NANODIAGNOSTICS

REPORT n° 17/2009

21st September 2009

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REPORT

n° 17/2009

NANODIAGNOSTIC EVALUATION OF ENVIRONMENTAL SAMPLES THROUGH SCANNING ELECTRON MICROSCOPY AND ENERGY-DISPERSIVE X-RAY SPECTROSCOPY.

Customer: Municipal Council of Montegrotto Terme (Padova)

Test material: filters from the Systemlife srl filtering station

Manager: Dr. Stefano Montanari

Signature:

Date: 21st September 2009

This report comprises 86 (eighty-six) pages including the cover sheet.

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1. Introduction

Nanodiagnostics srl is a scientific consultancy firm operating in the medical, industrial and ecological fields. Its main activity is the survey, using an innovative environmental electron-microscopy technique, of inorganic micro- and nano- particles in any medium (biological tissues, food, drugs, cosmetics, environmental samples, etc.).

Studies principally use a specially modified FEG-ESEM (Field Emission Gun - Environmental Scanning Electron Microscope).

The entire range of each sample is observed under the microscope in various ways: SED (secondary electrons) and BSE (backscattered electrons), low vacuum (0.98 Torr), 25-30 KV and various spots, depending on the magnification (from 6 to 4), with a distance of 10 mm.

The microanalytical X-ray probe is focused on the identified particulate in order to determine its chemical composition. By measuring the peculiar energy returned in the form of X-rays by the various elements in the sample after being hit by the electronic beam emitted by the source, energy dispersive X-ray microanalysis (EDS - Energy Dispersive Spectroscopy) calculates the elementary chemical composition of the particulate. Each sample is photographed and archived together with the spectrum of the identified elements.

The analysis is not destructive and is repeatable, with the sole exception of fluids, which are often impossible to recover.

These studies can be carried out on samples of biological origin, such as biopsies, autopsies, organic liquids or food, but they can also be performed on many other kinds of materials, such as environmental samples, drugs or cosmetics. As the study mainly involves inorganic and non-biodegradable elements, it is not difficult to identify particulate of this kind in both fresh and archive samples.

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2. Materials

The following samples were received by the Laboratory:

Sample n° 1 (Lab. code STD 951-B)

Date: 28/08/09

Material: prefilter 1

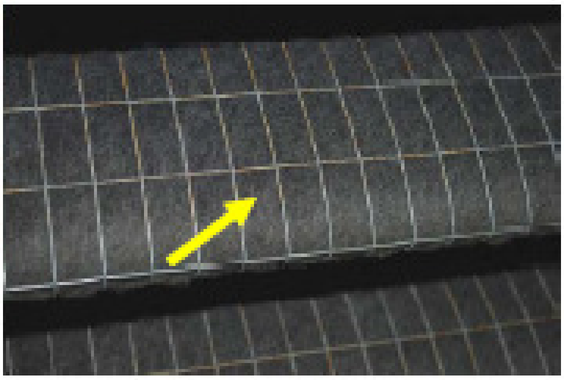
Description of sample: fragment of filtering fabric from the **flow inlet side** (side opposite to that with the metal mesh).

Sample n° 2 (Lab. code STD 951-A)

Date: 28/08/09

Material: prefilter 1

Description of sample: particulate captured by the filtering fabric on the **flow outlet side** (side with metal mesh).



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Sample n° 3 (Lab. code STD 952-A)

Date: 28/08/09

Material: prefilter 2

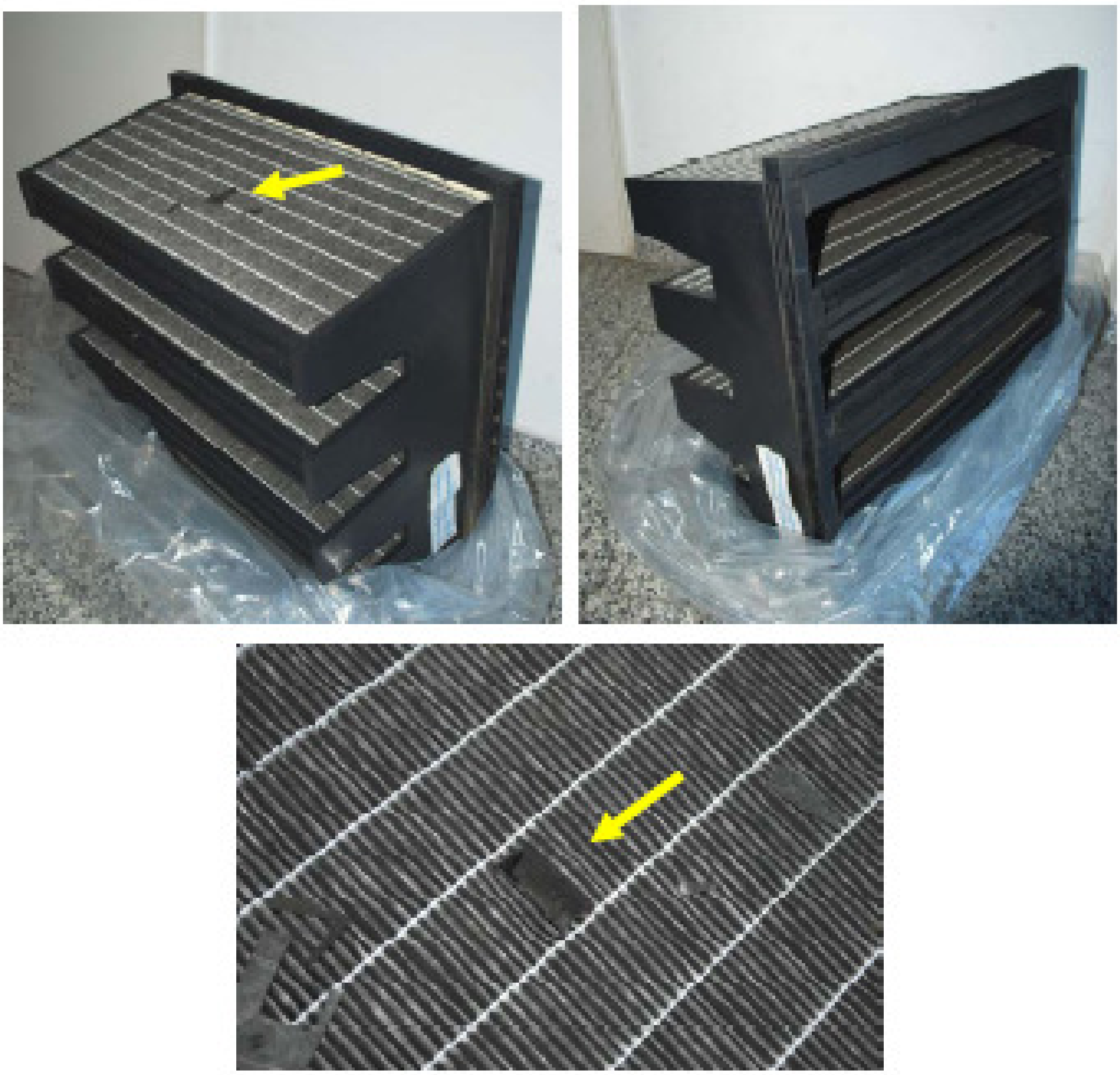
Description of sample: fragment of paper filter - **upper side** - taken from upper support.

Sample n° 4 (Lab. code STD 952-B)

Date: 28/08/09

Material: prefilter 2

Description of sample: fragment of paper filter - **lower side** - taken from upper support.



Prefilter 2 – the arrow indicates the point where the sample was taken from.

Sample n° 5 (Lab. code STD 950-A)

Date: 27/08/09

Material: electrofilter

Description of sample: fragment of strip with particulate captured by the electrofilter (4th blade from the top).

Sample n° 6 (Lab. code STD 950-B)

Date: 27/08/09

Material: electrofilter

Description of sample: fragment of strip with particulate captured by the electrofilter (2nd blade from the top).

Sample 5 Sample 6

Electrofilter – the arrows indicate the points where the samples were taken from.

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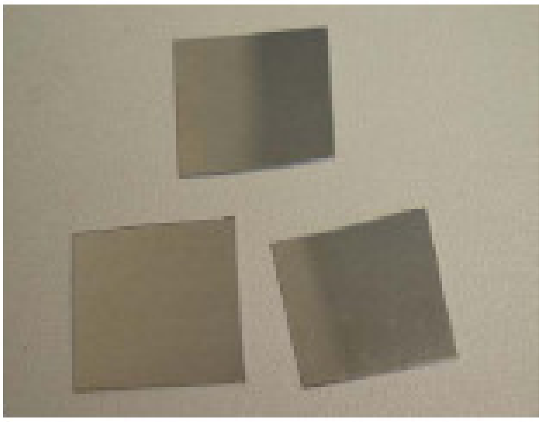
Sample n° 7 (Lab. code STD 953)

Date: 02/09/09

Material: unused electrofilter

Description of sample: fragment of strip from an unused electrofilter.



3. Types of analysis performed

The study used the environmental scanning electron microscopy (FEG - ESEM) technique to verify the possible presence of environmental particulate, and X-ray micro-analysis (SED) to assess its chemical composition, as described in section 1. No processing was performed on the sample which was therefore examined in its original state. The method, as established in the European Community project QLRT-2002-147 (Nanopathology), also made it possible to evaluate the presence of nanometric particles (see bibliography 1- 56).

4. Preparation of samples

The samples were taken at the Nanodiagnostics Laboratory from the Città Model of Filtering Station used at Montegrotto Terme for a total of 4,093 hours.

Fragments of strips were taken from the electrofilter and samples of fabric, filter paper and particulate were collected from the prefilters.

The strip fragments and prefilter samples (fabric and paper) with particulate (samples 1, 3, 4, 5 and 6) and without particulate (sample 7) were deposited on an adhesive carbon disk and mounted on an aluminium support (stub).

The particulate (sample 2) was collected by placing adhesive carbon disks mounted on aluminium stubs.

The samples were immediately put into a clean sample container and then examined under the electron microscope with no additional preparation or treatment procedures.

5. Results

Electron microscope analysis revealed the presence of particulate with different chemical compositions (see Tables I-VII).

The morphological and chemical results of the sample are shown in the following images. The spectrum peaks that do indicate the element to which they refer are secondary peaks of an element whose main peak has already been marked.

N.B.: the list of elements in the tables observes the criterion of greater representativeness of the element in the EDS spectrum. The sequence in the list of the elements starts from those with the highest EDS peak.

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Sample 1, prefilter 1 - fragment of filtering fabric from the flow inlet side STD 951-B

The table summarises the most significant analyses performed on the sample.

Tab. I. EDS analysis of sample 1,

Analysis n°	Description	Elements present
1	filter fragment	morphological image - 100x
2	filter fragment	morphological image - 500x
3	filter fragment	morphological image - 2000x
4	fragments 3-5 µm	Fe,O,Si,Ca,C,Al,K,Mg,S,Cl,Na,P,Mn,Zn
	background signal around fragments	Ca,C,O,Si,Cl,Al,K,Fe,Na,S,Mg,P,Zn,Cu
5	accumulation of fragments	Fe,O,Si,Ca,C,Al,K,Mg,S,Cl,Na,P,Mn,Zn
		detail
6	accumulation of fragments	Fe,Ca,Si,O,C,Al,K,S,Cl,Mg,P,Na,Zn,Ti,Cu,Mn
	background signal around fragments	Si,C,O,Ca,Al,K,Fe,Mg,Cl,S,Na,P,Ti,Zn,Mn,Cu
		detail
7	accumulation of fragments	Fe,Ca,Si,O,C,Al,K,S,Cl,Mg,P,Na,Zn,Ti,Cu,Mn
8	spherule 0.3 - 5 µm	Fe,O,Ca,Si,Al,Mn,C,Mg,K,Cl,S,Cr,Zn
		detail
9	spherule 2.5 µm	Fe,Ca,O,Si,C,Zn,Al,K,S,Cl,Mg,Mn,
	background signal around spherule	Ca,Si,Fe,O,C,Al,K,S,Cl,Mg,Zn,Mn
10	aggregate of fragments	Fe,O,Si,Ca,C,Al,K,Mg,S,Cl,Na,P,Mn,Zn
11	debris 2-4 µm	O,Si,C,Fe,Ca,Al,S,Mg,K,Ba,Sn,Cl,P,Cu,Zn,Cr
	background signal around fragments	O,Si,Al,C,Ca,Fe,Mg,K,Na,Cl,S,P,Ti,Zn,Cu
12	spherule 3 µm	O,Fe,C,Si,Ca,Al,Mg,S,Cl,Na,K,P,Ni,Ti
	background signal around spherule	O,Si,Ca,Al,Ti,C,Mg,Na,Fe,K,S,Cl,P,Zn
13	crystalline formations	O,Fe,Si,Ca,C,Al,Cl,K,S,Mg,Na,P,Zn,Mn
	background signal around crystals	Ca,O,C,Si,Mg,Al,Cl,K,Fe,Na,S,P,Zn
		detail
14	accumulation of fragments 1-2 µm	Ca,Si,Ba,O,Fe,S,C,Al,K,Cl,Mg,Na,Zn,P,Cu
	background signal around debris	Ca,Si,O,C,Fe,Al,S,Cl,K,Mg,P,Ba,Na,Zn,Cu
15	spherule 1.5 µm	Fe,Ca,O,Si,C,Cl,K,Al,S,Mg,Zn,Ti,Na,Cu
	grey debris around spherule	Ca,O,Si,C,Fe,Cl,K,S,Al,Zn,Mg,Ti,P,Cu
16	spherule 1 µm	Fe,Ca,O,Si,C,Cl,K,Al,S,Mg,Zn,Ti,Na,Cu
17	accumulation of fragments	Fe,O,Ca,Si,C,S,Al,Mg,Cl,K,Ba,Na,Cu,Mn,Zn
	grey debris around accumulation	Ca,Si,O,C,Al,Fe,K,Mg,Cl,S,Na,P,Ti,Zn,Cu
		detail
18	spherule 2 µm	Fe,O,Si,C,Ca,Al,K,Mg,S,Na,P,Ti,Zn
	background signal around spherule	Si,O,Al,Ca,C,Na,Fe,Mg,K,S,Cl,Ti,Zn

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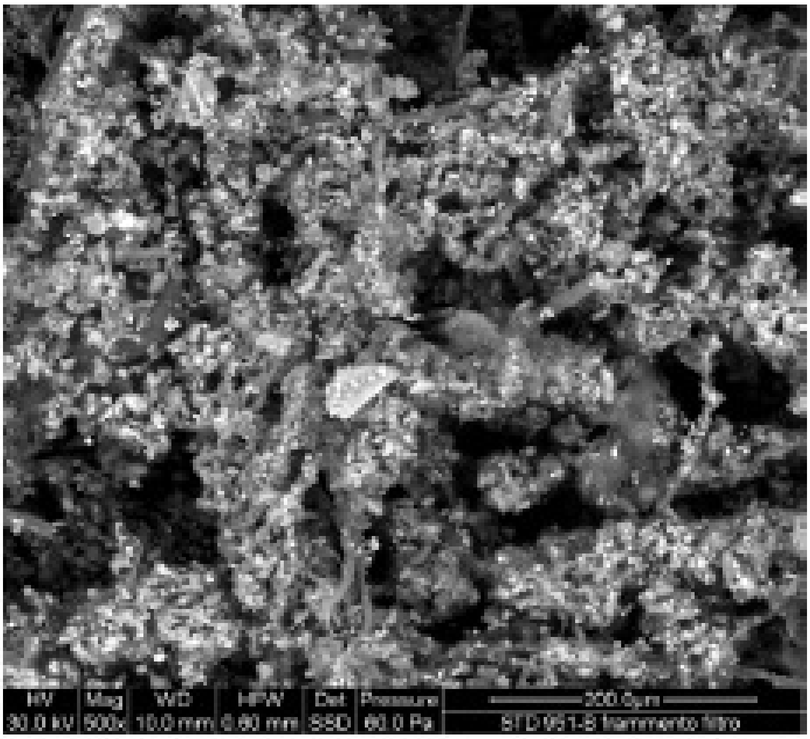

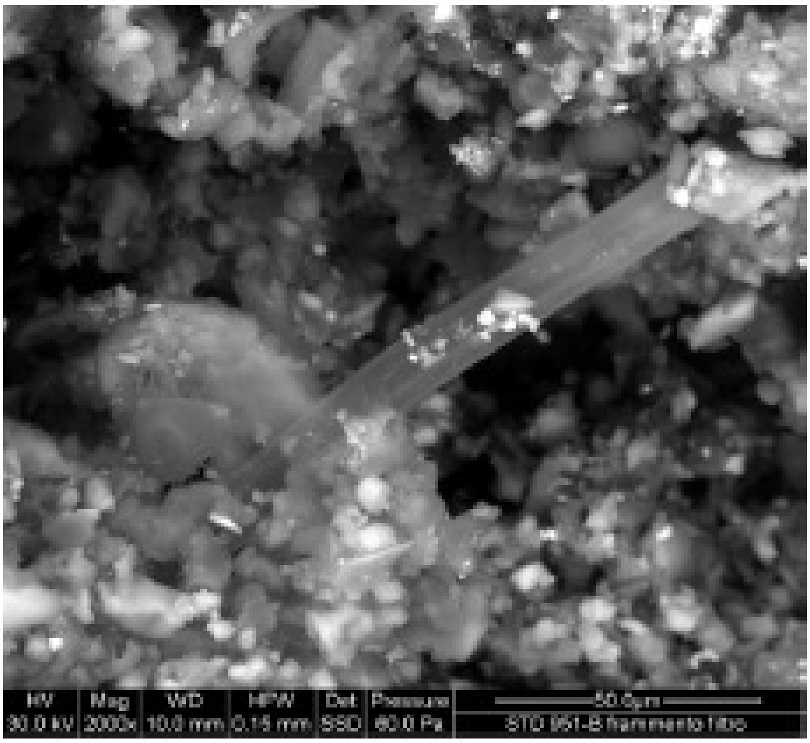

Analysis 2 and 3 of table I. The figures show the morphology of the surface of the sample at various magnifications on which numerous fragments of different shapes and sizes are clearly visible.

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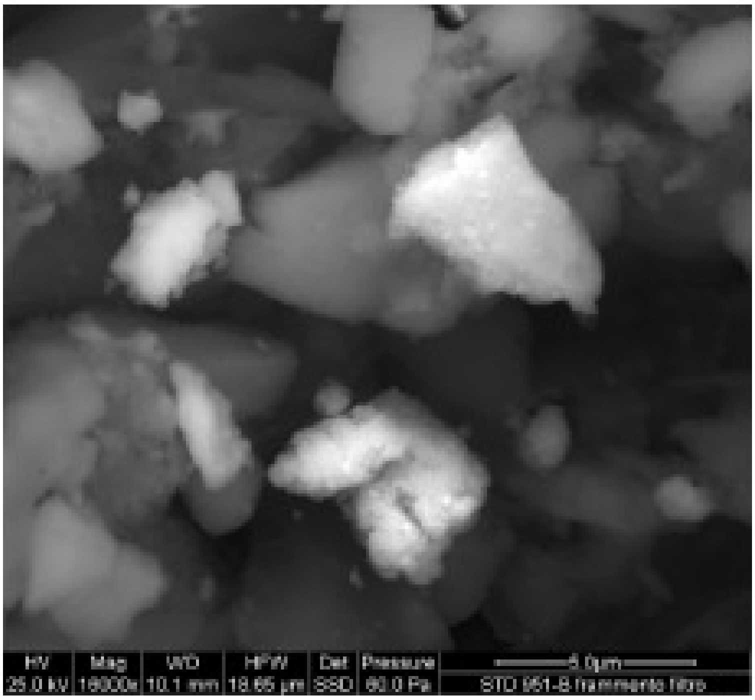

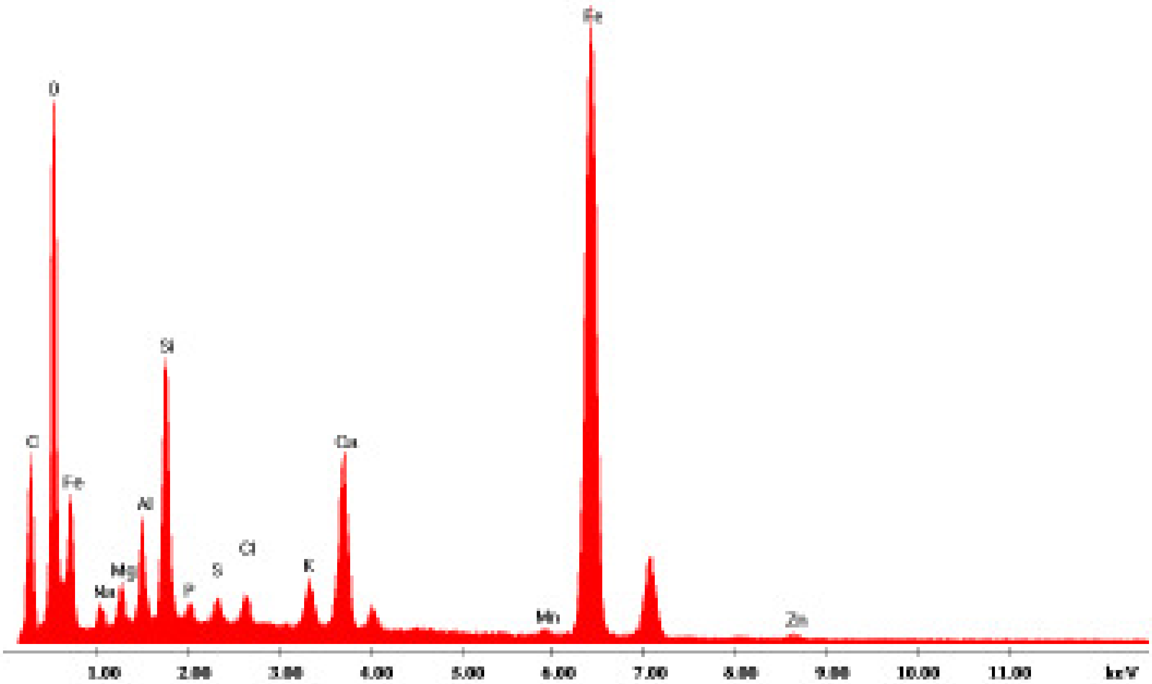

Analysis 4 of table I. The figure shows a high resolution image of some fragments ranging in size from 3 to 5 microns. EDS analysis shows that these comprise Iron, Oxygen, Silicon, Calcium, Carbon, Aluminium, Potassium, Magnesium, Sulphur, Chlorine, Sodium, Phosphorous, Manganese and Zinc.

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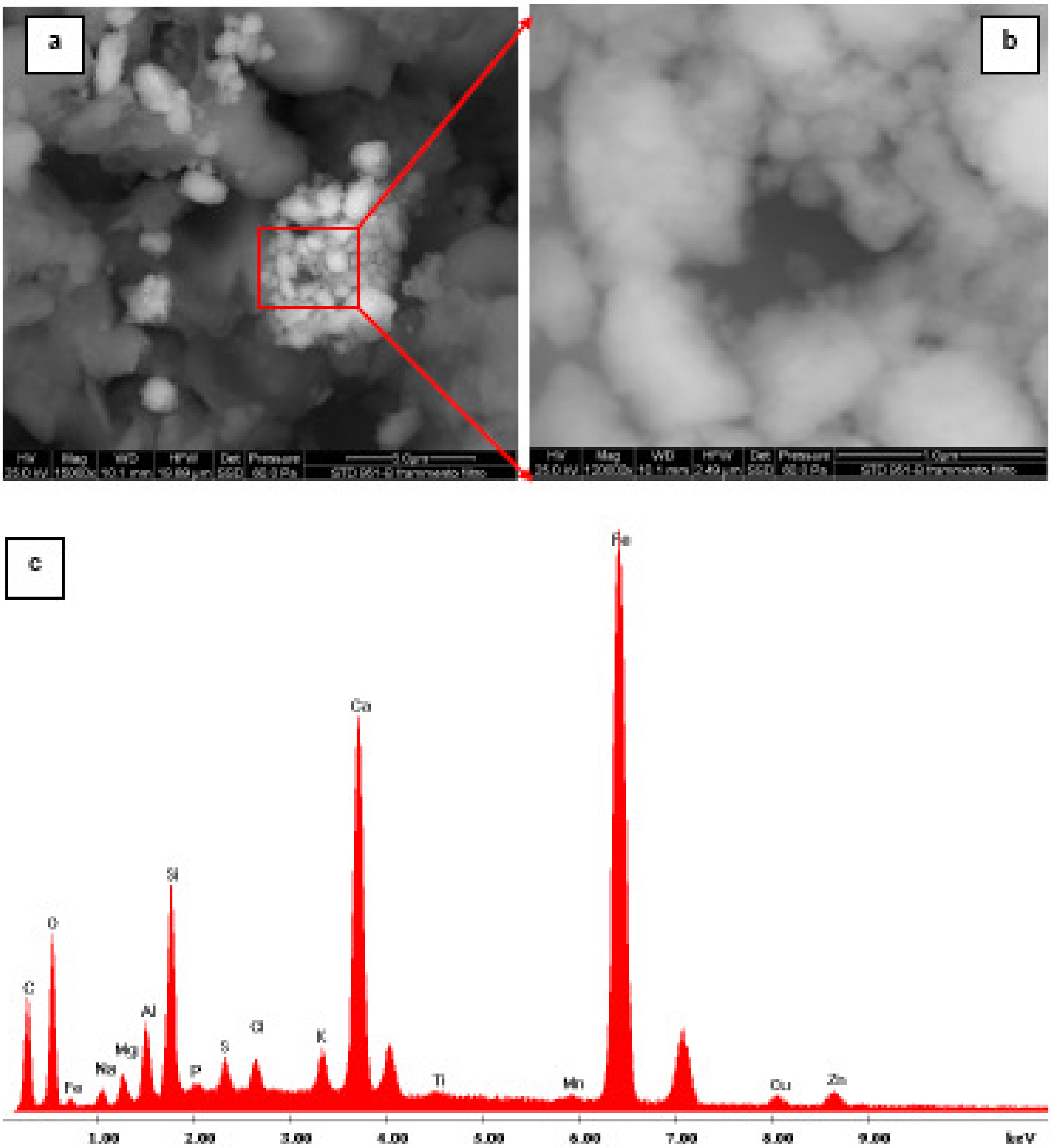

Analysis 6 of table I. The figures show a accumulation of fragments at two different magnifications (Figs. a and b). EDS analysis shows that these comprise Iron, Calcium, Silicon, Oxygen, Carbon, Aluminium, Potassium, Sulphur, Chlorine, Magnesium, Phosphorous, Sodium, Zinc, Titanium, Copper and Manganese.

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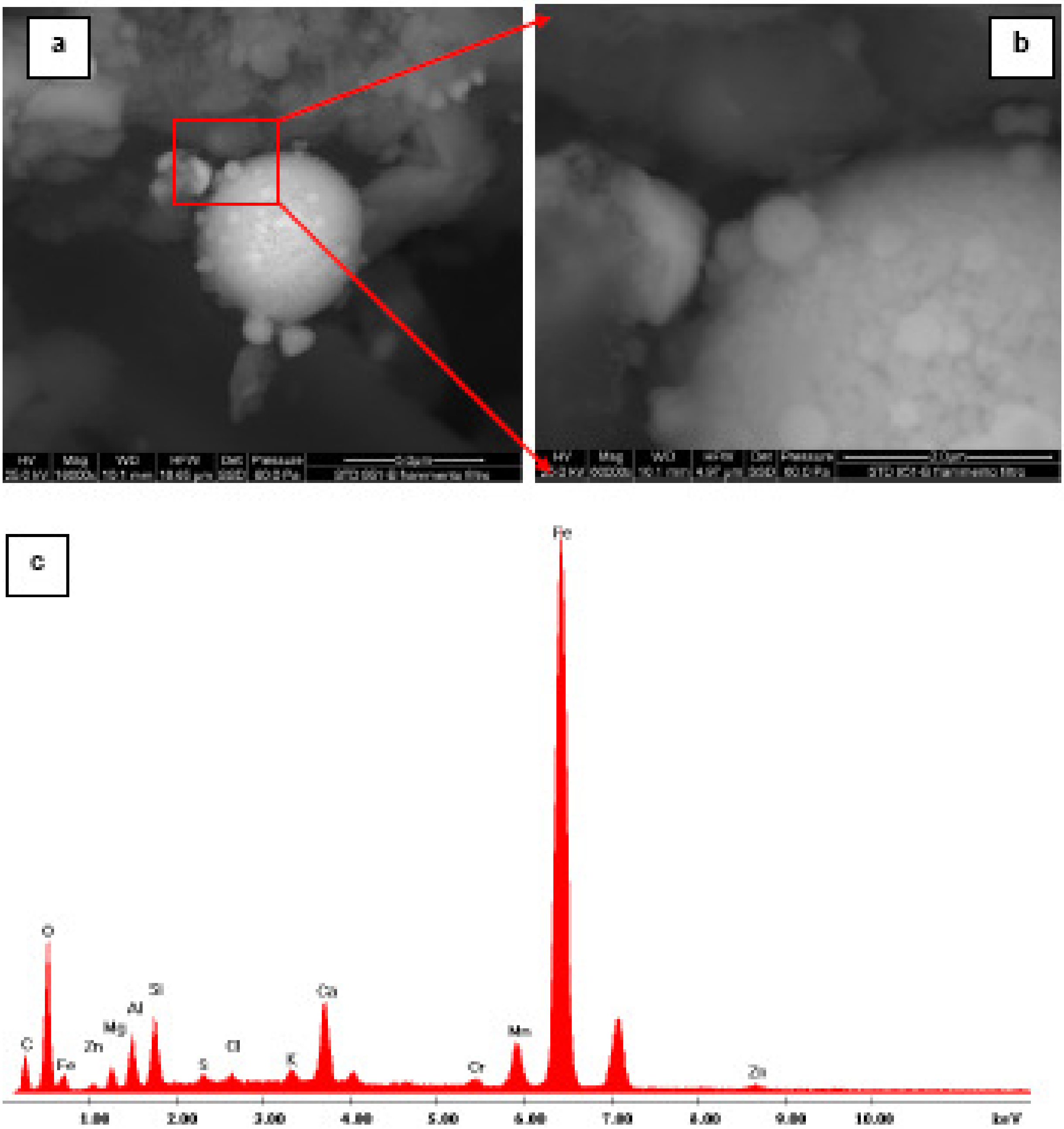

Analysis 8 of table I. The figures show a accumulation of spherules with diameters ranging from 0.3 to 5 microns at two different magnifications (Figs. a and b). EDS analysis (Fig. c) shows that these spherules comprise Iron, Oxygen, Calcium, Silicon, Aluminium, Manganese, Carbon, Magnesium, Potassium, Chlorine, Sulphur, Chromium and Zinc.

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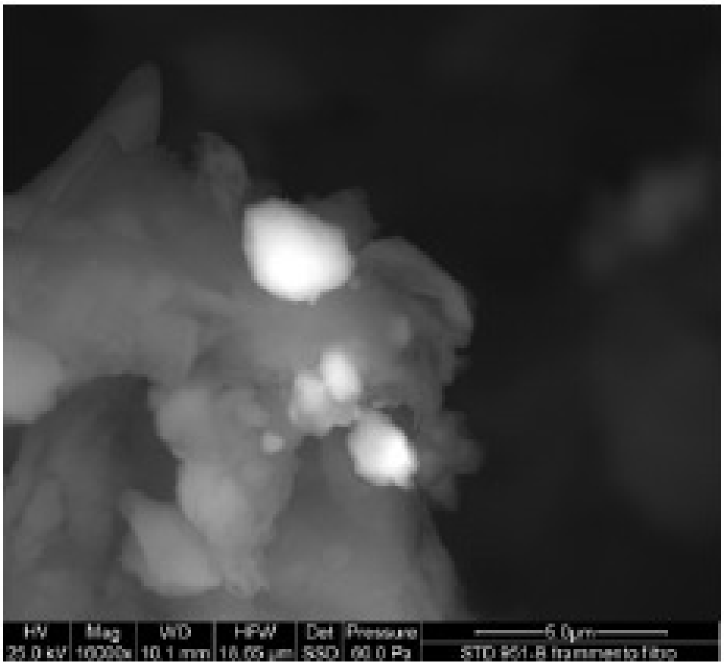

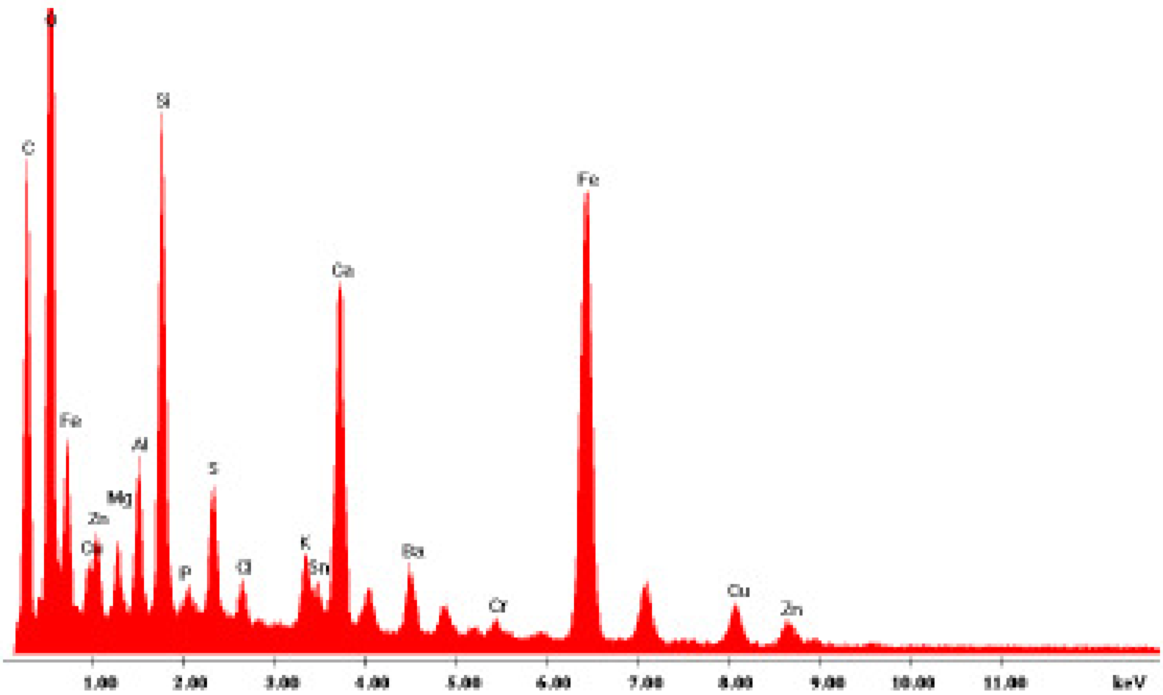

Analysis 11 of table I. The figure shows a high resolution image of some fragments ranging in size from 2 to 4 microns. EDS analysis shows that these comprise Oxygen, Silicon, Carbon, Iron, Calcium, Aluminium, Sulphur, Magnesium, Potassium, Barium, Tin, Chlorine, Phosphorous, Copper, Zinc and Chromium.

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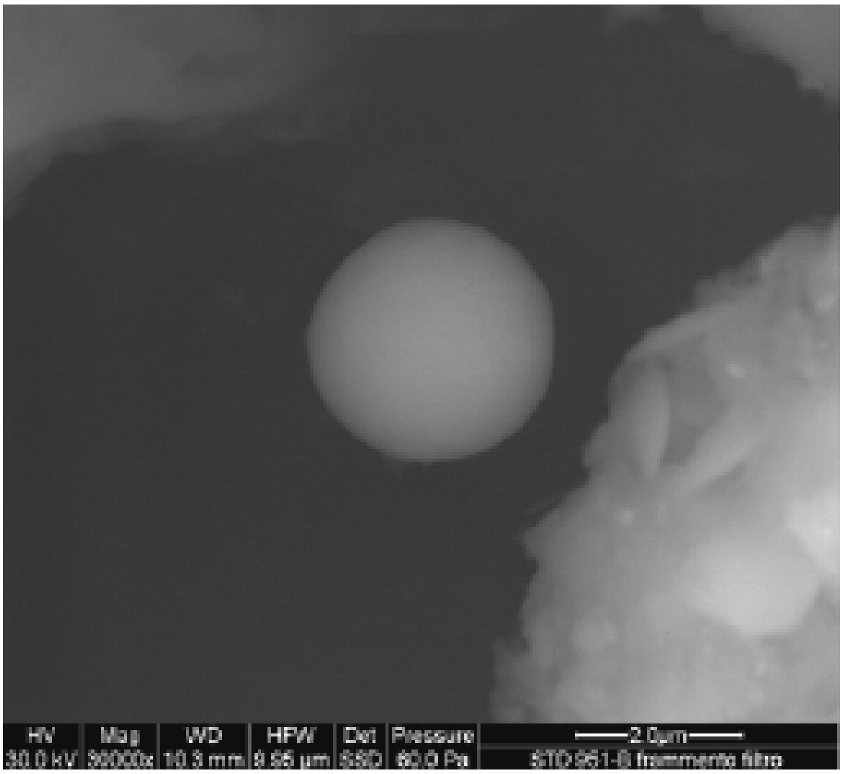

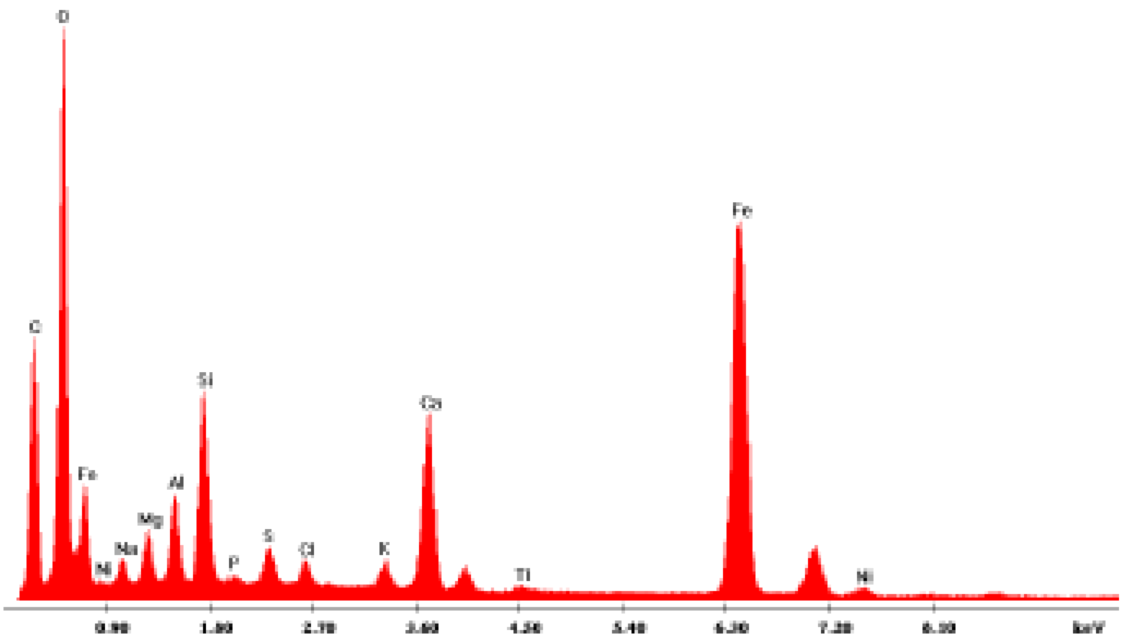

Analysis 12 of table I. The figure shows a high resolution image of a spherule with a diameter of 3 microns. EDS analysis shows that these comprise Oxygen, Iron, Carbon, Silicon, Calcium, Aluminium, Magnesium, Sulphur, Chlorine, Sodium, Potassium, Phosphorous, Nickel and Titanium.

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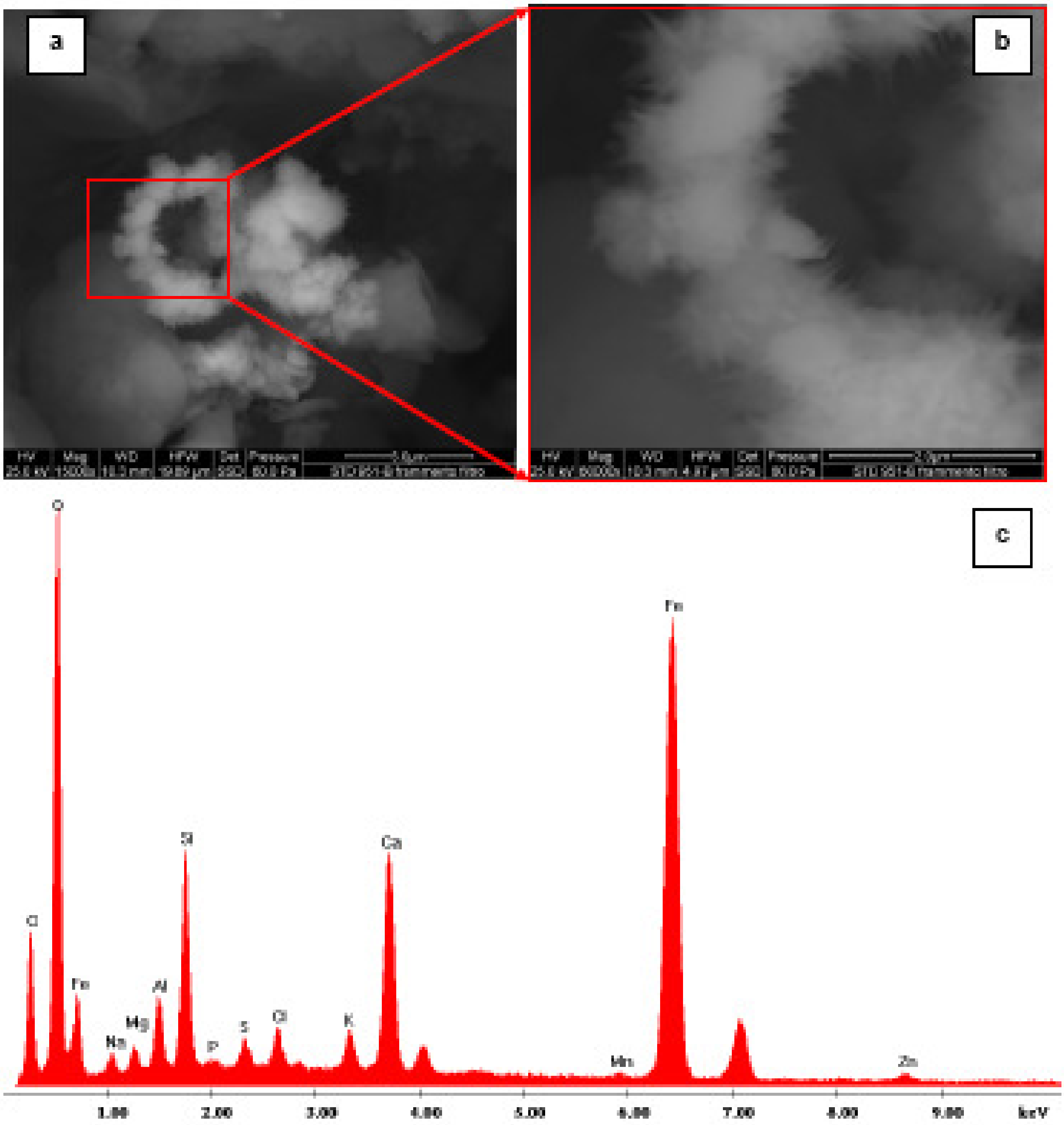

Analysis 13 of table I. The figures show the images of some crystalline formations at two different magnifications (Figs. a and b). EDS analysis (Fig. a) shows that those comprise Oxygen, Iron, Silicon, Calcium, Carbon, Aluminium, Chlorine, Potassium, Sulphur, Magnesium, Sodium, Phosphorous, Zinc and Manganese.

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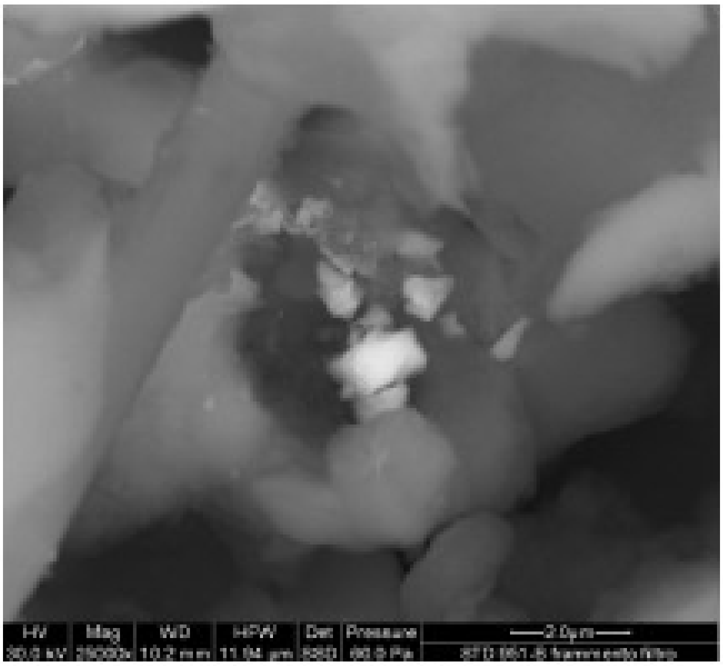

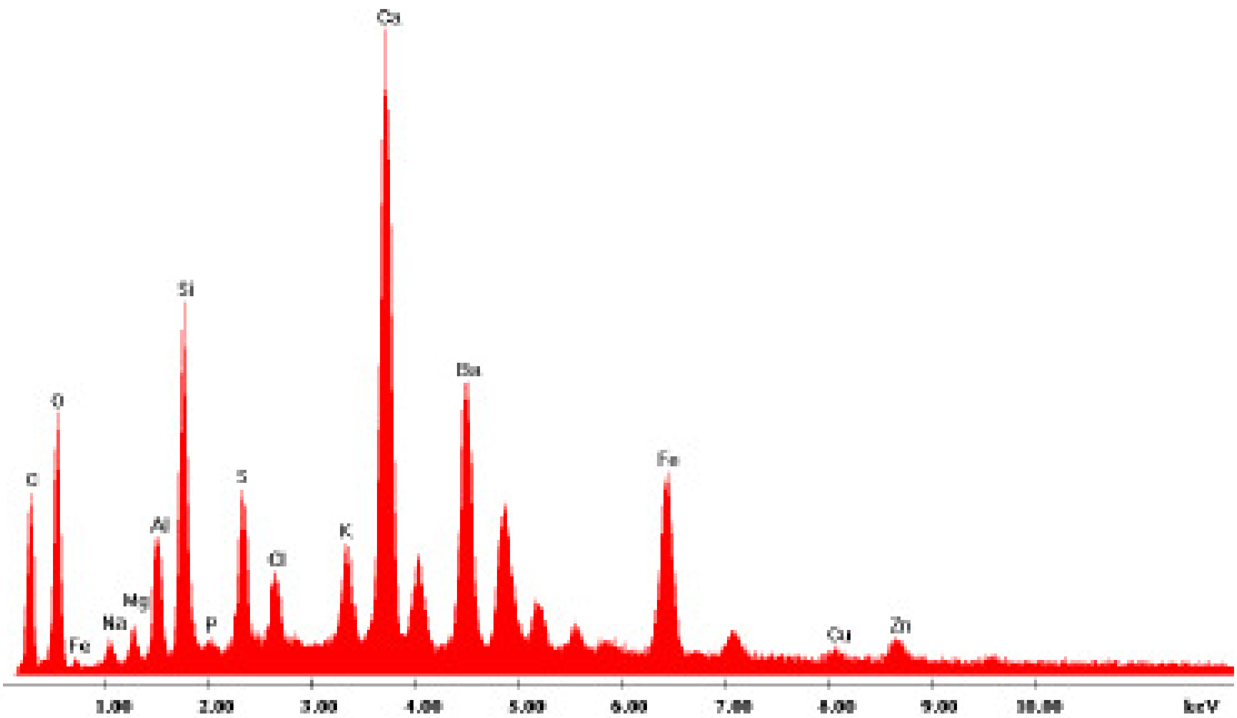

Analysis 14 of table I. The figure shows a high resolution image of an accumulation of fragments ranging in size from 1 to 2 microns. EDS analysis shows that these fragments comprise Calcium, Silicon, Barium, Oxygen, Iron, Sulphur, Carbon, Aluminium, Potassium, Chlorine, Magnesium, Sodium, Zinc, Phosphorous and Copper.

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6. Conclusions

One characteristic that is common to all the particulate captured by the various filters, each with a different selectivity, is the complexity of their composition, so much so that it was not at all rare to find micro- and nano- particles comprising as many as 16 different elements. Similar compositions cannot be found in chemical and metallurgy literature and are the fruit of totally random combinations caused by uncontrolled combustions such as those occurring in wartime explosions, in refuse incinerators or in cement works that also handle waste.

The frequency with which spherical forms, typical of the combustive origin of numerous particles, are seen is further proof of the above. In numerous circumstances, smaller irregular particles, and therefore more aggressive, seem to derive from the fragmentation of the above-mentioned spherical particles, particles that are very fragile.

It should be considered that the distance from the location of the system from which the test filters were removed and the probable sources of the combustive pollution is probably a few kilometres, a route that the particulate covers in a short time both though diffusion and by wind transport. It goes without saying that a significant part of the particulate probably originates from vehicular traffic.

Several of the elements found are notoriously toxic, and this toxicity is synergically added to the aggressiveness of non-biodegradable or non-biocompatible particulate that can easily penetrate all organs and tissues both by inhalation and ingestion. The latter possibility occurs when the particulate falls by gravity onto fruit, vegetables and cereals.

By no there is no doubt as to the capacity of particles such as the ones observed to cause illnesses ranging from simple bronchial or allergic pathologies to much more serious conditions such as cardiovascular diseases and cancer. Also to be considered are spontaneous abortions or foetal malformations, given the proven capacity of particulate to pass from mother to foetus, or endocrinous illnesses (e.g.: diabetes or thyroiditis) as a consequence of the “endocrine disruptor” activity of the particulate.

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In the opinion of the author and director of the analysis, it seems unavoidable, therefore, to take action on the atmosphere, particularly as regards the urban environment where most of the population and most of the particulate is concentrated.

This upline action, which removes the polluting particulate at the source, is certainly effective, but its implementation involves a drastic change in the lifestyle of society which very few people are prepared to accept. Is should also be highlighted that the particulate identified in this analysis is not biodegradable and therefore remains in the atmosphere for, as far as human-beings are concerned, eternity. Therefore, a mitigating action aiming exclusively at the source would be ineffective on the quantities of existing non-degradable pollutant which continues to grow, as indicated in the report of the European Environment Agency 2/2007.

Therefore, a system such as the one under examination is, in the author's opinion, an indispensable complement to upline action, with the advantage of already being available and not requiring any form of "education". It should be added that, in the author's experience, the system in question currently seems to be the only system capable of efficiently mitigating the particulate pollution that urban environments suffer from to a growing extent today.