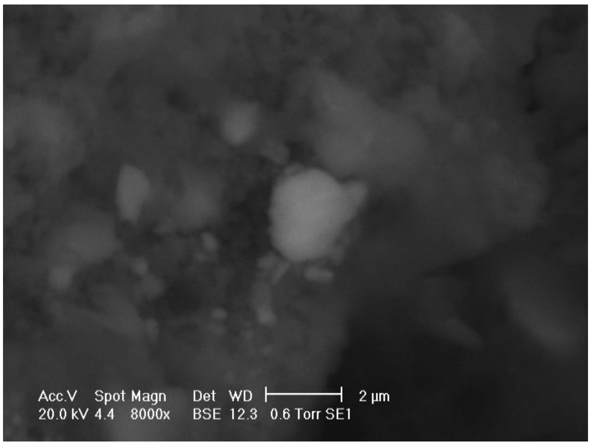

Figure 4.3.5 SEM micrograph showing an irregular particle (2.9 µm Feret diameter) that could be apportioned to the EAF steelmaking plant based on its composition. EDXS measured 39.6 wt% of Fe_2O_3 (only 4.5 wt% were detected in the background substrate by whole field measurement in a different field of view), 2.7 wt% of ZnO (absent in the substrate) and 0.7 wt% MnO (absent in the substrate).

Other particles that could be apportioned to the steelmaking plant with less certainty are those with other morphologies and containing elements characteristic of steelmaking emissions detected in all powder samples. An example is given in Figure 4.3.5.

Particles from brake and catalytic converter wear have been detected at site O where on the road in front of the façade there is a “stop” sign and all vehicles have to stop before merging into Via Molinari (Figure 4.3.6).

Figure 4.3.6 Picture showing the presence of a “stop” sign in front of the East façade of the building at site O where particles that can be apportioned to brake wear have been detected.

Particles from brake wear (Figure 4.3.7) were characterized by the presence either of Ba and S or of Si and Zr which are respectively due to the fillers of brake linings, commonly containing $BaSO_4$, and to the abrasive component sometimes made of zirconium silicate ($ZrSiO_4$) (Kukutschová *et al.*

2010, Kukutschová *et al.* 2011; Thorpe and Harrison 2008). For instance, the particle in Figure 4.3.7 has the following composition: 64.8 wt% SiO_2, 15.5 wt% TiO_2, 7.1 wt% CaO, 5.2 wt% ZrO_2, 2.1 wt% Al_2O_3, 1.7 wt% Fe_2O_3, 1.0 wt% SO_3, 0.6 wt% K_2O. A little bit of Si signal can come from the substrate as well as all Ti, Ca, Al, Fe, S and K as an EDXS spectrum measured in whole field mode detected 37.4 wt% SiO_2, 28.3 TiO_2, 22.3 CaO, 3.2 wt% Al_2O_3, 2.9 wt% Fe_2O_3, 2.4 wt% MgO, 1.5 wt% SO_3, 1.1 wt% K_2O, 0.6 wt% Na_2O.

Catalytic converter wear particles detected at site O were characterized by the presence of cerium that in its oxide form (ceria CeO) is used as an additive in the washcoat of the converter (Figure 4.3.8). Because of the presence of these traffic-related particles, others made of stainless steel or containing Fe_2O_3 also detected at the site may be related to wear of vehicles, but no certain apportionment can be done.

Looking at the external layers of two cross-sections of fragment samples collected at site SE particles containing Pt and Ce were detected. In this case the apportionment to the wear of catalytic converters is not convincing since the facades from which the samples were collected are not exposed to streets with road traffic nor other particles from vehicle wear have been identified. On one of the façades particles containing Pb were detected in the cross-section (www.appa.provincia.tn.it/binary/pat_appa/news/9_Contributo_report_DIMTI.1373474308.1374071392.pdf). This may support the hypothesis of a traffic contribution, if the Pb particles could be related to past pollution from leaded gasoline[24]. However, this again cannot be stated with certainty.

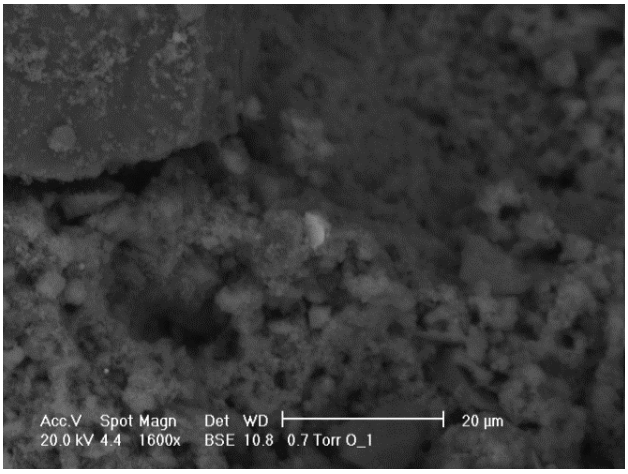

Figure 4.3.7 SEM micrograph showing a particle (4.9 µm Feret diameter) with composition measured by EDXS containing 64.8 wt% SiO_2 (partially from the substrate) and 5.2 wt% ZrO_2.

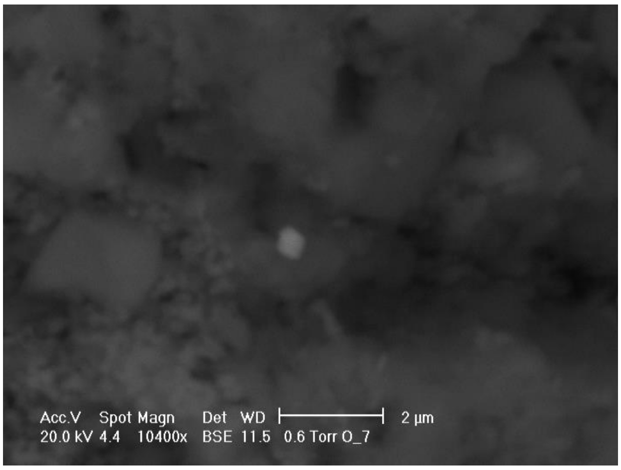

Figure 4.3.8 SEM micrograph showing a fine particle (0.7 µm Feret diameter) containing Ce.

The different type of building at site VB is reflected in a very different sample. The fragment collected passing in a street of the historical center, which was already detached from a stone portal, had the following general surface composition: SO_3 36.6 wt%, CaO 35.7 wt%, SiO_2 12.1 wt%, Al_2O_3 4.4 wt%, MgO 3.6 wt%, Fe_2O_3 3.6 wt%, K_2O 1.9 wt%. The composition as well as the morphology made of rose-like aggregates of gypsum lamellae (Lazzarini and Laurenzi Tabasso 1986) (Figure 4.3.9) highlights that the stone constituting the substrate has been altered in gypsum. This was confirmed by the observation and EDXS analysis of the layers visible in the cross-section (Figure 4.3.10 and Figure 4.3.11).

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[24] In the European Union leaded gasoline was banned in 2000, thus this building, not restored after 1994, may have collected some particles due to previous regime.

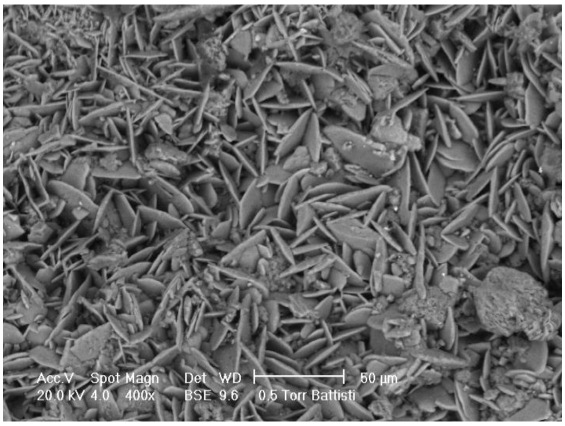


Figure 4.3.9 SEM micrograph showing the general aspect of the surface of the fragment sample collected in VB covered with gypsum.

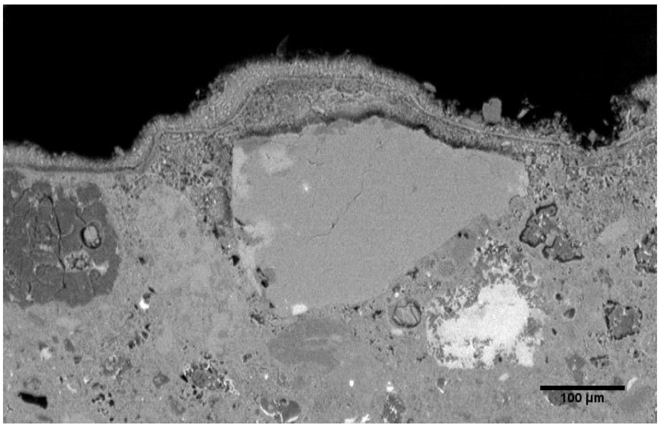


Figure 4.3.10 Micrograph of the cross-section of the fragment VB. A gypsum layer has formed on the external surface of the fragment. The identification of this crystalline phase was done both because of the morphology of the layer (Figure 4.3.9) and from the EDXS composition measured (Figure 4.3.11).

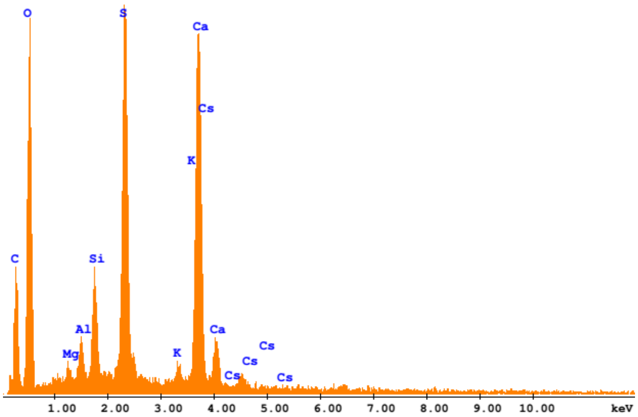


Figure 4.3.11 EDXS spectrum of the external layer of the fragment cross-section (Figure 4.3.10 and Figure 4.3.11). The layer is made of gypsum ($CaSO_4 \cdot 2H_2O$).

4.3.2 Spruce needles

The scope of this paragraph is summarizing the results of the study of the pollutants collected by spruce needles. A synthesis of the results obtained by both bulk and single particle analytical techniques is presented, along with the apportionment of the contaminants to a specific source, when possible, and to a specific kind of emissions of the EAF steelmaking process for those pollutants apportioned to this industry.

Spruce needles from the background locations, that have been exposed for 1 year (2012-2013, site PS) or for spring and summer 2011 (AK) were analysed to assess the goodness of the background selection. Both the samples, being of paramount importance in our study, were submitted to FKV Company for microwave-assisted closed-vessel digestion that was proven (see paragraph 3.4) to guarantee a more accurate determination of the elemental concentrations in the subsequent ICP-AES measurements. The concentrations measured in this way are used for comparison with concentrations given in the literature. However, for homogeneity with the other samples used in this study, two other samples from these sites were prepared with the open-vessel apparatus available in our laboratory and the concentrations measured after this procedure were used to calculate a maximum acceptable concentration value for a non-polluted site.

Table 6.6-1 (Appendix F) provides the elemental concentrations of the needles as sampled (not washed) of the two background locations AK and PS determined after digestion in the closed-vessel system (UltraWave).

The AK needles (exposed during spring and summer 2011) have most elements (Mn, Fe, Cu, Pb, Cd) in the expected concentration ranges (König *et al.* 2010). Mg and K are in concentrations lower than expected (Fürst 2010; König *et al.* 2010), while Ca and Zn concentrations are higher than normal (König *et al.* 2010). However, a similar Ca concentration (12330 ppm) was measured by Fürst (2010) in needles from Austrian spruce and a high concentration of Zn (50.08 ppm) was measured in spruce needles from Finland. Therefore, these measurements do not raise any concern regarding the suitability of the sample as background reference.

The PS needles (exposed from spring 2012 to spring 2013) have many elements in normal ranges (Ca, Fe, Cu, Zn, Pb, Cd), although they seem to have a very low Mn content, low K and high Mg concentrations (Fürst 2010; König *et al.* 2010).

To evaluate the impact of the EAF steelmaking facility the metals of interest are Mn, Zn, Fe, Cr and Pb (Appendix D), thereby the sites AK and PS seem suitable background locations from the total metal concentrations. Unfortunately, the data do not give the same indication if the concentrations obtained from the subtraction of the amount of an element present in the not washed needles and its amount in the washed subsample (both measured after digestion in the closed-vessel apparatus named UltraWave) are considered (estimate of the amount of metal coming from atmospheric deposition) (Table 6.6-1, appendix F). Both samples have a Mn atmospheric concentration which exceeds the expected 2% of the endogenous concentration (Wyttenbach *et al.* 1995). Moreover, while site AK seems to be affected by deposition of Ba, Sr, Fe, Zn and in lower amount of Pb, PS shows atmospheric uptake of Cr, Fe, Co, Ni, Cu and Pb. This means that if AK is used as a background reference any site where an atmospheric concentration of Cr, Cu, Ni, Co and Cd is measured can be considered as contaminated with those elements, while for Ba, Sr, Fe and Zn the possible presence of an atmospheric contribution ought to be supported by additional information associated with air pollution. Similarly, using PS as background reference would mean that any site where an atmospheric-related Zn, Sr or Ba concentration is measured on the spruce needles can be considered contaminated with those elements with respect to PS, while for the other metals further data supporting the hypothesis of air contamination must be gathered.

The needles collected at these two locations were examined with LVSEM-EDXS to look at the particles deposited on their surfaces. On the needles from Alaska only particles of natural origin were detected suggesting that it is possible to use this site as reference for a pristine location especially in a study directed toward the identification of the influence of a steelmaking plant. Only a spherical particle containing Mn, Fe, Zn and Cr was identified on the Alaska needle. On a 2012 needle from site PS, among many natural particles, HVSEM-EDXS identified a single Fe-rich spherical particle (Figure 4.3.12). As no Si or Al were present in the EDXS spectrum of this sphere, it is possible to hypothesize that it was emitted by the EAF steelmaking plant (Ault *et al.* 2012). This would confirm what was observed on the samples from the building present at the same site: although the analysis of organic contaminants, the dispersion model (www.appa.provincia.tn.it/news/pagina199.html) and the general bulk composition do not indicate pollution from the EAF plant, single particles emitted by this source reach the site. This is not surprising as industrial sources may affect areas of 20 km^2 (Machemer 2004) and PS is only about 2.4 km far away from the steelmaking plant. Other spherical particles (Figure 4.3.13) did not contain Fe but were mainly composed of Si and contained K. Because of the presence of K it can be hypothesized that they come from wood combustion (Appendix B).

To have another indicator of the goodness of site PS as a background location, the elemental enrichment factors were calculated with respect to the available data on local soil (EF_S). The results show that the needles are slightly enriched ($1 < EF_S < 10$ Feng *et al.* 2009) in Na, Ca and Cd and intermediately enriched ($10 < EF_S < 100$ Feng *et al.* 2009) in Mg and K (Table 4.3-1).

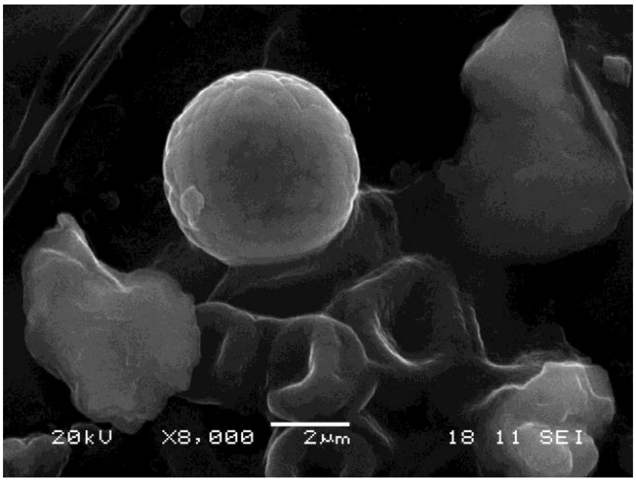


Figure 4.3.12 Micrograph of an Fe-rich spherical particle on the surface of a 2012 needle sampled at site PS (exposed one year to the atmosphere). No Si or Al was present in the EDXS spectrum.

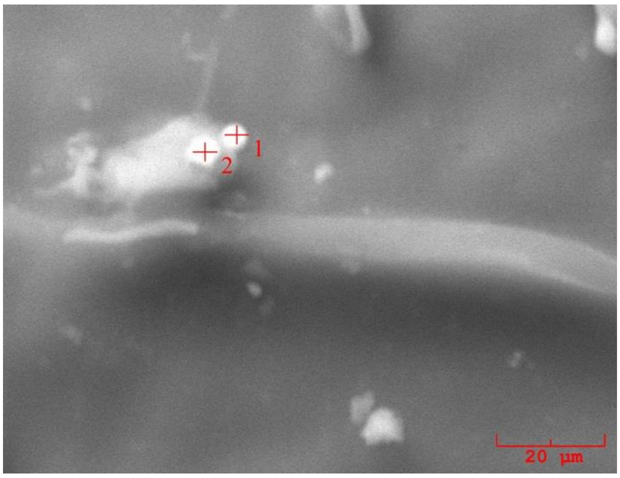


Figure 4.3.13 Micrograph of two spherical particles present on the surface of a 2012 needle sampled at site PS (exposed one year to the atmosphere). Both the particles are mainly composed of Si. Particle 1 is also rich in Al and contains a small amount of Fe and K. Particle 2 contains some K and Ca.

Table 4.3-1 Enrichment factors of 2008 needles collected at the local background site PS with respect to soil concentrations measured in 2009 (Cr, Co, Ni, Cu, Zn, Pb, Cd) (allegato 11 – analisi storiche www.appa.provincia.tn.it/news/pagina199.html) or in 1995-1996 (Na, Mg, Ca, Mn, K, Fe) (I.T.C. e G. "G. Gozzer" Borgo Valsugana, a. s. 95/96. c. 3A. 1995/1996).

Element	**Na**	**Mg**	**Ca**	**Cr**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**Pb**	**Cd**	**K**	**Fe**
EF_S	1.9	22.7	2.1	0.0	0.0	0.1	0.0	0.0	0.2	0.0	7.7	14.5	0.3

To establish if the concentration of an element might suggest that the site is polluted, a maximum acceptable value was calculated from the results of the analysis of these background samples as the upper limit of the background concentration plus three times its standard deviation (VDI 2007). The maximum acceptable values for atmospheric deposition-apportioned concentrations (subtraction of elemental concentrations of the needles not washed and washed in a 1:1 v/v toluene:tetrahydrofuran solution) are also reported in Table 6.6-1 (Appendix F) and are based on the concentrations determined after digestion in the open-vessel microwave apparatus. In this work it is suggested to take the maximum acceptable concentrations only as references, as the washed and not washed samples are in reality two different samples (ICP-AES analysis is destructive), thus the natural heterogeneity of the needles may play a significant role in determining whether an element seems to come from the atmosphere in a sample.

Since site PS was directly sampled by the Author, also needles from 2008 were collected and a maximum acceptable concentration for each element on older needles was calculated (Table 6.6-1, appendix F). It can be observed that to state that a site is polluted using 2008 needles for most elements (Li, K, Mg, Sr, Ca, Mn, Fe, Co, Zn, Cd) it is necessary to detect higher concentrations than the pollution threshold for 2012 needles. This may be due to the fact that a longer exposure means higher concentrations due to atmospheric deposition no matter the level of pollution at a site.

As this study was developed in different moments there has been more than a sampling campaign for needles of the same age cohort at different sites. Hence, although the needles were produced the same year they were exposed for a different number of years to conditions that is uncertain whether were similar. This is especially true for 2008 and 2012 needles sampled at sites P, F, L and M that would have no correspondent background sample. It is then necessary to estimate what background concentrations of the elements of interest would have been measured if sampling would have been coeval. For internal elemental concentrations, that is for the concentrations due to root uptake, biodynamic functions are available that would allow the prediction of the concentration of washed needles of a certain age group exposed for a certain number of years (Wyttenbach *et al.* 1995). Unfortunately, being interested in the component of elemental concentrations due to atmospheric deposition we cannot rely on any available biodynamic function. For Fe a function describing surface loading of spruce needles with reference to 1 g of dry needles was proposed by Wyttenbach and Tobler (2000):

$$Fe\ [ppm] = 28.5[1 - \exp(-0.61AC)]$$

where AC is the exposure time. Attempting to apply this formula we do not obtain the same atmospheric concentrations for 2012 and 2008 needles calculated from experimental data. We experimentally obtained a value that is the 29% of that calculated with the given formula, which means that we underestimated Fe atmospheric concentration of about 71%. This discrepancy could be due to the open-vessel sample preparation procedure. Looking at the available data on atmospheric concentrations at site PS it can be observed that Cr, Co, Ni, Zn and Pb were absent both on 2008 and 2012 needles sampled in 2013. Therefore, we can assume that if the needles would have been sampled in 2012 still there would have been no contribution from atmospheric deposition to their concentration. Mn, Fe and Cd showed an increasing behaviour with needle age class. Likely the increasing behaviour is not linear as deposition and washout are not constant with time. However, as the atmospheric concentrations were already 0 in 2012 needles exposed one year, we can assume they were 0 also when they were exposed for spring only. Therefore, the year more of exposure did not affect the amount we would have measured on 2008 needles if they were sampled in 2012. Cu, on the contrary, shows a decreasing behaviour, again likely not linear as not even its internal concentration has a linear behaviour in the work of Wyttenbach *et al.* (1995).

To validate the estimates made on the concentrations of the elements of interest, it was decided to sample 2013 needles in fall 2013 and measure the elemental concentrations (Table 6.6-2, appendix F). This analysis suggests that the value we could have predicted (6.507 ppm) for Fe atmospheric related concentration using Wyttenbach and Tobler's (2000) equation is wrong. Moreover, it is evident that Fe and Cu atmospheric concentrations have not a linear behaviour with needle age class (Figure 4.3.14Figure 4.3.15). From this it can be concluded that it is not possible to extrapolate needle atmospheric concentrations, especially for elements such as copper and iron that could be emitted by a variety of sources. Hence, from a methodology point of view, the background site must be sampled together with the other sites and for the same age cohorts.

HV-SEM-EDXS analysis was employed to further prove the suitability of the background location. The particles analyzed on the 2008 needles collected at site PS were of natural origin containing Si, Ca, Mg, Ti and K. Small particles (1-3 µm Feret diameter) (Figure 4.3.16) mainly composed of Fe were detected but their source was not identified as they did not present any specific morphology. As Fe is widely present in the environment it is likely that also these particles are of natural origin.

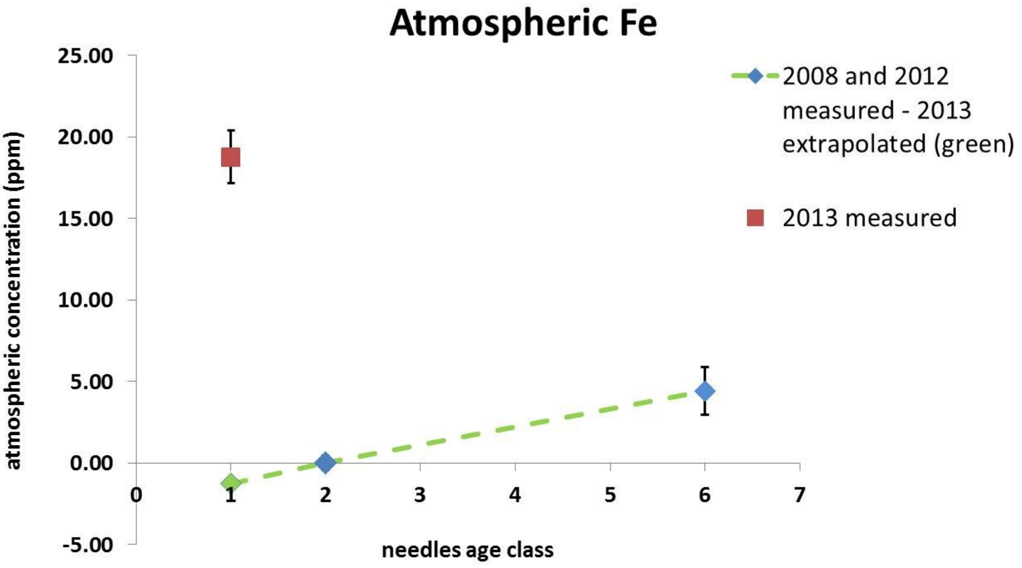


Figure 4.3.14 Fe concentration (ppm) in 2008 (age class 6), 2012 (age class 2) and 2013 (age class 1) needles sampled at site PS in May (2008 and 2012) and November 2013 (2013). From a linear extrapolation from 2008 and 2012 concentration one would have expected a negative class 1 needle which means that no atmospheric Fe should have been present on the needles.

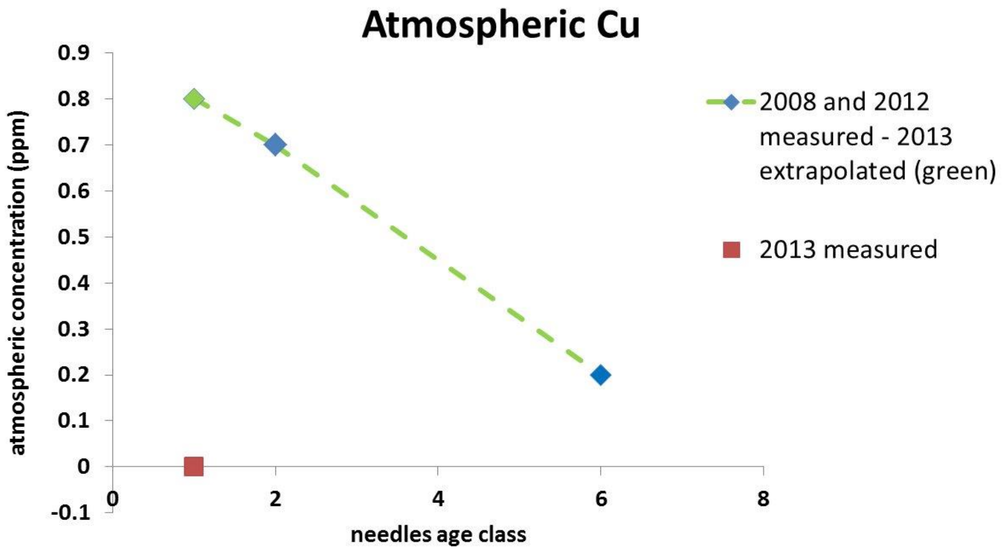


Figure 4.3.15 Cu concentration (ppm) in 2008 (age class 6), 2012 (age class 2) and 2013 (age class 1) needles sampled at site PS in May (2008 and 2012) and November 2013 (2013). From a linear extrapolation from 2008 and 2012 concentration one would have expected a class 1 needle concentration of 0.801 ppm.

The fact that site PS is relatively not polluted was also confirmed by TEM-EDXS. As the site is relatively far from the steelworks particles in smaller size ranges could have been present. Instead, there were in general very few particles on the TEM grid when a 100 µl drop taken from the DCM residual washing solution of 1.5 g of 2012 needles was deposited. Even after evaporation of the residual washing solution to concentrate the particles, still the number was quite low compared to other samples prepared with the same procedure. The particle type most detected, thus characteristic of the site, was rich in CaO. Very few agglomerates contained some Zn-rich areas.

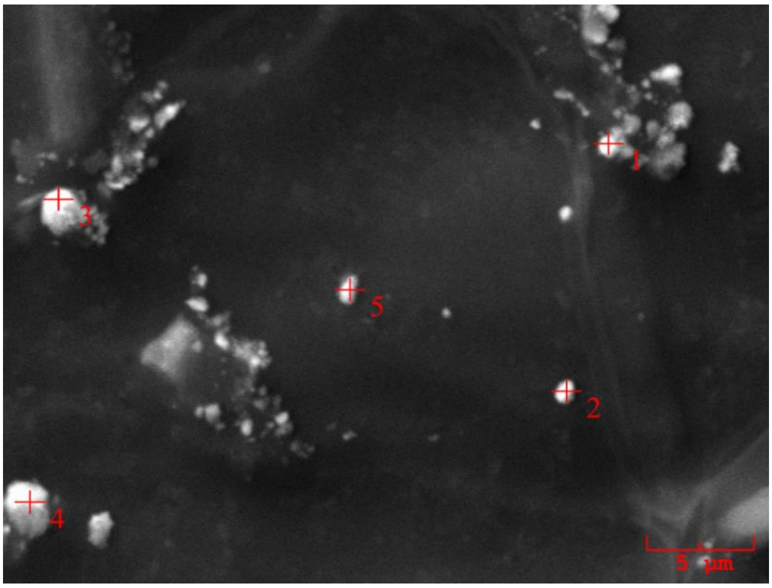


Figure 4.3.16 SEM micrograph of particles deposited on the surface of a needle exposed from 2008 to 2013 at site PS. All the numbered particles were mainly composed of Fe.

Having established the suitability of the background samples and calculated the maximum acceptable atmospheric concentrations, it is possible to discuss the data gathered from the study of the samples collected at the other sites.

From bulk analysis (ICP-AES) of 2008 needles site B and site P seem the most polluted: a variety of metals that can be related to anthropogenic sources are there present (Table 4.3-2).

Within the limitation of the different exposure periods of certain samples, it seems that the concentrations of certain metals measured by ICP-AES on 2008 needles have similar behaviors with respect to their distance from the EAF steelmaking plant: Cr and Co appear at the same sites and Co is in comparable amounts of Cu at these. Cu concentration decreases with distance from the steelmaking plant along the NW transect, and so does Mn concentration which, however, is absent from the site 500 m far (site F) and present elsewhere in much higher amount. This may suggest that if the source is the EAF plant these metals are located in different type of particles (size, stage of steel production, etc.) spreading in association with the exact emission point (tall chimney, fugitive ground-level) and meteorology. Sr in 2008 needles has a concentration trend that seems to be not related with orientation and distance from the steelmaking plant.

ICP-AES analysis of 2012 needles (Table 4.3-3) seem to confirm the association between Cu and Mn, present at the same sites.

Although different strategies of ICP-AES data interpretation (pollution threshold, EF_B, EF_S) lead to slightly different conclusions on EAF-induced pollution, there is a general agreement among the methods regarding these data (Table 4.3-4):

- site B atmosphere between 2008 and 2012 was polluted by Cr, Mn, Co, Cu and Fe, while later Cr was absent; comparing the concentration measured on the unwashed sample of 2011 needles with the ranges provided in the literature for spruce needles of comparable age class (König *et al.* 2010) it is evident that this site is polluted: Cr (18.5 ppm d.m.), Fe (740 ppm d.m.) and Cu (11.4 ppm d.m.) concentrations are higher than normal ranges, nonetheless the concentrations were measured after digestion in the open-vessel system which could have led to underestimate their amounts (see paragraph 3.3).
- site F atmosphere between 2008 and 2012 was polluted by Mn and Cu; Cu was absent in spring 2012;
- site L atmosphere between 2008 and 2012 suffered of excess Mn that still seem to be present in spring 2012;

- site P atmosphere was polluted by Cr, Mn, Co and Cu between 2008 and 2012 of which only Mn and Cu were still present in excess amount in spring 2012;
- site M was polluted by Mn between 2008 and 2012 but not in spring 2012;
- different interpretation strategies never agreed at site O in suggesting a specific contaminaton problem.

Table 4.3-2 ICP-AES metal concentrations due to atmospheric deposition on **2008 needles** as a function of the distance and orientation of the site from the EAF steelmaking plant: 120 NW (site B), 500 NW (site F), 1000 NW (site L), 1000 E (site P), 1100 S-SE (site M), 1910 SE (site O), 2370 E (site PS, local background location). In bold the concentrations that are above the threshold calculated as the 95% upper confidence limit plus 3 times the standard deviation of the concentration at the local background location (PS) for needles of the same age. Sample from site B was washed in DCM while the others in a 1:1 (v/v) solution of toluene:tetrahydrofuran.

Element	**site**	**B**		**F**	**L**	**P**	**M**	**O**	**PS**
	distance (m)	**120**		**500**	**1000**	**1000**	**1100**	**1910**	**2370**
	direction	**NW**		**NW**	**NW**	**E**	**S-SE**	**SE**	**E**
	exposure	5 years and 1 month ca		4 years and 2months ca	4 years and 2months ca	4 years and 2months ca	4 years and 2months ca	5 years and 1 month ca	5 years and 1 month ca
	pollution threshold (ppm d.m.)	**atmospheric concentrations (ppm d.m.) = not washed - washed**							
Na	7								
Mg (mg/g)	1,42	0,6			0,08				1,3
Ca (mg/g)	2,00	0,5	0,01	0,5	1,7	**2,9**		1,01	1,8
Al	8,60	536,0	**75**	**68,0**		**203**	2		5,0
Li	0,2	**0,2**				**3,71**			0,2
Sr	7,750	**0,4**			3,80	3,69	0,81	2,32	7,6
Ba	3,7			**4,3**			**20,9**	**19**	
Cr	0,0	**6,3**		**0,8**		**3,2**			
Mn	0,50	**103,0**	0,3	**4,8**	**2,4**	**15,8**	**9,5**		0,4
Co	0,016	**5,1**		**0,4**		**4,0**			
Ni	0,0								
Cu	0,24	**5,4**	**3,1**	**1,0**	0,1	**3,9**			0,2
Zn	11,55	2,0							
Pb	0,0								
Cd	3,86								3,8
K (mg/g)	0,09	0,3	**0,6**						
Fe	12	**733,0**						0,2	4,0

Table 4.3-3 ICP-AES metal concentrations due to atmospheric deposition on **2012 needles** as a function of the distance and orientation of the site from the EAF steelmaking plant: 120 NW (site B), 500 NW (site F), 1000 NW (site L), 1000 E (site P), 1100 S-SE (site M), 1910 SE (site O), 2370 E (site PS, local background location). In bold the concentrations that are above the threshold calculated as the 95% upper confidence limit plus 3 times the standard deviation of the concentration at the local background location (PS) for needles of the same age. Sample from site B was washed in DCM while the others in a 1:1 (v/v) solution of toluene:tetrahydrofuran.

most likely source type	**element**	**Site**	**B**	**F**	**L**	**P**	**M**	**O**	**PS**
		distance (m)	**120**	**500**	**1000**	**1000**	**1100**	**1910**	**2370**
		Direction	**NW**	**NW**	**NW**	**E**	**S-SE**	**SE**	**E**
		Exposure	1 year and 1 month ca	2 months ca	2 months ca	2 months ca	2 months ca	1 year and 1 month ca	1 year and 1 month ca
		pollution threshold (ppm)	**atmospheric concentration (ppm) = not washed - washed**						
crustal	Na	31,9			12,0			0,4	30
	Mg (mg/g)	0,06	0,04	0,03				0,04	
	Ca (mg/g)	0,3						**1,1**	
	Al	24,3	**114**	1,6	23,0	24		11,3	22
	Li	0,0	**0,205**						
anthropogenic	Sr	0,06						**2,46**	
	Ba	9,31		0,1	4,2	2,70		5,97	9,46
	Cr	0,0							
	Mn	0,0	**2,46**		**3,1**	**0,5**			
	Co	0,0	**0,806**						
	Ni	0,0							
	Cu	0,709	**1,37**		**0,9**	**1,7**			0,70
	Zn	11						0	
	Pb	0,0							
	Cd	0,0							
ambiguous	K (mg/g)	0,06		0,04		**2,43**	0,01	0,00	
	Fe	1,9	**21**	**11**	**23,0**		3,6	**23**	

Table 4.3-4 Summary of the atmospheric pollution at each site evaluated through the calculation of the enrichment factor (EF_B) for each element (that may be related to the EAF plant emissions) with respect to the concentration at the local background location (PS) for the needles of the same year or through comparison with a threshold value calculated from the same background sample. The EF_B atm was calculated between atmospheric concentrations, that is those obtained by subtraction of the concentration measured on solvent-washed needles from the not washed ones while the EF_B tot was calculated only from the concentrations of not washed needles. A cross indicates that the $EF_B > 2$, thus that the atmosphere at the site may have an excess (pollution) of the element (Mingorance *et al.* 2007).

year	Site		B			F			L			P			M			O		
	distance (m)		120			500			1000			1000			1100			1910		
	Direction		NW			NW			NW			E			S-SE			SE		
	most likely source type	Element	pollution threshold	EF_B atm	EF_B tot	pollution threshold	EF_B atm	EF_B tot	pollution threshold	EF_B atm	EF_B tot	pollution threshold	EF_B atm	EF_B tot	pollution threshold	EF_B atm	EF_B tot	pollution threshold	EF_B atm	EF_B tot
2008	EAF steelmaking plant	Cr	X	X	X	X	X	X				X	X	X						
		Mn	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
		Co	X	X	X	X	X					X	X	X						
		Ni																		
		Cu	X	X	X	X	X	X				X	X	X						
		Zn		X	X															X
		Pb																		
		Cd																		
	ambiguous	Fe	X	X	X															
2012	EAF steelmaking plant	Cr																		
		Mn	X	X	X			X	X	X	X	X	X	X						
		Co	X	X	X															
		Ni																		
		Cu	X						X		X	X	X	X						
		Zn																		
		Pb																		
		Cd																		
	ambiguous	Fe	X	X	X	X	X		X	X					X	X		X	X	
2013	EAF steelmaking plant	Cr				not sampled			not sampled			not sampled			not sampled					
		Mn	X	X	X															X
		Co	X	X	X															
		Ni																		
		Cu	X	X	X															
		Zn																		
		Pb																		
		Cd																		
	ambiguous	Fe	X	X	X															

Principal component analysis (PCA) was conducted on ICP-AES data from all samples (19 cases) considering as variables the total (unwashed) concentrations of 12 elements (Na, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Cu, Zn, Al). The ratio between the number of cases and variables is not optimal for the technique, as generally the number of cases should be three times the number of variables. It was decided to attempt the analysis anyway to define whether such a method could help in data interpretation and especially in combining data from single particle analysis. Two explicative variables, distance from the steelmaking plant and needle duration of exposure to the atmosphere, were used as supplementary variables, which means that they did not contribute to the calculation of the principal components but they were plotted in the space defined by them in the outputs of the analysis. Some data pre-treatment was necessary. Four concentrations were estimated from the value provided by the ICP-AES software (>number) as they were above the IDL and after dilution they strangely became below the IDL. These four concentrations were Fe in samples F2008, P2008, P2012 and Zn in B2005 (the latter sample has never been diluted). Other four concentrations were estimated although they were below the LOD or the LOQ: Zn in M2008, P2008, B2011 and L2008. The reason why these operations were undertaken is to avoid to have too many 0 values which do not express variance and do not reflect the real situation especially in the case of the first four samples mentioned. The explanatory variables were inserted in the data matrix after the categorizations described in Table 4.3-5 and Table 4.3-6.

Table 4.3-5 Criterion to categorize the distance from the steelmaking plant for upload in the PCA data matrix

distance (m)	Numerical value assigned
<500	0
500 ≤ d< 1000	1
1000 ≤ d< 1500	2
1500 ≤ d< 2000	3
> 2000	4

Table 4.3-6 Criterion to categorize the exposure to the atmosphere for upload in the PCA data matrix

exposure (months)	Numerical value assigned
< 10	0
10 ≤ e < 30	1
30 ≤ e <50	2
50 ≤ e <70	3
> 70	4

The correlation matrix showed that Mn, Cr, Fe, Co, Cu, Al and Zn are correlated. Hence, there will be a principal component grouping these elements. Three factors have eigenvalues above 1 and explain about 87.9% of the total variance of the data. Following the Kaiser criterion (StatSoft Inc. 2013) these are the factors to be considered principal components and used for further interpretation. The scree plot confirmed this choice.

Therefore, the PCA was performed with a 3-factor solution. The coordinates of the first three principal components were computed. Looking at factor loadings (Table 4.3-7), factor 1 could be associated with the steelmaking plant, factor 2 with natural sources and factor 3 with traffic on the basis of the elements they are more correlated with (Appendix C). It is interesting to observe that Al and Cu seem to be associated with the steelmaking component although they are not traditionally apportioned to this emission source. The samples more important in determining factor 1 (steelmaking plant) are 2008, 2011 and 2012 needle concentrations from site B and in lower amount the 2012 concentrations from site M, F and P (Table 4.3-8). This is an interesting data as it would have been expected a higher contribution from the 2008 needles from these other sites. Associating factor 3 with traffic based on its correlation with Ba concentration (Appendix C), makes sense looking at Table 4.3-8, as SEM-EDXS detected particles from brake wear samples on 2008 needles from site P and on the building located at site O.

Table 4.3-7 Factor loadings.

*supplementary variables

	Factor 1 = steelmaking	Factor 2 = natural	Factor 3 = traffic
Na	-0.81	0.06	-0.16
Mg	-0.64	0.47	-0.35
Ca	-0.61	0.65	-0.04
Sr	-0.74	0.56	0.26
Ba	-0.53	0.01	0.77
Cr	-0.94	-0.12	0.06
Mn	-0.92	-0.31	0.00
Fe	-0.95	-0.17	-0.19
Co	-0.96	-0.20	-0.12
Cu	-0.93	-0.22	-0.05
Zn	-0.85	-0.12	0.34
Al	-0.94	-0.11	-0.24
***distance**	0.53	0.36	0.20
***exposure**	-0.52	0.32	0.00

Table 4.3-8 Factor coordinates of the cases.

	Factor 1 = steelmaking	Factor 2 = natural sources	Factor 3 = traffic
M 2008	1.41	0.48	0.09
M 2012	2.30	-1.27	-0.06
F 2008	1.29	0.06	-0.61
F 2012	2.30	-1.13	-0.60
P 2008	-0.61	1.38	-2.49
P 2012	2.11	-1.39	0.04
L 2008	0.51	1.52	0.03
L 2012	1.43	-0.67	0.81
O 2012	1.58	0.15	0.14
B 2008	-4.58	-0.31	-1.51
B 2012	0.81	-0.28	-0.01
PS 2012	1.14	0.07	0.64
PS 2008	0.22	2.12	-0.27
O 2008	0.17	1.21	2.74
PS 2013	1.36	-0.04	0.08
O2013	1.66	-0.52	-0.31
B 2013	0.71	-0.70	0.04
B 2005	-7.96	-1.88	0.55
B 2011	-5.84	1.20	0.70

Looking at the case score plot of factor 1 versus factor 2 (Figure 4.3.17) it is evident that B2005, B2008 and B2011 samples are related to the steelmaking component (factor 1) and that more recent samples from the same site (B2012 and B2013) are grouped with the other samples. This could suggest a reduction of fugitive emissions that reach this site very close to the plant. Both from the case (Figure 4.3.17) and from the variable (Figure 4.3.18) score plots, it emerges that the accumulation of elements which can be apportioned to natural sources is related to needle exposure period.

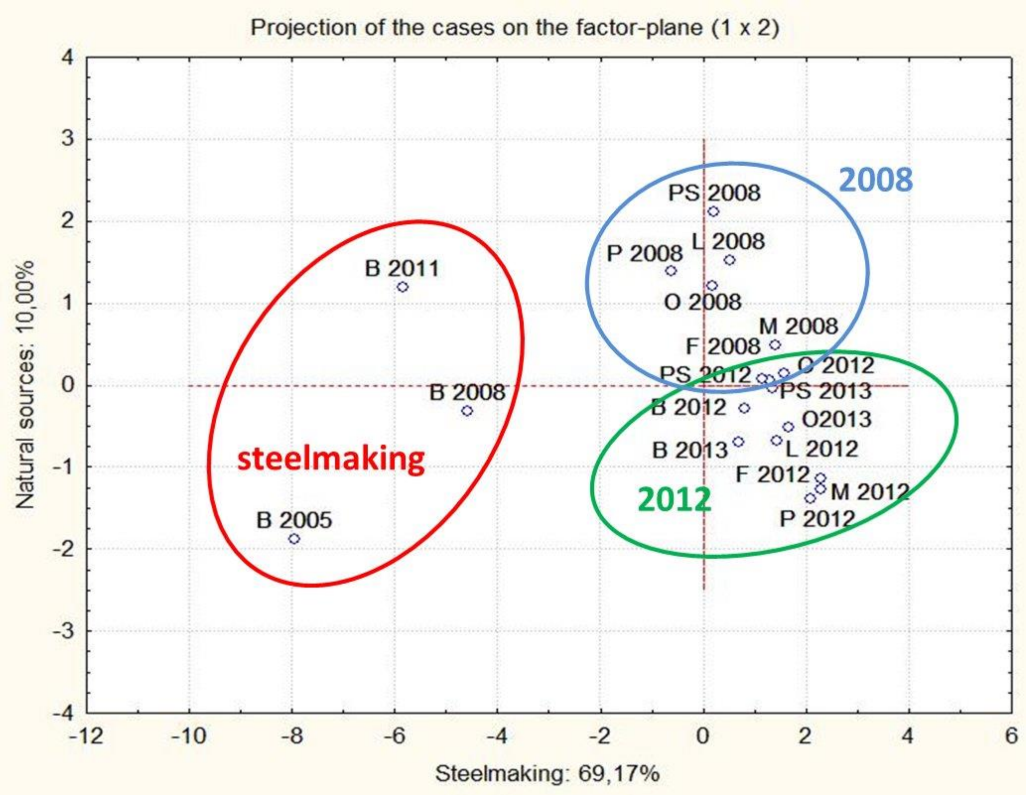


Figure 4.3.17 Case score plot of factor 1 (steelmaking plant) vs factor 2 (natural) for the PCA performed on 12 total element concentrations (Na, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Cu, Zn, Al).

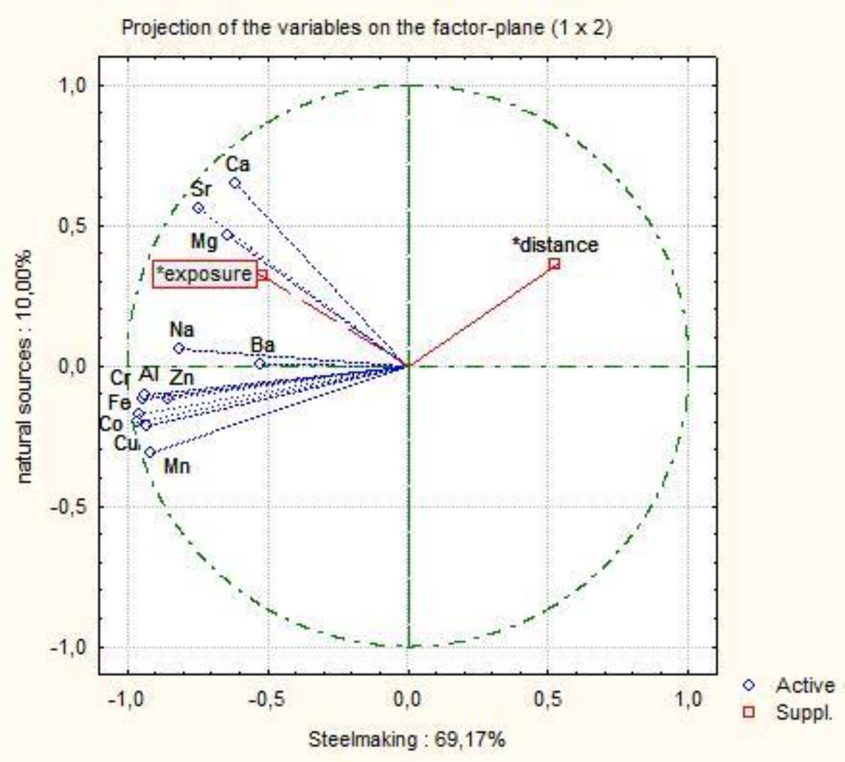


Figure 4.3.18 Variable score plot of factor 1 (steelmaking plant) vs factor 2 (natural) for the PCA performed on 12 variables and 19 cases.

The samples more affected by traffic are 2008 needles collected at sites P and O (Figure 4.3.19). The loadings plot of factor 1 versus factor 3 (Figure 4.3.20) shows that Zn and Sr are related to both factors. This suggests, that Zn cannot be used as a tracer of the steelmaking plant.

The study of the case and the variable score plots for factor 2 versus factor 3 does not add to the previous observations.

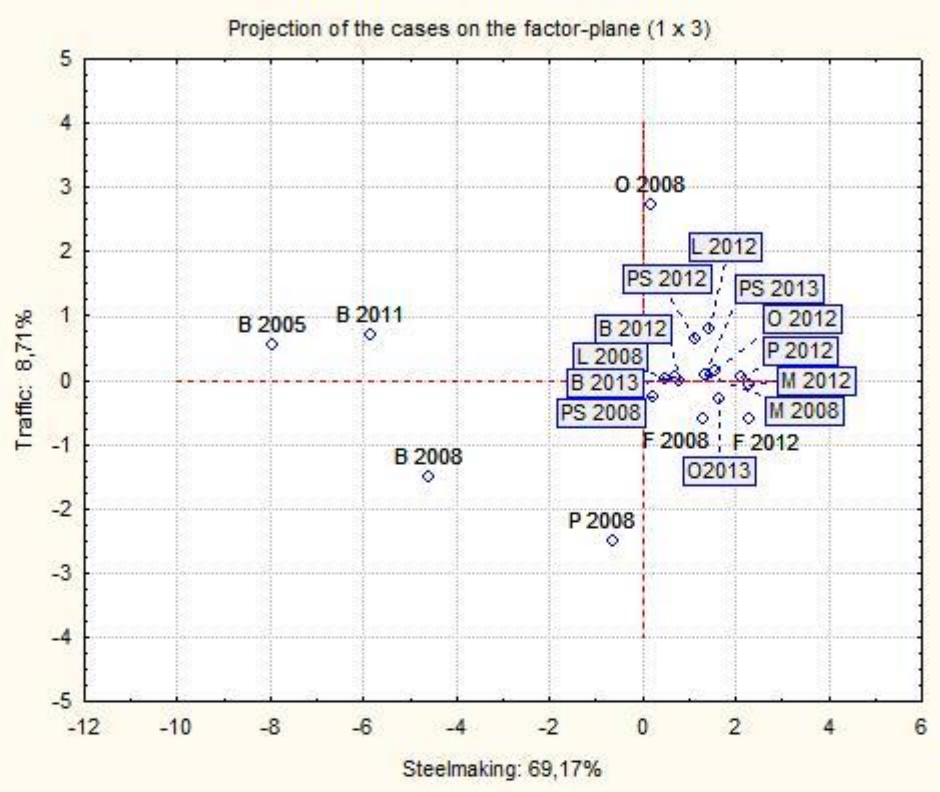


Figure 4.3.19 Case score plot of factor 1 (steelmaking plant) vs factor 3 (traffic) for the PCA performed on 12 total element concentrations (Na, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Cu, Zn, Al).

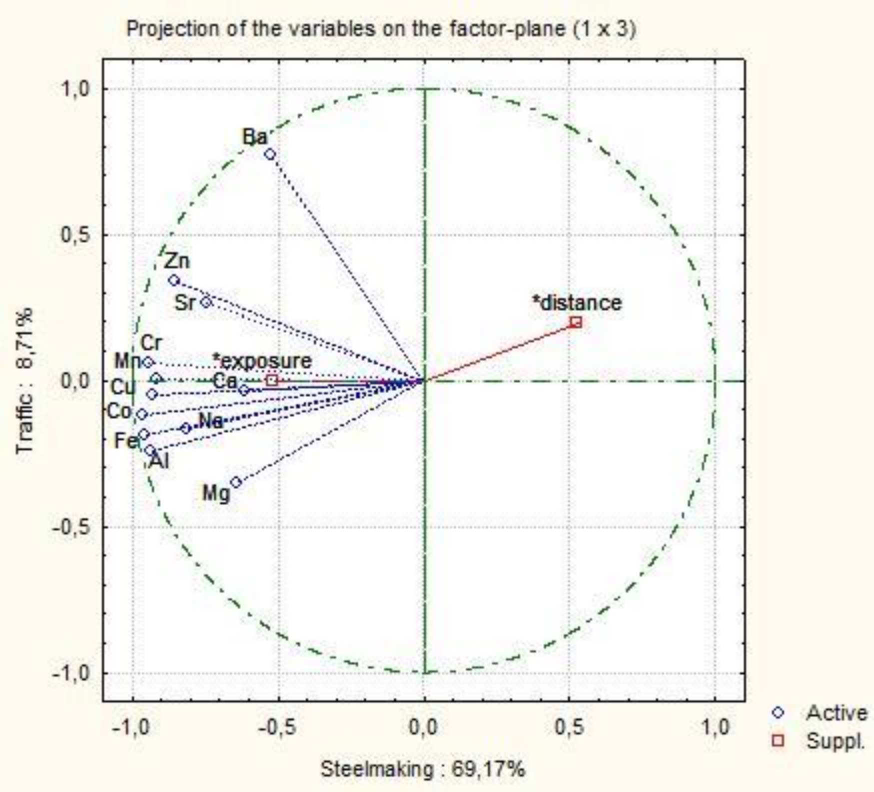


Figure 4.3.20 Variable score plot of factor 1 (steelmaking plant) vs factor 3 (traffic) for the PCA performed on 12 variables and 19 cases.

Within the limitation that needles of different age cohort were not collected in the same sampling campaign at all sites, it was attempted to trace temporal trends for the concentration of the metals that could be related to the steelmaking emissions.

At site P Mn, Cr and Cu concentrations have decreased (Figure 4.3.21 - A).

At site M while Mn atmospheric deposition decreased, Fe concentration increased (Figure 4.3.21 - B).

Temporal trends at site L show that Fe deposition decreased, while Mn and Cu concentrations seem to have slightly increased (Figure 4.3.21 - C).

At site F Mn, Cu, Cr and Co concentrations decreased with time, while, on the contrary, Fe concentration increased (Figure 4.3.21 - D).

At site B there has been a decrease in Pb, Ni and Mn deposition (Figure 4.3.21 – E and F). Cu, Co and Fe concentrations are lower than in the past, but it seems that recently they increased (Figure 4.3.21 – E and F). The drastic reduction of these elements in 2012 may be due to the long periods of shut down that the plant underwent before a new property acquired it. Cu and Co have a very similar trend that suggest an association for these elements (Figure 4.3.21 – F). Cr and Zn also decreased in recent years although in the past their concentrations did not present a clear trend (Figure 4.3.21 – F).

At site O the estimated atmospheric depositions on the needles draw a decreasing trend for Ba concentration, while for Fe and Sr the trend is not evident (Figure 4.3.21 – G).

In the years it seems that at the local background location (PS) Sr, Cd and Mn concentrations decreased, Fe and Cu concentrations increased and Ba trend is not clear (Figure 4.3.21 – H).

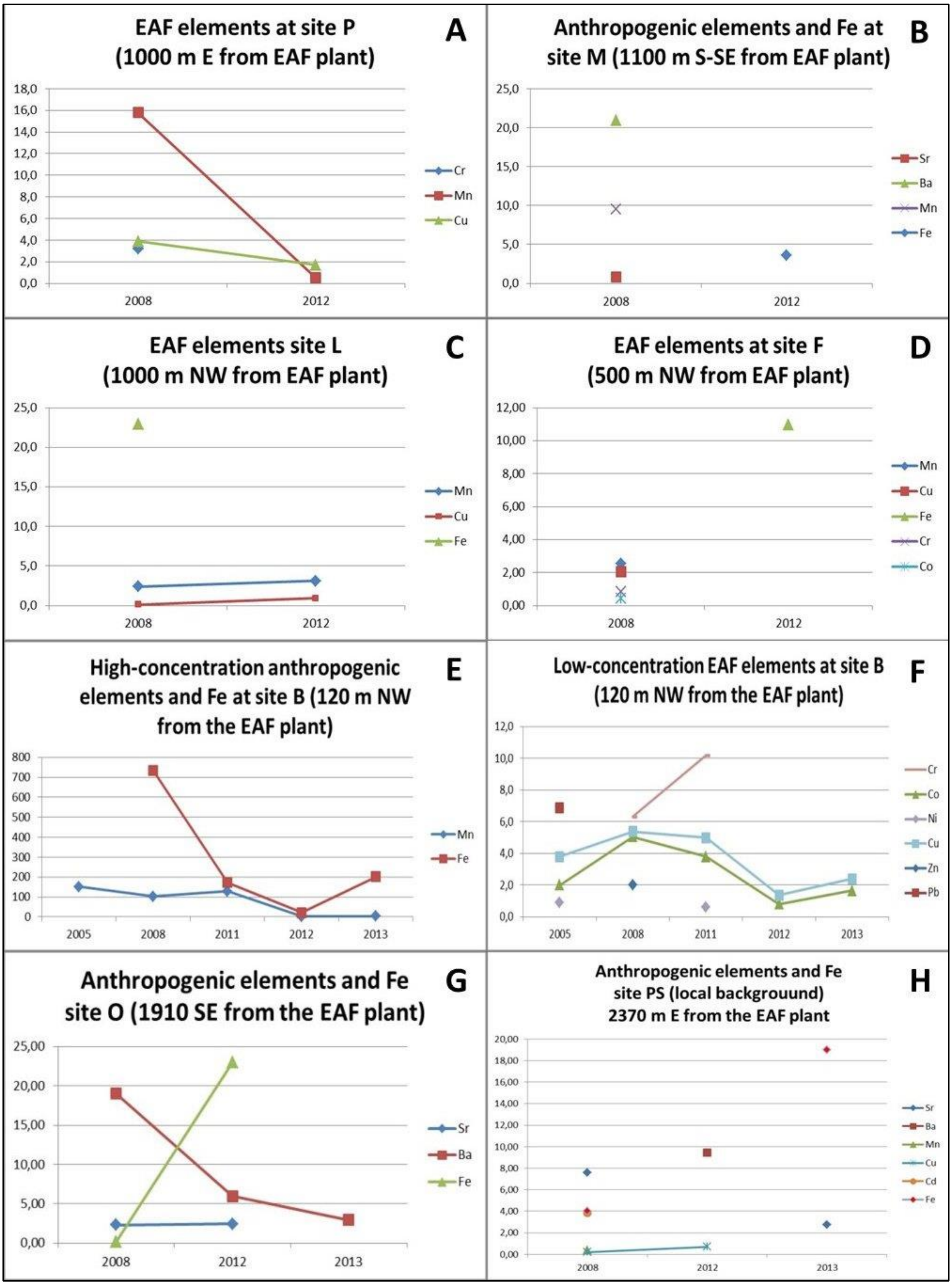
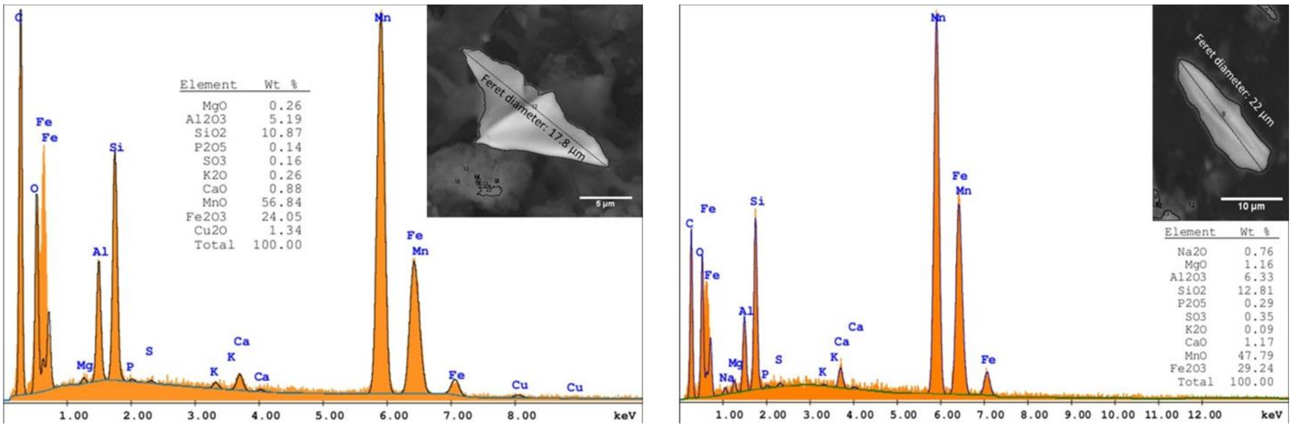


Figure 4.3.21 Variation of atmospheric concentrations (total-internal) of anthropogenic emissions-related elements and Fe between needles collected: A-site P; B-site M; C-site L; D-site F; E-site B Fe and Mn; F-site B other EAF-related elements; G-site O; H-site PS (background location).

SEM-EDXS and TEM-EDXS identified the following classes of particles:

- particles apportioned to the EAF steelmaking plant
    - irregularly shaped particles which can be apportioned to the steelmaking plant on the basis of their chemical composition (Fe, Mn, Cr, Zn) (Figure 4.3.22). The typical Feret diameters of these particles were 2-28 µm. Some of the coarsest, especially detected at site B, could be apportioned to the fugitive emissions of the steelmaking plant. These particles were identified in samples B2005, B2008, B2011, P 2008, P2012, M2008, L2008, F2008 and F2012



Element	Wt %
MgO	0.26
Al2O3	5.19
SiO2	10.87
P2O5	0.14
SO3	0.16
K2O	0.26
CaO	0.88
MnO	56.84
Fe2O3	24.05
Cu2O	1.34
Total	100.00

Element	Wt %
Na2O	0.76
MgO	1.16
Al2O3	6.33
SiO2	12.81
P2O5	0.29
SO3	0.35
K2O	0.09
CaO	1.17
MnO	47.79
Fe2O3	29.24
Total	100.00

Figure 4.3.22 SEM micrographs and relative EDXS spectra of particles emitted by the steelworks rich in Mn and Fe detected on 2005 needles collected at site B. The quite large (17.8 µm and 22 µm) size of the particle is consistent with the fact that the site is close to the plant (120 m).

-
    - combustion spheres and subspherical particles which can be apportioned to the steelmaking plant on the basis of their chemical composition (Fe as major element and eventually smaller amounts of Zn or other elements characteristic of the steelmaking emissions) (Figure 4.3.23 and figure 2B of Bertolotti *et al.* 2014). On a 2011 needle an ipertitaniferous (EDXS: 48.9 wt% TiO_2 and 33.6 wt% Fe_2O_3) one was detected. They are the transformation of projected liquid droplets resulting from the burst of CO-bubbles during steel refining (appendix D). The Feret diameters of these particles are in the range 1-25 µm. Sometimes the particles are found in agglomerates of 3-20 µm diameter (Figure 4.3.24) described in the past both by Li and Tsai (1993) and by Guézennec *et al.* (2005). The agglomerates form due to the high temperature either inside the furnace or in the fume extraction ducts (Guézennec *et al.* 2005). These particles were identified in samples B2005, B2008, B2011, P 2008, M2008, F2008, F2012 and PS2012

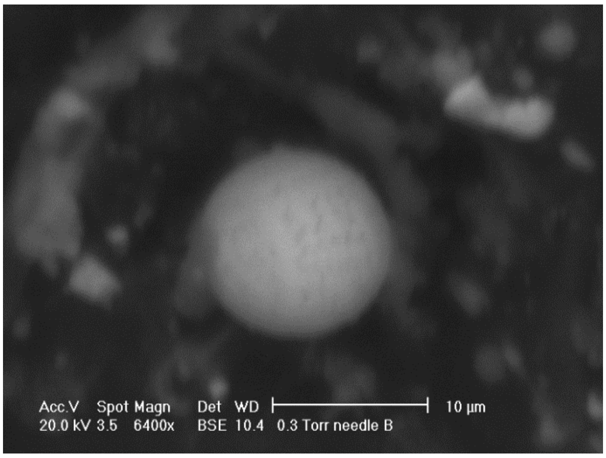


Figure 4.3.23 SEM micrograph of a ferrosphere of 12.4 µm Feret diameter detected on 2005 needle collected at site B.

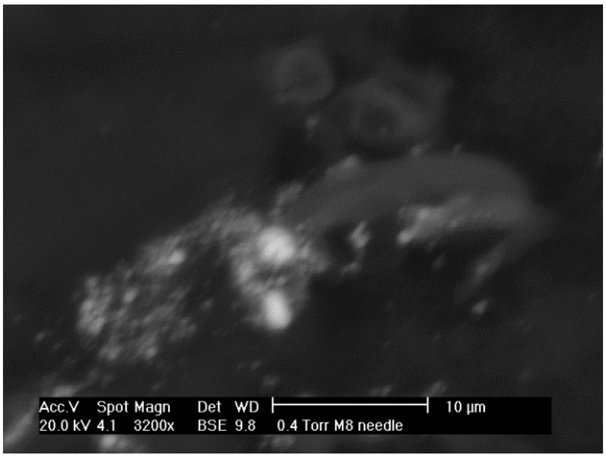
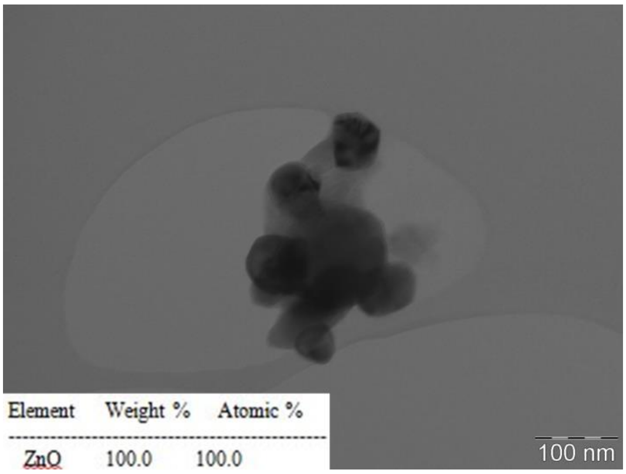


Figure 4.3.24 SEM micrograph of 2 spherules (Feret diameter 2 µm) in an agglomerate emitted by the steelworks found on a 2008 needle collected at site M.

  - agglomerates of subspherical particles rich in Zn in the size range investigated by TEM-EDXS-SAED (Figure 4.3.25) which can be apportioned to the steelmaking plant
  - elongated zincite monocrystals apportioned to the EAF emissions through comparison with the description by Guézennec *et al.* (2005) who characterized electric arc furnace dust (Figure 4.3.26). Their Feret diameter was in the 3.5-5 µm range. They were detected in samples O2008 and B2008



Element	Weight %	Atomic %
ZnO	100.0	100.0

Figure 4.3.25 TEM micrograph of an agglomerate of subspherical particles and relative composition in oxide measured by EDXS detected in the residual washing solution of 2012 needles collected at site P.

Figure 4.3.26 SEM micrograph of a zincite particle on a 30 µm nylon membrane through which the residual DCM washing solution used for 2008 needles collected at site O was filtered.

- particles apportioned to other anthropogenic sources
  - silicoaluminate combustion spheres of uncertain origin (Figure 4.3.27) (appendix B)
  - wood combustion spheres (Figure 4.3.28) apportioned to this fuel as in addition to the major presence of Si and Al they have a certain amount of K (EDXS measured in the particle in Figure 4.3.28 3.1 wt% K_2O), and eventually of P (EDXS measured in the particle in Figure 4.3.28 less than 1 wt% P_2O_5) (appendix B)

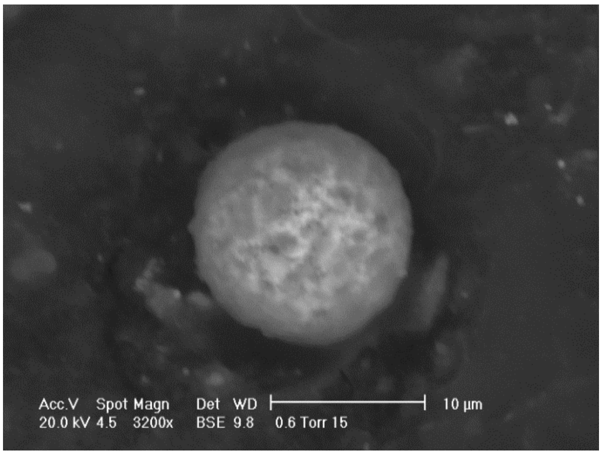


Figure 4.3.27 SEM micrograph of 14.7 µm Feret diameter combustion particle detected on a 2005 needle collected at site B.

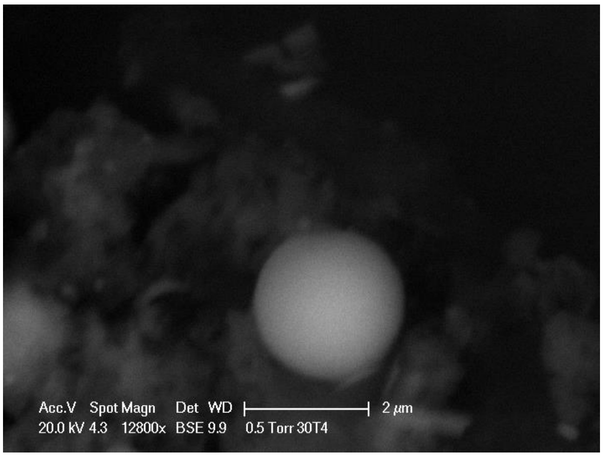


Figure 4.3.28 SEM micrograph of 2.6 µm Feret diameter combustion particle on a 30 µm nylon membrane through which the residual DCM washing solution used for 2008 needles collected at site B was filtered.

- particles related to brake and catalytic converter wear which witness that traffic is a pollution source at certain sites. These particles were identified at site P (both 2008 and 2012 samples) where the sampled trees are located at an intersection among three roads where vehicles have to stop or slow down: in one direction as they find a *give way* sign, in another one as they find a *stop* sign. The particles were in two size ranges: one of 2-3 µm with elements only from brake linings (Ba, S, Cu, Zn, K, Ti) and another one of coarser particles (7-12 µm) contaminated with Pt from catalytic converters. Barite-containing particles were also detected on 2012 needles from site L and on 2008 needles from site B (Figure 4.3.29). A particle of of zirconium silicate (Figure 4.3.30) was detected on 2005 needles collected at site B. This particle can be also apportioned to brake wear as $ZrSiO_4$ is used as an abrasive component in brake linings (Kukutschová *et al.* 2010; Kukutschová *et al.* 2011; Thorpe and Harrison 2008). Its shape is identical to a smaller particle (4.9 µm Feret diameter) identified on a sample from the building in Olle (Figure 4.3.7) where cars have to stop, thus the apportionment can be confirmed for this particle. Reasonably, since the entrance of the town with lower speed limit is further on this way, some cars may slow down here. Alternatively, the little car parking area along the cycling path near the site may be the reason because such a brake wear particle has been detected.

Figure 4.3.29 SEM micrograph of a barite particle on a 30 µm nylon membrane through which the residual DCM washing solution used for 2008 needles collected at site O was filtered.

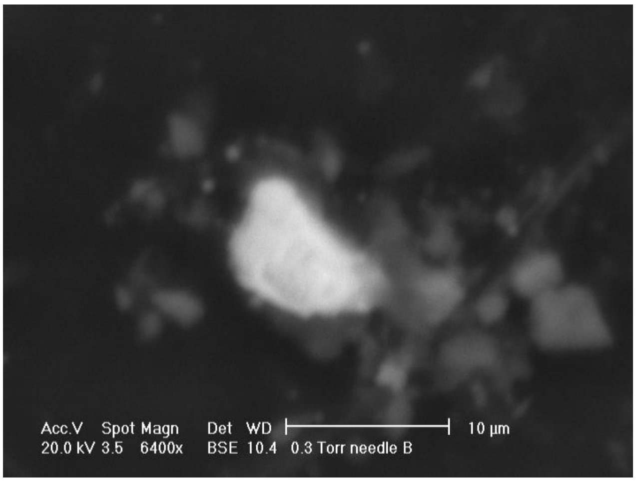


Figure 4.3.30 SEM micrograph of a Zr-rich coarse particle (10.4 µm Feret diameter) deposited on 2005 needle collected at site B.

- edgy natural particles. They were observed in all samples but analyzed only in few cases as in BSE mode it is easy for the microscope operator to select only the particles containing elements with high atomic number. Natural particles can also be very coarse, such as that shown in Figure 4.3.31.

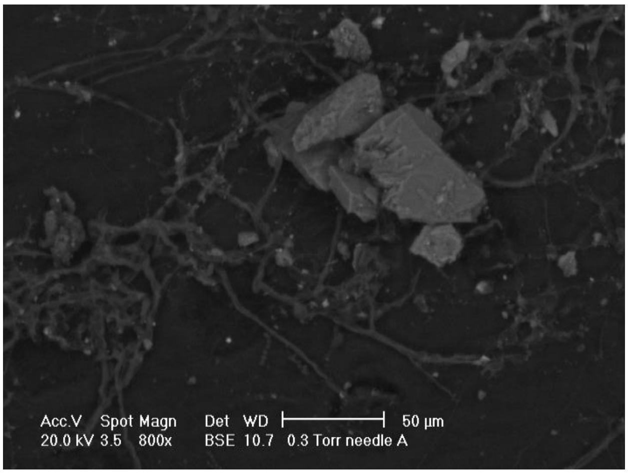


Figure 4.3.31 SEM micrograph of a natural coarse particle (71 µm Feret diameter) of quartz (SiO_2 94.6 wt%) deposited on a 2011 needle collected at site B.

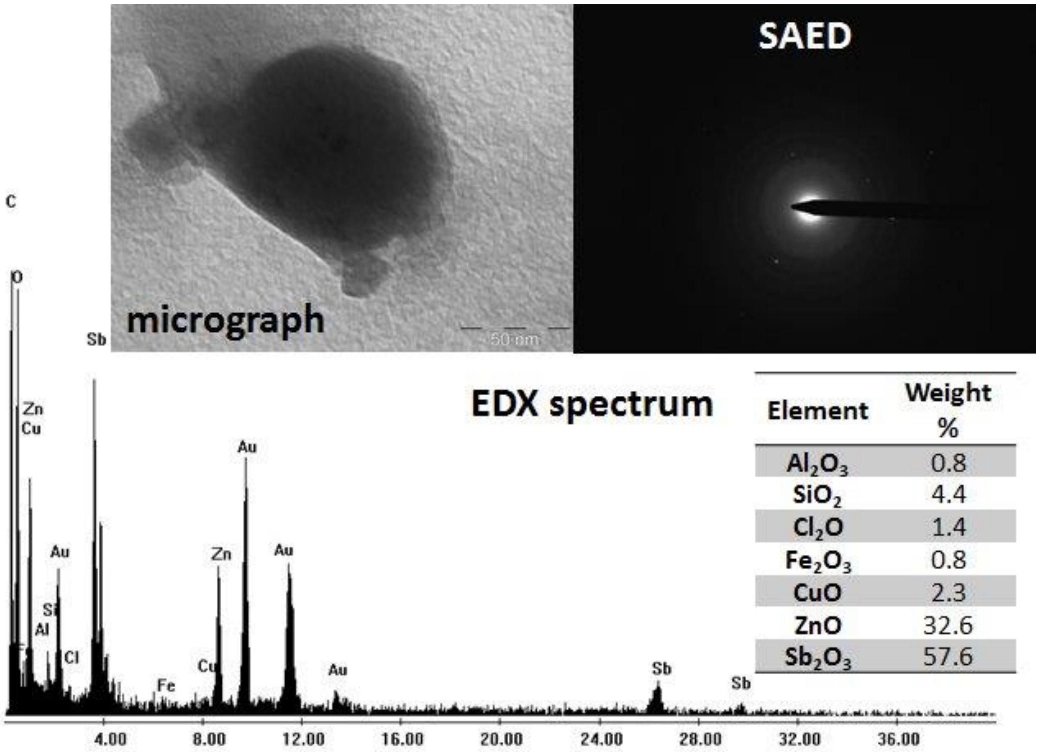
SEM-EDXS analysis of particles on needles collected at site L leads to hypothesize the presence of other pollution sources closer to this site than the steelmaking plant. On 2008 needles many combustion spheres contained small amounts of nickel (0.3-0.8 wt% calculated by EDXS as NiO) and particles containing heavy metals characteristic of steels were too coarse to have been emitted from the EAF plant. On 2012 needles SEM-EDXS detected Fe-rich particles (Fe_2O_3 80 wt%) containing other metals (Mn, Cr, Cu, Zn) among which small amounts of Ni (NiO 0.7-1.6 wt%). In both ICP-AES analysis Ni was not among the elments of atmospheric provenance being in one case below the IDL and in the other one below the LOD (Table 6.6-5, appendix F). Ni was detected only in few particles on the surfaces of 2005 needles from site B, sample where also ICP-AES indicated atmospheric deposition of this element (Table 6.6-1 part 1, appendix F), and within Sn-rich particles on 2008 needles from the same site, where, on the contrary, no indication of Ni provenance from the atmosphere was given by ICP-AES. If it would be possible to exclude the existence of another source for these dusts, a deeper investigation of the dispersion of the particles emitted by the steelworks and their Ni content would be appropriate.

Certain elements which were suggested by ICP-AES analysis to be of atmospheric provenance were never detected in particles analyzed by SEM-EDXS. Co should have been found in particles deposited on 2008 needles from site P and from site F, and on 2005, 2008 and 2011 needles from site B (appendix F). Sr should have been found in particles deposited on 2008 needles from site P, site B and site O and on 2011 needles from site B (appendix F). Li should have been found in particles deposited on 2008 needles from site B (Table 6.6-1 part 1, appendix F). Ba was not dected in particles deposited on 2008 needles from site F (Table 6.6-7, appendix F). Co concentration presents a spatial trend so similar to that of other metals likely emitted from the EAF plant and it seems associated to them in PCA (Table 4.3-7) that it is most likely apportioned to this source even if no particles containing it have been characterized. Hypothesis explaining why particles containing the listed elements were not found when expected are that a not sufficient number of particles was analyzed, that the manual mode of our electron microscope leads to a bias in particle selection for analysis, that the particles would have been found on other samples from the same site or alternatively that they would not have been found if the ICP-AES measurement would have been repeated for another sample from the same site. This latter situation occurred for the duplicate samples analyzed at site F: Co and Ba were listed among elements of atmospheric provenance only on one of the two replicates (Table 6.6-7). As Co is a missing element in many samples no conclusion can be drawn from this situation. However, Ba-containing particles are generally detected and their absence may suggest that the sample indicating an atmospheric provenance for this element is contaminated. The last hypothesis explaining the not detection of certain elements by SEM-EDXS would be that they are present in particles too fine to be observed by this analytical technique.

To verify this latter hypothesis and to assess which of the classes previously described exists also in the ultrafine range, samples P2012, L2008 and B2008 were characterized by TEM-EDXS-SAED.

The TEM grid obtained from sample P2012 was covered by more particles than the other two. No Mn-containing particles were detected nor those related to catalytic converter or brake wear.

On the grid for sample L2008, a sufficient number of particles was present but among those observed none contained Mn, Cu, Ni or Sr neither was spherical. Zn was instead present in many of them. To have more particles the washing solution was left under the hood to concentrate the solution by evaporation. It seemed that the number of agglomerates increased. Again even after concentration no particles were spherical or contained Mn, Ni or Sr. Particles containing Sn were detected. Since site B is the closest to the EAF steelmaking plant the TEM-EDXS-SAED analysis of the particles deposited on the 2008 needles was strategic to check whether particles related to this industrial source are present also in the ultrafine range. This seems to be true as particles containing Fe and small amounts of Zn and Mn were detected and also particles rich in Zn. Particles containing Sn and spherical particles containing Sb were also detected. Sb was foud also in association with Zn in a particle with a very weak electron diffraction pattern, nonetheless clearly indicating the presence of Zn in oxide form (Figure 4.3.32 Figure 4.3.33). It is not surprising to find antimony-containing particles in the submicron range and in a spherical shape, as the emission of this element is associated with high-temperature processes and in particular with fossil fuel and waste combustion (Christian *et al.* 2010; Filella *et al.* 2009; van Velzen and Langenkamp 1996). As antimony has a low boiling point it is likely to be emitted as submicron particles for instance by small industrial facilities (García *et al.* 2006). This could suggest an apportionment of the particle to the steelmaking plant which might be supported by the presence of zinc in the same particle (appendix D) and by the fact that this particle was detected at site B which is very close to the plant. Tha rare detection of Sb-containing particles supports the data obtained from bulk deposimeters which did not signal excess Sb concentration in any of the monitored sites (www.appa.provincia.tn.it/news/pagina199.html).



Element	Weight %
Al_2O_3	0.8
SiO_2	4.4
Cl_2O	1.4
Fe_2O_3	0.8
CuO	2.3
ZnO	32.6
Sb_2O_3	57.6

Figure 4.3.32 TEM micrograph of an Sb-rich particle with relative EDX spectrum and SAED pattern.

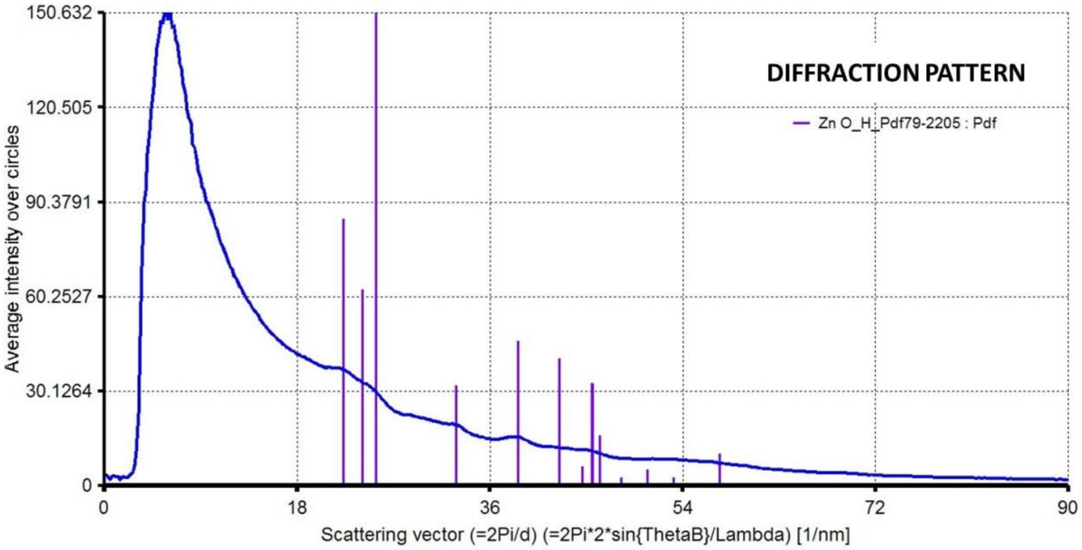


Figure 4.3.33 Diffraction pattern extracted from SAED analysis of the particle in Figure 4.3.32 showing the presence of ZnO.

4.4 *Discussion and suggestions for future work*

This case study represents another proof of the support that unconventional monitoring techniques can provide in complex situations. In particular, it showed that it is possible to infer information about:

- how large was in the past and it is nowadays the area of impact of a stationary emission source;
- the relative significance, and its eventual change in time, of process and fugitive emissions for the air quality around an industrial plant.

These two aspects will be thoroughly discussed in this paragraph for what concerns this case study. Furthermore, this research highlighted the potential and different characteristics of two "unconventional" samplers of PM: conifer needles and building facades. The advantages and drawbacks of the two systems are summarized in Table 4.4-1.

Table 4.4-1 Comparison between conifer needles and building facades as passive samplers of air pollutants

CONIFER NEEDLES	BUILDING FACADES
Temporal information is always available: different time averages can be defined and continuous monitoring is possible (conifer needles can be readily and repeatedly collected from trees at a selected location)	Precise temporal data are rare; unless there are chronologically defined layers, only one time average can be defined
The maximum temporal limit is species-dependent (e.g. 11 years for Norway spruce, 3 years for Scots pine)	They can go as back in time as their construction date (Roman buildings…) given that they did not undergo any later conservation treatment
Conifers are diffuse in all the Northern hemisphere but they are not always present in urban contexts	They are present in urban areas in any climatic region
The substrate is organic (it does not interfere with the study of inorganic PM)	The substrate is inorganic (it can sometimes interfere or at least raise data interpretation issues when inorganic pollutants are studied). This is especially true for modern buildings having plaster facades containing heavy metals
They collect: particulate matter (PM), dioxins, dichlorodiphenyltrichloroethane (DDT), polychlorinated n-alkanes (PCAs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons	They collect: particulate matter (PM), polycyclic aromatic hydrocarbons, nitrates, sulphates
collaboration and exchange of information is possible with scientists studying forest health and plant response to environmental pollution	collaboration and exchange of information is possible with architects and conservation scientists studying decay mechanisms of building materials or planning preventive conservation strategies and conservation treatments

Information on far past come exclusively from the SEM-EDXS analysis of the building samples. They showed that particles emitted by the EAF steelmaking plant are present at all investigated sites both in urban and suburban areas (Figure 4.4.1). The detection of steelmaking emission particles in the historical center is in agreement with the fact that since 1995-6 the steelworks is working mainly at night when the wind blows along the main valley direction from the plant to the town (www.appa.provincia.tn.it/news/pagina199.html). EAF emission particles were identified also on the builing at the sport centre area of Borgo Valsugana that showed no steelworks-related organic contamination (www.appa.provincia.tn.it/news/pagina199.html). The fact that these particles were found on top of the gypsum layer characteristic of the sample VB witnesses the existence of other major sources of air pollution before the beginning of the activity of the steelmaking plant. In particular, sources of SO_2 had to be present on the territory.

Only combining the information obtained from the analysis of building samples with that obtained on the co-located spruce needles it is possible to exclude that the deposition of the EAF particles is recent. SEM-EDXS analysis of particles on the 2012 needles collected at the sport centre observed a combustion ferrosphere poor in silicon and aluminum that can be apportioned to the steelmaking plant (Ault *et al.* 2012). This observation suggested that even recently low amounts of particles emitted by the plant reach the site. Similarly, since steelmaking-related particles were detected both on the samples from the building in site O and among the particles removed by washing the surface of 2008 needles collected nearby, it cannot be excluded that this kind of particles were emitted in recent times. However, no combustion particles that could be apportioned to the steelmaking process were found in the same samples of needles.

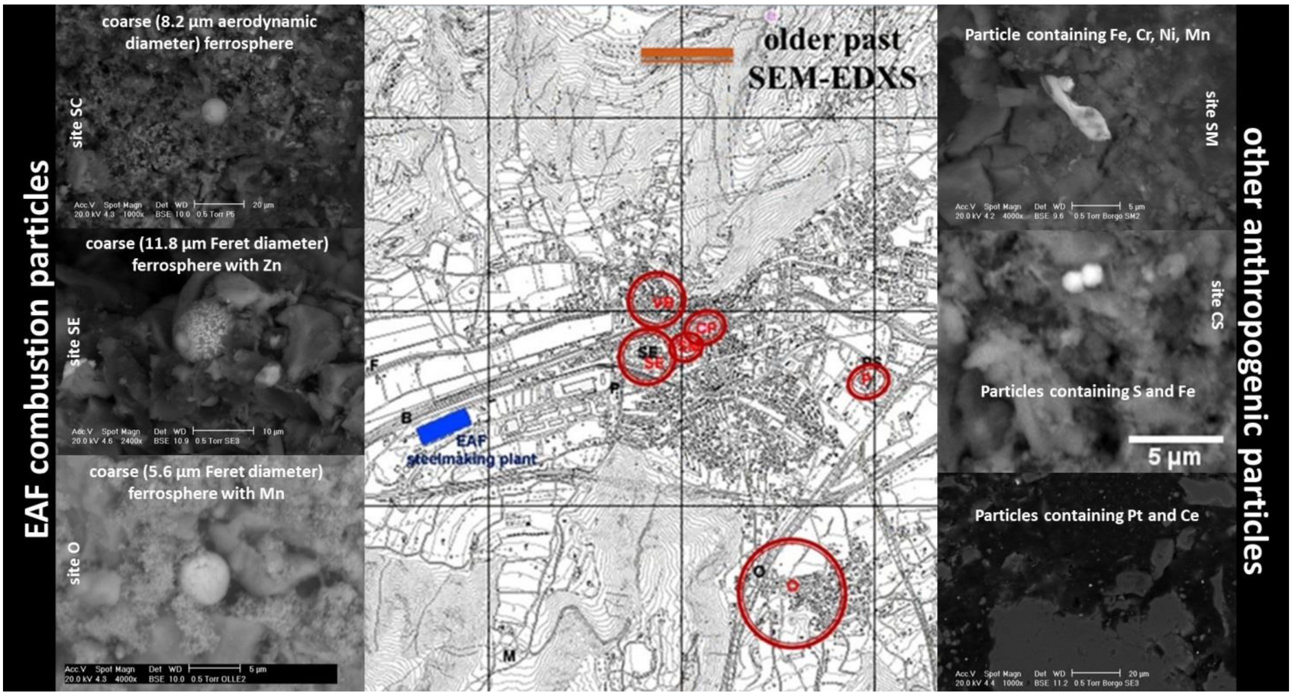


Figure 4.4.1 Map of the EAF steelmaking plant impact based on building results and examples of particles detected on building samples. A larger circle indicates that more particles that can be apportioned to the steelmaking plant were detected at the site by electron microscopy. The buildings have been exposed to the atmosphere for different time periods.

Recent time periods including years before 2009, when the plant adopted the best available technologies for the control of emissions, were studied through spruce needle analysis.

ICP-AES analysis of current year needles suggested that the steelworks contribute to PM only at site B and in lower amounts at sites L and P. The elements present in PM at sites M, F and O suggest natural sources at these sites. At first, Cu was used to hypothesize a contribution from traffic (appendix C). Cu seems to be present at the same sites where there is an influence from the steelworks with higher concentration at site B and lower in order at site P and L. It must be highlighted in providing these conclusions that the needles collected at site B and O have been exposed for about one year and a month, while those collected at the other sites have been exposed for a few months. Hence, the higher concentration of contaminated particles at site B could be a result of a longer exposure.

ICP-AES analysis of needles exposed to the atmosphere for multiple years provided time weighted averaged elemental compositions of PM deposited on the needles. The needles collected at site B and O have been exposed to the atmosphere for about five years and a month, while those collected at the other sites for about four years and two months. This biased the results as in the case of current year needles. Given that principal component analysis (PCA) highlighted that Zn is emitted by multiple sources in the studied area, Mn concentrations were used as tracers of the emissions from the steelworks. Differently from current year needles, it seems that all the sites besides O are affected by the presence of the plant in this decreasing order: B, P, M, L and F. Using again Cu as a tracer for traffic emissions an unlikely scenario where traffic is also present at site F is derived. This leads to believe that other sources of Cu in the environment exist. Even the steelworks might contribute to Cu contamination as in the PCA Cu concentration contributed to the same factor as the elements characteristic of steelmaking (Table 4.3-7).

It must be reminded here that we are likely underestimating atmospheric concentrations on the needles both because it was shown by Nieminen *et al.* (2004) that the washing procedure does not remove all the dry deposition contribution and because an open-vessel digestion procedure was utilized (see paragraph 3.4). In addition, a steelmaking plant is a difficult source to be traced through bulk analysis with reference to elements such as Cr. Pöykiö *et al.* (2000) stated that lower concentrations of Cr than real ones are often measured as a consequence of the fact that this element is present in the dust emitted by steelmaking plants as $FeO-Cr_2O_3$ that is very difficult to digest.

Another issue experienced in this work is related to ICP-AES calibration: in certain samples (2008 and 2012 needles from site P, 2008 needles from site F) Fe and Zn concentrations were above the instrument detection limit and after dilution, although a concentration was measured, this was below the limit of detection or the limit of quantification. This could be solved by preparing standards with higher concentrations of these elements so that the instrument detection limit is not exceeded in first place and quantification is possible.

Taking as tracers of process (primary and secondary) emissions spherical particles with Fe or Zn as main component and eventually containing also Mn, Cr, Ni, Pb and Cu, it can be concluded that their presence is less significant in needle samples that have been exposed to the atmosphere only during spring 2012 than in needles exposed for a longer period (2008-2012/13). This may be due either to the temporary shutdowns of the production in recent years, or to the improvements in the off-gas abatement system undertaken in 2012 or to a season being a not sufficiently long period to collect representative data by manual single particle analysis. Unfortunately, the data are also partially masked by the different exposure time of the needles in the different samples.

We seize here the opportunity to support the statement already made by Willis *et al.* (2002) that manual analysis has a too low particle throughput to guarantee a statistically representative analysis of numerous samples such as those necessary for environmental monitoring. Therefore, we recommend, whether possible, the use of computer controlled systems for quantification of the differences among samples. In this context, it could be taken as a reference that Deboudt *et al.* (2008) experimentally demonstrated that it is necessary to analyse by CCSEM-EDXS minimum 500 particles to obtain representative size distributions and 1000 particles for representative chemical description. We also suggest if a CCSEM-EDXS is available to program a dedicated particle-search by identifying on a sample apportioned with high certainty to the source under investigation the brightness level for the particles of interest in backscattered electron mode and setting it as a minimum threshold for object analysis.

Fugitive emissions could be considered coarse particles with element composing the raw materials and irregular morphology as they should be detached fragments. Particles having Feret diameter larger than 10 µm containing elements characteristic of steels were found only at site B (many) and at site L (2 particles). The two particles found at site L may have a different source as this site is quite far from the plant to be affected by fugitive emissions. Particles having Feret diameter in the range 2.5-10 µm and composition that may suggest a relationship with the steelmaking process are found at site B, P, F, M, L. At site B they are found on 2005, 2008 and 2011 needles, with coarser size on the 2011 needles. At site F on 2008 needles, at sites L and P mainly on 2008 needles, while at site M on 2012 needles. Smaller fragments but larger than 1 µm were detected on 2008 needles at site B (4 particles), 2008 needles at site M (1 particle), 2012 needles at site P (3 particles) and on 2008 needles at site O (1 particle). Since even site O is reached by particles with steel elements and Feret diameter in the range 1-2.5 µm it is likely that this size range includes process rather than fugitive emissions. From this data it seems that fugitive emissions were more significant in the past at all sites except B and M. In B they have been always present while in M they recently increased.

From previous consideration it can be concluded that both from bulk (Figure 4.4.2) and single particle analysis (Figure 4.4.3) it seems that the area of impact of the plant has been reduced over the years.

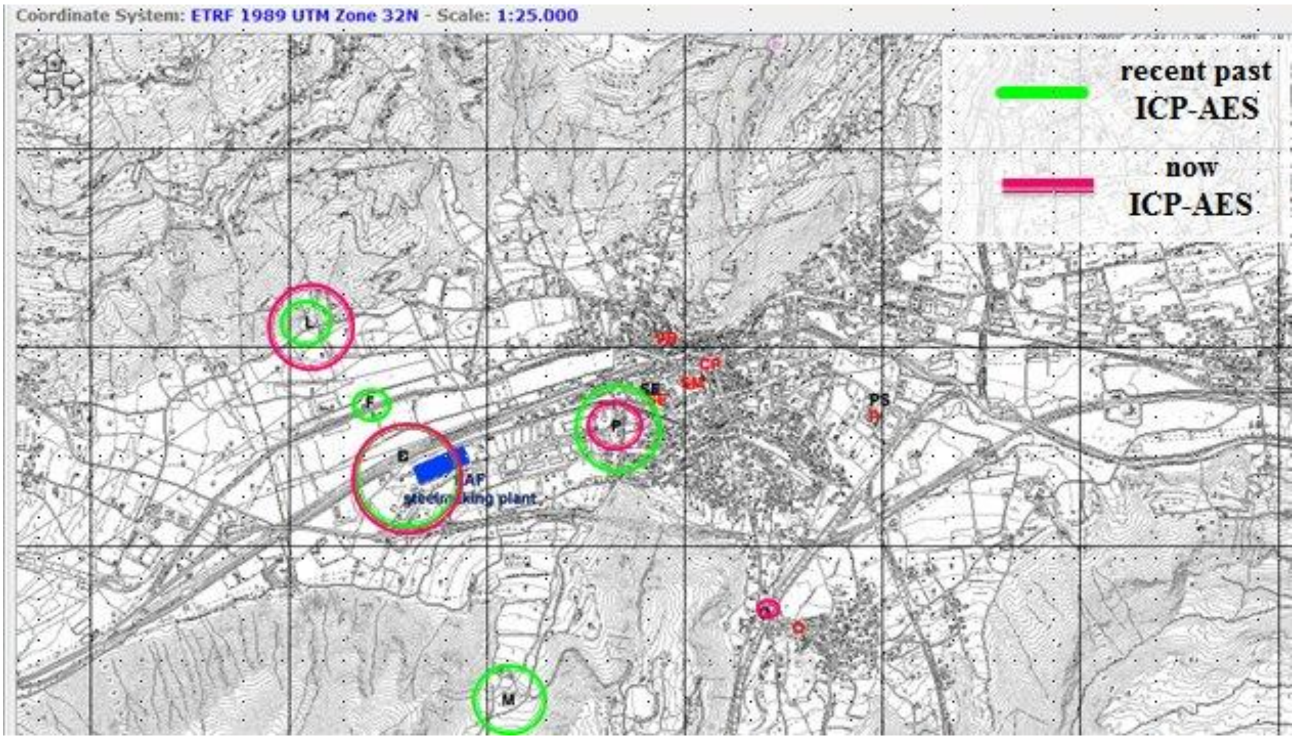


Figure 4.4.2 Map (edited from Carta Tecnica Provinciale) of the impact of the EAF steelmaking plant hypothesized on the basis of ICP-AES analysis of conifer needles. Recent past: needles belonging to the 2008 age cohort (no matter their exposure time). Now: needles belonging to 2012 age cohort (no matter their exposure time). A larger circle indicates a higher concentration of Mn, Fe and Co for recent past, a higher concentration of Mn, Fe and Cu for current samples (now). 2012 age cohort was not analysed at site O.

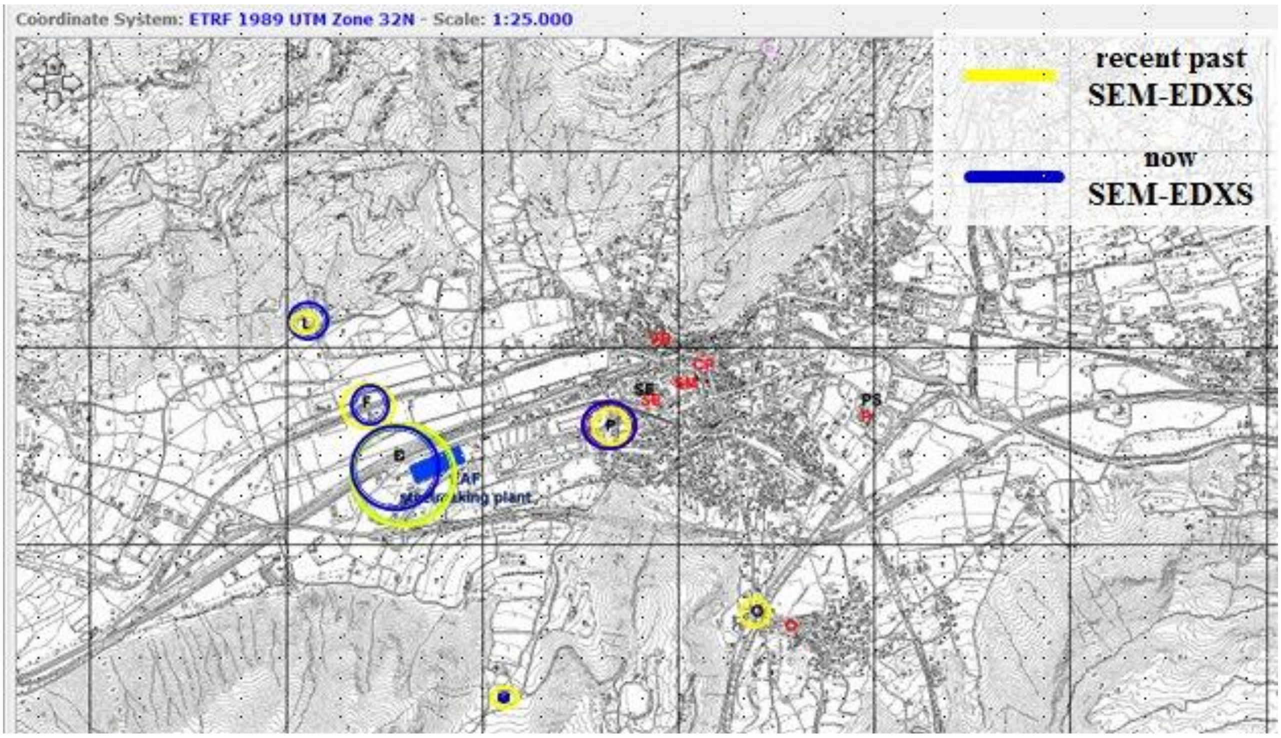


Figure 4.4.3 Map (edited from Carta Tecnica Provinciale) of the impact of the EAF steelmaking plant hypothesized on the basis of SEM-EDX analysis of conifer needles. Recent past: needles belonging to the 2008 age cohort (no matter their exposure time). Now: needles belonging to 2012 age cohort (no matter their exposure time). A larger circle indicates that more particles that can be apportioned to the steelmaking plant were detected at the site.

A decrease in the size of the particles reaching farther locations was observed in building samples with SEM-EDXS. TEM-EDXS was then used to check whether it would be possible to extend the area of impact of the plant by detecting ultrafine particles which are known to be able to travel longer distances. While close to the site (site B, 120 m far from the plant) ultrafine particles containing Mn were found, at further locations (sites L – 1000 m from the plant - and PS – 2370 m from the plant) no particle contained this element. Zn-rich ultrafine particles, however, were found also at the farthest sites investigated (sites L and PS). No combustion particles seem to be present in the ultrafine range, besides an Sb-rich subspherical particle found at site B. Further investigations are required to make sure that there are no other sources of ultrafine Zn-rich particles and it would be good to characterize the ultrafine component of the EAF dust of the specific plant in order to be able to compare the morphology of the particles found in the environment with that of particles apportioned with certainty to this industrial activity.

Both building and spruce samples suggested that traffic is a source of particles at many sites and that these samplers are suitable for the study also of this type of source as it was possible to identify particles from brake and catalytic converter wear. The possibility to investigate the issue of catalytic converter-emitted PM, at least through single particle analysis[25], is particularly significant as

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[25] The analysis of platinum group elements (PGEs: Pt, Pd, Rh) is challenging (Bencs *et al.* 2003; Djingova *et al.* 2003; Godlewska-Żyłkiewicz 2004; Niemelä *et al.* 2004; Petrova *et al.* 2010) as:

- spectral interferences with other transition metals could obstacle the determination of PGE concentrations in the needles
- the compounds of PGEs easily undergo transformations even with slight changes of conditions (pH, temperature, etc.):
    - Pt and Pd tend towards hydrolysis and it was observed that standard solutions are not stable as new species form even after only 2 hours;

catalytic converters contribute to the environment elements rare in nature (Pt, Rh, Pd) whose effect on the environment and humans is still unclear. Particles from brake wear can be divided into two groups: particle with Feret diameter of 2-3 µm containing only elements from brake lining materials (Cu, Ba, S, Zn, Ti, K) and coarser particles in which there is also Pt from the catalytic converters. The larger size would suggest a different process for the formation of the particles in the wear mechanism, such as mechanical abrasion. The smaller sized-particles are in agreement with the fact that in Italy brake pads lining commonly contain $BaSO_4$ and their wear particles might be of 2-3 µm of diameter (Thorpe and Harrison 2008). It has to be highlighted that the presence of Zn, Ti or K alone does not allow the apportionment to brake wear as these elements may be emitted by many sources (see Appendix C).

This case study highlighted also that source apportionment of combustion spherical particles is very difficult. Most of the spheres detected in this work were Fe-rich (Figure 4.4.4). The literature apportion ferrospheres to high-temperature technological processes using Fe-containing raw materials or burning Fe-containing fuels. Therefore, the EAF steelworks in Borgo Valsugana very likely emits these particles considering it uses steel scraps and anthracite as raw materials. Other factors supporting the apportionment of the detected ferrospheres to the EAF plant are that many had low Si and Al content (Figure 4.4.4) (Ault *et al.* 2012), that some contained also Zn, Mn, Cu, Pb and Cr (Magiera *et al.* 2011) and that some of the finest were observed in agglomerates (appendix D). To our knowledge, no other sources of these particles, such as power plants or other industrial facilities, are present on the territory, thereby all the ferrospheres, except the few very coarse ones found too far from the source, were apportioned to the EAF emissions.

  - Pt and Pd tend to be adsorbed onto the wall of storage vessels and to undergo reduction of their oxidation state. Polystyrene and quartz containers are recommended for storage and mineralization of the samples. However, even with quartz vessel it is necessary to employ multiple steps washing treatments between a sample preparation and another one as wall memory effect was observed
  - the ease of transformation favors analyte loss or biases in the measurements due to alterations of standard solutions used for instrument calibration
- catalyst-derived particles carrying PGEs are in a form difficult to digest
- Pt compounds have low reactivity towards certain chemicals, thus they require long reaction times and drastic reaction conditions
- Pt and Pd are chemically similar, thus difficult to separate
- their concentrations are not provided in the certified reference materials of the matrix of interest (NIST 1575a Trace Elements in Pine Needles) used for the determination of the accuracy of the measurement.

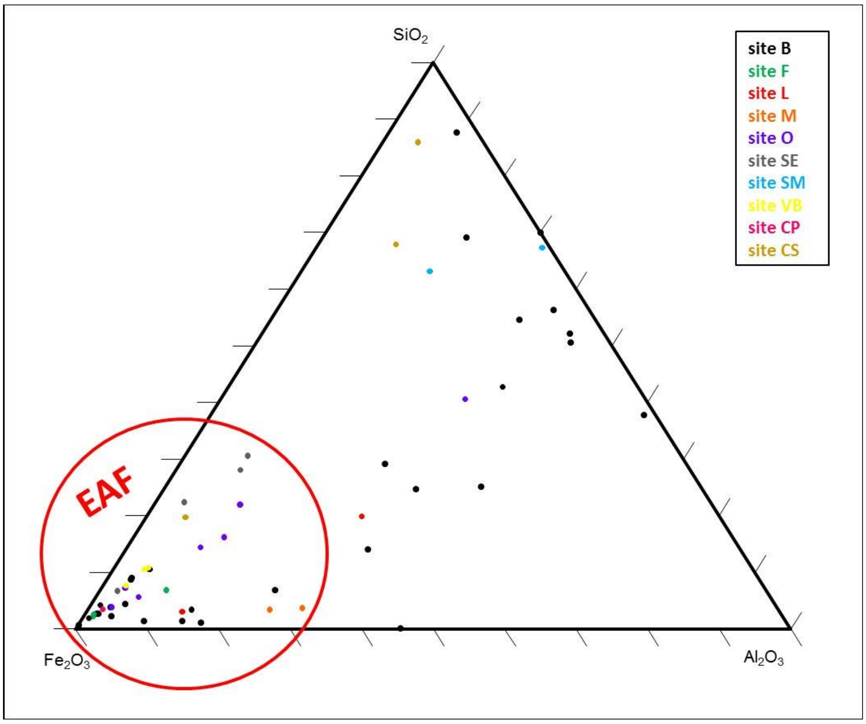

Figure 4.4.4 Ternary diagram of the composition of combustion spheres detected in the case study. The diagram was produced using the Excel sheet developed by Graham and Midgley (2000) and EDXS concentrations of Si, Al and Fe expressed as oxides and normalized to 100.

A small fraction of the spherical particles was apportioned to wood combustion on the basis of the presence in them of 2-6 wt% of potassium (measured as K_2O by EDXS) (Bucko *et al.* 2011; Tissari *et al.* 2008). Interestingly, most of these spheres were identified mainly at the closest site from the plant (B) which leaves open the hypothesis that they are also emitted by the plant and not by wood burning.

From this apportionment it looks like the EAF steelmaking plant is the dominant source of combustion particles (Figure 4.4.4). However, it can be inferred from Table 4.4-2, taken from the work of Kochbach Bølling *et al.* (2009), that most wood combustion appliances emit organic rather than inorganic particles.

Table 4.4-2 Emission characteristics for the different classes of wood combustion appliances (from Kochbach Bølling *et al.* 2009)

Type of combustion source	Particle class(es) dominating the emissions
open fireplaces	organic carbon/soot
conventional wood stoves	organic carbon/soot
masonry heaters	organic carbon/soot
conventional boilers for wood logs	organic carbon/soot
modern wood stoves	inorganic ash/organic carbon/soot
modern boilers for wood logs	inorganic ash/organic carbon/soot
pellet stoves and boilers	inorganic ash

A better evaluation of the contribution of wood combustion to local air pollution requires the study of organic particles, nonetheless wildfires can be excluded as significant sources given the limited number of events recorded in the past years in the area (Figure 4.4.5).

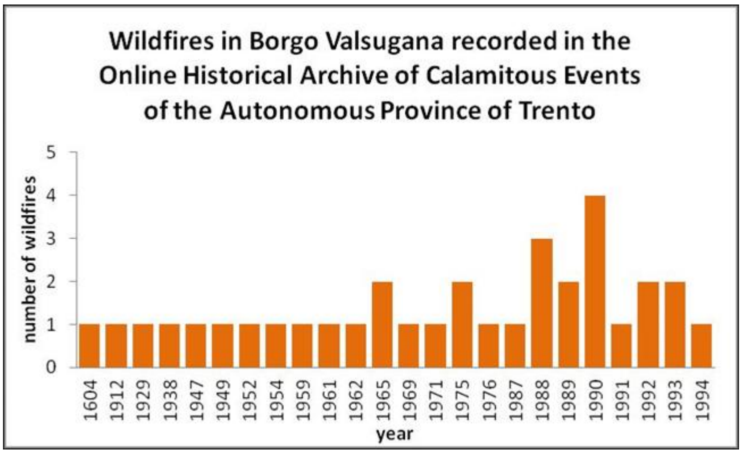

Figure 4.4.5 Number of wildfires in Borgo Valsugana (TN) from 1604 to 1994 (194.105.50.156/arca/).

Regarding inorganic spherical particles, more studies comparing combustion spheres from specific sources and identifying diagnostic morphological, chemical and crystallographic features are required to assist in the apportionment of particles collected in the field in multi-source situations.

This work alone cannot be considered conclusive on the effectiveness of the improvements of the off-gas abatement system in contributing to a good air quality in Borgo Valsugana. It is reminded here that bioavailability of the elements was not investigated, thus even if the data would have been quantitative, which are not because of the unconventional samplers used, no information on the real potential hazard caused by the presence of certain metals could have been provided. Magiera *et al.* (2011) suggested that in Fe-rich fly ash if a metal is bound in the crystal lattice has low mobility while if it is adsorbed on the surface of iron oxides or connected with the amorphous phase of iron oxides or hydroxides it is more bioavailable. Therefore, we believe that TEM-EDXS-SAED could provide important information through crystallographic data if samples from a specific source (the EAF plant) are characterized. However, from samples coming from the field and having a variety of sources we believe that the conventional sequential extraction procedures or chemical speciation analysis employed on PM samples collected on filters (Feng *et al.* 2009) are better.

Future developments of this study could be the selection of a certain number of spruces as samplers to collect needles at regular time intervals and the employement of magnetic measurements as another tool to correctly apportion the particles to the EAF steelmaking plant (Hansard *et al.* 2012).

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5 Concluding remarks and suggestions for further work

The study was set out to contribute to the improvement of the characterization of air pollutants and has identified particulate matter as the object of the investigation considering that a better definition of its properties in relation to their impact on human and environmental health may lead to better regulating, thus managing, air quality, as well as to better develop technological solutions for emission reduction (paragraph 2.1). The study has also sought to advance the field of passive sampling of particulate matter as a way to monitor wider areas and face the costs of active sampling. In particular, by exploiting existing surfaces exposed to the atmosphere (conifer needles and building facades) passive sampling becomes a way to reach also remote locations and obtain everywhere information on past air quality, even if no instrumental monitoring stations were there installed. The literature on this subject, and specifically on the use of conifer needles, is limited to the evaluation of metal concentrations on the whole sample, for instance through inductively coupled plasma atomic emission spectroscopy (ICP-AES). This leads to incur in the same problem as for official PM monitoring of obtaining bulk data averaged over a population of particles which may be composed of individual units with different origin, dimensions, composition, morphology, crystal structure, thus different chemical and physical properties (Bertolotti and Gialanella 2014). Hence, this study was specifically aimed at evaluating whether combining data collected by electron microscopy and related spectroscopies on single particles with the bulk information obtained from the same passive sampling strategy would offer a more complete characterization of the pollutants leading to a better source apportionment of the particles (paragraph 2.2). The case study presented in chapter 4 well exemplified the stated research questions. The case study regarded the monitoring of a single source: an electric arc furnace steelmaking plant. Being an industrial source, characterized by emissions at different heights from the ground and in a sector were raw materials and production technology employed make every monitoring project a single case, it required monitoring a wide territory. Monitoring a single source covering a large area would be expensive by active sampling. As the plant is located in the bottom of a valley, the wide territory includes mountainous areas and is affected by a complex meteorology. The use of conifer needles allows the investigation of these locations. From the beginning of the operations of the plant the off-gas abatement system and the production cycle have changed, but before emission regulations became more stringent no measurement at receptors were undertaken and even those at the stacks were accomplished at irregular time intervals. Inferring information on the past scenario would offer a complementary tool to the evaluation of the effectiveness of the improvements in the emission control strategies undertaken. Hence, the temporal complementarity of conifer needles (recent past) and building facades (older period) seemed a suitable monitoring strategy.

The main experimental findings related to the development of a new streamline analytical method for the characterization of PM deposited on conifer needles were summarized within chapter 3, while those specific to the case study were discussed in chapter 4. This section will synthesize what emerged from both in relation to the above discussed aims.

Concerning the use of conifer needles this study highlighted the importance of collecting the samples of different age cohorts and different sites on the same day to be able to define temporal and spatial trends of pollutant dispersion. The pattern identified in the case study cannot be taken as definitive indication of pollution reduction, as it was proven that it is not possible to confidently estimate bulk elemental concentrations that would have been measured if needles were sampled on a different sampling campaign. The sampling requirement becomes than strict also considering the difficulty of extending the results from samples collected at one point to larger areas given the heterogeneity of wide territories (Wagner 1995), existing as well using other samplers.

ICP-AES was confirmed a good technique for the bulk characterization of the needles and the contaminants they collected. It is multi-elemental and sensitive enough to detect most pollutants. This study highlighted that special care must be taken in data interpretation due to the concentration subtraction technique. Even the enrichment factor approach with respect to a background location has to be applied on both total and atmospheric concentrations.

Electron microscopy was confirmed a successful tool to completely characterize individual particles for their size, shape and chemistry. As already highlighted by other Authors, such as Kemppainen *et al.* (2003), it was also confirmed a good tool for source apportionment in a multi-source scenario, where particles from steelmaking emissions, wood combustion and traffic were distinguished in the case study. The origin of some particles, however, was identified with high uncertainty. This suggests that the possibility to accomplish source apportionment of particles collected in the field by electron microscopy would benefit from the development of atlas with micrographs and relative EDX spectra for particles taken directly at different sources.

The advantages offered by the multi-technique approach emerged when the atmospheric provenance of an element was just suggested by a single data interpretation approach of ICP-AES results. The correct information was selected using electron microscopy verifying whether particles containing that element were present on the surface of the needles. If particles containing the element are detected on the surface of the needles or in the residual washing solution of the needles, then the element could have reached the needle from the atmosphere. Another positive outcome of the combination of ICP-AES and electron microscopy is related to the great screening capability of the latter, able to identify particles in which metals in low relative concentration are present. For instance, in a few samples, Ni- or Pb-containing particles were detected by SEM-EDXS although their concentrations were below the detection limit of ICP-AES.

The case study in which the emissions of an industrial point source were studied both in a temporal and in a spatial frame is an example, but not the only possible field of application. As elements and particles apportioned to traffic were identified, it is here suggested that linear sources might be investigated through this sampling approach as well. The large diffusion of conifers on the territory would allow the collection of field data, for instance, to evaluate the effect produced by being at different distances from major roadways. This would be in agreement with the tendency in current epidemiology studies to abandon central monitoring strategies in favour of measurements of personal exposure (Grahame and Schlesinger 2007).

The main issues emerging from the developed method and application in the field are the difficulty for ICP-AES analysis of distinguishing between root and atmospheric element uptake. The washing treatment conducted on a subsample for the determination of the element amount taken up from the roots may be biased by the natural heterogeneity of the needles and an incomplete removal of the wax and deposited materials from the surface. The use of electron microscopy to validate or solve doubts raised by the bulk technique was not always successful. For instance, in our case study Co and Sr seemed a recurring exception to the good agreement and complementarity between the data obtained from the two techniques. ICP-AES suggested an atmospheric provenance for these elements in certain samples, but no particles containing them were detected by electron microscopy neither in coarse and fine range, by SEM, nor in the ultrafine range, by TEM. Different strategies could be adopted in these situation: the analysis of co-located soil samples to verify the enrichment of the needles in the elements of interest as a consequence of atmospheric deposition; the exposure of plants grown in a greenhouse at a subset of monitoring sites to evaluate experimentally soil contribution following the approach suggested in the VDI 3957 Blatt 5 (VDI 2001); the analysis of co-located samples of mosses or lichens, known to take up elements exclusively from the atmosphere.

The second issue in the application of the proposed method is related to the current qualitative nature of electron microscopy data, which limits the possibility to compare sites and validate or even improve the dispersion models suggesting sources that were not taken into account in their development. Obtaining quantitative data is the direction in which there are more opportunities to advance this work. The first step would be furthering the standardization of the sample preparation procedure and selection of the area for analysis by electron microscopy. The use of filters and membranes on which particles washed away from many needles are deposited guarantees higher sample representativeness than the analysis of the particles on a single needle. Developing a filtration procedure or apparatus which guarantees homogeneous particle deposition on the filter is the first step to design an area selection criteria whose analysis allows the extension of the results the whole filter surface. Making possible to obtain quantitative data from PM sampling through conifer needles, would truly set these biomonitors in the passive sampler category. A role for instrumental passive samplers in survey studies for the strategic selection of the sites in which active samplers of the official monitoring network should be placed was already proposed by Ott (2007) and the Author feels that the large diffusion of conifers on the territory would be an attractive feature of their application in this research area.

Other aspects deserving attention to develop an even more extensively applicable method, are the comparison of ICP-AES results with those from other bulk analytical methods with simpler sample preparation, such as x-ray fluorescence, the automation of SPA using suitable electron microscope attachments and the obtainment of satisfactory statistical reliability of the collected data, to be effectively used in multivariate statistical analysis.

Using conventional sampling approaches guarantees to be able to evaluate compliance with air quality regulations and often allows the comparison of the data obtained in a context with many other as the sampling, analytical, and quality control protocols are well defined. However, the definition of these protocols and regulations as well could definitely benefit from using unconventional sampling approaches and analytical techniques, such as electron microscopy, that are at the moment only research instruments. This work showed how conifer needles could fill the gaps in conventional monitoring given by the absence of data before the installation of a monitoring station and by the difficulty in bringing active samplers to remote locations and in spreading them enough on the territory to have data proving the predicted dispersion and deposition of pollutants. This work was also a first step to show that a better standardization of micro-analytical methodologies for the characterization of airborne inorganic pollutants collected on unconventional substrates could lead to the obtainment of quantitative data complementing those obtained with conventional approaches.

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6 Appendices

6.1 Appendix A: Guidelines, standards and regulations related to this research

This appendix summarizes existing guidelines, regulations, recommendations, standards and official methodologies relevant to this thesis work. Guidelines and recommendations are to be intended as informative documents issued by an institution or a group of scientists working in the field as a result of research work or as a basis for interlaboratory collaborations to guarantee the comparability of the data. Regulations are issued by states, unions of states or local authorities delegated by their state to issue the regulation. Standard methods are developed and agreed by "members of a standard-setting organization (e.g. ASTM) which operates on a consensus principle, i.e. all members (and sometimes non-members) who have an interest in the product, including producers, government officials and consumers are permitted to have input into the generation of the standard" (Dux 1986). Official methods "are produced by an organization (e.g. government agency or professional society) with a mandate of some sort to produce testing methods" (Dux 1986). The government agency that provides more methods in the field of environmental analysis is the Environmental Protection Agency (EPA) of the USA and often this thesis used the analytical protocols developed by this agency as a reference. If a standard method is adopted by a government agency it becomes official.

Regulations and guidelines cited or employed in this thesis

Regulations and guidelines on air quality

The first standard to mention with the purpose of using the correct terminology, is the vocabulary defining the most important terms to be used in the field that was published in 2005 by ASTM International, formerly known as the American Society for Testing and Materials (ASTM), under the name **ASTM D1356 "*Standard Terminology Relating to Sampling and Analysis of Atmospheres*"** (ASTM2005). For the same purpose it is important to refer to the regulation issued by the International Organization for Standardization (ISO) and adopted in Italy by the Ente Nazionale Italiano di Unificazione (UNI) under the name **UNI ISO 7708 *Qualità dell'aria – Definizioni delle frazioni granulometriche per il campionamento relativo agli effetti sanitari*** (UNI1998) which provides the scientific terms of the inhalable fraction of PM and its subfractions (thoracic, respirable and tracheobronchial) in four languages: English, German, Italian and French.

The **World Health Organization (WHO)** issued in 2005 the ***Air Quality Guidelines*** (WHO 2006) that, summarizing the available scientific knowledge, aimed at serving as a worldwide basis to implement environmental policies aimed at the reduction of the impact on human health of air pollution, in particular of particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Regarding PM, the guidelines highlight that no complete protection against its drawbacks can be guaranteed by regulations. Nonetheless, limit values are provided on the basis of studies using PM_{10} and $PM_{2.5}$ as indicators (PM_{10} includes also $PM_{2.5}$) and a $PM_{2.5}/PM_{10}$ ratio of 0.5 which is close to that observed in urban areas of developing countries and at the bottom of the range (0.5-0.8) measured in urban areas of developed countries. Two limits are given one annual and one for 24 hours. The second one serves to avoid dangerous peaks of pollution. An annual limit of 10 $\mu g/m^3$ is given for $PM_{2.5}$ to reduce, although not to eliminate, health risks. The guidelines suggest also target values to gradually reach the desired reduction of pollutants. $PM_{2.5}$ has this series of target values steps: 35 $\mu g/m^3$, 25 $\mu g/m^3$ that would reduce risks of long-term exposure by about 6% relative to the first target value and 15 $\mu g/m^3$ that would reduce risks of long-term exposure by about 6% relative to the second target value. In the document, no guidelines on the concentration of ultrafine particles are given, as the existing epidemiological knowledge is not sufficient to establish a clear relationship between exposure to these particles and adverse health effects. In this booklet references to many existing regulations are also given. In particular, it is reported that only four institutions, the European Union, the United States Environmental Protection Agency (EPA), the State of California and Japan, established emission limits in grams per kilometer or grams per mile for pollutants (carbon monoxide, nitrogen oxides, VOCs and particulate matter) emitted by road vehicles sold inside their territory of competency. The limits were not established testing each new vehicle but testing some samples of a new model submitted by manufacturers on rolling roads by simulating a standard driving cycle. Generally, neighboring countries, such as Canada for the US and European non EU countries for EU, adopt the same measurements.

The European Parliament and the Council of Europe issued in 2008 the **Directive 2008/50/EC** on ***ambient air quality and cleaner air for Europe*** (EU 2008), which has been adopted by Italy through the **Decreto legislativo 13 agosto 2010, n.155** (Decreto Legislativo 2010). This directive highlights the importance to have standardized measurement techniques and criteria to have representative and comparable data on air quality. In Italy the task of guaranteeing the compatibility of the data acquired in all the countries and to promote the development of standardized sampling and analytical protocols is assigned to the Agenzia per la protezione dell'ambiente e per i servizi tecnici (APAT) (2003). The European directive indicates the need to characterize and regulate fine particulate matter ($PM_{2.5}$) as it has a significant adverse effect on human health and no threshold is identifiable below which it is not harmful. Although, to protect the larger part of the population a reduction of $PM_{2.5}$ concentrations in urban areas is hoped, it is also highlighted the need of measurements of fine particulate matter at rural background locations. The measurement of $PM_{2.5}$ includes the total mass concentration and the concentrations of the most suitable compounds to determinate its chemical composition and always the concentrations of these chemical species: SO_4^{2-}, NO_3^-, Na^+, K^+, NH_4^+, Cl^-, Mg^{2+}, Ca^{2+}, elemental carbon (EC), organic carbon (OC) (Italian d.lgsl). The standard methods for the measurement of the regulated pollutants are provided both in the directive and in its Italian adoption. In this directive the standards to follow to measure the regulated pollutants (PM_{10}, $PM_{2.5}$ and Pb/Cd/As/Ni) are also mentioned. The directive allows member states to use other methods but their equivalence with those described in the standard has to be demonstrated.

In the **United States of America** PM is also regulated based on size as there is not sufficient scientific evidence to exclude certain components as not hazardous or to identify certain else as the clear cause of adverse health effects. The importance of conducting research aimed at identifying the association between health effects and various fine particle components and types of source categories was highlighted (EPA 2006). Chemical speciation of suspended PM is needed for the US EPA's $PM_{2.5}$ monitoring program as set forth in the 40 CFR part 58 "Ambient Air Quality Surveillance for Particulate Matter" (old standard) while PM_{10} chemical speciation may be desirable, but not required (Chow and Watson 1998). It is interesting to note that although in Europe $PM_{2.5}$ standard is not regulated on a 24-hour base (Table 6.1-1), EPA states that "there is a growing body of studies that provides additional evidence of effects associated with exposure periods shorter than 24-hours" and established both 24-hours and annual limits (Table 6.1-1) (EPA 2006).

Table 6.1-1 Limit values for PM_{10} and $PM_{2.5}$ established by European Union (2008/50/EC) and American regulations (71 FR 61144, Oct 17, 2006)

pollutant	Europe	USA
PM_{10}	**24-hour** average: 50 µg/m^3 not to be exceeded more than 35 times in any calendar year **annual** average: 40 µg/m^3	**24-hour**: 150 µg/m^3 not to be exceeded more than once per year on average over a three-year period masured at each monitor within an area **no annual** standard (the available evidence does not suggest an association between long-term exposure to coarse particles and health effects)
$PM_{2.5}$	**annual** average: 25 µg/m^3 (to be met by January 1, 2015) and 20 µg/m^3 to be met by January 1, 2020 (this will be reviewed in 2013 in the light of further information on health and environmental effects, technical feasibility, etc.)	**24-hour**[26]: 35 µg/m^3 98th percentile, averaged over 3 years **annual**[27]: 15 µg/m^3 annual mean, averaged over 3 years

Standardized methods and guidelines for the collection and characterization of airborne pollutants

Very general principles regarding sampling of ambient air are given by the standard **ASTM D1357** ***Standard Practice for Planning the Sampling of the Ambient Atmosphere*** (ASTM1995). This document does not describe a specific sampling procedure but highlights which criteria guide the selection of a sampling procedure (statistical, objectives of the study, meteorological and topographical considerations, etc.).

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[26] Primary standard: set limits to protect public health, including the health of "sensitive" populations such as asthmatics, children, and the elderly (http://www.epa.gov/ttn/naaqs/)

[27] Secondary standard: set limits to protect public welfare, including protection against visibility impairment, damage to animals, crops, vegetation, and buildings (http://www.epa.gov/ttn/naaqs/)

In the USA the determination of pollutant metals is standardized by the EPA's *"Compendium of methods for the Determination of Inorganic Compounds in Ambient Air"*, section **IO-3** ***"Chemical species analysis of filter-collected suspended particulate matter (SPM)"***:

- method **IO-3.1** (the European standard EN14902:2005 on the determination of Pb, Cd, As, Ni in PM10 (UNI 2005) says to follow this method for filter cutting): "***Selection, preparation and extraction of filter material***" (EPA 1999). This method suggests how to select the filter media, extract the metals from them and determine their amount:
    - the type of filter medium depends on the purpose of the test and is specified for each sampling method. For high-volume samplers 4 types of filters are recommended: cellulose fiber, quartz/glass fiber, mixed fiber and membrane filters. Filters should: remove more than 99% of suspended PM drawn through them, regardless of particle size or flow rates, be mechanically stable to minimize leaks, not chemically react with the trapped PM, retain their porosity and structure regardless of temperature and not contain high concentrations of target compounds analytes. It was already highlighted by EPA (Chow and Watson 1998) that no single filter medium is appropriate for all desired chemical analyses and it is often necessary to sample on multiple substrates when chemical characterization is desired.
    - microwave or hot acid extraction of 1/9 of the filter. The filter can be hold before extraction maximum 180 days in protective envelopes and after it must be analyzed within 180 days
    - metal analysis by ICP, FAAS, ICP-MS or GFAAS (the results of the analysis have to be multiplied by a factor of 9 to obtain the total µg of each metal present on the entire filter). A blank filter must be also analysed to obtain its background metal concentrations.
- Method **IO-3.4**: ***"Determination of metals in ambient particulate matter using inductively coupled plasma (ICP) spectroscopy"*** (EPA1999b) describes the procedure to analyze the elemental metal components in ambient air PM collected on high volume filter material (TSP or PM_{10} sampler). The analyst should have a minimum of 6 months experience with ICP instrumentation.
    - Hot acid extraction or microwave extraction (see IO-3.1)
    - Preparing calibration curve standards, initial calibration verification standard, interference check standard, laboratory control spike, matrix spike and reagent blank
    - processed ICP-AES data give results in µg of metals/m^3 of air sampled.

In 1995, the **National Institute for Occupational Safety and Health (NIOSH)** published the "Guidelines for Air Sampling and Analytical Method Development and Evaluation". These guidelines should be followed while developing new sampling and analytical methods for the study of airborne pollutants (NIOSH 1995).

Guaranteeing the quality of the measurements conducted in a monitoring campaign is important and standards exist that must be followed for this purpose:

- **UNI EN ISO 14956** ***"Qualità dell'aria – Valutazione dell'idoneità di una procedura di misurazione per confronto con un'incertezza di misura richiesta"*** (UNI 2004) shows how to determine the uncertainty on a single measurement result (average defined in time in dynamic and static conditions) using the performance characteristics of the measurement method and verifying that it complies with the requirements of the measurement itself.
- **UNI EN ISO 20988** ***"Air quality – Guidelines for estimating measurement uncertainty (ISO 20988:2007)"*** (UNI 2007) highlights how air quality measurements (measurements of ambient air, stationary source emissions, indoor air, workplace atmospheres and meteorology) are affected by bias due to the presence of random effects, thus cannot be treated following the Guide to the Expression of Uncertainty in Measurement (GUM) that regulates series of unbiased observations.
- the Italian **d.lgs. 155/2010** (Decreto Legislativo 2010) establishes that the procedures of quality assurance and instrument approval are given on the basis of the technical guidelines issued by the **Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA)**.

The EPA published the third draft of the "***Guideline on Speciated Particulate Monitoring***" (Chow and Watson 1998). In this document some indications regarding individual particle analysis are given:

- optical microscopy is useful for coarse particles with sizes much larger than the wavelength of light (0.3-0.7 microns) which are well separated one from another. It is possible to magnify coarse particles (mainly minerals, pollens and metal fragments) and visually compare them with known standards for colour, refractive index, birefringence and crystallographic properties. If the standards underwent chemical analysis before, it is possible by comparison to infer the chemical composition of the samples;

- scanning electron microscopy (SEM) is needed to characterize particles in the $PM_{2.5}$ fraction for which particle size is comparable to visible light wavelength. Computerized scanning electron microscopy (CCSEM) is needed to characterize a number of particles sufficient to represent $PM_{2.5}$ sample distribution. It is possible to determine the chemical composition of the analysed particles with the associated technique of energy dispersive x-ray spectroscopy (EDXS);
- transmission electron microscopy (TEM) is ideal to characterize ultra-fine particles and it has been used extensively to analyze asbestos fibers.

Analysis on botanical materials for air monitoring purposes

The ***use of bioindicators*** to evaluate the effects on ecosystems of certain pollutants (As, Cd, Ni, PAHs and Hg) as reported in d.lgs.155/2010, is regulated in Italy by the **decreto legislativo 28 agosto 1997 n. 281** (Decreto Legislativo 1997).

The United Nations Economic Commission for Europe, in the frame of the Convention on Long-Range Transboundary Air Pollution and in particular of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, issued the "***Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests***" (Rautio *et al.* 2010). The fourth part of this manual regards sampling and analysis of needles and leaves and gives the criteria to be used to select the sample trees, sample preparation by digestion and their analysis by titration, colorimetry, turbidimetry, X-ray method (RFA), atomic emission spectroscopy (AES), atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and elemental analyzer. It suggests the best analytical technique to provide the concentrations of specific elements and provides information on how the data should be expressed.

The Commission on Air Pollution Prevention of the Verein Deutscher Ingenieure (VDI) establishes guidelines that serve as a decision-making aid in the preparatory stages of legislation and application of legal regulations and ordinances and that are considered the common German point of view in the establishment of technical rules by the European Committee for Standardization (CEN) and by the International Organization for Standardization (ISO) (VDI 2001). The subdivision IV of the commission is called Environmental measurement techniques and deals also with the measurement of PM. VDI issued the following guidelines related to the use of conifer needles as air pollutant samplers:

- **VDI 3957 Blatt 5** (2001) ***Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication) - Standardised exposure of spruce***
- **VDI 3957 Blatt 10** (2004) ***Biological measuring techniques for the determination and evaluation of effects of air pollutants on plants (bioindication) – Source-related measurements of ambient air quality using bioindicators***
- **VDI 3957 Blatt 11** (2007.10.01) ***Biological measuring techniques for the determination and evaluation of effects of air pollution on plants (bioindication) – Sampling of leaves and needles for a biomonitoring of the accumulation of air pollutants (passive biomonitoring)***

Finland issued a standard on the use of conifer needles as air pollutant samplers which is unfortunately not available in English (SFS 1990).

Certified standard reference materials

Certified standard reference materials can be employed also in environmental analysis to calibrate instruments, validate analytical procedures, assess the quality of the analytical measurements and allow interlaboratory comparisons as required by UNI CEI EN ISO/IEC17025 for accreditation (APAT 2003). There are different kinds of reference materials:

- pure substances characterized by their chemical purity or presence of impurities
- standard solutions and gas mix prepared with gravimetric methods from pure substances which are used to calibrate instruments
- reference materials in matrix characterized by the composition of a component. They can be prepared from matrixes that contain the component of interest or from synthetic mixtures and are used to evaluate the accuracy and precision of all the steps of an analytical protocol
- reference objects with characteristic properties such as smell, colors, etc..

The National Institute of Standards and Technology (NIST) produces Standard Reference Materials®. The number **1575a** is the standard for trace elements in **pine needles**. The 1575a Pine Needles was released in 2002 to replace NIST SRM 1575 Pine Needles that was out of stock. The needles were collected from loblolly trees (*Pinus taeda*) in North Carolina, USA, coarse-ground and dried, jet milled, blended and radiation sterilized (Mackey *et al.* 2004; NIST 2002). The standard is sold in bottles containing approximately 50 g. Twelve element concentrations (P, K, Ca, Al, Ba, Cd, Cl, Cu, Fe, Hg, Rb, Zn) are certified expressed as mass fraction on a dried basis and were determined by many independent analytical techniques, with the exception of mercury that was determined with only one method. The analytical techniques used were: instrumental neutron activation analysis (INAA), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), prompt gamma-ray activation analysis (PGAA), radiochemical neutron activation analysis (RNAA) and cold vapor isotope dilution mass spectrometry (CV-IDMS). In Mackey *et al.* (2004) the analytical procedure used for each technique is described together with the method used to combine the data from different techniques in order to provide the certified value. For other eleven elements (Mg, As, B, Cs, Co, Pb, Mn, Ni, Sc, Se, Na) reference values based on one analytical technique only are provided. The certificate accompanying the standard includes the instructions for a correct use and storage of the material: minimum 250 mg of a sample should be desiccator dried or freeze-dried and analyzed. The user should determine the moisture content on separate individual sample portions from each bottles for each use. It is an agricultural certified reference material. Prior to release this material was used as the test material for an international interlaboratory comparison (15 laboratories from 8 countries) and the result of this interlab comparison are reported in Mackey *et al.* (2004).

Regulations, guidelines and recommendations related to stone surfaces deterioration patterns, sampling and analysis

EN 16085:2012 ***Conservation of Cultural property – Methodology for sampling from materials of cultural property – General rules*** describes general sampling criteria and operations, defines documentation and storage requirements and provides a template for a sampling data sheet.

EN 16096:2012 ***Conservation of Cultural property – Condition survey and report of built cultural heritage*** describes how to assess, document and record the condition of built cultural heritage. Professionals are asked to gather preliminary information on the property before going on site to record current preservation condition. Based on the condition survey and risk assessment the professional can then recommend maintenance, preventive and simple repair measures or further investigations to develop solutions to major damages.

NOR.MA.L. 3/80 ***Campionamento – Prelievi di Materiale in Opera e non più in Opera per gli Studi Mineralogici, Chimici, Fisici e Biologici*** (NORMALa)
The whole sampling procedure should be conducted directly by or under the responsibility of the same person that will analyze the collected materials. The owner of the artifacts or the Institution in charge of it should be informed of the sampling procedure and release a written authorization to conduct it. At the end of the sampling procedure a copy of the documentation of the activity should be provided to whom released the authorization. A minimal but sufficient number and amount of samples should be collected to guarantee representativity and when possible also statistical criteria and to obtain the information needed to fullfill the purpose of the study. The factors influencing the characteristics of the material collected should be all considered in the sampling process. Samples should be taken at different heights, exposure to sunlight and rain, etc.. Changes in the aesthetic of the artifact due to sampling should be avoided. Before sampling it is necessary to research in the literature information regarding the nature and provenance (quarry) of the materials used. Moreover, the conservation state of the artwork should be evaluated and all the decay patterns should be described using the terms provided in NORMAL 1/80. Photographs of the artwork as a whole, of different alteration patterns and areas from which samples are taken, areas after sampling and samples should be acquired as documentation. Different types of samples should be collected with different tools (brushes and scalpels) chosen depending on the consistency of the materials:

- surface samples: all the strata present on the artefacts are sampled in sequence including the original not altered substrate possibly on the side of a depth sample;
- depth samples: samples of all the strata together collected by a core drill with the minimum diameter possible and low number of revolutions (ca 100 revolutions/min) working, if possible, in dry conditions. The hole left on the wall must be closed with a material compatible with the surrounding one.

The samples have to be stored in inert containers, previously weighted, tightly closed and labeled. The samples should be weighted as soon as possible to estimate the water content at sampling. In this standard are also described: sampling methodologies for biological studies, sampling of detached parts not anymore reinstatable in the artifact and sampling in quarries. For this last task, sampling should be aimed at finding the material most similar to the one constituting the artifact possibly in the original quarry or in quarries of the same geological formation and with similar mineralogical and petrographical characteristics.

NOR.MA.L. 8/81 ***Esame delle Caratteristiche Morfologiche al Microscopio Elettronico a Scansione (SEM) – Materiale Lapideo di Cava o da Manufatti, Sano e Degradato, Trattato e Non Trattato*** (NORMALb)
Different kind of studies can be conducted by SEM on stone samples collected from quarries and artifacts that may have been treated or not and might be decayed or not:

1. mineralogical study aimed at the identification of the minerals present in the sample as well formed crystals: the study by SEM should be coupled with optical microscopy, X-ray diffraction and microprobe; observation of the following parameters should be made:
    - shape: prismatic (squat or oblong), acicular, etc.
    - habitus: cubic, rhombohedral, etc.
    - corrosion figures and growing defects
    - cleavage and perfection degree: rhombohedral (perfect, good, poor), etc.
    - fracture: irregular, conchoidal, etc
    - twinning and type: polysinthetic, lamellar, etc.

   Samples from quarry must be observed on parallel an perpendicular sections with respect to the stratification. The sections must be obtained with diamond wheel avoiding to use water if soluble compounds are present.
2. study of the conservation state of the material (also resulting from accelerated aging tests) as a whole and of its components: increase of specific surface, decohesions, micro and macrofracture holes, delaminations, deformations, compounds of new formation, deposited materials, depth distribution of the alterations. The study is conducted on the external surface of samples collected from the artifacts and on planes perpendicular to it. Morphological characteristics of deposits and efflorescences are observed directly on sampled material while the characteristics of crusts, laminae, fragments are studied only on the external surfaces and on sections perpendicular to them.
3. study of the effects of stone working with tools: modifications of the morphology of the material and its components and depth distribution of these modifications. The study is conducted on the external surface of samples collected from the artifacts and on sections perpendicular to it.
4. study of the effectiveness of cleaning procedures: check the removal of undesired elements. The study is conducted on the external surface of samples collected from the artifacts and on sections perpendicular to it.
5. study of the effectiveness of protective and consolidation treatments: surface characteristics of the coating, adhesion, penetration, distribution. The study is conducted on the external surface of samples collected from the artifacts and on sections perpendicular to it.

In this document is also described how to prepare the sections and to metal-coat the samples as well as criteria for analysis and comparison of different samples.

Regulations and guidelines complementary to this thesis

Standardized methods and guidelines for the collection and characterization of airborne pollutants

The European sampling and measurement reference method for PM_{10} is described in **EN 12341:1999** ***"Air Quality - Determination of the PM10 fraction of suspended particulate matter - Reference method and field test procedure to demonstrate reference equivalence of measurement methods"***. This method has been adopted in Italy through the UNI EN 12341 Qualità dell'aria – Determinazione del particolato in sospensione PM10. Metodo di riferimento e procedimento per prove in campo atte a dimostrare l'equivalenza dei metodi di misurazione rispetto al metodo di riferimento (UNI2001). This standard regulates the performance requirements of the instruments to sample PM_{10} and provides a protocol to compare the results obtained with a field sampler to those of the described instrument. It is addressed especially to European institutions and companies that want to evaluate sampling systems in ambient conditions.
Three designs of the sampling instrument are accepted and described also through schemes: low-volume system (LVS-PM10), high-volume system (HVS-PM10) and very-high-volume system (WRAC-PM10). They all have air entrance orifices at a height between 1.5 and 8 m from the ground. It is established that the study of the equivalence of alternative instruments must be conducted with 1 reference instrument and 2 samplers to be tested. A minimum of 40 measurements based on the same sampling time must be conducted, generally for 24 hours each, using quartz fiber filters with separation efficiency higher than 99.5%. The equivalency of the sampling instruments is based on:

- uncertainty of the measurements obtained by the two samplers located in the same place $\leq 5\mu g/m^3$ at 95% confidence level (average mass concentrations of the two samplers $\leq 100\ \mu g/m^3$) or $\leq 5\%$ (average mass concentrations of the two samplers $> 100\ \mu g/m^3$)
- function of equivalency to the reference that describes the ratio between the mass concentrations measured by the reference sampler and the new instrument $\leq 10\ \mu g/m^3$ (reference concentration $\leq 100\ \mu g/m^3$) or $\leq 10\%$ with respect to the reference concentration (reference concentration $> 100\ \mu g/m^3$).

ASTM D 4096 ***Standard Test Method for Determination of Total Suspended Particulate Matter in the Atmosphere (High-Volume Sampler Method)*** (ASTM1991) is a standard method that if applied guarantees intersite comparisons. It thoroughly describes the instrument to sample PM, including its protective housing. It gives recommendations on how to place more samplers in an area to monitor air quality, stating, in particular, that samplers should be placed at least 2 meters apart in order to avoid reciprocal influences in the measurements. The standard describes the characteristics of different types of filters that can be placed in the instrument (glass-fiber, silica fiber and cellulose paper) and states that the glass fiber one is the most used for mass loading determination. The filters are preweighted and after the period of exposure, generally 24 hours, they are weighted again and the result, that is the particulate mass collected per unit volume of air sampled is given in $\mu g/m^3$. The sampling procedure is entirely described, from calibration of the instrument to calculations and estimate of precision (3.0-3.7%). It is highlighted that it is not possible to provide an indication of the accuracy of the method since a standard atmosphere to be used as a primary standard for measurement comparisons does not exist.

EPA released a compendium of methods for the determination of inorganic compounds in ambient air in 1999. In this compendium the method number **IO-2.1** ***"Sampling of ambient air for total suspended particulate matter (SPM) and PM10 using high volume (HV) sampler"*** recommends the use of quartz fiber or glass fiber filters to collect particles through a high volume sampler for TSP and PM10 exposed usually for 24 hours. It explains installation, calibration of the sampler and filter recovery procedure as well as calculations, validation, data reporting and maintenance procedures. In addition it reminds that it is important to select a monitoring site or more where the collected particulate mass is representative of the whole monitored area and lists some requirements for the sites (EPA1999c). In the same compendium, method number **IO-1.3** ***"Determination of PM10 using a continuous Rupprecht and Patashnick (R&P) TEOM® particle monitor"*** explains that this instrument calculates mass rate, mass concentration and total mass accumulation on exchangeable filter cartridges and provides hourly and daily averages (EPA1999d).

The Italian and European sampling and measurement reference method for $PM_{2.5}$ is described in UNI **EN 14907:2005** ***"Ambient air quality – Standard gravimetric measurement method for the determination of the PM2.5 mass fraction of suspended particulate matter"*** (UNI2005a). Measurements are made over a sampling period of 24 hours and expressed in $\mu g/m^3$. Two designs of the sampling instrument are accepted and described also through schemes: the low volume (LVS) or the high volume (HVS) design. The filters allowed are made of glass fibre, quartz fibre, PTFE or PTFE coated glass fibre and should have a separation efficiency of at least 99.5% at an aerodynamic diameter of 0.3 µm. The annex provides an equivalence test procedure for non-standard measurement methods but it is not normative yet.

The EPA recommends the use of an impactor-type separator (WINS impactor) or a cyclone-type separator (VSCC™) to collect $PM_{2.5}$ (EPA 2006). Measurements are acquired over 24-hour periods on Teflon-membrane filters (Chow and Watson 1998).

The EPA is finalizing the federal reference method for $\mathbf{PM_{10\text{-}2.5}}$ based on separate concurrent measurements of PM_{10} and $PM_{2.5}$ even though the National Ambient Air Quality Standard (NAAQS) for $PM_{10\text{-}2.5}$ is not being adopted. Using an inertial particle size separator, PM is collected on a polytetrafluoroethylene (PTFE) filter (EPA 2006).

The Italian and European **EN 14902:2005** ***"Ambient air quality – Standard method for measurement of Pb/Cd/As/Ni in the PM10 fraction of suspended particulate matter"*** describes the reference method for sampling these metals as part of the PM_{10} aerosol, the microwave digestion of the samples using nitric acid and hydrogen peroxide and their analysis by graphite furnace atomic absorption spectrometry (GFAAS) or by inductively coupled plasma (quadrupole) mass spectrometry (ICP-MS). In the annex at the end of the document also some examples of digestion procedures are provided. The same elements can be measured in atmospheric depositions following the standard **UNI EN 15841** ***"Ambient air quality - Standard method for determination of arsenic, cadmium, lead and nickel in atmospheric deposition"*** (UNI2010). This standard specifies methods for sampling wet-only and bulk deposition (wet-only collector, bulk collector, Bergerhoff collector sampler) of the indicated metals, sample treatment procedures (digestion process) and analysis by graphite furnace atomic absorption spectrometry (GF-AAS) or by inductively coupled plasma mass spectrometry (ICP-MS). It also provides recommendations for the selection of the sampling sites.

In the USA the determination of these metals is standardized by the EPA's *"Compendium of methods for the Determination of Inorganic Compounds in Ambient Air"*, section **IO-3 *"Chemical species analysis of filter-collected suspended particulate matter (SPM)"***:

- Method **IO-3.3 *"Determination of metals in ambient particulate matter using X-ray fluorescence (XRF) spectroscopy"*** (EPA1999a). The analyst should have been trained by a scientist with an advanced degree in the physical sciences and a minimum of 5 years experience in x-ray spectroscopy.
    - Dichotomous Sampler collection of ambient air particles on thin membrane filters (Teflon® with a pore size of 2 µm, or 0.6 µm pore size Nuclepore® if electron microscopy is planned for the coarse fraction) for a duration that produces an ideal mass loading on the filter (maximum target mass is about 100 µg/cm^2). The sample collected must be uniformly distributed.
    - Thin film standards are used for calibration because they most closely resemble the layer of particles on a filter. The background files which are used for background fitting are created at calibration time. Thirty clean Teflon® and Nuclepore® blanks are kept sealed in a plastic bag and are used exclusively for background measurement.
    - Spectra acquired in sets of 15 samples each, up to 7 spectra for each sample depending how many secondary excitation targets are selected.
    - Elemental intensities are determined by spectral deconvolution with a least squares algorithm which utilizes experimentally determined elemental shape functions and X-ray attenuations corrections and interference corrections. Application of a computer to calculate the metal concentrations (µg/m^3) with uncertainty calculated with 68% confidence level.

The analysis of asbestos is defined by many regulations depending on the field of application (e.g. occupational exposure). The general terminology for sampling and analysis is given in **ASTM D7712 – 11** – **Standard Terminology for Sampling and Analysis of Asbestos**. The standards describing the methodologies to use electron microscopy techniques are here briefly summarized:

- **UNI EN ISO 16000-7 - Aria in ambienti confinati. Parte 7: Strategia di campionamento per la determinazione delle concentrazioni di fibre di amianto sospese in aria** is referred to indoor environments and provides the criteria for the selection of the analytical method suggesting that each country should follow its national regulations or in their absence one of four ISO analytical standard methods based on phase contrast microscopy (ISO 8672), scanning electron microscopy after direct sample preparation (ISO 14966), transmission electron microscopy after direct (ISO 10312) or indirect sample preparation (ISO 13794);
- **ASTM D 5756 – 02 (Reapproved 2008) – Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Mass Surface Loading** describes the use of a microvacuum dust sampling technique of a certain area followed by indirect sample preparation and identification of asbestos by transmission electron microscopy (TEM) on the basis of morphology, selected area electron diffraction (SAED) data and energy dispersive X-ray analysis (EDXA). The application of this method provides an estimate of the mass loading of asbestos per unit area vacuumed or per mass of sampled dust;
- **ASTM D5755 – 09 Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading** describes the use of a microvacuum dust sampling technique of a certain area followed by indirect sample preparation and identification of asbestos by transmission electron microscopy (TEM) on the basis of morphology, selected area electron diffraction (SAED) data and energy dispersive X-ray analysis (EDXA). The application of this method provides an estimate of the number of asbestos structures per unit area vacuumed;
- **ASTM D6480 – 05 (Reapproved 2010) – Standard Test Method for Wipe Sampling of Surface, Indirect Preparation and Analysis for Asbestos Structure Number Surface Loading by Transmission Electron Microscopy** describes the use of a wipe sampling technique of a certain area followed by indirect sample preparation and identification of asbestos by transmission electron microscopy (TEM) on the basis of morphology, selected area electron diffraction (SAED) data and energy dispersive X-ray analysis (EDXA). The application of this method provides an estimate of the number of asbestos structures per cm^2;
- **ASTM D7201 – 06 (Reapproved 2011) – Standard Practice for Counting Airborne Fibers, Including Asbestos Fibers, in the Workplace, by Phase Contrast Microscopy (with an Option of Transmission Electron Microscopy)** refers to the field of occupational hazard. The application of this method provides the number of fibers per ml of air and transmission electron microscopy is optionally employed to refine the analysis;

- **ASTM D7200 – 12 Standard practice for Sampling and Counting Airborne Fibers, Including Asbestos Fibers, in Mines and Quarries, by Phase Contrast Microscopy and Transmission Electron Microscopy** refers to the field of occupational hazard. Following the more general ASTM D7201 – 06 previously listed, also in this standard Transmission Electron Microscopy is not mandatory but recommended to assist in fiber identification.

Analysis on botanical materials for air monitoring purposes

Air monitoring with mosses is a recognized technique. Besides the guidelines issued by VDI (VDI 2009a; VDI 2009b) and the finnish standard (SFS 1994), a European standard method has already been approved and will be soon available: **CEN PREN 16414** (draft 2012.03.01) ***Air quality – Biomonitoring with mosses – Accumulation of atmospheric contaminants in mosses collected in situ: from the collection to the preparation of samples***.

Also for lichens a European standard method will be released: **EN 16413** ***Air quality - Biomonitoring with lichens - Assessing epiphytic lichen diversity***.

Certified standard reference materials

NIST produced also the standard **1648** for **urban particulate matter** to evaluate the accuracy of elemental metal concentrations of PM collected on filters.

In Italy the Agenzia per la Protezione dell’Ambiente e per i servizi Tecnici (APAT) has a laboratory for the production and characterization of reference materials that are provided for free at all the national environmental agencies in charge of official monitoring activities (APAT 2003). For gaseous pollutants mixtures, reference samples are certified by the Istituto nazionale di ricerca metrologica (I.N.RI.M.) (Decreto Legislativo 2010).

The use of standards is not always easy and in the literature limits are highlighted for many of them. For instance, EPA reported the following problems regarding the use of NIST 1648 (urban particulate matter): absence of a filter substrate, relatively large amount (100 mg) necessary to overcome problems of apparent inhomogeneity, which in turn necessitates dilutions not required in normal application of the quantification of metals in urban PM on filters and different element ratios from those found in real samples (EPA 1999b).

Recommendations related to cultural heritage and pollution

The importance of cultural heritage in the European context emerges from the analysis of the recommendations formulated by the Committee of Ministers of the Council of Europe to members states (Ballester 2000). Cultural heritage has to be considered by governments not only when implementing strategies for conservation and protection, but also when regulating air pollution. Although the mentioned **recommendation, number R (97) 2 “on sustained care of the cultural heritage against physical deterioration due to pollution and other similar factors”**, was adopted many years ago, in 1997, it seems that it has been taken into consideration recently and only in the research environment (see the European 7th Framework project TEACH - Technologies and tools for the assessment of air pollution impact on immoveable and moveable cultural heritage”). This recommendation replaced a pre-existing one, the number R (88) 5 “on control of physical deterioration of the architectural heritage accelerated by pollution” and enlarged the request for consideration from buildings to all kinds of cultural heritage.

Regulations, guidelines and recommendations related to stone surfaces deterioration patterns, sampling and analysis

UNI 11182:2006 ***Beni Culturali – Materiali lapidei naturali ed artificiali – Descrizione della forma di alterazione – Termini e definizioni*** (UNI2006) substitutes NOR.MA.L. 1/88 and defines alteration and degradation as well as the different decay patterns. It also provides examples through pictures. On an International level it corresponds to the more detailed ICOMOS-ISCS (International Council on Monuments and Sites-International Scientific Committee for Stone)’s “***Illustrated glossary on stone deterioration patterns***” (ICOMOS-ISCS2008). Each term in this booklet is accompanied by similar terms and terms with which it should not be confused.

NOR.MA.L. 10/82 macroscopic description of the sample

NOR.MA.L. 14/83 thin section (also cross-section) and polished section

NORMAL 16/84 ***Definizione della Sequenza Analitica per la Caratterizzazione Chimico-Mineralogico-Petrografica di Materiali Lapidei in Opera e del loro Stato di Conservazione***

These protocols are aimed at obtaining the highest number of information possible from small samples in amounts and dimensions. All these analysis are necessary before a conservation treatment of stone monuments.

- Analysis of non-decayed fragments of materials aimed at the obtainment of the mineralogical-petrographical and chemical (qualitative) classification of the material and determination of its provenance (quarry)
    1) observation at OM (stereomicroscope) without any preparation + metal coating of the external fracture surface and SEM observations: mineralogical-petrographical macroscopic description, identification of crystalline species with well defined habitus and exam of the traces of the working techniques;
    2) flattening of a surface: XRF and SEM-EDXS to have elemental composition and provenance information (Ca/Sr, etc.)
    3) thin section and transmitted light microscopy: mineralogical, petrographical and textural characterization and observation of decay traces
    4) pulverization: XRD, thermal analysis and calcimetry to identify crystalline phases and degree of crystallinity, quantitative determination of carbonates (NORMAL 1985)
- Analysis of fragments collected on the original surface of the material to study its state of conservation and the ongoing alteration processes:
    1) observation at OM (stereomicroscope) without any preparation + metal coating of the external fracture surface and SEM-EDS observations aimed at determining the macroscopic morphology of decay and identifying and localizing decay products
    2) thin cross-section observed by optical microscopy in reflected and transmitted light, UV and phase contrast microscopy in order to obtain a mineralogical analysis, determine the neoformation phases and observe the stratification of decay and of past conservation treatments
    3) polished cross-section symmetrical to that in 2 observed with an optical microscope + chemical analysis of decay products by instrumental microanalysis
- Decayed material in fragment or powder form collected at different depths, from black crusts, salt efflorescences, conservation stucco in order to study the conservation state and the ongoing degradation processes
    1. powder XRD and OM to obtain mineralogical composition, determine neoformation phases and past treatments
    2. powder and eventual preparation of pellets for XRF to have quantitative elemental composition and highlight eventual past treatments
    3. powder + metal coating and analysis by SEM-EDXS to have quantitative elemental composition and highlight eventual past treatments
    4. Solubilization and AAS, spectrophotometry, flame photometry, chromatographic techniques to have a quantitative analysis of soluble salts, a qualitative analysis of organic compounds and highlight eventual past treatments.

6.2 Appendix B: Characteristics of fly ash observed by SEM-EDXS and TEM-EDXS in relation to the fuel used in the combustion source

The compositions obtained by EDXS do not take into account C concentration.

Source	**fuel**	**spherical carbonaceous micro-soot** **20-50 nm** **associated in chains (µm) or agglomerates**	**Fly ash** **1-hundreds µm** **Morphology related to chemical composition** **Size not related to chemical composition**
analytical technique		**TEM-EDXS**	**SEM-EDXS**
	Natural gas		Not emitted
	Domestic fuel		
Diesel engines (vehicles, railways, etc)	Diesel fuel	S	
Biomass burning, open fireplaces, wood and pellet stoves, masonry heaters and boilers, forest, bushes and savannahs fires, paper industry, domestic heaters, agricultural practice	Wood charcoal wood logs wood chips pellet	Mainly C with associated K Sometimes also Si, Na, Al, Cl, Ca, Zn 50-600 nm spherical organic carbon particle (electron microscopy measure) produced because of incomplete combustion (Burning wood of poor quality - high moisture content-, overloading the firebox, insufficient air supply) at low-temperatures (300-500°C) in conventional stoves and open fireplaces originating from thermal degradation of cellulose, hemi-cellulose and lignine. They are poor in K, S and Cl. Aging in the atmosphere they become insoluble tar-balls poor in elemental carbon. 20-50 nm (electron microscopy measure) soot (elemental carbon) aggregates consisting of a large number of primary spherical carbon particles that may have internal turbostratic microstructure (concentric arrangement of layer planes with a two dimensional graphitic structure) and are insoluble. Produced because of incomplete combustion at high temperatures (800-1000°C) in conventional stoves, masonry heaters, open fireplaces, boilers for wood, wood chips and pellets	Smooth microspherules with lower size than coal fly ash. Due to the melting of silica contained in wood. They could be either hypersiliceous (SiO_2 = 90-96%) or alumino-silicates, potassic and ferriferous (SiO_2 = 60-62%, Al_2O_3 = 26-30%, K_2O = 4-5%, Fe_2O_3 = 5-6% PM_1size spheres rich in K, S and Zn resulting from the volatilization of the alkali metals in wood 50-125 nm (electron microscopy measure) soluble spheres made of alkali salts (mainly KCl and K_2SO_4 with small amounts of trace elements such as Zn) produced during complete combustion at high temperatures (higher than 900°C) in pellets stoves, boilers for wood, wood chips and pellets. Content of organic and elemental carbon is less than 1% of the particle mass. They may also contain P. Coarse inorganic fly-ash particles (> 1 µm) containing Ca, Mg, Si, P and Al from large scale grate fired biomass boilers and wood chip burners

Source	**fuel**	spherical carbonaceous micro-soot 20-50 nm associated in chains (µm) or agglomerates	Fly ash 1-hundreds µm Morphology related to chemical composition Size not related to chemical composition
analytical technique		**TEM-EDXS**	**SEM-EDXS**
Stationary: industries and power plants	Heavy fuels (fly ash rich in S; produce acid aqueous solution)	not emitted	Cenospheres (hollow spheres) (10-50 µm): with smooth surface eventually covered by very fine deposits or incrustations; aluminosilicate composition (SiO_2 55%, Al_2O_3 30%) independent from size with sometimes V (V_2O_5 2%) and other elements (SO_3 5%, FeO 2%, Na_2O 2%, TiO_2 1%, CaO 1%). They can contain also Mn, Cu, Zn, Pb, Cd, Cr. They are produced by the fact that the injector of the fuel burner produces an aerosol of fine droplets that burns in the beginning on the surface producing an external crust that after is broken by the emissions of gases trapped inside the droplet
			Microspherules with **rough surface** due to an internal heterogeneous structure (10-50 µm), 20 times less abundant than cenospheres; aluminosilicate composition (SiO_2 7%, Al_2O_3 6%) richer in **S**, Na, Mg (SO_3 55%, Na_2O 15%, MgO 2%)
			Porous or spongy micro-particles (10-400 µm) le più piccole (fino a 50 µm) sferiche le altre più irregolari; **S**O_3 77%
	Coal (fly ash rich in Si and Al, in some cases also K; soluble silicates cause an alkaline leachate if the fly ash are leached with water)	not emitted	**Smooth** microspherules mainly **alumino-silicate** in composition and rich in Fe and K with diameter < 10 µm
			Microspherules with hyperferriferous (from the melting of pyrite, FeS_2, iron oxides, FeO and Fe_2O_3) or titaniferous (from the melting of rutile, TiO_2) compositions
			Cenospheres (hollow spheres) that form because gas (SO_2, from sulfate decomposition, N_2, from air trapped in the melt, CO_2 from either oxidation of C by Fe oxide or carbonate minerals decomposition) is generated inside a molten droplet. 75-90% **aluminosilicate**, 7-10% Fe oxide, 0.2-0.6 Ca oxide. 80-110 µm mass median diameter
			Plerospheres (spheres filled with other spheres resulting from encapsulation during particle formation: the **aluminosilicate** particle is heated thus a molten surface layer surrounds the core that will boil away because of gas formation) (70% in numero): Microspherules with smooth surface; composition SiO_2 50%, Al_2O_3 32%, FeO 6%, K_2O 4%, SO_3 1%. They do not contain Ni or V.
			Rough-surface microspherules (10% in number): rich in FeO 66%, CaO 6%, K_2O 4%, SO_3 2%. They do not contain Ni or V. The proportion of other oxides does not change the proportion of SiO_2 e Al_2O_3
			Spongy microspherules (20% in number) SO_3 18%, fixed alumino-silicate matrix: mullite or quartz or mix of the two in fixed proportions
	Fe-containing raw materials and Fe-containing fuels used in high-temperature technological processes (e.g. steelmaking)		Fe-rich, low Si and Al content. In the crystal structure of Fe oxides during cooling other elements with volatile behavior in high temperatures can be entrapped

6.3 Appendix C: Toxicity and sources of metals in the atmosphere with special regard to the steelmaking process

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Aluminum (Al)	Beneficial element					geological			crustal sources
						additive in catalytic converters			
						tyre wear	coarse particles		
Antimony (Sb)			antimony trioxide is possibly carcinogenic to humans (IARC)			brake wear (3-7% of brake pads as stibnite (Sb_2S_3))			Cu:Sb Cu:Mo Sb:Mo ratio almost constant in high traffic areas (brake pads)
						waste incineration			
						metal smelting			
						combustion of fossil fuels			
Arsenic (As)		X	As and inorganic As compounds are carcinogenic to humans both for inhalation and ingestion (IARC, 2008/50/CE)	could be emitted by scrap coatings		coal combustion (coal power plants)			
						mining industry			
						glass manufacturing			
						Cu metallurgy			
						non-ferrous metal smelting			
						fertilizers			
						use of pesticides			
						wood preservatives			

Metal	**Plant health**		**Human toxicity and eventual regulatory limits**	**Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products**		**Other sources**		**Main form in the air**	**Tracer of**
	Normal amounts	**Toxicity**		**Phase in the production process when emitted**	**Characteristics of the particles**	**source**	**Characteristics of the particles**		
Barium (Ba)						brake wear: barite ($BaSO_4$) is used as a filler in brake linings			brake dust
						wood preservatives			
						combustion of fossil fuels			
						smoke suppressant in diesel engines (not common			
Cadmium (Cd)		5 ppm (4-8 ppm for spruce)	Listed among the hazardous air pollutant substances (HAPs) or air toxic by the USA Clean Air Act. It accumulates in the liver producing its apoptosis and necrosis. Adverse effects on kidney, bones and lungs, Carcinogenic both for inhalation and ingestion (2008/50/CEand IARC), more than 20 years of biological life in humans	Furnace loading (diffuse emissions): Cd can be in paints and coatings of the scraps used as raw materials		coal and petrol combustion (found at sub-ppm level in petroleum in amounts depending on the origin of the fuel)			
						non-ferrous metal smelting			
						tyre wear (impurity –less than 0.1% - in ZnO)			
						brake linings wear			
						fertilizers			
						exhaust emissions from gasoline			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Cesium (Ce)						catalytic converters wear (additive in the washcoat)			
Chromium (Cr)		X	while Cr(III) is essential for human nutrition (glucose metabolism), Cr(VI) compounds are toxic, carcinogenic and mutagenic (IARC). Cr^{6+} is listed among the hazardous air pollutant substances (HAPs) or air toxic by the USA Clean Air Act.	scraps may contain Cr: stainless steels or chrome plated steels	primarily emitted in the form of chromic oxide (Cr_2O_3)	galvanic industries (electroplating)			
						mechanical industries			
						leather tanning			
						cement producing plants			
						wearof Cr-based catalytic converters			
						wear of brake linings			
						dyeing (textiles)			
						paint and pigments			
						wood preservatives			
				it can be emitted during tapping, casting or other production steps if it is added as an alloying element		coal and petrol derivatives combustion			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Cobalt (Co)	beneficial but not essential for higher plants (very low concentration)		listed among the hazardous air pollutant substances (HAPs) or air toxic by the USA Clean Air Act. Co and Co compounds (evaluated as a group) are possibly carcinogenic to humans (IARC)			combustion of fossil fuels (coal)			
Copper (Cu)	Micronutrient: minimum amount in spruce 0.7 ppm. Sufficiency: 3-7 ppm d.w. in leaves	20-30 ppm				mechanical industries			vehicular pollution: Cu:Sb or Cu:Mo:Sb ratios are almost constant in high traffic areas due to brake pad wear
						combustion of fossil fuels			
						metal mining, smelting and refining, alloying			
						fertilizer industry			
						petrochemical industry			
						waste incineration			
						agriculture and zzotechnique: fungicide			
						volcanoes			
						exhaust emissions from gasoline			
						brake wear			
						tyre wear			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Iron (Fe)	micronutrient. Sufficiency: 50-75 ppm d.w. in leaves. Normal amounts 10-1000 ppm d.w. in leaves. The majority of Fe is in the ferric form (Fe^{3+}) as ferric phosphoprotein		increase cytokine production in human airway epithelial cells	primary emissions (melting)		geological/crustal/soil			crustal and vehicular sources
						coal combustion			
						gasoline operated vehicles (additives in lubricating oils)	below 0.02 µm		
						brake wear			
						catalytic converters wear (additive in the washcoat)			
						tyre wear	coarse particles		
Lanthanum (La)						catalytic converters wear (additive in the washcoat)			

Metal	**Plant health**		**Human toxicity and eventual regulatory limits**	**Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products**		**Other sources**		**Main form in the air**	**Tracer of**
	Normal amounts	**Toxicity**		**Phase in the production process when emitted**	**Characteristics of the particles**	**source**	**Characteristics of the particles**		
Lead (Pb)	Normal concentration: <3/10 ppm	30 ppm	Listed among the hazardous air pollutant substances (HAPs) or air toxic by the USA Clean Air Act. Pb inorganic compounds are probably carcinogenic to humans (IARC). Cumulative toxicant affecting multiple body systems including neurological, hematological, gastrointestinal, cardiovascular and renal systems. Children particularly vulnerable to neurotoxic effect even low levels of exposure can cause neurological damage.	diffuse emissions (oven loading): scrap pollutant (leaded steel, automatic steels, Pb batteries associated with car scraps)		vehicle exhaust (leaded gasoline (in Italy phased out in January 2002)		PbO (produced during oxidation and condensation of metals at high temperature) or $PbCl_2$	
						non-ferrous metallurgical plant			
						demolition of structures with leaded paint			
						volcanoes			
						loss of Pb wheel weights			
						brake wear (not common although Pb is present in the linings)			
						tyre wear (impurity in ZnO – less than 0.1%)			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Lithium (Li)						practically not emitted by human activities			
Manganese (Mn)	micronutrient - Sufficiency: 10-50 ppm d.w. in leaves		listed among the hazardous air pollutant substances (HAPs) or air toxic by the USA Clean Air Act. Neurotoxical effects with Parkinsonlike symptoms. Inhalation of Mn oxides may cause manganic pneumonia.	primary emissions (melting, tapping, casting)	70% of MnO particles < 5 µm in diameter	holigoelement in soil (12th most abundant element in the Earth's crust)	Coarse particles ≥ 2.5 µm median aerodynamic diameter. Oxides or hydroxides of oxidation state +2, +3 or +4, carbonates, silicates		
						plants producing Mn metal and Mn chemicals			
						fossil fuel combustion	Fume or ash < 2.5 µm		
						unleaded gasoline (methylcyclopentadienylmanganese tricarbonyl (MMT) was used as antiknock agent especially in 1977-1978)	Mn_3O_4 particles < 1 µm		
						diesel exhaust			
Mercury (Hg)		X	Listed among thepriority metals in the Clean Air Act Amendements (1990)	Primary emission (melting): from vehicle scrap (before 2003 vehicles had Hg-containing switches)	Vapor (not controlled by PM control devices)	coal combustion			
						waste combustion			
						chlorine-alkali process			
						battery production			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Molibdenum (Mo)						brake wear (1% of brake pads as MoS_2)			Cu:Mo or Sb:Mo ratios almost constant in high traffic areas due to brake wear
						fossil fuel combustion			
Nickel (Ni)	beneficial		listed among the hazardous air pollutant substances (HAPs) or air toxic by the USA Clean Air Act. carcinogenic both for inhalation and ingestion (2008/50/CE; IARC). Increase cytokine production in human airway epithelial cells	primary emissions (melting, tapping, casting)		gasoline exhaust emissions			
						fertilizer industry			
						petrochemical industry			
						brake wear			
						catalytic converters wear (additive in the washcoat)			
						waste incineration			
						mining and smelting			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Palladium (Pd)						catalytic converters wear			vehicular pollution
						jewelry manufacture			
						dental and medicinal devices			
Platinum (Pt)						catalytic converters wear			vehicular pollution
						electrical and electronic industry (computer hard disks)			
						glass industry (special glass and glass fibre production)			
						fuel cell industry (Pt-coated electrodes)			
Rhodium (Rh)						three-way catalytic converter wear			vehicular pollution
Sodium (Na)						sea salt			
						de-icing substances (NaCl)			
						biomass combustion			
Strontium (Sr)			Not toxic	primary emissions (melting): radiocontaminant		crustal			
Tin (Sn)						brake wear			
						combustion of fossil fuels			
Titanium (Ti)			Ti dioxide is possibly carcinogenic (IARC)	from painted scraps	smooth surface fly ash rich in Ti	crustal			crustal
						coal combustion			
						catalytic converters wear (additive in the washcoat)			
						tyre studs wear			
						tyre wear	Coarse particles		
						semi-metallic brake wear (potassium titanate fibers)			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Tungsten (W)						tyre studs wear (tungsten carbide components)			
						snowplough wear wear (tungsten carbide components)			
						catalytic converters wear (additive in the washcoat)			
Uranium (U)						natural			
						coal combustion			
						waste incineration			
						uranium mining			
						testing of nuclear weapons			
						nuclear accidents			
Vanadium (V)		X	Increase cytokine production in human airway epithelial cells. Vanadium pentoxide is possibly carcinogenic (IARC)			soil			Industrial/fuel oil combustion (power plants and domestic heating)
						oil combustion and oil refineries (oil-fired power plants)			
						catalyzer			
Yttrium (Y)						catalytic converters wear (additive in the washcoat)			

Metal	Plant health		Human toxicity and eventual regulatory limits	Production of steel from scraps with an EAF furnace or subsequent transformation of steel into final products		Other sources		Main form in the air	Tracer of
	Normal amounts	Toxicity		Phase in the production process when emitted	Characteristics of the particles	source	Characteristics of the particles		
Zinc (Zn)	micronutrient. Normal concentration: 10-100 ppm. Sufficiency: 15-50 ppm d.w. in leaves	threshold of Zn phytotoxicity of 230 µg/g for aerial parts	soluble Zn in urban PM from Ottawa (Ontario, Canada) was proven to induce lung inflammatory response on mice. One of the least harmful metals	diffuse emissions (oven loading) and primary emissions (melting): painted and coated scraps 20% of EAF dust	insoluble phase: Zn-Fe oxide similar to franklinite	tyre wear	Coarse particles	ZnO (produced during oxidation and condensation at high temperature)	vehicular pollution and steel mills
						brake wear (2^{nd} most abundant metal in brake lining materials)			
						corrosion of glavaized structures (safety fences in the roads, roofs, rain gutters, etc.)			
					soluble phase containing Ca and probably associated with carbonates	combustion of fossil fuels			
						gasoline exhaust emissions (additive in lubricating oils)	in mixture with other elements in particles > 0.02 µm		
						volcanoes			
						non-ferrous metal smelting: Zn metal production, Zn galvanization, brass production, alloy and die casting			
						dyestuff production (ZnO basic component especially of white colors)			
						waste incineration	< 2 µm		
						batteries	ZnO		
						cigarette filters	ZnO		
Zirconium (Zr)						brake wear (sphalerite is used as abrasive in brake linings)			
						catalytic converters wear (additive in the washcoat)			

6.4 Appendix D: Emissions from EAF steelmaking plants

There are three kinds of emissions from an EAF steelmaking plant:

- **primary emissions**: collected from the fourth hole located on the roof of the furnace when this is close. These emissions are collected at very high temperature (about 1650°C), thus are rich in oxides;
- **secondary emissions**: collected by a hood and eventually by the dog-house that surrounds the furnace. These emissions come from the ladles, and from the lifting of the vault of the furnace to charge the scrap inside;
- **fugitive emissions**: released when the abatement system is not operating because the roof of the furnace is open for charging or when the furnace is tilted for tapping. During charging they result from vaporization of oil, grease and dirt introduced with the scrap, oxidation of organic matter adhering to the scrap and vaporization of water from wet scrap (EPA 2007). Fugitive emissions from backcharging are higher than those caused by the first charge because of the reactions favoured by the heat of the molten steel. Fugitive emissions also result from the movement of the scraps, the movement of the dust collected by the fume abatement system and the demolition of the refractory materials of the ladles. The dust emitted by all these processes is re-suspended by the wind and by the trucks moving in the area of the plant.

Considering the different stages of steel production with EAF technology that constitute a cycle lasting from 1 to 5 hours (APAT 2003) a distribution of the emissions between primary and secondary can be hypothesized as shown in Table 6.4-1, while fugitive emissions could be produced at every stage and even before and after the production of the steel by trucks moving around the plant and wind resuspending contaminated soil, etc. The majority of emissions are due to the melting and refining stages of the process, while charging and tapping emissions represent about the 5% each of the total emissions in a cycle (EPA 2007). The emission volumes depend on oxygen inputs, electric power (higher power means faster combustion of organic substances and faster increase of evolved gas temperatures) and pre-heating (reduces the emissions) (APAT 2003).

The health risks associated with the presence in a territory of an EAF plant are due to the release of carcinogenic compounds. EAF workers are exposed to a higher pulmonary cancer risk (International Agency for Research in Cancer (IARC); Panizza 2011). The pollutants emitted by an EAF furnace are both organic and inorganic. The **organic component of the emissions** includes CO, hydrocarbon vapours, soot, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), formed during the combustion of the scrap contaminated by varnishes, plastic, oils, and PCBs (especially scrap such as condensers, transformers and mineral oil containers). A study by Onofrio *et al.* (2011) investigated organic pollution from an Italian EAF steelmaking facility located in Piedmont. Inorganic pollutants emitted by EAF will be described here in more detail as they are the subject of the investigation presented in chapter 4.

The **inorganic component of the emissions** is made of particles of different sizes that are drops of metal and slag and also volatile metals fumed from the steel melt (Whichterle *et al.* 2010). The exact composition of this dust depends on the scrap used as raw material. All components of the scrap (steel, possible coating of the steel, materials accompanying the scrap that was not separated from it such as plastics and sometimes lead batteries) end up in the fumes. Low melting temperature components are the first to pass in the fumes but also iron itself and metals with high melting temperature are drag in the fumes by the boiling of the melted material. This implies that all the scrap must be examined to establish its suitability to be introduced in the furnace. It happened in the past that radioactive components hidden in the scraps caused contamination of the whole furnace.

Table 6.4-1 Stages in the production of steel in an EAF plant and emissions associated to them. Information in the table were taken from EPA (2007), APAT (2003) and Guézennec *et al.* (2005)

stage	primary emissions	secondary emissions
preparation and charge of the furnace: Fe and steel scrap are loaded to a specified weight into a drop-bottom (clam-shell type) charge bucket with an electromagnet that is suspended from an overhead crane. Alloying materials, lime (fluxing agent to reduce S and P content in the molten steel and form the slag), sand, Fe ore and coke are also eventually added. The roof of the furnace is opened and the charge is dropped into the furnace from the charge bucket		100%
melting of the charge. The electric arc is created between the graphite electrodes and the scrap. The temperatures reached are in the order of 3500°C. Vaporization of Fe and transformation of mineral additives. A steel bath is formed covered by a slag layer. The emissions escape through electrode holes, slag door, roof ring and sometimes the tap spout	100% metallic (Fe, Mn) and mineral oxide particles, CO, hydrocarbons	10%
charge of another bucket full of scrap (**backcharge** scrap) when the first charge is almost entirely molten. Reddish-brown fumes		100% (Zn, Pb, Cd)
melting of the other bucket full of scrap	100%	10%
Refining and slag foaming: addition of limestone, lime and fluorspar that fluidize basic slags obtained by lime addition (double slagging process). Slags are removed before beginning oxidizing refining. C is oxidized by adding Fe ores or plate scales to reach the desired C content or by oxygen blowing (fast decarburation) (increased bath and gas temperatures, gas evolution and generation of particulates). Bubbles of CO are formed in turn favouring the removal of other dissolved gases. The CO-bubbles crossing the slag layer make it foam especially if coal powder is added. Oxidizing slags are removed, new addition of lime and production of slags P, Si, Mn, C and other elements in the scrap are oxidized and slag formed. Eventually slags are de-oxidized with Al drops, or FeSiMn alloy, or FeSi, $CaSi_2$, SiC, coke and anthracite.	100%	30%
tapping of the steel into a ladle. Sometimes ferromanganese, ferrosilicon, aluminium and other alloying agents are added to the ladle to adjust the oxygen content of the steel.		100% (fugitive: Fe oxides and other oxide fumes resulting from alloys added to the ladle. Chromic oxide can be emitted if stainless steel is produced)
Ladle metallurgy and casting: the steel is transferred to a ladle metallurgy facility, additionally refined to obtain the requested properties and formed into semi-finished shapes in the continuous caster. The final steel product is obtained processing the semi-finished shapes in rolling mills.		

Nowadays, the quality of the scrap is decreasing due to the increase of coatings on steel. Some companies made a business out of treating scraps to increase their quality for use as raw materials in EAF production. Among their activities there is the removal of the zinc coating from galvanized sheet iron and the treatment of electrical household appliances (refrigerators, washing machines) and cars to remove components that are either toxic for the environment (such as chlorofluorocarbons and polyuretanic foam) or obstacles for the metallurgical process (APAT 2003). Other factors influencing the pollutants emitted by the EAF are the type of furnace and its size, the number of backcharges in each production cycle, the refining procedure, the duration of tapping and the melting temperature (EPA 2007). On the basis of the work of Guézennec *et al.*

(2005) and Mantovani *et al.* (2004) the mechanisms of formation of particles during the production of steel (process emission) in an EAF plant are listed in order of importance:

- solidification of steel and slag droplets projected as a result of the bursting of CO-bubbles produced during the decarburization of the steel bath. When the CO-bubble, with an external steel layer that will form spinel ferrite, emerges from the bath the liquid film cap on top reaches a critical thickness and breaks. While emerging, the bubble passes through the slag, thus calcium silicate glass can form from CaO and SiO_2 (60% of the dust produced through this mechanism)
- volatilization of the liquid steel where the arc or the oxygen jet impinge or in the CO bubbles (27% of the dust produced through this mechanism) producing submicrometric particles rich in the metals with a high vapour pressure at steelmaking temperature (Zn, Pb, Cd)
- direct fly-off of particles from the raw materials when they are introduced in the furnace
- solidification of droplets projected where the arc or the oxygen jet impinge (generally they fall back into the liquid bath)
- disintegration of larger particles due to the explosion of CO gas resulting in the formation of broken spheres and angular particles.

Another mechanism of formation of droplets was proposed but not proved. It consists in the formation of droplets as a result of the contact with the oxidizing atmosphere. Once produced some physical (condensation, agglomeration) and chemical (oxidation) transformations may occur both within the furnace and within the fume extraction ducts producing chemically complex phases. For example, due to high temperature, fine spherical particles of less than 1 µm may form agglomerates of 1-30 µm diameter, rarely of 50 µm (Guézennec *et al.* 2005; Li and Tsai 1993; Machado *et al.* 2006; Mantovani *et al.* 2004; Sofilić *et al.* 2004).

Different kind of particles can be emitted by the plant depending on the originating mechanism and subsequent transformations. However, spherical shapes, including broken spheres and cenospheres, predominate (Ault *et al.* 2012; Mantovani *et al.* 2004; Sofilić *et al.* 2004). Cenospheres are formed when gas is present inside the particle that is solidifying from a liquid. These are the typical morphologies of the particles due to process emission (Ault *et al.* 2012; Guézennec *et al.* 2005; Trimbacher and Weiss 1999):

- zincite (ZnO) monocristals with facetted aspect and size below a few hundred nanometers resulting from the condensation of the Zn vapors contained in the EAF fumes;
- spherical particles enriched in Zn with size in the range 0.2 – 20 µm:
  - spheres with composition either of the slag or of the steel bath resulting from the solidification of liquid droplets projected when CO-bubbles burst during the decarburization of the steel; the composition is homogenous all over the sphere; if larger than 2-3 µm these spheres are often hollow
  - spheres made of a slag phase and a zinc phase (the composition is heterogeneous all over the sphere) sometimes displaying a Fe-rich dendritic structure (iron spinels) inside a vitreous phase (Ca-Fe silicate glass) resulting from the solidification of liquid droplets projected when CO-bubbles burst during the decarburization of the steel
  - zincite submicrometric spheres resulting from the condensation of the Zn vapors contained in the EAF fumes
- spherical particles rich in Fe with low aluminium and silicon in which Fe and O are evenly distributed throughout the particle
- if the process or the abatement system is not well controlled, spherical particles with the chemical composition of the slag (Ca, Al, Fe, Si, etc.) and size between 20 and 200 µm that are solidified droplets projected from the bath where the arc or the oxygen jet impinge;

- if the process or the abatement system is not well controlled, irregular particles of coal and lime ranging between 20 and 500 µm from direct fly-off during addition of powder materials.

Most of the particles have diameter below 5 µm but it is possible to have also coarse particles with diameters of 20-80 µm (Mantovani *et al.* 2004).

From a chemical point of view, Fe constitutes between 15 and 37% of the dust, Zn constitutes about 20%, Mn 2-4%, Pb about 5% and Cr about 15% (EPA 2008; Li and Tsai 1993). Also Cd (up to 3%), As, Ni (up to 4%) and Cu might be present. Appendix B describes the precise source in the EAF process of each metal. If the plant produces carbon steel the dust will be richer in Zn and Pb, while if the plant produces stainless steel the dust contains low amounts of Zn and Pb but it is richer in Cr and Ni (Mantovani *et al.* 2004). Manganese and zinc have been identified by many studies as markers of steelmaking emissions (Baiutti *et al.* 2007). The most common form in which these elements are detected is as oxides because oxidation is favoured by the oxygen in the furnace and the high temperature (APAT 2003; Machado *et al.* 2006): Fe_2O_3, Fe_3O_4, $FeCr_2O_4$, ZnO, SiO_2, CaO, Al_2O_3, Cr_2O_3, MnO, Mn_3O_4, PbO, SiO_2, $Ca_{0.15}Fe_{2.85}O_4$, MgO, Mn_2O_3, FeO, Mn-Zn ferrite ($(Mn, Zn)Fe_2O_4$), $ZnFe_2O_4$ (franklinite), $ZnCl_2 \cdot 4Zn(OH)_2$. Jenq *et al.* (1992) defined a source profile for the EAF fugitive emissions and observed that S was mainly in the fine ($PM_{2.5}$) fraction, Zn, Mn and Cu were almost equally distributed between fine and coarse fraction (PM_{10}) while Fe, Si and Al were mainly in PM_{10}.

From a mineralogical point of view the most common form is a solid solution of iron spinels and zincite (Li and Tsai 1993). More Zn is present in the dust higher the content of zincite. Zinc ferrite ($ZnFe_2O_4$) in the mineralogical form of franklinite with spinel structure forms when Zn particles are in contact with Fe particles at high temperature in the oxidizing atmosphere (Machado *et al.* 2006; Whichterle *et al.* 2010). Iobstraibizer *et al.* (2014) analysed fugitive dust around the EAF steelmaking plant described in this chapter by XRD and detected a magnetic component made of magnetite-franklinite, hematite and wüstite and a non-magnetic component made of calcite, quartz, larnite, portlandite, zincite and periclase. Metallic iron particles can be found although not often enclosed within a matrix made of complex phases. For what concern the halogen bearing phases, the most commonly detected are soluble NaCl and KCl (Mantovani *et al.* 2004).

From a crystallographic and texture point of view, the rapid cooling characteristic of EAF dust formation produces a variety of internal crystalline configurations characterized by skeletal textures. For example, iron spinels can assume dendritic texture or rhombohedron morphology depending on the cooling rate (Mantovani *et al.* 2004). Paragraph 4.3 provides sample micrographs of particles emitted by EAF plants.

The density of EAF dust range between 2.96 and 4.12 g/cm^3 (Mantovani *et al.* 2004).

6.5 Appendix E: Comparison of air pollution studies based on the collection of conifer needles

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
1986	Finland, California, Austria, Switzerland		Picea abies Pinus attenuata Pinus coulteri Pinus muricata Pinus sylvestris	from slightly polluted to heavily polluted	study the erosion of epicuticular wax due to pollution and identify possible role of O_3	SEM	Karhu and Huttunen 1986
1987	Germany	Cu, Fe, Pb, Sb, Al, Cd, Cs, Sc, B, Ba, Ca, Co, K, Mg, Mn, N, Rb, S, Th, Zn	Picea abies		Establish whether is necessary or not to wash the needles before elemental analysis	INAA, AAS, microtitrimetry, elemental C-H-N analysis	Krivan et al. 1987
1988	Switzerland	20 elements	Picea abies		determine seasonal changes in the concentration of different elements in the needles over two years	INAA	Wyttenbach and Tobler 1988
1989	Germany	C, Si, Al, Fe, K, Ca, Mg, Na, Pb, Ni, Zn	Picea abies	forest		XRD, XPS, LA-FD-MS, ICP-AES	Simmleit et al. 1989
1989	USA	28 trace elements	Picea rubens, Abies fraseri	forest in mountain area	foliar elemental analysis to contribute to the understanding of forest decline	FAAS, ICP-AES, INAA	Robarge et al. 1989
1991	Finland	Cr, Ni		vicinity of ferrochrome and stainless steel works	comparison of different natural samplers: mosses, lichens, barks of conifers, needle litter, earthworms, moths, snow	AAS, ICP-AES	Kansanen and Venetvaara 1991
1991	Germany	acid precipitation, ozone	Picea abies		distribution of Ca oxalate in the needles and its relationship with the impact of pollutants	light microscopy, polarizing light microscopy, TEM	Fink 1991
1992	Russia		Picea abies			ICP-MS, ICP-AES, INAA	Markert and Wtorova 1992
1993	New Zealand	organochlorine pollutants	Pinus radiata	coastal sites, large plantation forests, farmlands far from larger municipal and industrial areas	identify compounds of natural or anthropogenic origin that interfere with the determination of organochlorine pollutants in pine needle wax	GC-FID, GC-MS	Franich et al 1993
1994	Switzerland, Germany	Sc, La, Ce, Sm, Eu, Tb, Yb, Lu	Picea abies		accumulation of REE and Sc in different needle age classes of Norway spruce also compared to other species	INAA	Wyttenbach et al. 1994
1994	Switzerland, Germany	Cs, Rb	Picea abies		determine endogenous concentrations and their changes with needle age; the eventual relationship between Rb and Cs concentrations	INAA	Tobler et al. 1994
1995	Greece	Cu, Zn, Pb	Pinus brutia	urban	Comparison with different tree species, lichens and mosses; distribution of contaminants in the city and differences between leaves collected in spring and fall	GF-AAS	Sawidis et al. 1995

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
1995	Poland	S, Zn, Cd, Pb, Cu, As	Pinus sylvestris	all Poland randomly selected points	danger for vegetation, animals, humans	distillation-colorimetric, AAS, colorimetric	Dmuchowski and Bytnerowicz 1995
1995	Switzerland, Germany	Ca, Cl, Cu, Fe, K, Mg, Mn, N, P, Zn, Al, As, Ba, Br, Co, Cr, Cs, Hg, I, La, Na, Rb, Sb, Sc, Si, Sr	Picea abies		describe with algebraic functions (biodynamic functions) the changes in the concentrations of different elements as a function of time in needles from individual trees	INAA, ICP-AES, combustion analysis	Wyttenbach et al. 1995
1995	Switzerland, Germany, Sweden	Ca, Sr, Ba, Mn	Picea abies		changes in the concentrations in successive needle age classes	ICP-AES, INAA	Wyttenbach et al. 1995
1996	Finland, Russia	K, Ca, Mn, Fe, Ni, Cu, Zn, Al, P, S, Cl, Mg	Pinus sylvestris	Cu-Ni smelters, Fe, apatite and mica ores, fertilizer factory, Al smelter, pulp and paper mills, steel mill	characterize needle surface structure and wettability; identify ecological factors responsible for the geographical variation in needle surface characteristics	SEM, contact angle measurements, sequential X-ray spectrometer	Turunen and Huttunen 1996
1996	Germany	Cr, Co, Ni, Cu, Zn, Cd, Ba, Pb	Picea abies and Pinus sylvestris		Study the pattern of heavy metal abundance in buds, needles, twigs and cones from single trees and to compare the results with the integrated (composite, homogenized) ESB material of the same origin.	ICP-MS	Rossbach and. Jayasekera 1996
1996	UK	PCBs	Pinus sylvestris	rural sites	spatial and temporal distribution of PCBs	GC	Tremolada et al. 1996
1997	Austria		Picea abies	forest (background sites)	develop method for quantifying changes in the epicuticular wax structure of spruce needles	SEM, S analyzer, ICP, AAS	Trimbacher and Eckmüllner 1997
1997	Russia	S, Ni, Cu, Fe, Ca, P	Pinus sylvestris	Cu-Ni smelters, open-cast apatite and mica mines, Al smelter	study the effects of S and heavy metal deposition on the physico-chemical characteristics of needle surfaces. The response is investigated in relation to the distance from the CU-Ni smelter	SEM, GC-FID, contact angle measurement, sequential X-ray spectrometer	Turunen et al. 1997
1999	Austria	N, S, P, K, Ca, Mg, Mn, Zn, Cu, Fe, Al	Picea abies	background, industry, conurbation, motorway	if wax quality, dust and gypsum crystallites and nutrient elements content reflect pollution level	SEM, ICP	Trimbacher and Weiss 1999
1999	Finland		Pinus sylvestris		develop an image analysis method to score the wax tube distribution of pine needles in response to increased UV-B	SEM	Kinnunen et al. 1999
1999	Latvia	P, S, K, Ca, Mn, Fe, Cu, Zn, Rb, Sr, Cr, Co, Ni, Cd, Pb	Pinus sylvestris	polluted and unpollutes areas	development of a database of exogenous and endogenous elemental concentrations in Scots pine needles; determination of the natural background levels of various elements in Scots pine needles	TRXRF, GF-AAS	Viksna et al. 1999

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
2000	Canada	Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn	Pinus banksiana	smelters, reference sites	understand ecosystem recovery	ICP-MS	Gratton et al. 2000
2000	Czech Republic	PAHs, chlorinated pesicides, PBCs	Pinus sylvestris	background site and 2 industrial ones	evaluate mosses and pine needles in the study of spatial differences in atmospheric burden of a range of POPs depending on landuse and sources of pollution	HRGC-MSD	Holoubek et al. 2000
2000	Lithuania	As, Cd, Cr, Mn, Pb, V, Zn	Picea abies		comparison with mosses and precipitation	AAS, GF-AAS	Ĉeburnis and Steinnes 2000
2000	Germany, Switzerland	Fe, Zn, Co	Picea abies		changes in the concentrations in successive needle age classes	INAA	Wyttenbach and Tobler 2000
2000	Finland		Picea abies	highway, street and local road	response of epicuticular wax structure to traffic pollution	SEM	Viskari 2000
2000	Finland		Picea abies	laboratory	effects of exhaust gas exposure on the epistomatal wax structure and mesophyll ultrastructure	SEM, light microscopy, TEM, porometer	Viskari et al. 2000
2000	Italy	Br, Ca, Co, Cr, Fe, K, La, Mo, Na, Sb, Sc, Sm, Al, Mg, P, Sr, Mn, Cu, Pb, Zn, Pb isotope composition	Pinus pinea	urban	identify sources of heavy metals in atmospheric aerosol and study the influence of air pollution on the morphological and cytological structure damage observed in pine needles	INAA, ICP-MS	Alaimo et al. 2000
2000	Poland	22 elements	Taxus baccata	urban and industrial areas	comparison different trees; comparison of digestion procedures	ICP-AES	Leśniewicz and Żyrnicki 2000
			Picea abies				
			Picea galuca				
			Pinus sylvestris				
2001	Russia, Finland, Norway	38 elements	Pinus sylvestris	near nickel smelter and refinery	comparison among different plants, relationship between element ratios in the emissions and in the plants	ICP-MS, ICP-AES, CV-AAS	Reinmann et al. 2001
			Picea abies				
2002	Greece	Cr, Mn, Fe, Ni, Cu, Cd, Pb	Pinus nigra	around a lignite power plant	Aerial and root uptake in different plants (lichens, mosses, trees)	GF-AAS	Tsikritzis et al. 2002
2002	South Korea	PCDD, PCDF	pine	urban	investigate the degree of contamination by PCDDs/DFs	HRGC-HRMS	Ok et al. 2002
2002	Nigeria	Zn, Cd, S	Pinus sylvestris	urban	danger for vegetation	AAS, turbidimetric method	Adekola et al. 2002
2002	Poland	N, P, K, Ca, Mg, S, B, Fe, Mn, Zn, Al, Cu, Pb, Cd	Pinus sylvestris	forest	forest condition: comparison 1997 and 2001, different locations and neighbouring countries	ICP-AES	Forest Research Institute 2002
			Picea abies				
2003	Russia, Finland, Sweden	Al, Ca, Cu, Fe, K, Mg, Mn, Ni, P, Pb, S, Zn	Pinus sylvestris	close to a smelter and far from pollution sources	investigate contribution of PM deposition to foliar chemistry and evaluation of soil analysis to estimate plant-available elements in the soil	XRF	Rautio and Huttunen 2003

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
2003	Italy	Pt, Pd, Au	Pinus pinea	urban	study presence and dispersion of Pt, Pd, Au and Pb in urban area affected by traffic	ICP-MS, INAA	Dongarrá et al. 2003
2003	Japan	U, Na, Mg, Al, Cl, K, Ca, Ti, V, Mn, Br, Sc, Cr, Fe, Co, La, Eu, Sm	Pinus thunbergii		investigate the feasibility of using pine needles to monitor uranium	ICP-MS, INAA	Saito et al. 2003
2003	Japan	PM	Pinus thunbergii	heavy traffic roads in urban areas	usefulness as indicator of atmospheric heavy metal particles	FE-SEM-EDS	Iwasaki and Tainosho 2003
2003	Korea, Mexico, USA	PAHs	Pinus densiflora	rural and urban/indutrialized	comparison between different sites	GC	Hwang et al. 2003
			Pinus thunbergii				
			Pinus maximartinezii				
			Pinus taeda				
2003	Italy	DDT	Picea abies	chemical company	spatial and temporal trends of accumulation	SEM	Di Guardo et al. 2003
			Pinus nigra				
2003	Poland, Norway	As, Bi, Sb, Sn	pine	urban	methodological: 1st application of an.technique	HG-ICP-AES	Pohl et al. 2003
			Picea abies				
2003	Sweden (also other Northern Europe countries)	SOCs	Pinus sylvestris	forest	uptake of pollutants in pine		Hellström 2003
			Pinus mugo				
2004	China	EOX	Pinus tabulaeformis	urban	evaluate distribution of EOX, tree contamination, contribution of OCPs and PCBs to the total EOCl, understand sources	INAA	Xu et al. 2004
2004	Finland		Pinus sylvestris				Nieminen et al. 2004
2004	Germany	PM, PAHs, Fe, Cd, Pb, Ni, Cr, Cu	Pinus nigra	parks, residential areas, major roads, railways, airport, industrial	test eveergreen species for suitability as biomonitor	IRM, ARM, SEM-EDX, AAS	Urbat et al. 2004
2004	Germany	PAHs	Pinus nigra	urban and suburban	evaluation of usefulness	SEM, GC-MS	Lehndorff and Schwark 2004
2004	Japan	dioxins	Japanese black pine	urban	involvement of citizens in environmental monitoring activities	HRGC-HRMS-SIM	Ikeda et al. 2004
2004	Poland	Pt, Pd, Rh	Pinus sylvestris	Forest (non polluted) and high traffic	Evaluate pine needles to monitor PGEs from catalytic converters	HR-ICP-MS, Q-ICP-MS, TXRF	Leśniewska et al. 2004
2004	Spain	Al, As, Cd, Cu, Cr, Fe, Hg, Mn, Ni, Pb, Zn	Pinus pinaster	rural areas	comparison with other biomonitors: oak leaves and mosses	FA-AAS, GF-AAS, AFS	Aboal et al. 2004

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
2004	Turkey	Cu, Zn, Pb	Pinus sylvestris	randomly selected nonindustrialized regions	danger for vegetation	AAS	Yilmaz and Zengin 2004
2005	CA, USA	SOCs	Abies concolor	National Park	comparison with lichens and species	GC	Deskin 2005
			Pinus contortas				
2005	Finland		Pinus sylvestris		effect of elevated CO_2 on photosynthetic capacity, anatomy and chemical composition		Luomala 2005
			Picea abies				
2005	Korea	dioxins	pine	different distances from an industrial waste incinerator	effect on different receptors	HR-GC, HR-MS	Kim et al. 2005
2005	Italy	Al, As, Ca, Ce, Cu, Fe, K, La, Mg, Mn, Mo, Na, Ni, Pb, Pd, Pt, Sb, Sc, Se, Sn, Th, Ti, V, Zn	Pinus halepensis	urban, industrial, rural	identify markers of petrochemical plant, traffic	ICP-MS	Bosco et al. 2005
2005	Italy	PCDD/PCDF, PAH, PCB, heavy metals	pine	around incinerator power plant and surrounding main roads	evaluate pollution caused by the plant	ICP, HRGC-MS	Capuano et al. 2005
2005	Spain	K, Ca, Mn, Fe, Cu, Sr, Pb, Zn	Pinus sylvestris	waste landfills surrounding an abandoned PB-Zn ore concentration factory	validation of the EDXRF method	EDXRF, ICP-AES	Marguí et al. 2005
2005	Tenerife, Canary Island	25 elements	Pinus canariensis		survey of potential pollutant elements in foliage and their sources	HPCL, AAS, ICP-MS	Tausz et al. 2005
2006	China	PCDD/F, PCB	Cedrus deodara	residential areas, heavy traffic, parks, island	first study in China using pine needles	HRGC-HRMS	Chen et al. 2006
2006	China	magnetic properties of PM	Pinus pumila	Highroad leading to airport far from industries and residential areas	Study magnetic properties of traffic PM	magnetic susceptibility, ARM, IRM, SEM-EDXS	Zhang et al. 2006
2006	Germany	magnetic PM	Pinus nigra	far from point sources and minimal influence of traffic	evaluation of enviromagnetic biomonitoring for the assessment of PM pollution	IRM, ARM, SEM-EDX	Lehndorff et al. 2006
2006	Germany, Czech Republic	PCDD/F	Picea abies	forest (national parks)	transboundary organic compounds in the Bavarian and Bohemian forest	GC-HRMS	Kirchner et al. 2006
2006	Spain	Al, Ba, Cr, Cu, Fe, Pb	Pinus pinea	indutrial area and background	suitability of different plant parts (leaves, bark, wood) as biomonitors (comparison also with oleander)	ICP-AES	Rossini Oliva and Mingorance 2006
2007	Germany, Czech Republic	PCDD/F	Picea abies	forest	comparison with active and passive samplers	HRGC-HRMS	Levy et al. 2007

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
2007	Jordan	Pb, Cd, Cu, Zn	Pinus halepensis	industrial, residential, high way, uncontaminated	usefulness as biomonitor in the arid environment	ICP-AES	Al-Alawi and Mandiwana 2007
2007	Croatia	PCB, pesticides		urban, semiurban	comparison with ambient air samples	GC	Romaniac and Krauthacker 2007
2007	ME, USA	Cd, Ni, Pb, Zn	Abies balsamea	forest	potential food chain transfer to herbivores	HR-ICP-MS	McGee et al. 2007
2007	Finland	N, Al, Fe, Cu, Zn, Mn, Ni, S, P, Ca, Mg, K	Picea abies	forest	evaluation of the threat posed by contaminated soils to the forest ecosystem	CHN analyzer, ICP-AES	Lindroos et al. 2007
2007	Germany	PCDD, PCDF, PCB	Picea abies, Pinus sylvestris	urbanized and rural areas	retrospective monitoring. Archived samples. Levels and time trends	HRGC-HRMS	Rappolder et al. 2007
2008	China	PCDD/Fs, PCBs	Cedrus deodara	urban (residential, heavy traffic, parks, island)	source identification	statistical	Tian et al. 2008
2008	China	magnetic properties	Sabina chinensis, Pinus bungeana	Industrial area	identify the relationship between magnetic properties and heavy metals in order to develop a method for fast quantification of environmental pollution	magnetic susceptibility, ARM, IRM	Hu et al. 2008
2009	Czech republic	PAHs, PCBs, OCPs	Pinus sylvestris	background station	evaluation of the feasibility of using pine needles to assess long-term trends of atmospheric contamination (comparison with HV and passive samplers)	GC-ECD, GC-MS	Klánová et al. 2009
2009	Italy	Co, Cr, Cu, Mn, Ni, Pb, V, Zn	pine	industrial, traffic, unpolluted	comparison with soils and other plant parts	ICP-AES	Danesino 2009
2009	Alps (Austria, Germany, Italy, Switzerland, Slovenia)	CPs	Picea abies	mountainous	develop a method for the detection of CPs using needles as passive samplers	GC-EI-MS/MS, ECNI-LRMS	Iozza et al. 2009
2009	Alps (Austria, Germany, Italy, Switzerland, Slovenia)	CPs	Picea abies	mountainous	Altitude profiles	GC-EI-MS/MS	Iozza et al. 2009
2009	Alps	PCDD/F, PCB	Picea abies	mountainous	Geographic distribution and altitudinal changes of pollutants; comparison with humus	GC-HRMS	Offenthaler et al. 2009
2009	Poland	PM containing metals	Pinus sylvestris	around flotation tailing ponds in a ore district	evaluate the potential use of dust particles trapped on pine needles for tracking dust migration around flotation tailing ponds	ESEM-EDXS	Teper 2009
2009	Poland	Cd, Ni, Pb, Zn, Fe, Mg, Mn, Ca, P, K, N	Abies alba	nature reserves	determine differences in the concentrations of elements with needle age	ICP-AES, FAAS, colorimetric	Szymura 2009

YEAR	COUNTRY	POLLUTANT/S and other elements measured	SPECIES	TYPE OF AREA	SCOPE	ANALYTICAL TECHNIQUE/S	REF
2010	Bulgaria	magnetic susceptibility, PAHs	Abies alba	areas polluted by metallurgical activities and background sites	study the magnetic signature of different plants suitable for biomonitoring	magnetic susceptibility, IRM, ARM, SIRM, OM, SEM-EDXS	Jordanova et al. 2010
2010	Finland	S	Pinus sylvestris	29 sites + 2 background	aerial distribution patterns around industrial plants	ICP-AES	Pöykiö et al. 2010
2010	Iran	Pb, Zn, Cu, Ni, Cr	Pinus eldarica	urban, industrial, highway, control site	usefulness as indicator for heavy metals pollution	AAS	Kord et al. 2010
2010	China	heavy metals, PAHs	Pinus massoniana	nearby industries	investigate contamination level by comparison with remote sites	ICP-AES, GC-MS	Sun et al. 2010
2010	China	PAHs	pine	woods near university	elucidate the effect of structural characteristics on the sorption properties of pine needle cuticular fraction in the interaction with POPs	CHN elemental analyzer, FTIR, solid state ^{13}C NMR	Li et al. 2010
2010	OH, USA	PAHs	Pinus strobes Pinus nigrus	urban	comparison with HV sampler filters	GC-MS	Tomashuk 2010
2010	Poland	Cr, Cd, Cu, Ni, Pb, Zn	Picea abies	along roads	determine influence of traffic density on heavy metal (Cr, Cd, Cu, Ni, Pb, Zn) concentrations along roads	AAS	Korzeniowska and Panek 2010
2011	Czech republic	39 elements	Picea abies	forest	comparison with moss and grass in studies at the scale of a whole country	ICP-MS, ICP-AES	Suchara et al. 2011
2011	Portugal	PAHs	Pinus pinea	from urban to remote mountain locations	differences in PAHs uptake among the two species	GC-MS	Ratola et al. 2011
2011	Salzburg (Austria), Belgrade (Serbia) and Thessaloniki (Greece)	Cr, Cu, Fe, Pb	Pinus nigra	urban	Estimate air pollution rate and the factors influencing it ; comparison with a deciduous tree and the use of bark	GF-AAS, SEM-EDXS	Sawidis et al. 2011
2012	France	PM, particularly radioactive particles	Chamaecyparis nootkatensis	near uranium-processing facility	characterize PM on the leaves and investigate whether or not radioactive particles were present; test the suitability of cypress leaves as passive biomonitors	SEM-EDXS, petrographic microscopy	Gieré et al. 2012
2012	Greece	Cd, Cr, Cu, Ni, Pb	Pinus brutia	urban and satellite communities	geographical distribution patterns and factors affecting toxic elements accumulation in trees	GF-AAS, SEM-EDXS	Sawidis et al. 2012
2013	Serbia	Cu, Pb, Zn, Mn	Pinus	urban-industrial, rural and background	correlation between the concentrations of the metals in different parts of the plant and their comparison with co-located soil composition, evaluation of air pollution biomonitoring ability near a Cu smelter and comparison with linden	ICP-AES	Serbula et al. 2013

6.6 Appendix F: ICP-AES data for the case study in chapter 4

Table 6.6-1 part 1 Elemental concentrations of needles collected at background sites (**AK and PS**) measured by ICP-AES.

Sample identification	**element**	**Li**	**Na**	**K**	**Mg**	**Sr**	**Ca**	**Ba**	**Cr**		**Mn**
	line	670.780	589.592	766.491	279.079	421.552	317.933	445.404	205.552	267.716	257.611
AK needles not washed digested with UltraWave	**concentration [ppm]**	670.8	590	766	279	57.753	13629	203.72	0.040	0.119	397.55
	ΔC [ppm]	0.3	58	2616	773	0.003	1	0.01	0.000	0.000	0.02
AK needles atmospheric concentrations digested with UltraWave	**concentration [ppm]**	0.000	0.00	4.8	0.0	4.43	951	3.24	0.000	0.000	28.66
	ΔC [ppm]	0.000	0.02	0.4	0.3	0.01	2	0.02	0.000	0.000	0.04
AK needles atmospheric concentrations (digestion with CEM)	**concentration [ppm d.m.]**	0.160	18	0	13	0.0	0	0.00	0.000	0.000	0
	ΔC [ppm d.m.]	0.005	2	22	13	0.1	51	0.05	0.000	0.000	4
	max acceptable	**0.186**	**28**	**121**	**84**	**0.3**	**280**	**0.26**	0.000	0.000	**24**
									0.000		
PS12 needles not washed digested with UltraWave	**concentration [ppm]**	0.240	13.909	2518.4	1688.2	18.785	9514	51.159	0.679	0.719	13.589
	ΔC [ppm]	0.000	0.005	0.2	0.1	0.001	3	0.003	0.000	0.000	0.001
PS12 needles atmospheric concentrations digested with UltraWave	**concentration [ppm]**	0.000	1.43	209.8	135.3	0.000	2132	0.00	0.278	0.278	1.551
	ΔC [ppm]	0.000	0.01	0.3	0.2	0.002	4	0.01	0.000	0.000	0.001
PS12 needles atmospheric concentrations (digestion with CEM)	**concentration [ppm d.m.]**	0.000	30.0	0	0	0.00	0	9.46	0.000	0.000	0.000
	ΔC [ppm d.m.]	0.000	0.6	15	13	0.03	67	0.04	0.000	0.000	0.000
	max acceptable	**0.000**	**33.2**	**81**	**73**	**0.15**	**370**	**9.70**	0.000	0.000	**0.000**
									0.000		
PS8 needles atmospheric concentrations (digestion with CEM)	**concentration [ppm d.m.]**	0.205	0.000	0.000	1324	7.58	1789	0.0	0.000	0.000	0.41
	ΔC [ppm d.m.]	0.001	1	17	18	0.03	38	0.7	0.000	0.000	0.02
	max acceptable	**0.210**	**7**	**91**	**1420**	**7.75**	**2000**	**3.7**	0.000	0.000	**0.50**
									0.000		

Table 6.6-1 part 2 Elemental concentrations of needles collected at background sites (**AK and PS**) measured by ICP-AES.

Sample identification	element	Fe	Co	Cu		Ni	Zn	Al		Pb	Cd	
	line	259.941	238.892	324.754	327.396	231.604	213.856	167.078	394.401	220.353	214.438	226.502
AK needles not washed digested with UltraWave	**concentration [ppm]**	35.601	0.198	1.661	1.582	1.305	54.193	66.812	57.753	0.079	<IDL	<IDL
	ΔC [ppm]	0.002	0.000	0.000	0.000	0.000	0.003	0.004	0.003	0.002		
AK needles atmospheric concentrations digested with UltraWave	**concentration [ppm]**	6.732	0.000	0.000	0.000	0.000	2.07	27.96	24.47	0.032	0.000	0.000
	ΔC [ppm]	0.004	0.000	0.000	0.000	0.001	0.01	0.01	0.01	0.002		
AK needles atmospheric concentrations (digestion with CEM)	**concentration [ppm d.m.]**	12	0.000	0.000	0.000	0.000	4	17	15	0.000	0.000	0.000
	ΔC [ppm d.m.]	1	0.000	0.000	0.000	0.000	1	2	2	0.000	0.000	0.000
	max acceptable	**18**	**0.000**	0.000	0.000	**0.000**	**9**	25	24	**0.000**	0.000	0.000
				0.000				**25**			**0.000**	
PS12 needles not washed digested with UltraWave	**concentration [ppm]**	65.548	0.560	2.918	2.878	0.240	35.572	64.708	53.957	0.040	<IDL	<IDL
	ΔC [ppm]	0.004	0.000	0.000	0.000	0.000	0.002	0.005	0.005	0.001		
PS12 needles atmospheric concentrations digested with UltraWave	**concentration [ppm]**	36.25	0.279	0.510	0.510	0.079	0.000	39.47	28.68	0.040	0.000	0.000
	ΔC [ppm]	0.01	0.000	0.000	0.000	0.000	0.004	0.01	0.01	0.001		
PS12 needles atmospheric concentrations (digestion with CEM)	**concentration [ppm d.m.]**	0.0	0.000	0.68	0.72	0.000	0	23.1	20	0.000	0.000	0.000
	ΔC [ppm d.m.]	0.4	0.000	0.01	0.01	0.000	2	0.5	1	0.000	0.000	0.000
	max acceptable	**2.4**	**0.000**	0.72	0.76	**0.000**	**11**	26.0	25	**0.000**	0.000	0.000
				0.74				**25.3**			**0.000**	
PS8 needles atmospheric concentrations (digestion with CEM)	**concentration [ppm d.m.]**	4	0.000	0.20	0.20	0.000	0	0	10	0.000	3.93	3.72
	ΔC [ppm d.m.]	1	0.003	0.01	0.01	0.000	2	1	1	0.000	0.01	0.01
	max acceptable	**12**	**0.016**	0.24	0.23	**0.000**	**12**	4	13	**0.000**	3.96	3.75
				0.24				**9**			**3.86**	

Table 6.6-2 2013 needles (sampled in fall 2013, exposed from spring 2013) collected at **site PS** concentrations and calculated max acceptable atmospheric concentration (pollution threshold).

	Total needle concentrations (not washed) (ppm d.m.)		**Atmospheric %**	**Total atmospheric (ppm d.m)**		**Max acceptable (ppm d.m.)**
Crustal elements	Na	10.0 ± 0.5	**39%**	Na	3 ± 1	6
	Ca	2.11 ± 0.02 mg/g		Ca	0.22 ± 0.04 mg/g	0.34 mg/g
	Mg	1.61 ± 0.01 mg/g		Mg	0.00 ± 0.02 mg/g	0.07 mg/g
	Al	21.4 ± 0.2		Al	19.9 ± 0.4	20.9
	Li	0.205 ± 0.000		Li	0.000 ± 0.001	0.002
Anthropogenic elements	Sr	9.02 ± 0.01	**2%**	Sr	2.77 ± 0.02	2.83
	Ba	13 ± 2		Ba	0 ± 2	6
	Cr	< LOD		Cr		0
	Mn	0.20 ± 0.02		Mn	0.00 ± 0.05	0.14
	Co	0.205 ± 0.000		Co	0.000 ± 0.001	0.003
	Ni	< LOD		Ni		0.09
	Cu	0.615 ± 0.002		Cu	0.00 ± 0.01	0.02
	Zn	32 ± 1		Zn	8 ± 2	14
	Pb	< IDL		Pb		0
	Cd	< IDL		Cd		0
Ambiguous elements	K	2.55 ± 0.01 mg/g	**59%**	K	0.35 ± 0.01 mg/g	0.39 mg/g
	Fe	49 ± 1		Fe	19 ± 2	24

Table 6.6-3 Summary of ICP-AES results at **site P**. The element in bold in the column total atmospheric concentration are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which following the Mingorance *et al.* (2007) criteria indicate pollution. The EF_S for 2008 needles was calculated with reference to the soil in the steelmaking plant (two different sides, NE and E-SE and Pergine (1995-6). The EF_S were evaluated following Feng *et al.* (2009): EF_S >100 highly enriched (h.e.), 10< EF_S <100 intermediately enriched (i.e.), 1< EF_S <10 slightly enriched (s.e.)

Needles age cohort and exposure time		**2008 (4 years and 2months ca)**							**2012 (2 months ca)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm dry matter)**	**atm (%)**	**atm (ppm dry matter)**	**atm EF_B**	**tot EF_B**	**EF_S NE**	**EF_S E-SE**	**total concentration (ppm dry matter)**	**atm (%)**	**atm (ppm dry matter)**	**atm EF_B**	**tot EF_B**
crustal	**Na**	90 ± 2	99			2.0	s.e.		<LOD	76		0.0	0.0
	Mg	2.36 ± 0.02 mg/g			0.0	0.8	i.e.		0.85 ± 0.01 mg/g				0.6
	Ca	7.7 ± 0.1 mg/g		**2.9 ± 0.1 mg/g**	1.6	1.7	s.e.		1.95 ± 0.03 mg/g				0.7
	Al	311 ± 6		**203 ± 7**	**40.6**	**7.4**			24 ± 1		24 ± 1	1.1	
	Li	4.28 ± 0.01		**3.71 ± 0.02**	**18.1**	**10.3**							
anthropogenic	**Sr**	9.15 ± 0.02	1	3.69 ± 0.03	0.5	0.6			1.73 ± 0.01	16			0.2
	Ba	<LOD				0.0			15.81 ± 0.02		2.70 ± 0.04	0.3	0.8
	Cr	3.2 ± 0.1		**3.2 ± 0.1**	**>2**	**>2**			<LOD				
	Mn	26.7 ± 0.1		**15.8 ± 0.1**	**38.5**	**65.1**			10.82 ± 0.04		**0.5 ± 0.1**	**>2**	**>2**
	Co	4.0 ± 0.1		**4.0 ± 0.1**	**>2**	**4.8**			< IDL				
	Ni	<LOD				0.0			< IDL				
	Cu	3.9 ± 0.1		**3.9 ± 0.1**	**19.5**	**4.2**			2.7 ± 0.1		**1.7 ± 0.2**	**2.4**	**2.1**
	Zn	<LOQ				0.0			<LOD				0.0
	Pb	<IDL				0.0			< IDL				
	Cd	<IDL			0.0	0.0			< IDL				
ambiguous	**K**	1.30 ± 0.01 mg/g	0			0.9	i.e.		5.20 ± 0.02 mg/g	8	**2.43 ± 0.03**	**>2**	**2.4**
	Fe	<LOQ			0.0	0.0			<LOD				0.0

Table 6.6-4 Summary of ICP-AES results at **site M**. The element in bold in the column total atmospheric concentration are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which following the Mingorance *et al.* (2007) criteria indicate pollution. The EF_S for 2008 needles was calculated with reference to the soil in Pergine (1995-6). The EF_S were evaluated following Feng *et al.* (2009): EF_S >100 highly enriched (h.e.), 10< EF_S <100 intermediately enriched (i.e.), 1< EF_S <10 slightly enriched (s.e.)

Needles age cohort and exposure time		**2008 (4 years and 2months ca)**						**2012 (2 months ca)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm dry matter)**	**atm (%)**	**atm (ppm dry matter)**	**atm EF_B**	**tot EF_B**	**EF_S**	**total concentration (ppm dry matter)**	**atm (%)**	**atm (ppm dry matter)**	**atm EF_B**	**tot EF_B**
crustal	**Na**	13 ± 1	6			0.3		<LOQ	0		0.0	0.0
	Mg	1.97 ± 0.01 mg/g			0.0	0.7	i.e.	1.10 ± 0.01 mg/g				0.7
	Ca	3.6 ± 0.1 mg/g			0.0	0.8	s.e.	1.51 ± 0.01 mg/g				0.5
	Al	10.1 ± 0.5		2 ± 2	0.4	0.2		<LOD			0.0	0.0
	Li				0.0	0.0						
anthropogenic	**Sr**	6.28 ± 0.01	94	0.81 ± 0.03	0.1	0.4		1.106 ± 0.002	0			0.1
	Ba	20.9 ± 0.2		**20.9 ± 0.2**	**>2**	1.2		15.85 ± 0.02			0.0	0.8
	Cr	<IDL				0.0		<IDL				
	Mn	12.07 ± 0.04		**9.5 ± 0.1**	**23.2**	**29.4**		9.35 ± 0.02				**>2**
	Co	<IDL				0.0		<IDL				
	Ni	<IDL				0.0		<IDL				
	Cu	<LOQ			0.0	0.0		1.0 ± 0.1			0.0	0.8
	Zn	<LOQ				0.0		<LOD				0.0
	Pb	<IDL				0.0		<IDL				
	Cd	<IDL			0.0	0.0		<IDL				
ambiguous	**K**	2.4 ± 0.2 mg/g	0			1.6	i.e.	2.69 ± 0.01 mg/g	100	0.01 ± 0.02 mg/g	**>2**	1.2
	Fe	19.3 ± 0.2			0.0	0.2		8.36 ± 0.03		**3.6 ± 0.1**	**>2**	0.2

Table 6.6-5 Summary of ICP-AES results at **site L**. The element in bold in the column total atmospheric concentration are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF$_B$s are those > 2 which following the Mingorance *et al.* (2007) criteria indicate pollution. The EF$_S$ for 2008 needles was calculated with reference to the soil at E from Roncegno (2009) or in Pergine (1995-6). The EF$_S$ were evaluated following Feng *et al.* (2009): EF$_S$ >100 highly enriched (h.e.), 10< EF$_S$ <100 intermediately enriched (i.e.), 1< EF$_S$ <10 slightly enriched (s.e.)

Needles age cohort and exposure time		**2008 (4 years and 2 months ca)**						**2012 (2 months ca)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm dry matter)**	**atm (%)**	**atm (ppm dry matter)**	**atm EF$_B$**	**tot EF$_B$**	**EF$_S$**	**total concentration (ppm dry matter)**	**atm (%)**	**atm (ppm dry matter)**	**atm EF$_B$**	**tot EF$_B$**
crustal	**Na**	10 ± 1	100	0 ± 2		0.2		19 ± 1	53	12 ± 3	0.4	0.4
	Mg	1.77 ± 0.02 mg/g		0.08 ± 0.03 mg/g	0.1	0.6	i.e.	0.94 ± 0.01 mg/g				0.6
	Ca	5.6 ± 0.1 mg/g		1.7 ± 0.2 mg/g	0.9	1.3	s.e.	2.69 ± 0.02				0.9
	Al	201 ± 1		0 ± 2	0.0	**4.8**		23 ± 1		23 ± 1	1.0	0.7
	Li				0.0	0.0						
anthropogenic	**Sr**	14.73 ± 0.03	0	3.80 ± 0.05	0.5	1.0		7.10 ± 0.02	12			0.8
	Ba	14.78 ± 0.02		0.0 ± 0.1		0.8		22.72 ± 0.03		4.2 ± 0.1	0.4	1.1
	Cr	<LOD						<LOD				
	Mn	24.2 ± 0.1		**2.4 ± 0.1**	**5.9**	**59.0**		22.8 ± 0.1		**3.1 ± 0.1**	**>2**	**>2**
	Co	<LOQ				0.0		<LOD				
	Ni	<IDL						<LOD				
	Cu	1.1 ± 0.1		0.1 ± 0.3	0.5	1.2		3.8 ± 0.2		**0.9 ± 0.3**	1.3	**2.9**
	Zn	<LOD				0.0		<LOD				0.0
	Pb	<IDL						<IDL				
	Cd	<IDL			0.0	0.0		<IDL				
ambiguous	**K**	1.85 ± 0.01 mg/g	0	0.00 ± 0.02		1.3	i.e.	1.83 ± 0.03 mg/g	35			0.8
	Fe	<LOD			0.0	0.0		37 ± 1		**23 ± 1**	**>2**	0.8

Table 6.6-6 part 1 Summary of ICP-AES results at **site B**. The element in bold in the column total atmospheric concentration are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which following the Mingorance *et al.* (2007) criteria indicate pollution. The EF_S for 2008 needles was calculated with reference to the soil in the NW side of the steelworks (2009) or in Pergine (1995-6). The EF_S were evaluated following Feng *et al.* (2009): EF_S >100 highly enriched (h.e.), 10< EF_S <100 intermediately enriched (i.e.), 1< EF_S <10 slightly enriched (s.e.)

Needles age cohort and exposure time		**2005 (ca 6 years and 9 months)**			**2008 (5 years and 1 month ca)**					
ICP-AES-based source apportionment	**element**	**total concentration (ppm dry matter)**	**atm (%)**	**atmospheric (ppm dry matter)**	**total concentration (ppm dry matter)**	**atm (%)**	**atmospheric (ppm dry matter)**	**atm EF_B**	**total EF_B**	**EF_S**
crustal	**Na**	268 ± 4	31	12 ± 7	757 ± 9	59	0 ± 13		**16.6**	i.e.
	Mg	2.41 ± 0.01 mg/g		0.06 ± 0.02 mg/g	2.69 ± 0.01 mg/g		0.61 ± 0.02 mg/g	0.5	0.9	i.e.
	Ca	4.81 ± 0.04 mg/g			3.6 ± 0.1 mg/g		0.5 ± 0.1 mg/g	0.3	0.8	s.e.
	Al	811 ± 15			756 ± 10		**536 ± 16**	**107**	**17.9**	
	Li	0.47 ± 0.02			0.816 ± 0.001		0.195 ± 0.002	1.0	2.0	
anthropogenic	**Sr**	16.7 ± 0.1	69		13.26 ± 0.02	4	0.43 ± 0.04	0.1	0.9	
	Ba	31.2 ± 0.3			21.42 ± 0.03		0.0 ± 0.9		1.2	
	Cr	20.9 ± 0.2			6.32 ± 0.03		**6.32 ± 0.04**	**>2**	**>2**	
	Mn	325 ± 3		151 ± 4	132 ± 1		**103 ± 1**	**251**	**322**	s.e.
	Co	14.7 ± 0.3		2 ± 1	7.75 ± 0.02		**5.06 ± 0.02**	**>2**	**9.4**	
	Ni	1.5 ± 0.1		0.9 ± 0.3	0.0 ± 0.2		0.0 ± 0.2			
	Cu	13.2 ± 0.2		3.8 ± 0.5	7.55 ± 0.01		**5.38 ± 0.02**	**27**	**8.1**	
	Zn	<LOD			41 ± 1		2 ± 2	**>2**	**2.6**	
	Pb	6.9 ± 0.4		6.9 ± 0.4	< IDL					
	Cd	<LOQ			< IDL			0.0		
ambiguous	**K**	2.44 ± 0.01 mg/g	0		2.15 ± 0.01 mg/g	37	0.30 ± 0.02 mg/g	**>2**	1.5	i.e.
	Fe	1238 ± 7			1047 ± 7		**733 ± 8**	**183**	**12.8**	s.e.

Table 6.6-6 part 2 Summary of ICP-AES results at **site B**. The element in bold in the column total atmospheric concentration are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which following the Mingorance *et al.* (2007) criteria indicate pollution.

Needles age cohort and exposure time		**2011 (9 months ca)**					**2012 (1 year and 1 month ca)**					**2013 (6-7 months)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B AK**	**tot EF_B AK**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**
Crustal	**Na**	654 ± 4	96		0.0	**25.2**	113 ± 1	85	0 ± 2	0.0	**2.5**	31 ± 1	2	**14 ± 1**	**4.7**	**3.1**
	Mg	2.02 ± 0.02 mg/g		**0.28 ± 0.03 mg/g**	0.0	**2.7**	1.61 ± 0.01 mg/g		0.04 ± 0.03 mg/g	**>2**	1.1	1.62 ± 0.01 mg/g		0.02 ± 0.02	**>2**	1.0
	Ca	9.6 ± 0.1 mg/g		**7.7 ± 0.1 mg/g**	**>2**	**3.2**	2.31 ± 0.02 mg/g		0.00 ± 0.05 mg/g		0.8	1.66 ± 0.01 mg/g		0.00 ± 0.03 mg/g	0.0	0.8
	Al	469 ± 13		**469 ± 13**	**29.3**	**12.2**	169 ± 2		**114 ± 3**	**5.2**	**5.1**	130 ± 3			0.0	**6.1**
	Li	0.32 ± 0.01		0.10 ± 0.03	**>2**	2.0	0.205 ± 0.000		**0.205 ± 0.000**	**>2**	**>2**	0.207 ± 0.000		**0.207 ± 0.000**	**>2**	1.0
Anthropogenic	**Sr**	19.6 ± 0.1	2	**14.6 ± 0.1**	**91.3**	1.3	7.70 ± 0.01	3	0.00 ± 0.03		0.9	7.02 ± 0.01	0	0.00 ± 0.02	0.0	0.8
	Ba	30.2 ± 0.2		**29.0 ± 0.2**	**>2**	**2.4**	18.17 ± 0.03		0.0 ± 0.1	0.0	0.9	18.6 ± 0.2		0.0 ± 0.3		1.4
	Cr	18.5 ± 0.3		**10.2 ± 0.5**	**>2**	**>2**	< LOD					< LOD				
	Mn	129 ± 1		**129 ± 1**	**>2**	0.4	2.46 ± 0.02		**2.46 ± 0.02**	**>2**	**>2**	3.10 ± 0.02		**3.10 ± 0.02**	**>2**	**15.5**
	Co	7.7 ± 0.3		**3.8 ± 0.5**	**>2**	**>2**	1.640 ± 0.002		**0.806 ± 0.004**	**>2**	**>2**	2.065 ± 0.003		**1.656 ± 0.004**	**>2**	**10.1**
	Ni	0.6 ± 0.3		**0.6 ± 0.3**	**>2**	**>2**	< LOD					< LOD				
	Cu	11.4 ± 0.4		**5 ± 1**	**>2**	**>2**	2.20 ± 0.01		**1.37 ± 0.01**	2.0	1.7	3.72 ± 0.01		**2.39 ± 0.01**	**>2**	**6.0**
	Zn	<LOQ			0.0	0.0	17 ± 1		0 ± 2		0.6	21 ± 1		0 ± 2	0.0	0.7
	Pb	<IDL					< IDL					< IDL				
	Cd	<IDL					< IDL					< IDL				
ambiguous	**K**	2.32 ± 0.02 mg/g	2			1.0	2.97 ± 0.03 mg/g	12	0.00 ± 0.07 mg/g		1.3	3.26 ± 0.02 mg/g	98	**1.29 ± 0.05 mg/g**	**3.7**	1.3
	Fe	740 ± 10		**172 ± 22**	**14.3**	**24.7**	107 ± 1		**21 ± 2**	**>2**	**2.3**	244 ± 3		**201 ± 4**	**10.6**	**5.0**

Table 6.6-7 Summary of ICP-AES results at **site F**. The element in bold in the column total atmospheric concentration are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which following the Mingorance *et al.* (2007) criteria indicate pollution. The EF_S for 2008 needles was calculated with reference to the soil on the NW side of the steelworks (2009) or in Pergine (1995-6). The EF_S were evaluated following Feng *et al.* (2009): EF_S >100 highly enriched (h.e.), 10< EF_S <100 intermediately enriched (i.e.), 1< EF_S <10 slightly enriched (s.e.)

Needles age cohort and exposure time		**2008 (4 years and 2months ca)**						**2008 bis (4 years and 2months ca)**						**2012 (2 months ca)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**EF_S**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**EF_S**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**
crustal	**Na**	<LOD	12			0.0		<LOD	98			0.0			37		0.0	0.0
	Mg	1.57 ± 0.01 mg/g			0.0	0.5	i.e.	1.44 ± 0.01 mg/g		0.00 ± 0.03	0.0	0.5	i.e.	1.12 ± 0.01 mg/g		0.03 ± 0.02 mg/g	**>2**	0.8
	Ca	3.23 ± 0.02 mg/g		0.01 ± 0.05 mg/g	0.0	0.7	s.e.	3.70 ± 0.04 mg/g		0.5 ± 0.1 mg/g	0.3	0.8	s.e.	1.38 ± 0.02 mg/g		0.00 ± 0.03 mg/g		0.5
	Al	107 ± 247		**75 ± 248**	**15.0**	**2.5**		100 ± 3		**68 ± 4**	**13.6**	2.4		1.6 ± 0.3		1.6 ± 0.3	0.1	0.0
	Li				0.0	0.0					0.0	0.0						
Anthropogenic	**Sr**	8.32 ± 0.02	0		0.0	0.0		8.26 ± 0.01	2	0.00 ± 0.03	0.0	0.6		3.16 ± 0.01	0	0.00 ± 0.02		0.4
	Ba	10.7 ± 0.1				0.6		15.08 ± 0.03		**4.3 ± 0.1**	**>2**	0.8		8.43 ± 0.01		0.10 ± 0.02	0.0	0.4
	Cr	<LOD				0.0		0.826 ± 0.001		**0.826 ± 0.001**	**>2**	>2		<IDL				
	Mn	10.60 ± 0.04		0.3 ± 0.1	0.7	**25.9**		15.1 ± 0.2		**4.8 ± 0.3**	**11.7**	36.8		19.4 ± 0.1		0.0± 0.1		**>2**
	Co	<LOQ				0.0		0.413 ± 0.001		**0.413 ± 0.001**	**>2**	0.5		<IDL				
	Ni	<IDL				0.0		<IDL						<IDL				
	Cu	3.1 ± 0.2		**3.1 ± 0.2**	**15.5**	**3.3**		1.033 ± 0.002		**1.033 ± 0.002**	**5.2**	1.1		1.0 ± 0.1		0.0 ± 0.2	0.0	0.8
	Zn	<LOD				0.0		<LOD				0.0		<LOD				0.0
	Pb	<IDL				0.0		<IDL						<IDL				
	Cd	<IDL			0.0	0.0		<IDL			0.0	0.0		<IDL				
ambiguous	**K**	5.0 ± 0.1 mg/g	88	**0.6 ± 0.1 mg/g**		**3.4**	i.e.	4.24 ± 0.04 mg/g	0	0.0 ± 0.1 mg/g		2.9	i.e.	2.61 ± 0.02 mg/g	63	0.04 ± 0.03 mg/g	**>2**	1.2
	Fe	<LOD			0.0	0.0		<LOD			0.0	0.0		23.6 ± 0.4		**11 ± 1**	**>2**	0.5

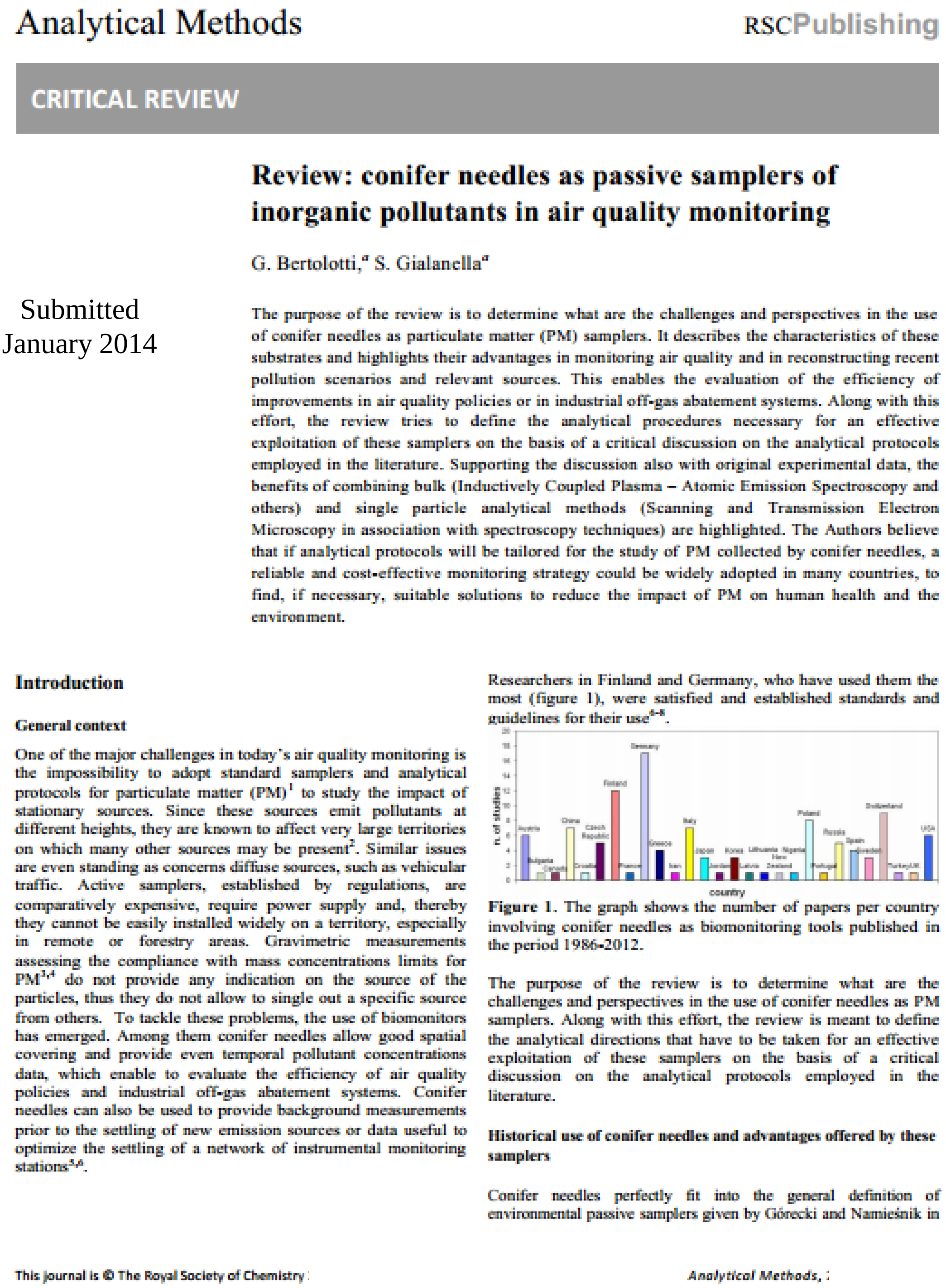
Table 6.6-8 part 1 Summary of ICP-AES results at **site O**. The element in bold in the column total atmospheric concentration (atm) are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which indicate pollution (Mingorance *et al.* 2007). The EF_S for 2008 needles was calculated with reference to the soil in Olle (2009) or in Pergine (1995-6) and evaluated following Feng *et al.* (2009): EF_S >100 highly enriched (h.e.), 10< EF_S <100 intermediately enriched (i.e.), 1< EF_S <10 slightly enriched (s.e.)

Needles age cohort (exposure time)		**2008 (5 years and 1 month ca)**						**2012 (1 year and 1 month ca)**					**2013 (6-7 months)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**EF_S**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**total EF_B**
Crustal	**Na**	22.5 ± 0.5	98	0 ± 1		0.5		0.4 ± 0.2	97	0.4 ± 0.2	0.0	0.0	< LOD	0	0.0 ± 0.3	0.0	0.0
	Mg	1.43 ± 0.01 mg/g		0 ± 13	0.0	0.5	i.e.	1.09 ± 0.01 mg/g		0.04 ± 0.02 mg/g	**>2**	0.7	1.31 ± 0.01 mg/g		0.00 ± 0.02 mg/g		0.8
	Ca	4.82 ± 0.04 mg/g		1.01 ± 0.06 mg/g	0.6	1.1	s.e.	3.69 ± 0.06 mg/g		**1.1** ± 0.1 mg/g	**>2**	1.3	2.02 ± 0.01 mg/g		0.00 ± 0.02 mg/g	0.0	1.0
	Al	18.0 ± 0.2		0 ± 6	0.0	0.4		16.4 ± 0.4		11.3 ± 0.6	0.5	0.5				0.0	0.0
	Li				0.0	0.0											0.0
Anthropogenic	**Sr**	15.70 ± 0.02	2	2.32 ± 0.04	0.3	1.1		9.73 ± 0.01	1	**2.46** ± 0.02	**>2**	1.1	7.10 ± 0.01	100	0.00 ± 0.02	0.0	0.8
	Ba	38 ± 1		**19 ± 1**	**>2**	**2.1**		10.54 ± 0.02		5.97 ± 0.02	0.6	0.5	7.30 ± 0.01		2.93 ± 0.02	**>2**	0.6
	Cr	0.00 ± 0.01		0.00 ± 0.03		0.0		<LOD					< LOD				
	Mn	0.00 ± 0.01		0.00 ± 0.02	0.0	0.0							3.65 ± 0.02		0.00 ± 0.06		**18.3**
	Co	< IDL				0.0		< IDL					0.405 ± 0.001		0.000 ± 0.002		2.0
	Ni					0.0		< LOD					< LOD				
	Cu	0.413 ± 0.003		0.0 ± 0.1	0.0	0.4		< LOD			0.0	0.0	0.811 ± 0.003		0.00 ± 0.01		1.3
	Zn	47 ± 1		0 ± 3		**2.9**		23 ± 1		0 ± 2		0.9	31 ± 1		0 ± 2	0.0	1.0
	Pb	< IDL				0.0		< IDL					< IDL				
	Cd	< IDL			0.0	0.0		< IDL					< IDL				

Table 6.6-8 part 2 Summary of ICP-AES results at **site O**. The element in bold in the column total atmospheric concentration (atm) are above the maximum concentrations not suggesting pollution calculated from needles of the same age collected at the local background location (PS). The values in bold in the column with the EF_Bs are those > 2 which indicate pollution (Mingorance *et al.* 2007). The EF_S for 2008 needles was calculated with reference to the soil in Olle (2009) or in Pergine (1995-6) and evaluated following Feng *et al.* (2009): EF_S >100 highly enriched (h.e.), 10< EF_S <100 intermediately enriched (i.e.), 1< EF_S <10 slightly enriched (s.e.)

Needles age cohort (exposure time)		**2008 (5 years and 1 month ca)**						**2012 (1 year and 1 month ca)**					**2013 (6-7 months)**				
ICP-AES-based source apportionment	**element**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**EF_S**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**	**total concentration (ppm d.m.)**	**atm (%)**	**atm (ppm d.m.)**	**atm EF_B**	**tot EF_B**
ambiguous	**K**	2.38 ± 0.01	0	0 ± 33		1.6	i.e.	2.48 ± 0.01 mg/g	2	0.00 ± 0.01 mg/g		1.1	1.96 ± 0.02 mg/g	0	0.00 ± 0.07 mg/g	0.0	0.8
	Fe	67 ± 6		0.2 ± 6	0.1	0.8		38 ± 1		**23** ± 1	**>2**	0.8	76 ± 1		0 ± 2	0.0	1.6

6.7 Appendix G: Scientific articles and abstracts of oral and poster presentations related to this thesis



Analytical Methods RSCPublishing

CRITICAL REVIEW

Review: conifer needles as passive samplers of inorganic pollutants in air quality monitoring

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Submitted
January 2014

The purpose of the review is to determine what are the challenges and perspectives in the use of conifer needles as particulate matter (PM) samplers. It describes the characteristics of these substrates and highlights their advantages in monitoring air quality and in reconstructing recent pollution scenarios and relevant sources. This enables the evaluation of the efficiency of improvements in air quality policies or in industrial off-gas abatement systems. Along with this effort, the review tries to define the analytical procedures necessary for an effective exploitation of these samplers on the basis of a critical discussion on the analytical protocols employed in the literature. Supporting the discussion also with original experimental data, the benefits of combining bulk (Inductively Coupled Plasma – Atomic Emission Spectroscopy and others) and single particle analytical methods (Scanning and Transmission Electron Microscopy in association with spectroscopy techniques) are highlighted. The Authors believe that if analytical protocols will be tailored for the study of PM collected by conifer needles, a reliable and cost-effective monitoring strategy could be widely adopted in many countries, to find, if necessary, suitable solutions to reduce the impact of PM on human health and the environment.

Introduction

General context

One of the major challenges in today's air quality monitoring is the impossibility to adopt standard samplers and analytical protocols for particulate matter (PM)[1] to study the impact of stationary sources. Since these sources emit pollutants at different heights, they are known to affect very large territories on which many other sources may be present[2]. Similar issues are even standing as concerns diffuse sources, such as vehicular traffic. Active samplers, established by regulations, are comparatively expensive, require power supply and, thereby they cannot be easily installed widely on a territory, especially in remote or forestry areas. Gravimetric measurements assessing the compliance with mass concentrations limits for PM[3,4] do not provide any indication on the source of the particles, thus they do not allow to single out a specific source from others. To tackle these problems, the use of biomonitors has emerged. Among them conifer needles allow good spatial covering and provide even temporal pollutant concentrations data, which enable to evaluate the efficiency of air quality policies and industrial off-gas abatement systems. Conifer needles can also be used to provide background measurements prior to the settling of new emission sources or data useful to optimize the settling of a network of instrumental monitoring stations[5,6]. Researchers in Finland and Germany, who have used them the most (figure 1), were satisfied and established standards and guidelines for their use[6-8].

Figure 1. The graph shows the number of papers per country involving conifer needles as biomonitoring tools published in the period 1986-2012.

The purpose of the review is to determine what are the challenges and perspectives in the use of conifer needles as PM samplers. Along with this effort, the review is meant to define the analytical directions that have to be taken for an effective exploitation of these samplers on the basis of a critical discussion on the analytical protocols employed in the literature.

Historical use of conifer needles and advantages offered by these samplers

Conifer needles perfectly fit into the general definition of environmental passive samplers given by Górecki and Namieśnik in

Aerosol and Air Quality Research, 14: 677–685, 2014
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ISSN: 1680-8584 print / 2071-1409 online
doi: 10.4209/aaqr.2013.10.0308

A Multi-Analytical Approach to the Use of Conifer Needles as Passive Samplers of Particulate Matter and Organic Pollutants

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ABSTRACT

The present work regards the analysis of airborne pollutants emitted by an electric arc furnace steel making plant at a test site in Northern Italy and collected by conifer needles. The spatial and temporal trends of accumulation of Mn, Zn, Fe, Cr, Pb, polychlorinated dibenzo-*p*-dioxins/dibenzofurans and polychlorinated biphenyls dioxin revealed that the contribution from the steel making plant has never been particularly high, whereas traffic emerged as a significant pollution source. The benefits of combining bulk and single particle analysis in air pollution studies from different sources are also discussed.

Keywords: Spruce needles; Air pollution; Steelmaking plant pollution; Heavy metals.

INTRODUCTION

Conifer needles can be considered passive samplers of particulate matter (Urbat *et al.*, 2004) and a number of semi-volatile organic compound (SVOC) pollutants (Eriksson *et al.*, 1989, Tremolada *et al.*, 1993, Kylin *et al.*, 1994, Tremolada *et al.*, 1996, Di Guardo *et al.*, 1999). For this reason, needles are used for air quality studies based on biomonitoring. The capturing mechanism depends on pollutant state. Gaseous organic pollutants can diffuse through the cuticle, penetrate through the stomata (Urbat *et al.*, 2004) or be trapped in the epicuticular wax (Iozza *et al.*, 2009). Inorganic particles or particle-associated organic pollutants deposit on needles via wet (Eriksson *et al.*, 1989) or dry mechanisms (Turunen *et al.*, 1997) and remain there thanks to the presence of sticky substances like wax or honeydew deposits (Sawidis *et al.*, 2012) and surface roughness (Teper, 2009).

As conifers are evergreen, they have the advantage of accumulating pollutants for several years also during wintertime (Lehndorff and Schwark, 2004). In countries like Finland, needles are routinely employed to evaluate sulphur and heavy metal deposition (Pöykiö *et al.*, 2000).

However, caution is required when using these passive samplers, since monitored concentrations depend on: species, age of the needles, nature and content of wax, aerodynamic factors, temperature and precipitations, location within the forest canopy (Di Guardo *et al.*, 2003). Moreover, concentrations may be underestimated due to particle removal by wind and rain (Urbat *et al.*, 2004).

Bulk analytical techniques, like inductively coupled plasma atomic emission spectroscopy (ICP-AES) and X-ray fluorescence (XRF), are widely used to investigate inorganic pollutants, particularly heavy metals. On the other hand, a few studies utilize single particle analysis (SPA) techniques, like electron microscopy (Iwasaki and Tainosho, 2003). This work aims at combining the benefits of the two approaches to identify spatial and temporal trends in the accumulation of particles emitted by a steel making plant located in a test site in Northern Italy, where the concentrations of polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) and polychlorinated biphenyls dioxin (like PCBs dl) were even measured.

The case of steelmaking plants utilizing electric arc furnaces (EAFs) is of general interest as they emit particulate matter containing hazardous heavy metals (Baiutti *et al.*, 2007), and represent a significant amount of the 60% of plants that in developed countries produce steel from scraps (APAT, 2003; Wichterle *et al.*, 2010). Chemical composition of the dust coming from EAFs depends on the type of scraps and on the type of production plant. Emitted dust would contain several oxide phases, some being characteristic of steel making plants, like zinc, manganese, chromium, lead and iron oxides (Baiutti *et al.*, 2007). Zinc comes from scraps with relicts of anticorrosion coatings. Manganese derives from silico-manganese, a de-oxidising alloying component (Yue *et al.*, 2006). Chromium is from stainless steel scarps; lead, not soluble in steel, may come from

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Electron Microscopy in Particulate Matter Analysis

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Keywords: scanning electron microscopy, transmission electron microscopy, particulate matter, air quality.

The application of electron microscopy in the field of environmental analysis has been quite immediate with respect to the development of the instruments. Already in 1956 the transmission electron microscope was used to characterize particulate matter [1]. At that time electron-microscope techniques were predicted to "remain essentially research techniques" [1]. Is today this prediction still true?

The present contribution tries to highlight the difficulties that made until recent times hard to loom ahead a different future for both scanning and transmission electron microscopy in the analysis of atmospheric particles: the necessity to have a suitable substrate and the impossibility to obtain the measurements currently required by regulations on air quality. Simultaneously, it summarizes the advantages that make these techniques fundamental to understand the environmental history of the particles and predict their impact on the environment (climate forcing, cloud formation, absorption and scattering of solar radiation) and human health. In relation to public health protection, two main roles of electron microscopy and associated techniques (energy dispersive X-ray spectroscopy and electron diffraction) will be presented: the characterization of the smaller sizes of particulate matter that are understood to be the most hazardous for human health [2] and the acquisition of the information on specific characteristics of particulate matter in one go that may be related to toxicity. Having these data may lead in the future to regulate precise types of particles of greatest concern to public health in place of current size-differentiated mass limits. Research on electron microscopy analytical protocols for particulate matter characterization is then to be considered as policy-relevant scientific research.

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Deterioration of stone building facade in suburban areas in Northern Italy: the case of the Engineering Faculty in Trento (Italy)

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Samples of the decayed stone cladding of the Engineering Faculty building of the University of Trento (Italy), made of Verdello marble, a calcium carbonate stone from the Rosso Ammonitico Veronese formation widely employed in Trento area, were taken from parts of the building showing soiling, staining and detachments. These samples were analyzed by x-ray powder diffraction (XRD), low-vacuum scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (LVSEM-EDXS) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Calcite, dolomite, muscovite, epidote, quartz, iron oxides, clay minerals and combustion residues were identified. The deposition of particulate matter and the consequent degradation of Verdello seemed to be influenced more by the intrinsic characteristics of the stone and its finish than by the exposition of the site. In particular, stylolites turned out to be the main weakening factor for the detachment of stone fragments. Although no gypsum was detected in the samples coming from the degraded outer layers of the facade, suggesting comparatively low sulphur dioxide concentrations in the atmosphere to which the Faculty building has been exposed over the years, the hypothesis of a future transformation of the areas showing soiling into black crusts cannot be excluded, as minor amounts of sulphur were detected. This study is a further proof of the fact that stone degraded layers on building facades might be taken as proxies recording changes in the environmental conditions and that information on stone deterioration processes can be obtained by characterizing even small amounts samples coming from these layers.

Air Pollution & Art

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Keywords: pollution, art conservation, public engagement, preventive conservation.

The history of air pollution can be traced through the examination of works of art and literary sources. For example, paintings by the Venetian Vedutisti, who strictly represented the reality as it was in front of them, clearly show the presence of black crusts on the buildings of the city suggesting at that time a significant level of pollution [1]. Similarly, literary descriptions made by writers already in Roman times may provide indications on air quality in certain locations [2]. Photography and movies often provide documentation of recent and current air pollution.

Contemporary art often expresses concern regarding air pollution and it is another evidence that also non-scientists have a negative perception of it and might want to take an action against it. Many artists support through their work environmental campaigns and exhibitions of works of art related to the theme of pollution are worldwide showcased.

Many environmental activists promoted actions against pollution to stress the importance of keeping air contaminants away from buildings and monuments. Such is the case of the Lanterna of Genoa (Italy) that can be proposed as an example of a monument located in a Mediterranean city rich of historical buildings threatened by pollutants emitted by a variety of sources (harbour, traffic, industries, power plants) located in the proximity of the urban inhabited area.

Monuments and historical buildings are not the only kind of artworks affected by pollution. Works of art stored in museum environments are exposed to indoor pollution. A specific sector of art conservation and art conservation science, called preventive conservation, is devoted to monitor environmental parameters in museums, historic buildings, libraries and archives, to the development of suitable showcases for museum objects and to the establishment of practical measures to be adopted by museum staff that help in guaranteeing the preservation of our cultural heritage for future generations. The challenge of preventive conservation lies in compromising between allowing access and use of the heritage and better conditions for its preservation.

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Airborne inorganic pollutants deposited on conifer needles and building facades: reconstruction of spatial and temporal variations of the impact of an EAF steelmaking plant on the surrounding territory

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Keywords: conifer needles, electron microscopy, steelworks pollution, particulate matter, heavy metals

In the evaluation of the improved efficiency in off-gas abatement systems of industrial plants it is not possible to rely only on instrumental measurements if no data are available regarding the past pollution scenario. Moreover, covering wide territories with instrumental monitoring stations could be cost and time consuming. Hence, an alternative approach was developed to evaluate how large was in the past and it is nowadays the area of impact on air quality of an electric arc furnace (EAF) steelmaking facility located in Northern Italy.

In a pilot study, bulk and single particle analytical techniques were combined to identify the distinctive characteristics of the particulate matter emitted by the plant. Iron-rich spherical particles often containing other heavy metals (Zn, Mn, Cr, Ni, Pb, Cu) were identified as markers of the dust emitted by the plant, while from the elemental point of view Mn, Zn, Cr and Pb and secondarily Fe and Cu were identified as possible indicators of contamination from the industrial activity. The pilot study has also been used to try to develop a methodology to provide quantitative data from the measurements obtained by these “passive” samplers that could support the calibration of dispersion models.

Then, conifer needles and building facades in the area surrounding the plant were sampled and used respectively as archive of recent and less recent information on local air quality. The markers of pollutants emitted by the steelmaking plant were detected and comparison were made between their concentrations in different areas and periods of time in relation with changes in the off-gas abatement system undertaken by the industry. An attempt to associate different kinds of detected particles with specific stages of the steel production process was made in order to understand the relative significance of process and fugitive emissions for air quality in the area.

To have a more detailed description of the spatial impact of the industrial activities particulate matter was studied with subsequent higher magnification. Where no traces of the steelmaking activities were detected by scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (SEM-EDXS), the samples were analyzed with the higher resolution of transmission electron microscopy coupled with energy dispersive x-ray spectroscopy (TEM-EDXS) and selected area electron diffraction (SAED) in order to make sure that no smaller particles, able to travel farther from their source, were present at the location that could be apportioned to the steelmaking plant.

All data provided by electron microscopy analysis were discussed in the context of elemental concentrations measured with bulk analytical techniques such as inductively coupled plasma atomic emission spectroscopy (ICP-AES). The benefits of combining the two analytical approaches emerged especially for the discrimination of the emissions from different sources.

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