New Nanodiagnostics Srl

REPORT No. 20/2021

Of the 15th of November 2021

REPORT

No. 20/2021

EVALUATION OF AN INORGANIC SAMPLE THROUGH AN ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY INVESTIGATION AND AN X-RAY MICRO-ANALYSIS

Client: Klagepaten DE

Sample checked: Swabs for PCR test

Analyses and conclusions by: Dr Antonietta M. Gatti, Dr Stefano Montanari

Signatures:

Date: 15/11/2021

This report is made up of 38 (thirty-eight) numbered pages including the cover.

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1. INTRODUCTION

New Nanodiagnostics Srl is a consulting company in the fields of medicine, biology, industry, environment, and ecology. Its main activity is detecting inorganic micro- and nanoparticles in any medium (biological tissues, food, drugs, cosmetics, environmental samples, garments, etc.), through environmental scanning electron microscopy and X-ray microanalysis. The present investigation is carried out by means of a FEG - ESEM (Field Emission Gun - Environmental Scanning Electron Microscope, QUANTA 650, Thermo Fisher Scientifics, USA) with suitable protocols according to the type of substrate.

This kind of analysis offers the possibility to observe biological samples in wet mode, i.e., in the condition of their normal hydration, at environmental pressure, without the need to dehydrate them or make them electro-conductive through a coating of Carbon, or metals like Gold and Palladium, a procedure that is necessary with the traditional scanning electron microscopes.

By applying appropriate protocols to be adapted to each kind of observation, this feature allows to check biological samples, including living cells, without impairing their integrity, and to repeat the test any time. The main objective of the study is the detection of inorganic micro- and

nano-particulate matter, should be any present in the sample, and this is achieved without the need to process the specimen.

An X-ray microprobe of an EDS (Energy Dispersive System, Thermo Fisher Scientifics, USA) supplements the investigation, as the spectrum thus obtained shows the chemical elemental composition of the particulate matter. In fact, it measures the energy characteristic of each element making up the particles in the specimen and returned as X-rays after the sample has been hit by the electron beam delivered by the FEG - ESEM.

So, through such an integrated analysis, the micro- and nano-sized inorganic particles are photographed, measured, and chemically characterized in a non-invasive, non-destructive, repeatable, way, the only exception being fluids, which are often hard to recover in their liquid form after some time.

The investigation can be carried out also on specimens of biological origin like biopsies, autopsies, and organic fluids, but is equally feasible on many other kinds of materials like, for example, environmental samples, food, drugs, or cosmetics. As the principal object of the investigation is inorganic and non-biodegradable, it is not particularly difficult to detect such particulate matter either in fresh or in archived samples.

2. MATERIALS

Three different swabs have been sent to our laboratory**.**

1- Transport Tube Polypropilene shaft Tipped Applicator Sterile EXPIRY 05/2026

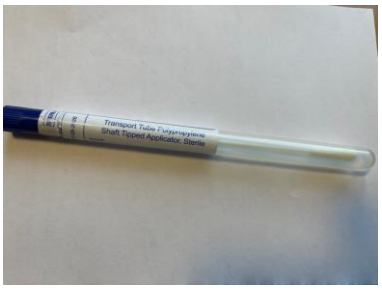
2- Sampling Swab SUNGO EUROPE LOT 20210601 EXPIRY 2024 05 31

3- Sampling Swab SYNOCURA LOT 20210422 EXPIRY 2024 04 21

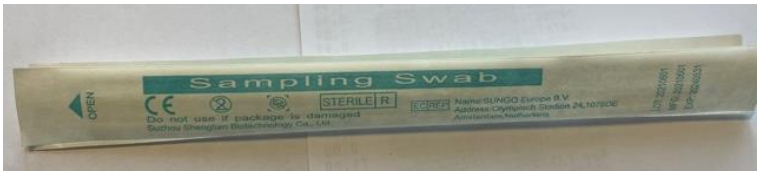
All the swabs were sterilized under Gamma rays.

They all have been re-numbered for our laboratory data bank as **STD1443A, STD1433V** and **STD1443B**.

STD 1443A LOT UNKNOWN, EXPIRY DATE 05/2026



STD1443V SUNGO EUROPE LOT 20210601, EXPIRY DATE 20240531



STD1443B SYNOCURA LOT 20210422, EXPIRY DATE 20240421

New Nanodiagnostics Srl was requested to evaluate the samples through the above-described method to detect the possible presence of an inorganic, foreign-body contamination. The search for foreign bodies included the evaluation of their elemental chemical composition.

3. TEST CONDITIONS

Intact samples were first observed under a Nikon stereomicroscope to check for anomalies. The samples were then opened, and the final portion of each swab was deposited on an Aluminium support covered with an adhesive Carbon disc. Since some of the fibres were broken, they were deposited on another disk of adhesive Carbon and analysed separately.

4. TYPES OF ANALYSES CARRIED OUT

The study of the exhibits in question verified the morphology and the possible presence of foreign bodies in the samples analysed using our environmental scanning electron microscopy technique (Quanta 650 microscope, Thermofisher, USA), while the X-ray microanalysis (EDS) evaluated the chemical elemental composition as described in paragraph 1. No processing was performed on the section of the exhibit which was therefore examined as it is. Foreign bodies, being atomically denser than the surrounding tissue, appear lighter than the biological tissue which, on the other hand, remains grey.

5. RESULTS

The electron-microscopy analyses of the sample clearly show the shape and morphology of the swab.

In the pictures that follow, the chemical and morphological results are shown.

It is necessary to underline that the peaks of the EDS spectra without an indication of the chemical element to which they are referred are secondary peaks of an already-reported element to understand the scientific meaning of the images. That means that only the principal peak of each element has been tagged.

The table in the following page indicates the analyses performed, as well as the electron-microscopy observations of the stub and other particles not belonging to the sample itself.

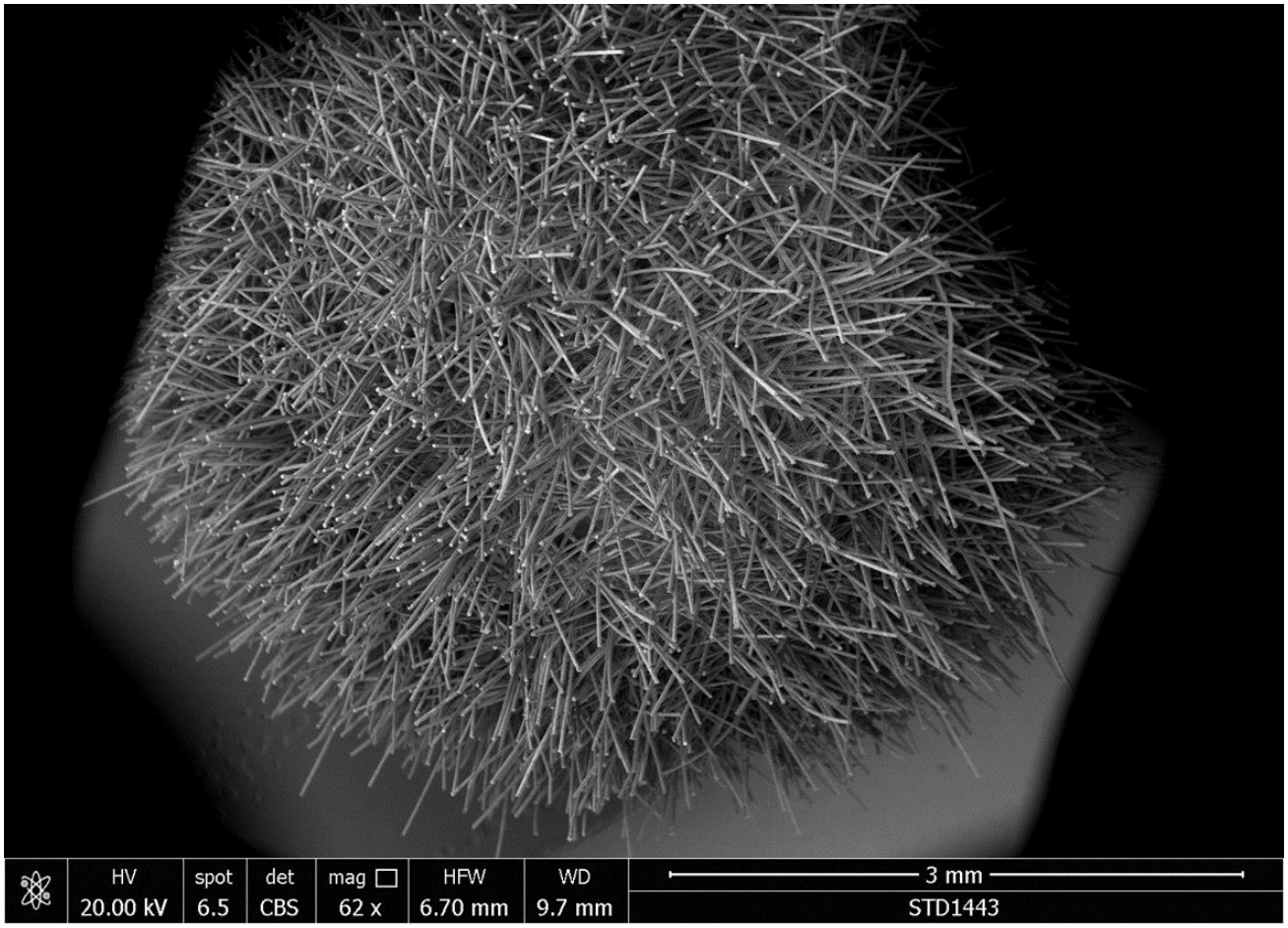
The list of the elements reported in the table is made according to their degree of representativeness in the EDS spectrum, starting from the highest to the lowest peak.

List of images and EDS analyses:

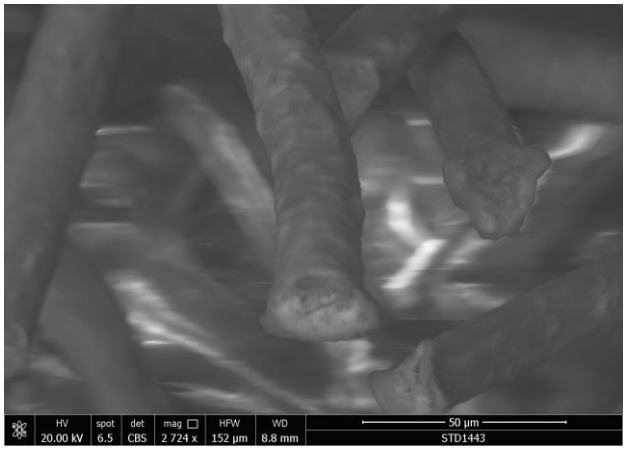
Analysis No. 1 STD1443A

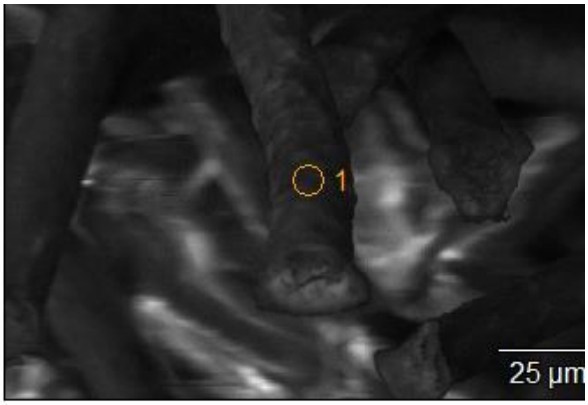
Table I containing all the analyses carried out on the sample.

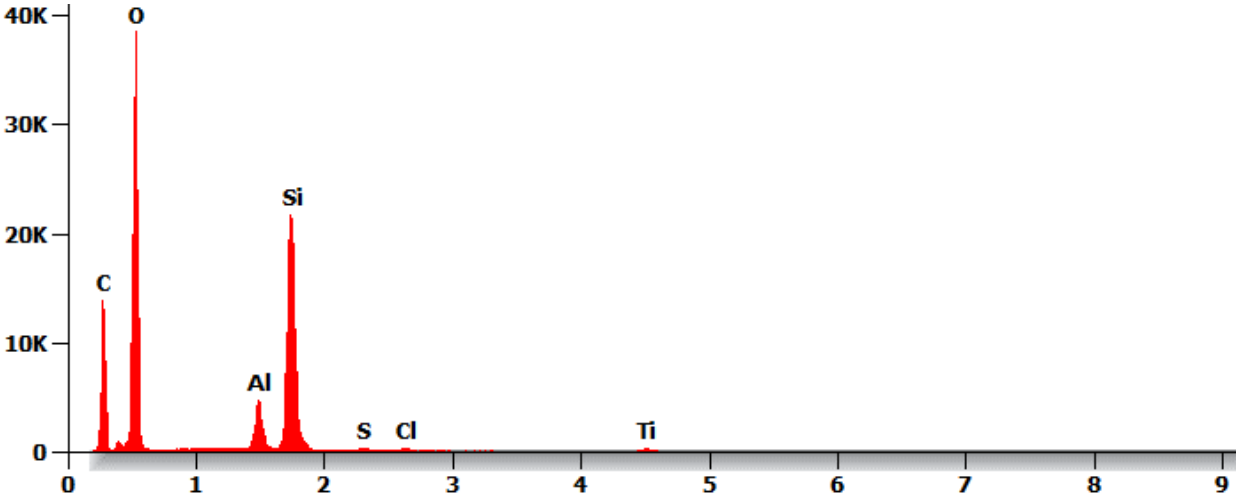
N. Analysis	Magnification	Elements
1	62x	Morphological Image
2	2724x	O Si C Al Cl S Ti
3	276x	Morphological Image
4	325x	C Ca O Si Al Mg
5	3087x	O Si C Al Na S Ca Ti Cl N
		C Si O Al S Ti N Cl Na
6	5982x	O Ca C S Si Na Al Mg
7	13332x	C O Ca Si Al N
		C O Si N Al



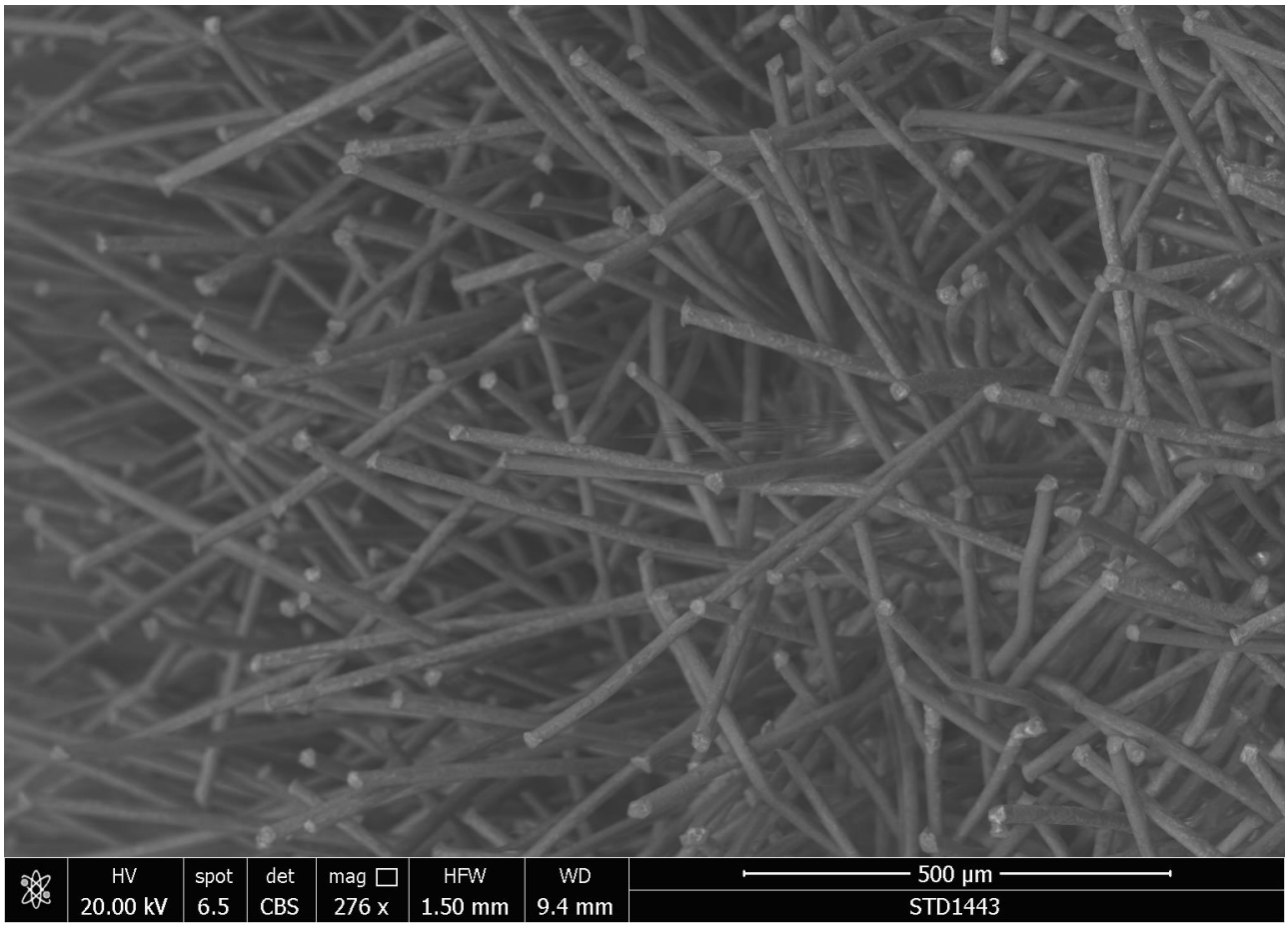
Analysis 1 of Table I. The image shows an area of the sample (magnification 62x). The structure of the swab resembles that of a porcupine.



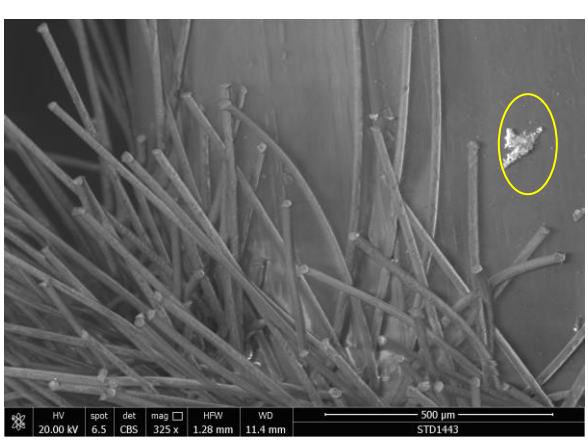


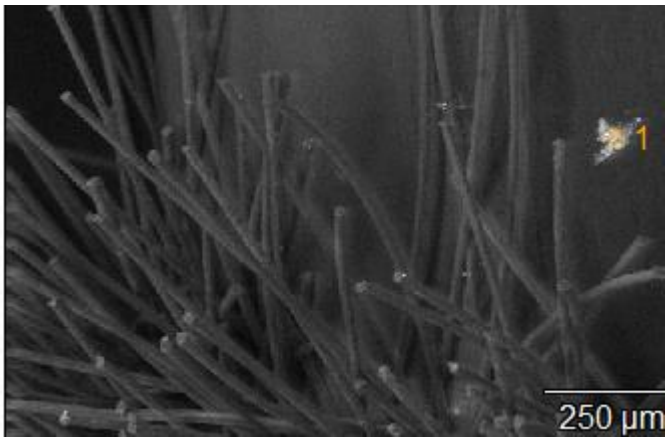


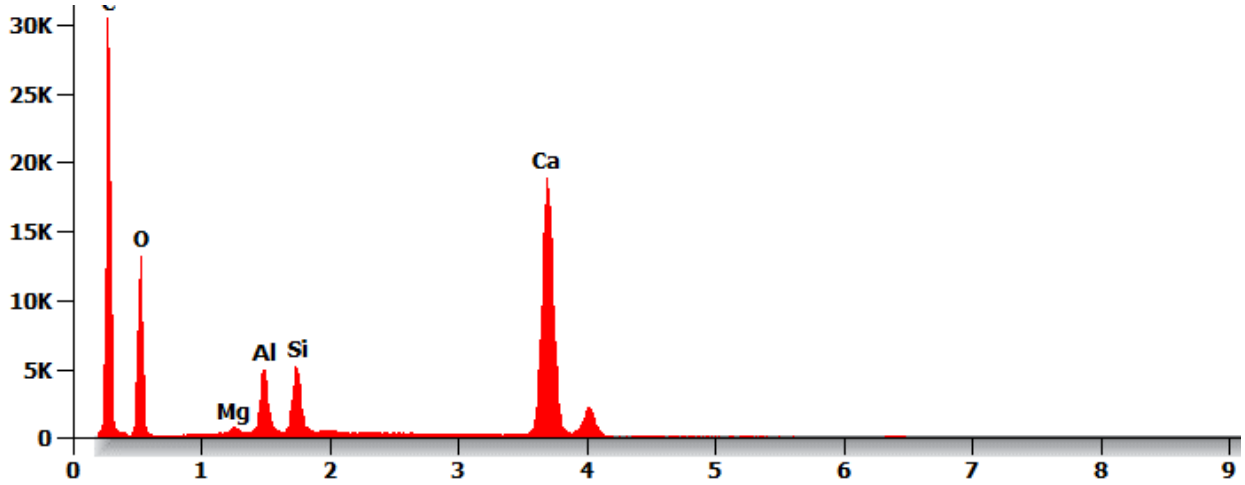
Analysis 2 of Table I. The image shows a particular of a swab's fibre (magnification 2724x). The circle defines the area where the EDS analysis was performed. The EDS spectrum shows that it is composed of Oxygen, Silicon, Carbon, Aluminium, Chlorine, Sulfur and Titanium. It is possible that the core of the swab is polymeric, but an additional coating of Silicon-Aluminium-Oxygen was applied.
Considering the interaction between the electron beam and the swab structure, it seems that at the base of the fibres there is a chemical compound added before the PCR analysis, for the biological conservation of the specimen.



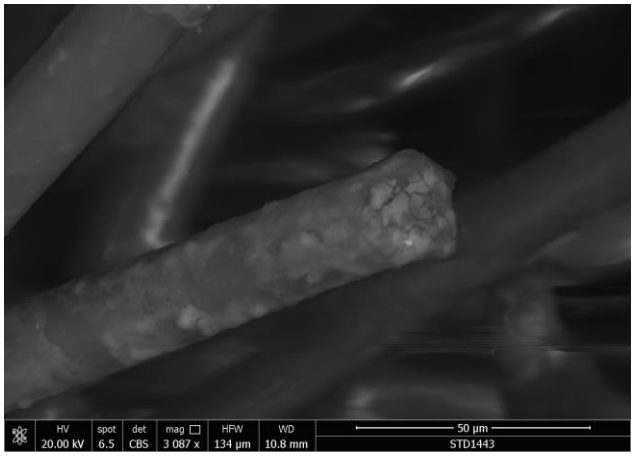
Analysis 3 of Table I. The image shows an area of the sample (magnification 276x), where the fibres distribution is visible.

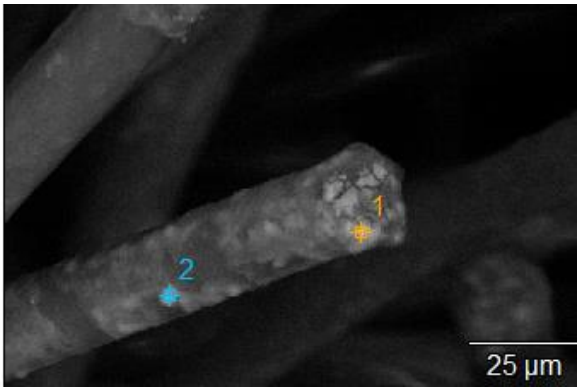


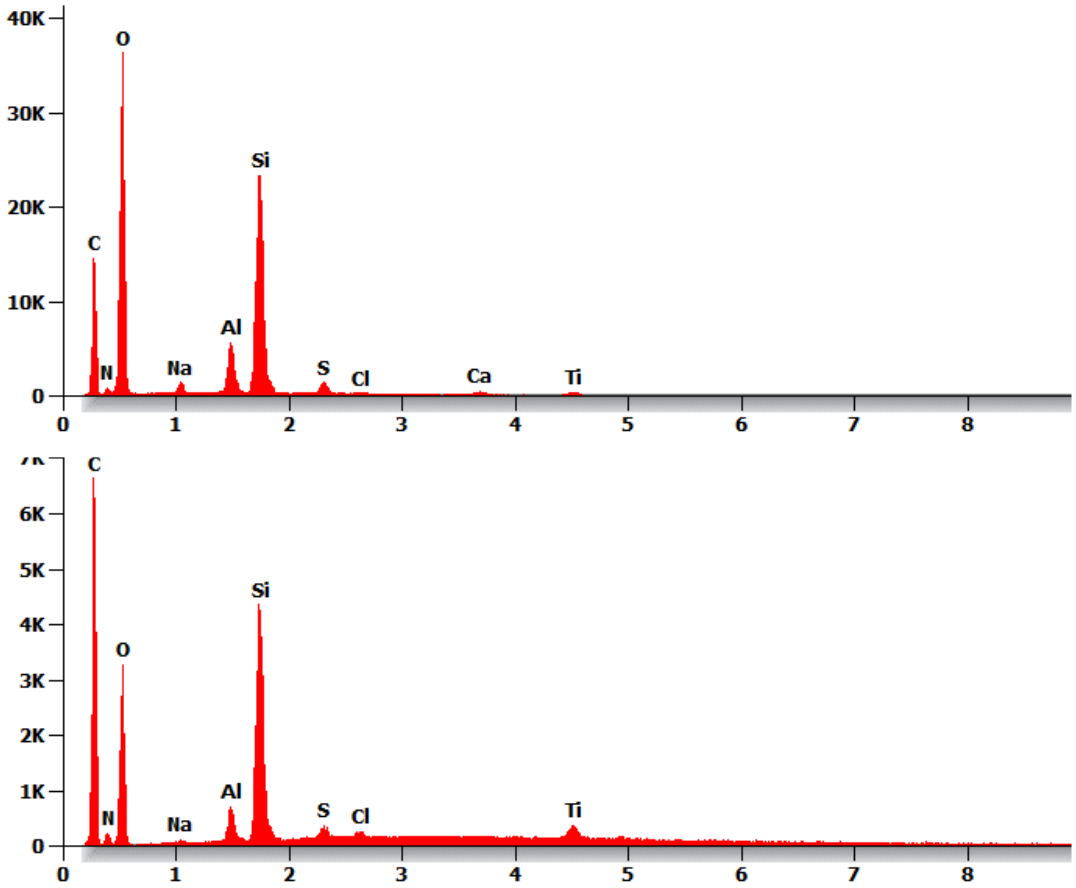




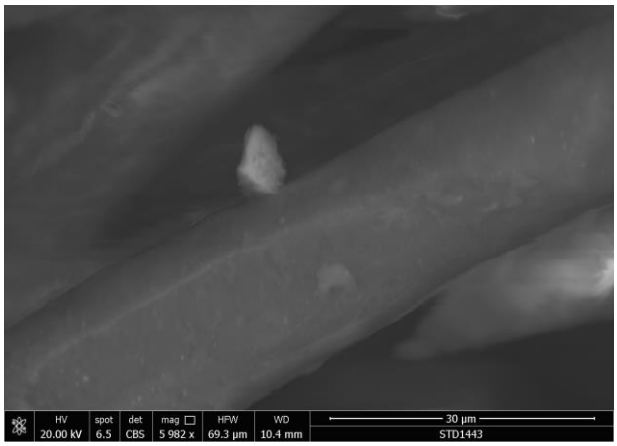
Analysis 4 of Table I. The image shows an area of the sample (magnification 325x) where a debris is visible. The EDS analysis shows that it is composed of Carbon, Calcium, Oxygen, Silicon, Aluminium and Magnesium. That debris does not belong to the swab: it is probably dirt. (Yellow circle).

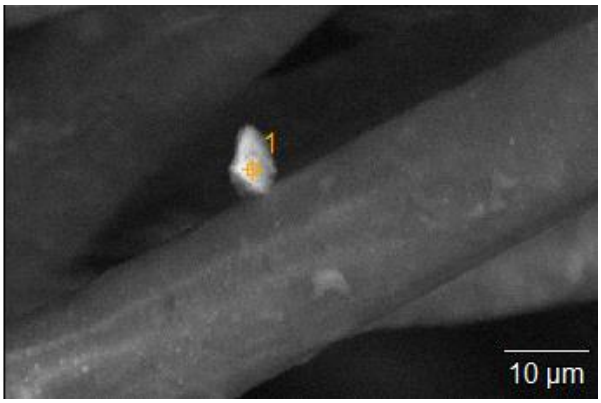


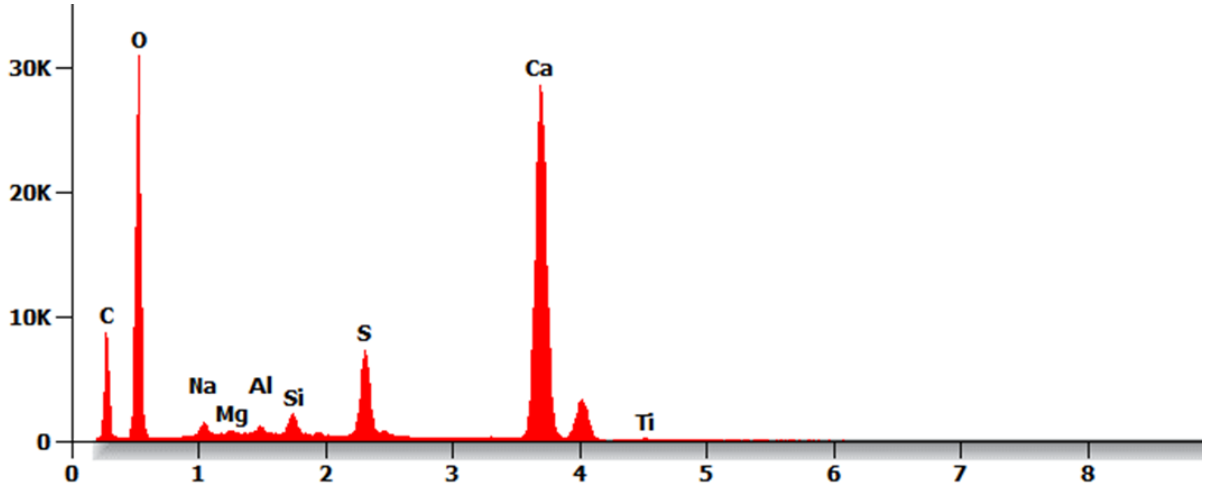




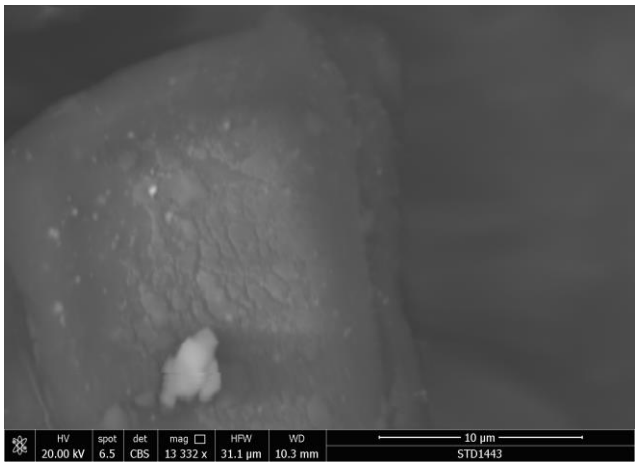
Analysis 5 of Table I. The image shows further details of the device (magnification 3087x) where two debris are visible. The EDS analyses show that the former is composed of Oxygen, Silicon, Carbon, Aluminium, Sodium, Sulfur, Calcium, Titanium, Chlorine and Nitrogen; the second of Carbon, Silicon, Oxygen, Aluminium, Sulfur, Titanium, Nitrogen, Chlorine and Sodium. They are similar, apart from a slightly difference in the Oxygen content. The spectrum represents the silicate coating of the fibres, a glassy one.

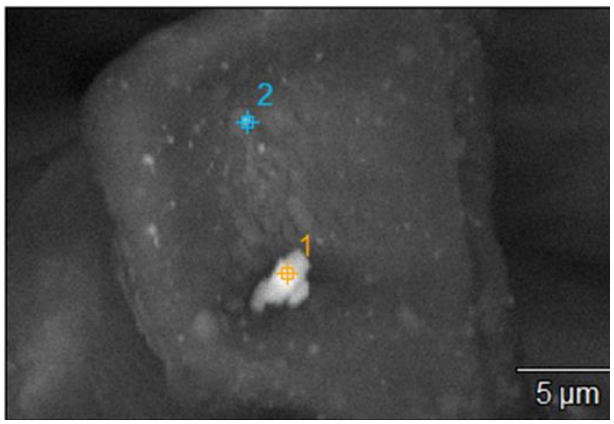


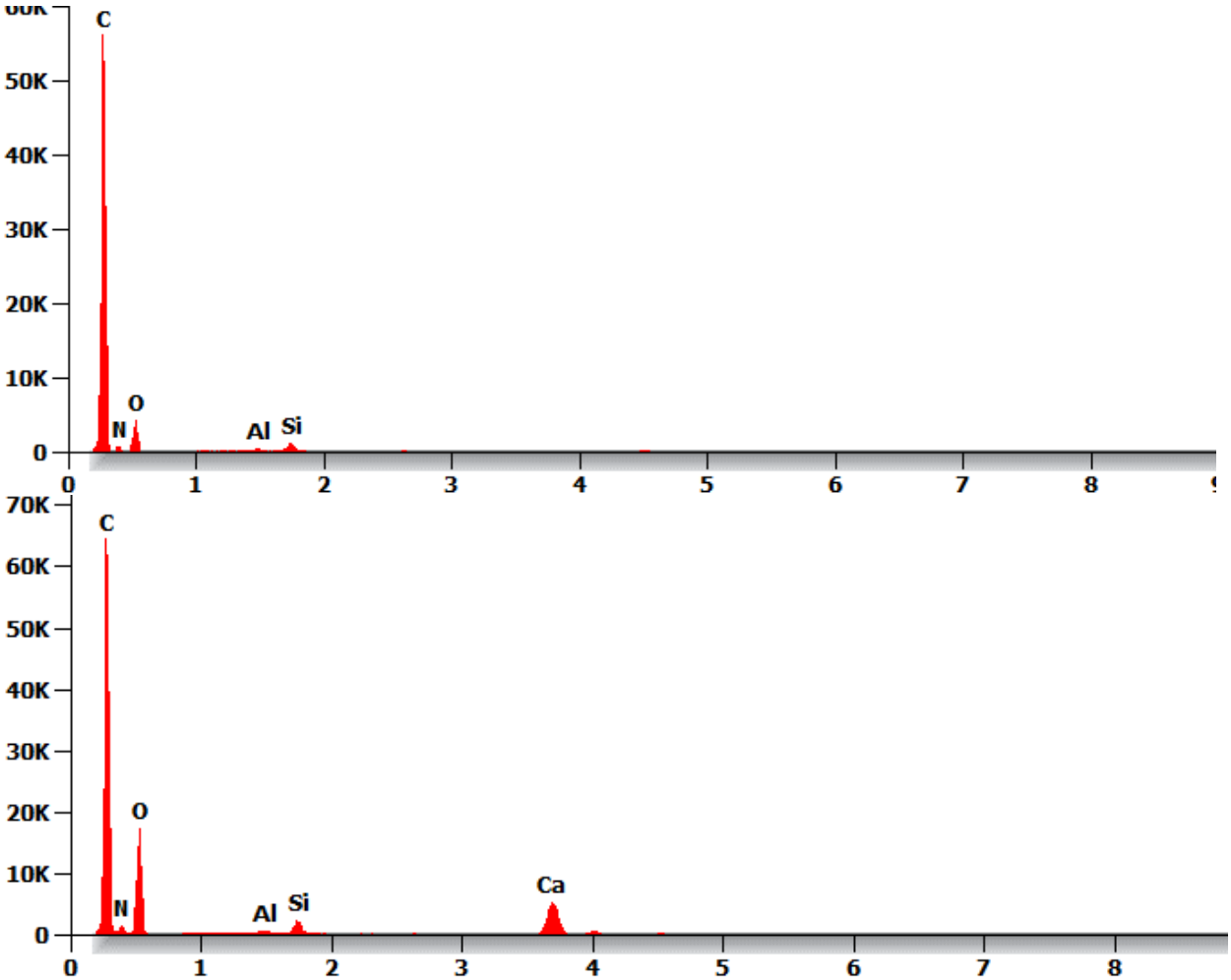




Analysis 6 of Table 1. The image shows an area of the fibre (magnification 5982x) where a debris is visible. That is composed of Oxygen, Calcium, Carbon, Sulfur, Silicon, Sodium, Aluminium and Magnesium. It is dirt. The images show that the coating is not homogenous and there are also debris not belonging to the coating process.







Analysis 7 of Table 1. The figure shows an area of the sample (magnification 3087x) where two debris are visible. The EDS analysis shows that the former is composed of Carbon, Oxygen, Calcium, Silicon, Aluminium, and Nitrogen; the second of Carbon, Oxygen, Silicon, Nitrogen and Aluminium. Fibres are dirty.

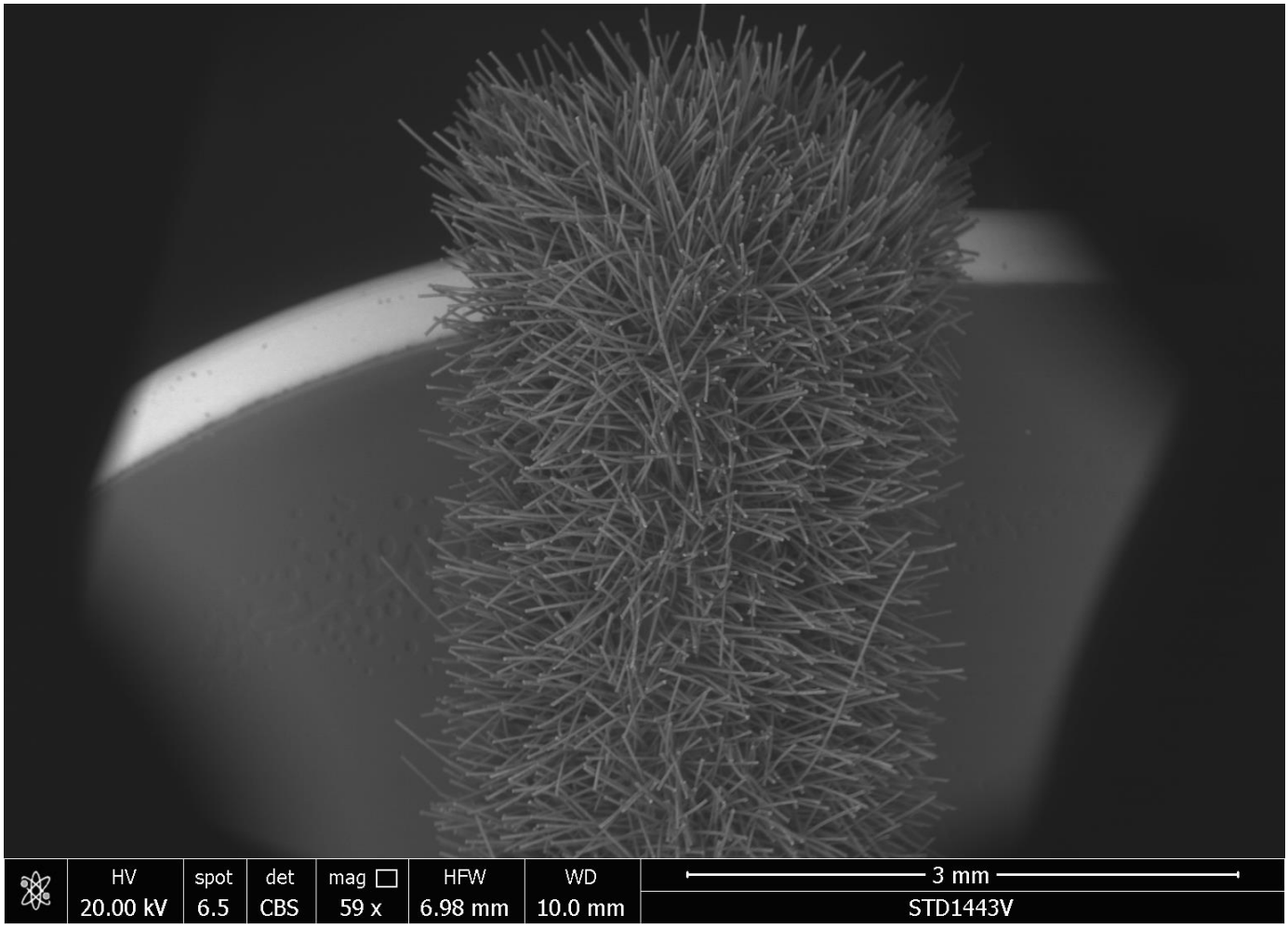
List of images and EDS analyses:

Analysis No.2

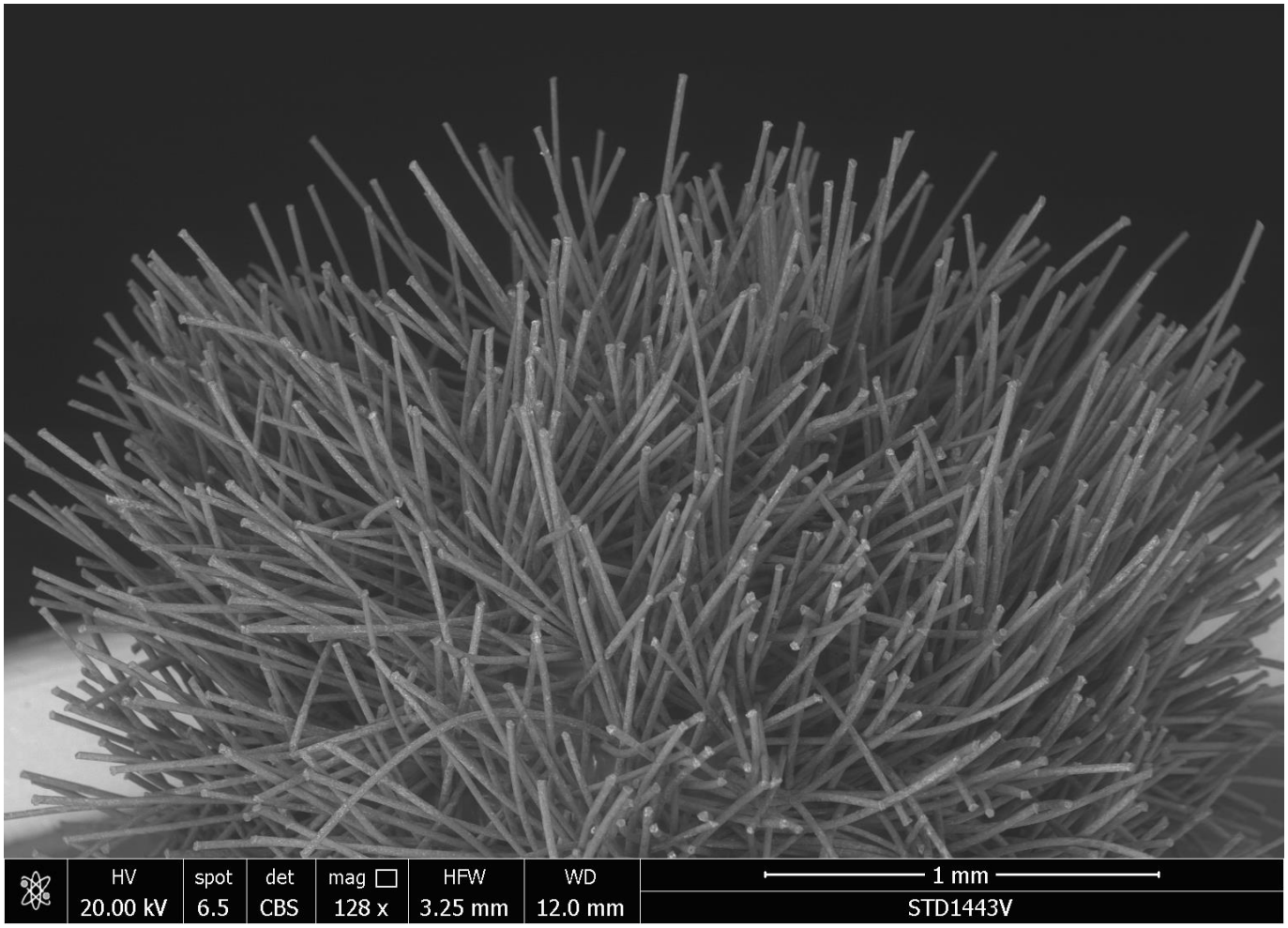
STD1443V SUNGO EUROPE

Table II containing all the analyses carried out on the sample.

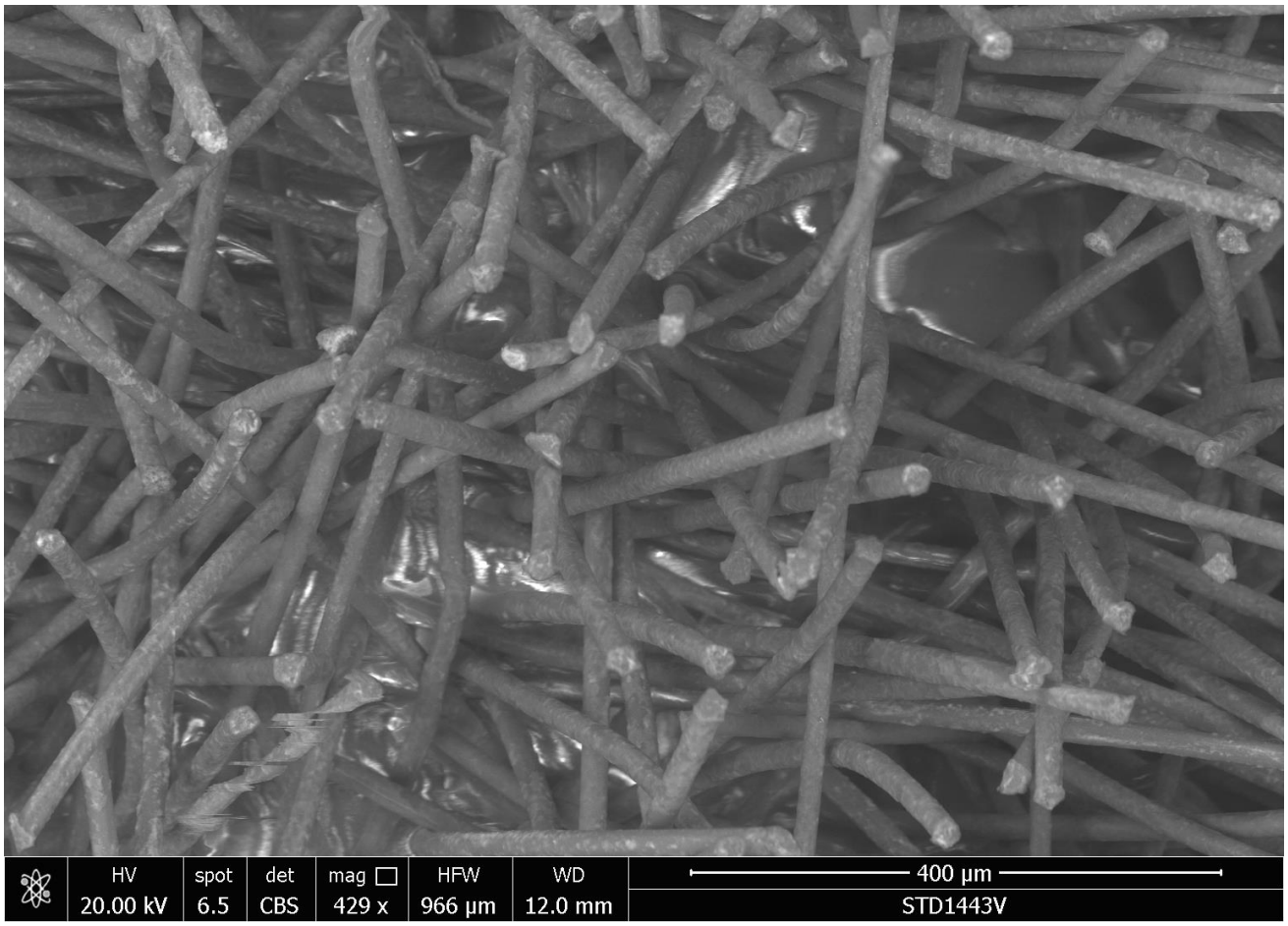
N. Analysis	Magnification	Elements
1	59x	Morphological Image
2	128x	Morphological Image
3	429x	Morphological Image
4	2423x	O Si C Al Ti Cl N
		C O Si N Al
5	875x	Si O C Al
		Si O C Al N
6	14583x	Si O C Al Ti N
7	93x	Morphological Image
8	1420x	C Au O Si Al Cu Ni Zn
		C O Si Al S
		C O Si
9	54302x	C Au O Ni Cu Al



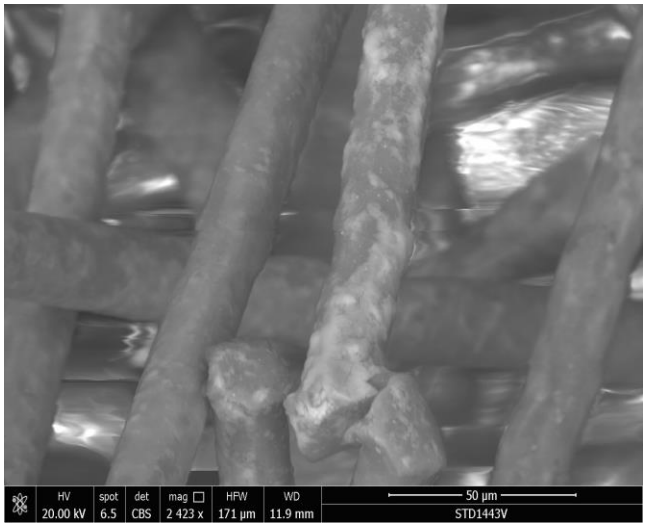
Analysis 1 of Table II. The image shows an area of the swab at low magnification (59x). The “porcupine” structure is well visible as well as one fibre already detached from the core.

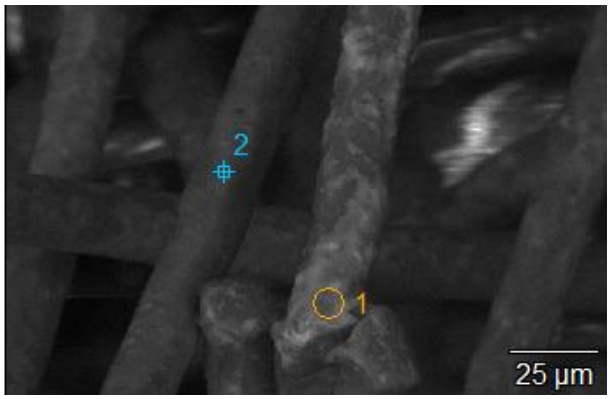


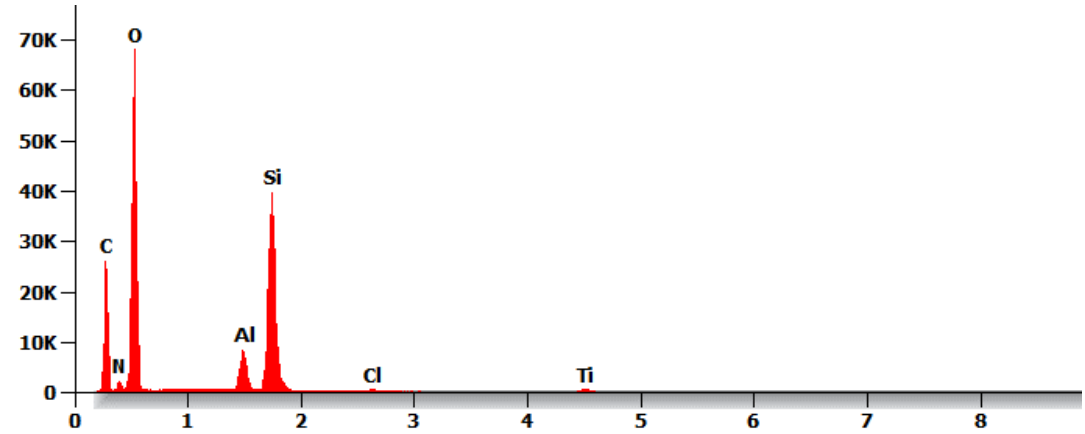
Analysis 2 of Table II. The image shows an area of the image (magnification 128x).

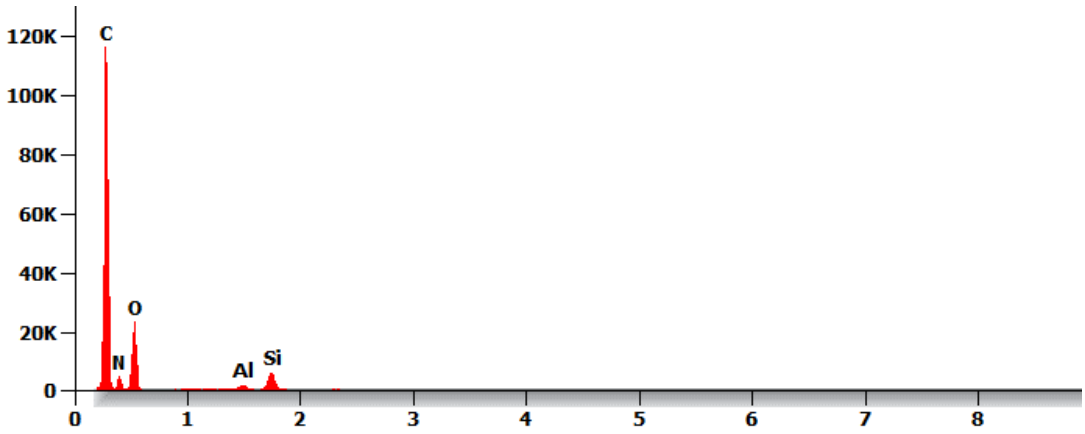


Analysis 3 of Table II. The image shows a detail of the swab with many fibres (magnification 429x).
Also in this case, it seems that at the base of the fibres a chemical compound was added that the FEGESEM is not able to identify, except that it is composed of Carbon and Oxygen.

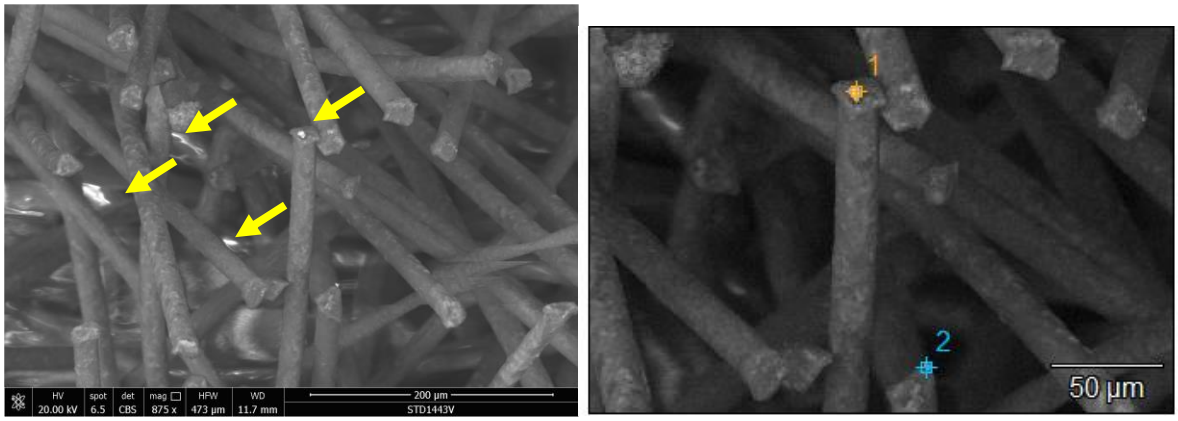


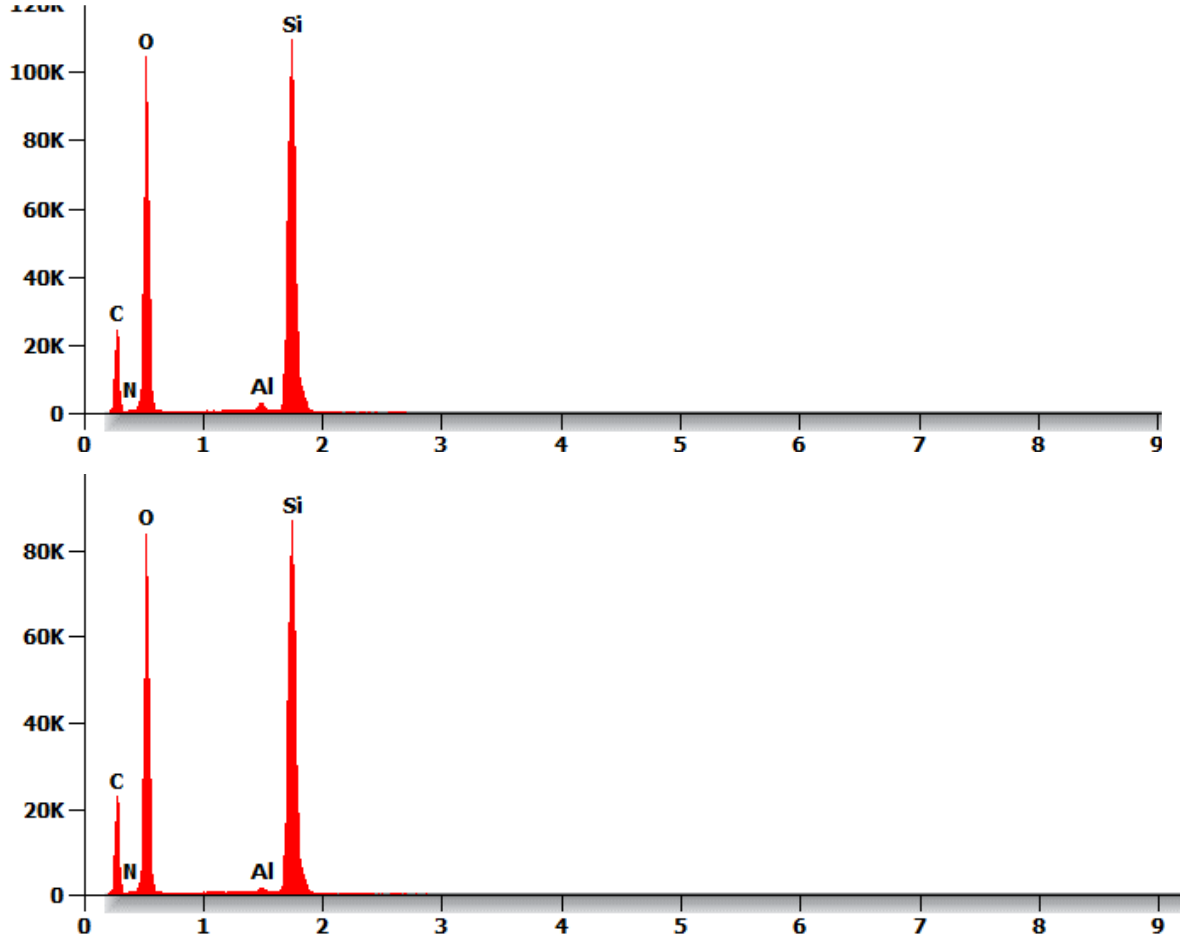




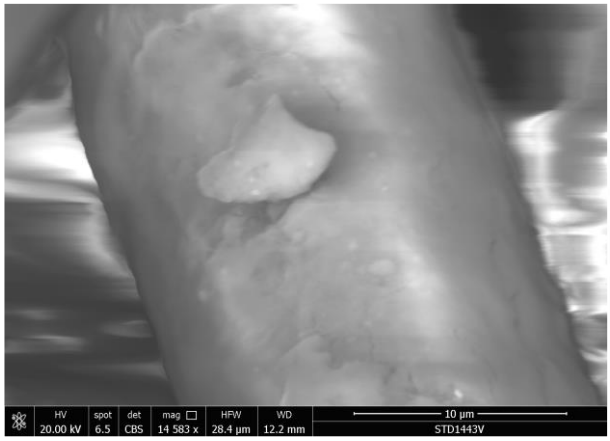


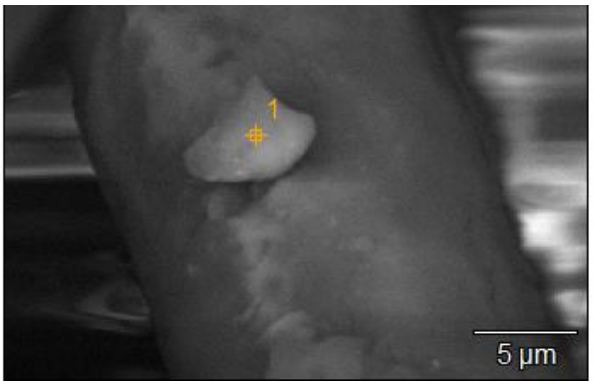
Analysis 4 of Table II. The figure shows an area of the image (magnification 2423x) where two particles are visible. The EDS analysis shows that the former is composed of Oxygen, Silicon, Carbon, Aluminium, Titanium, Chlorine and Nitrogen; the second of Carbon, Oxygen, Silicon, Nitrogen and Aluminium.
Also in this case, fibres were coated with a glassy material that was not deposited on them homogenously.

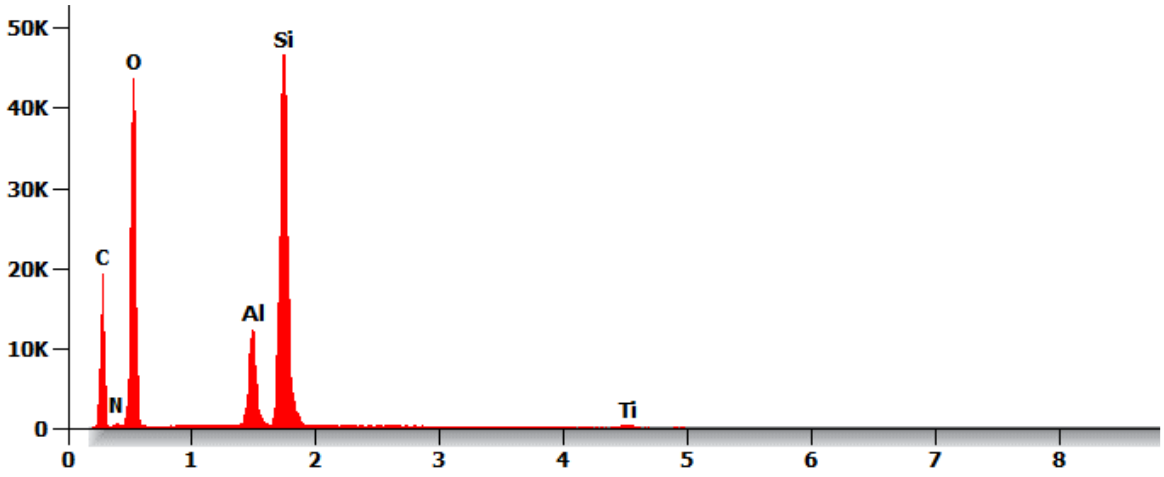




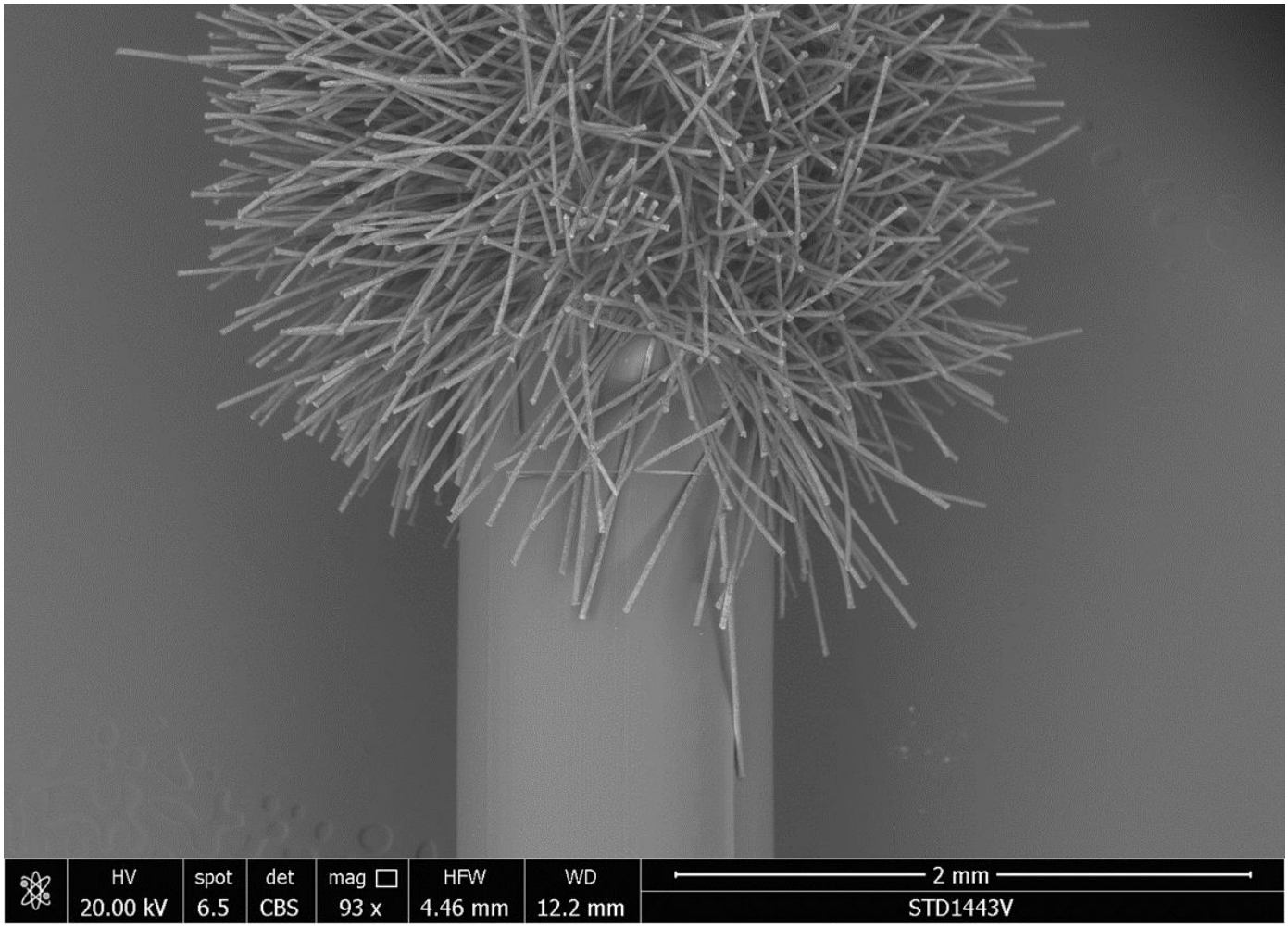
Analysis 5 of Table II. The figure shows an area of the image (magnification 875x) where two particles are visible. The EDS analysis shows that the former is composed of Silicon, Oxygen, Carbon and Aluminium; the second of Silicon, Oxygen, Carbon, Aluminium and Nitrogen. Debris of the glassy coating are detached from the fibre surface and lay free on its surface.



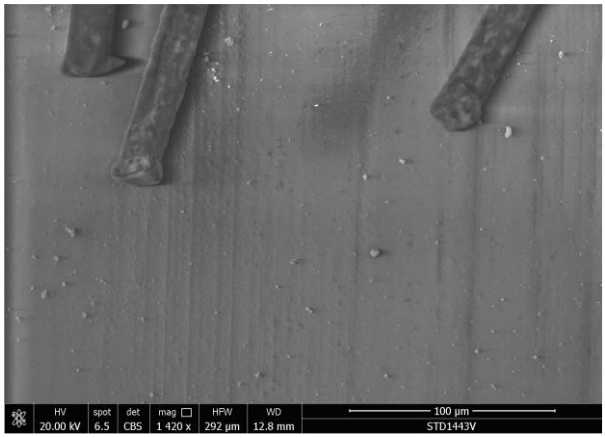


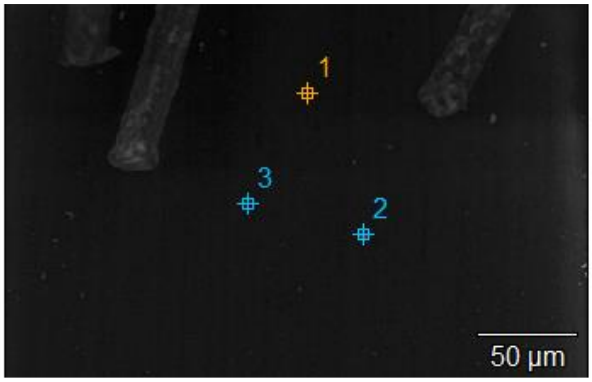


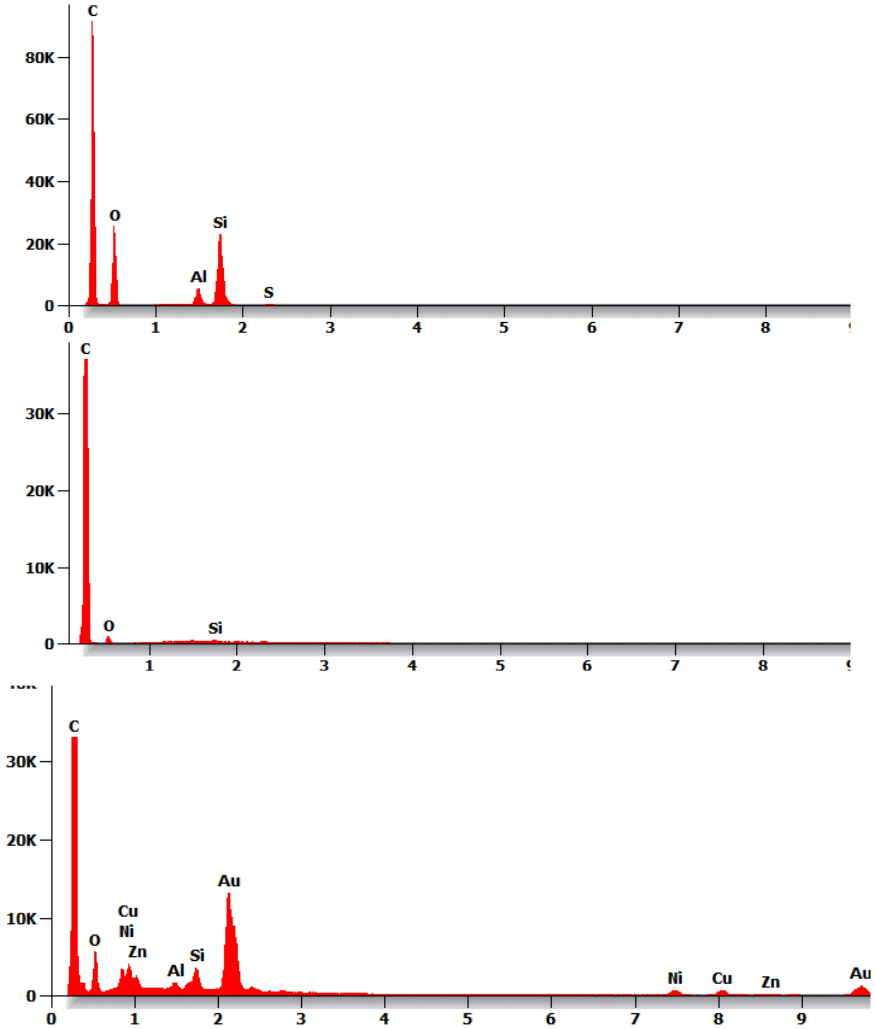
Analysis 6 of Table II. The figure shows an area of the image (magnification 14583x) where a particle is visible. The EDS analysis shows that it is composed of Silicon, Oxygen, Carbon, Aluminium, Titanium and Nitrogen.
Another particle of the coating lies free on the fibre surface.



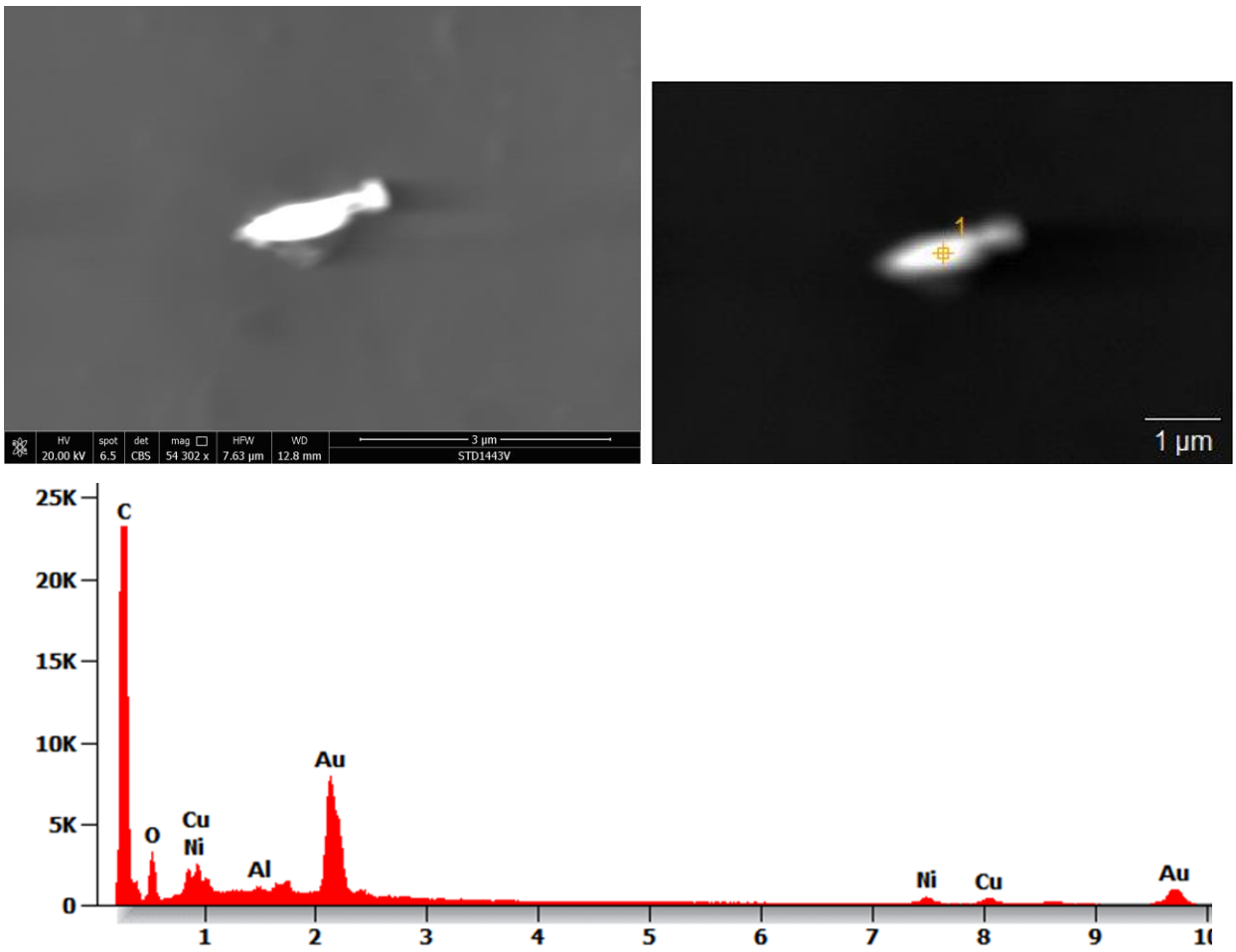
Analysis 7 of Table II. The image shows an area at the base of the swab (magnification 93x).







Analysis 8 of Table II. The image shows an area at the base of the swab (magnification 1420x) where three particles are visible. The EDS analysis shows that the first is composed of Carbon, Gold, Oxygen, Silicon, Aluminium, Copper, Nickel and Zinc; the second of Carbon, Oxygen, Silicon, Aluminium and Sulfur; the third of Carbon, Oxygen and Silicon. The particles identified are Gold-based. Nevertheless, they contain part of the coating but also Nickel Copper and Zinc.
It is a rather peculiar composition, not found in the manuals of materials. It is a rather unusual kind of dirt.



Analysis 9 of Table II. The figure shows an area of the image (magnification 54302x) where a particle is visible. The EDS analysis shows that it is composed of Carbon, Gold, Oxygen, Nickel, Copper and Aluminium. Another Gold-Nickel-Copper particle in a swab: a rather strange and “rich” kind of dirt.

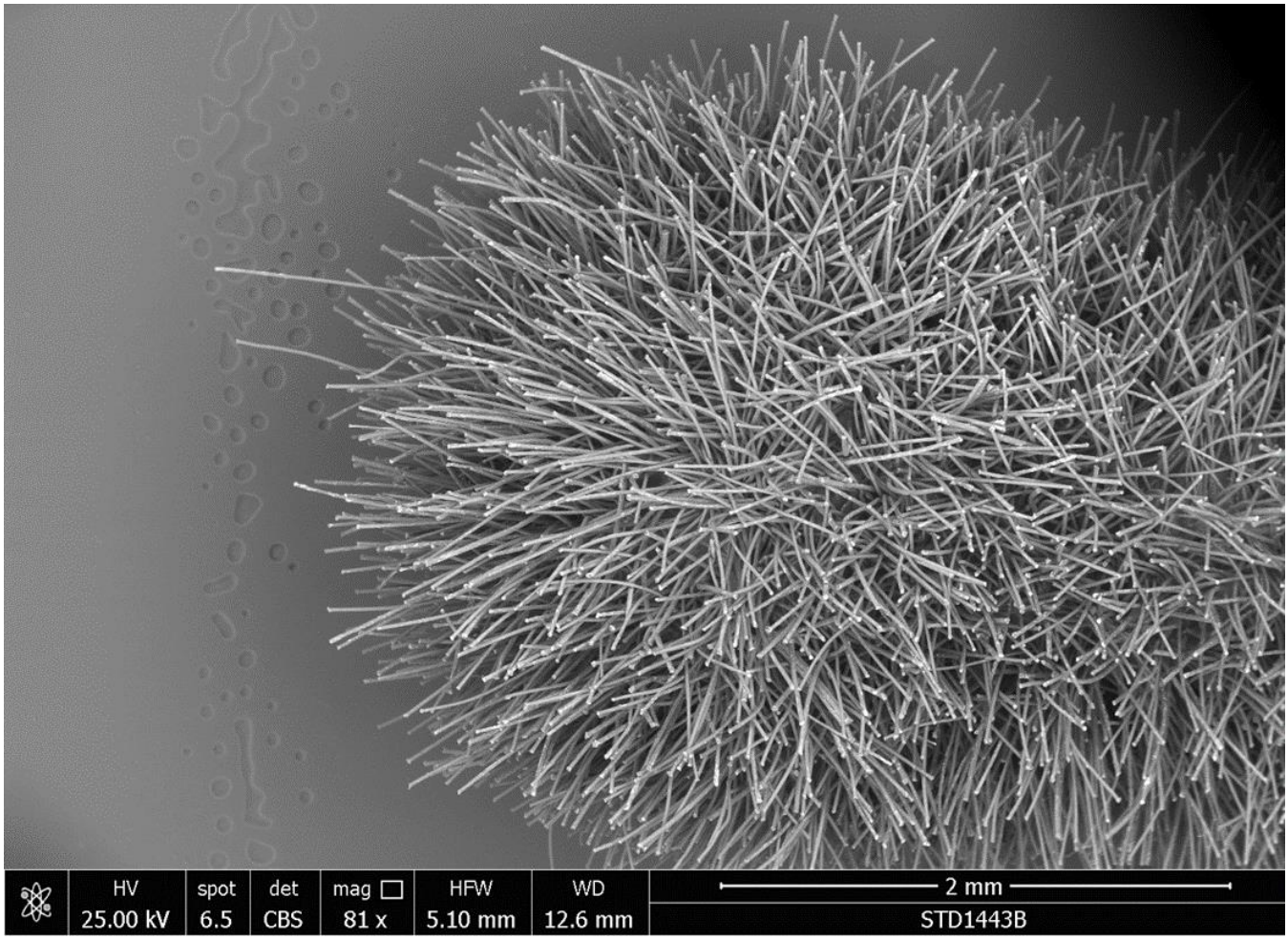
List of images and EDS analyses:

Analysis No. 3

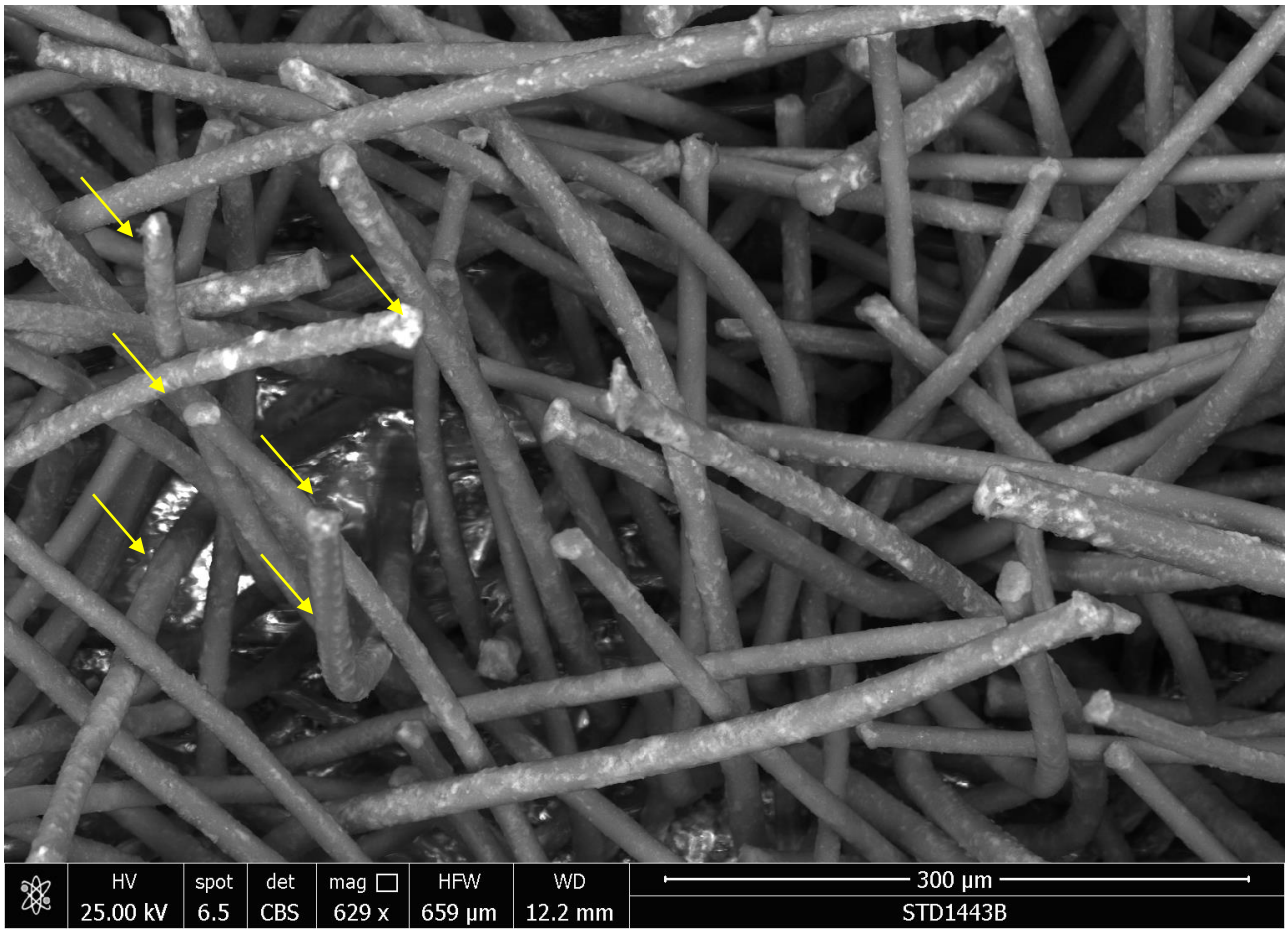
STD1443B SYNOCURA

Table III containing all the analyses carried out on the sample.

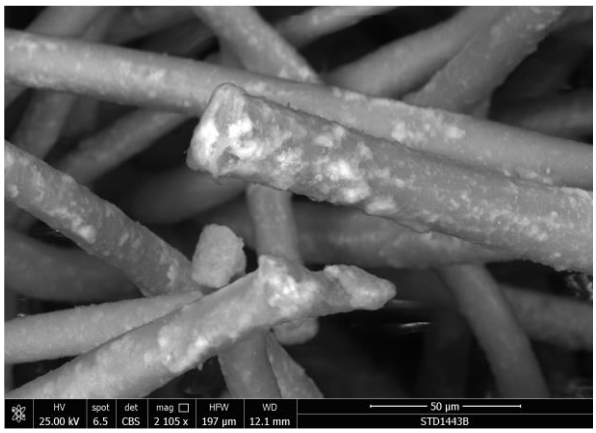
No. Analysis	Magnification	Elements
1	81x	Immagine morfologica
2	629x	Immagine morfologica
3	2105x	C O Si Al N Cl S Ti
4	585x	C Si O Al S N Na Cl Ti K Ca
5	100x	Immagine morfologica
6	100x	Immagine morfologica
7	251x	Immagine morfologica
8	1013x	C Al Si O S N Ti Cl Mg
9	227x	C Si O Al
		C O Si Al S Ca
		C O Al Si Ca S Na
		C Si O Al K

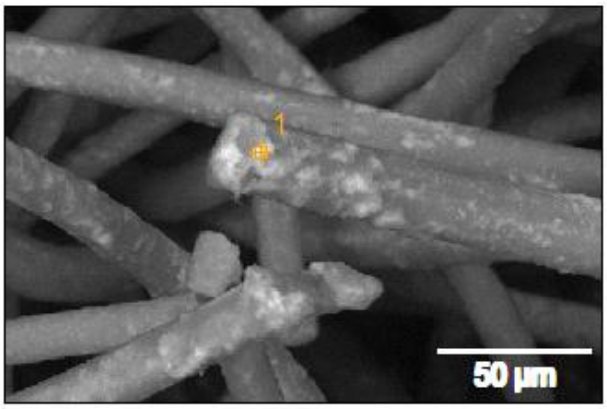


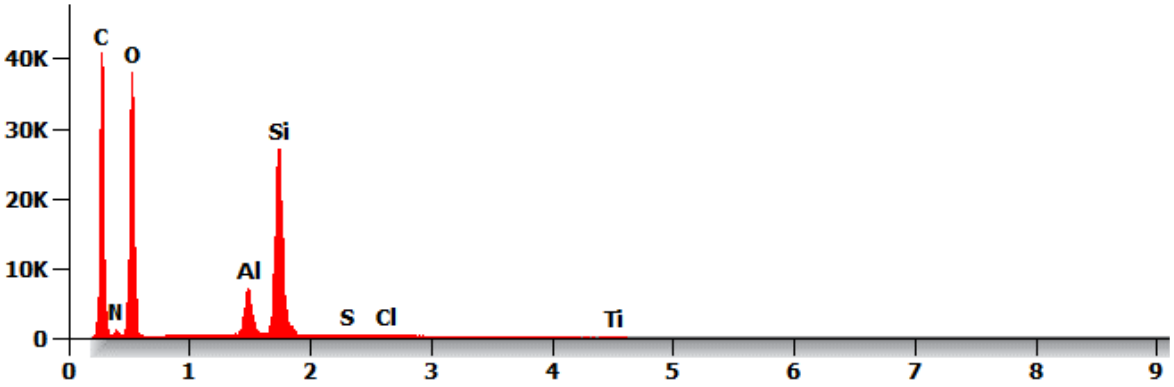
Analysis 1 of Table III. The image shows an area of the sample (magnification 81x).



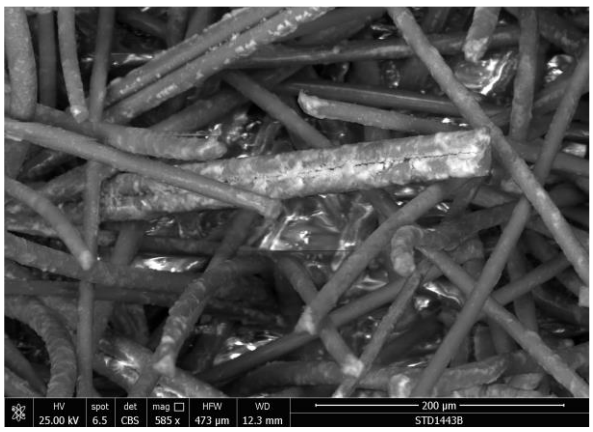
Analysis 2 of Table III. The image shows an area of the sample (magnification 629x). Yellow arrows indicate some contamination of the fibres.

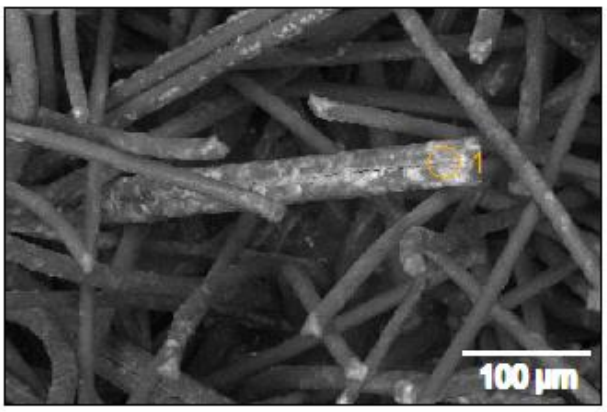


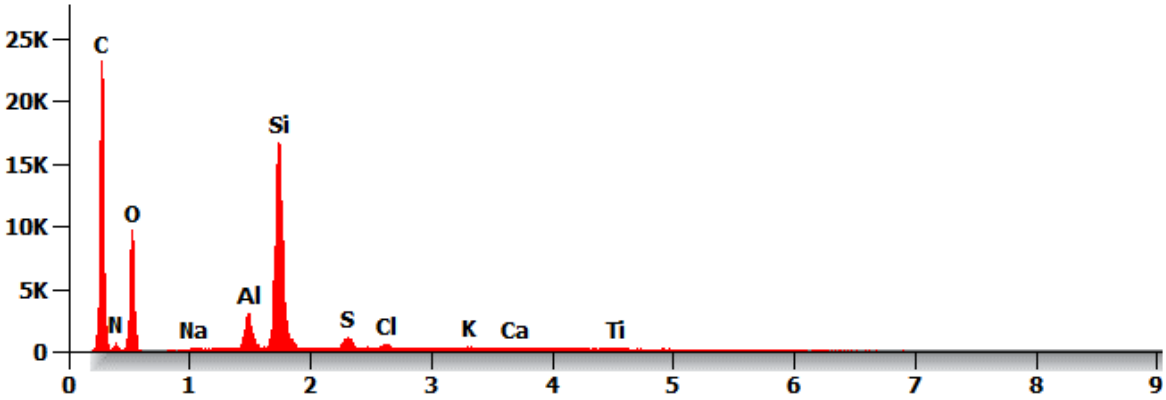




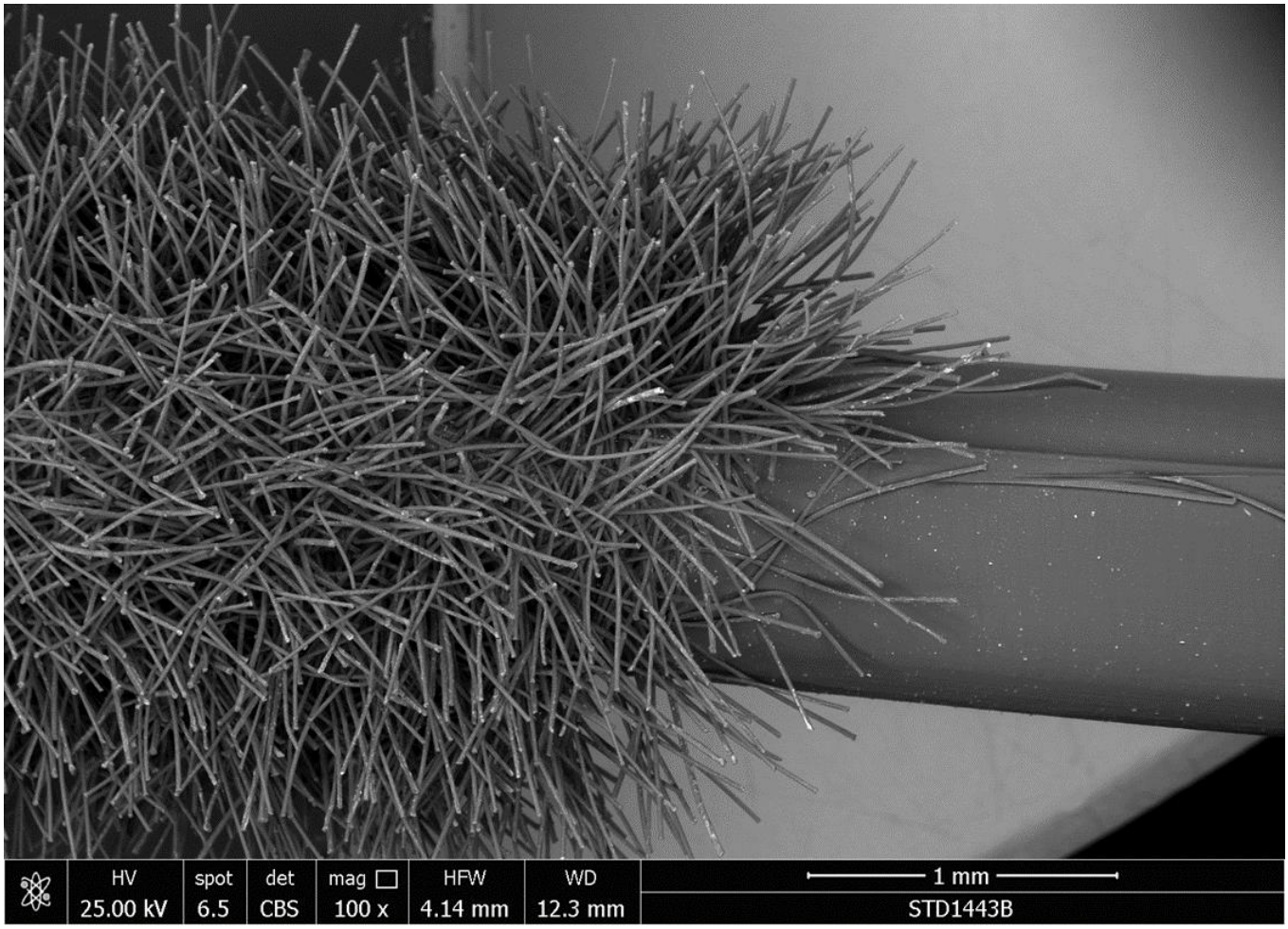
Analysis 3 of Table III. The image shows an area of the sample (magnification 2105x) where a particle is visible. The EDS analysis shows that it is composed of Carbon, Oxygen, Silicon, Aluminium, Nitrogen, Chlorine, Sulfur and Titanium. The image shows again the not-homogenous coating of the fibres.



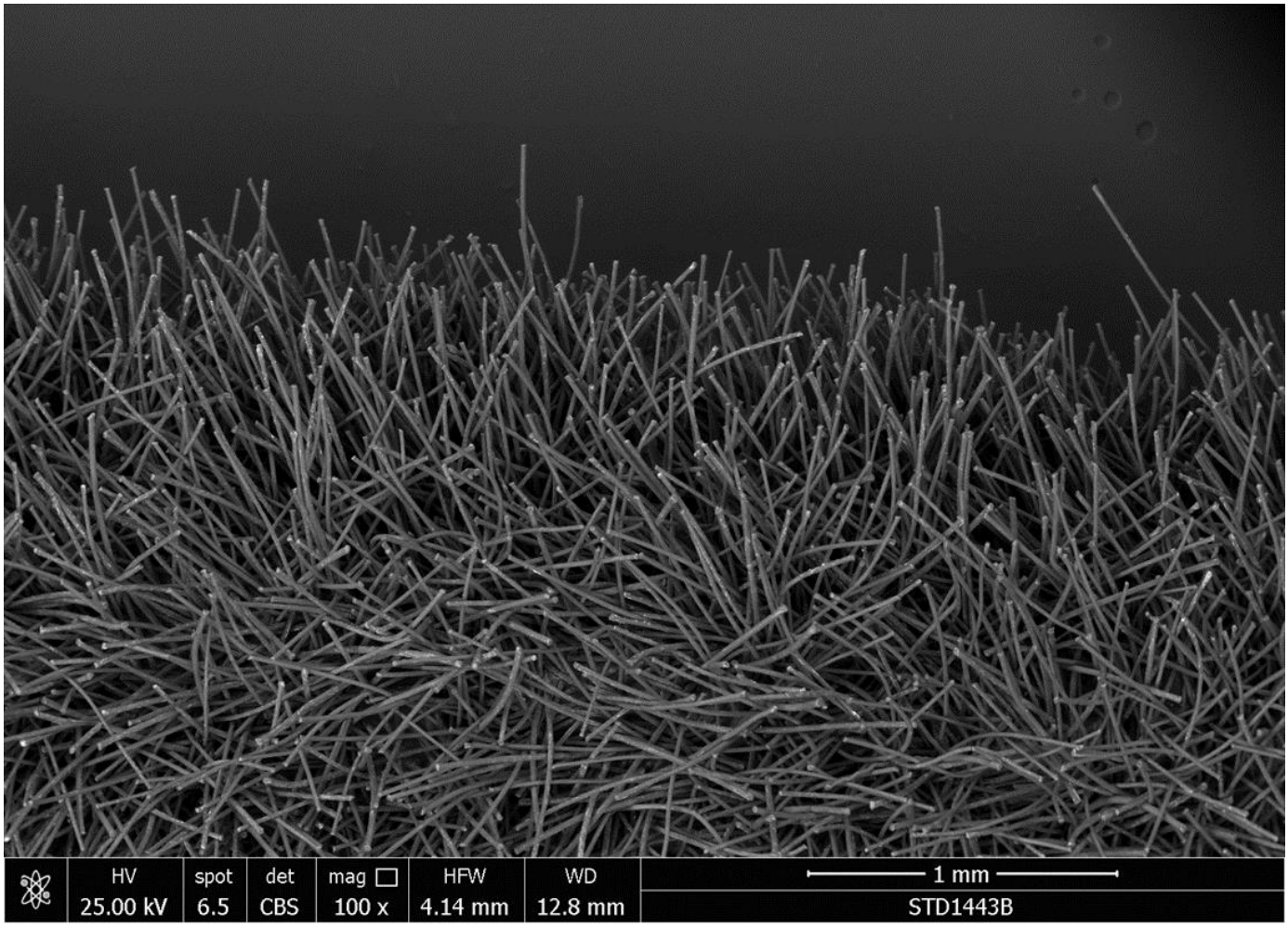




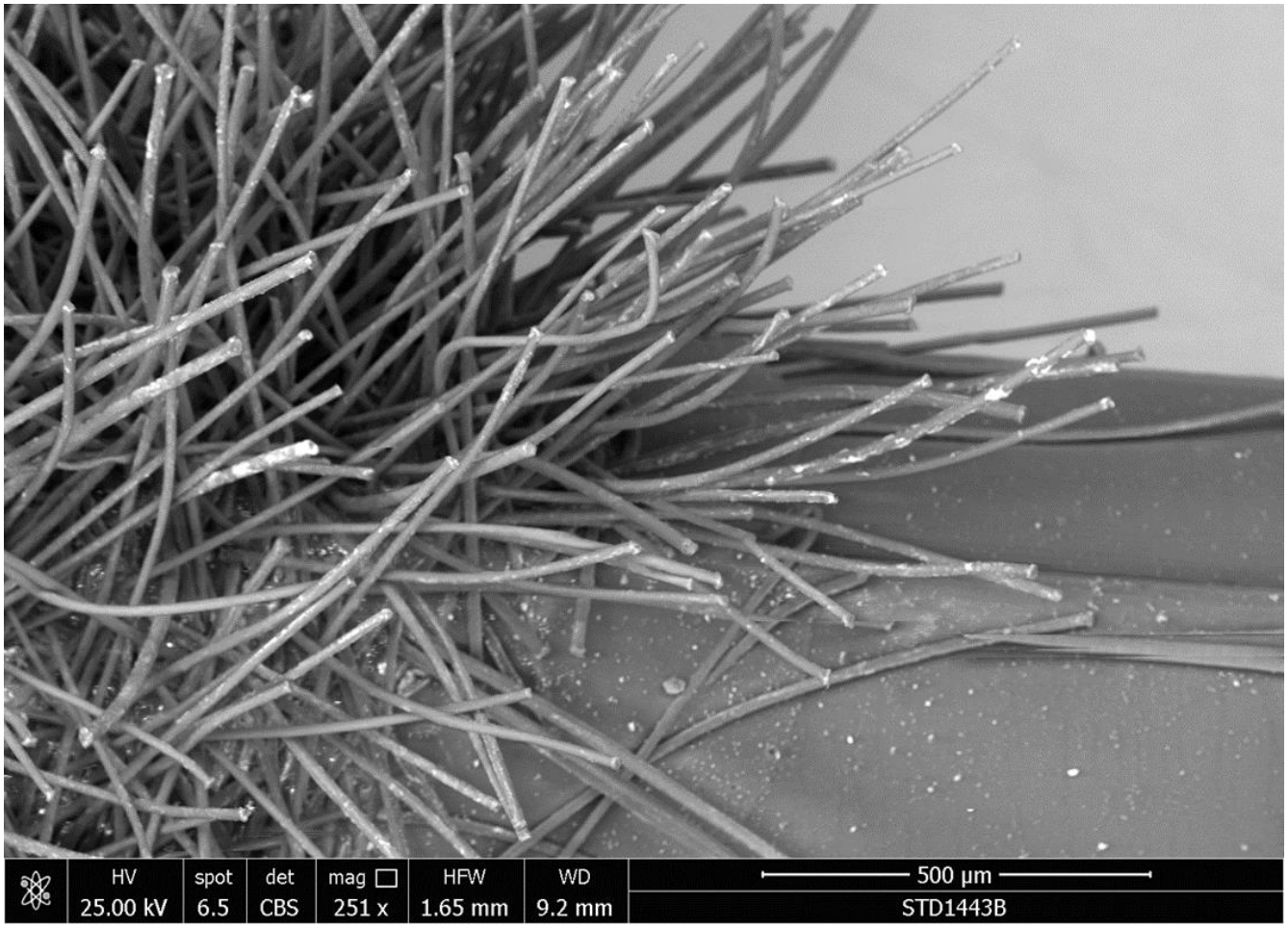
Analysis 4 of Table III. The image shows an area of the sample (magnification 2105x) where a particle is visible. The EDS analysis shows that it is composed of Carbon, Silicon, Oxygen, Aluminium, Sulfur, Nitrogen, Sodium, Chlorine, Titanium, Potassium and Calcium.



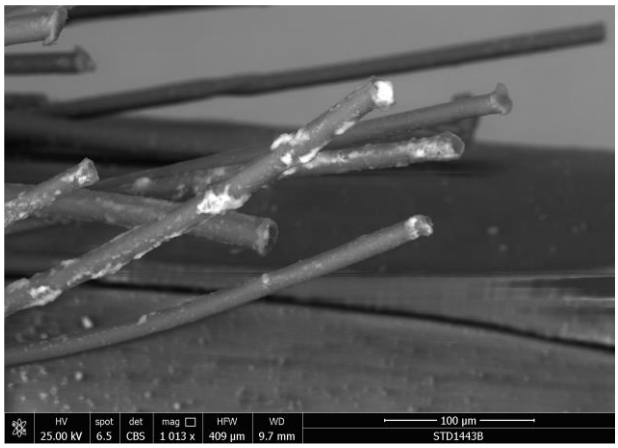
Analysis 5 of Table III. The image shows an area of the sample (magnification 100x).

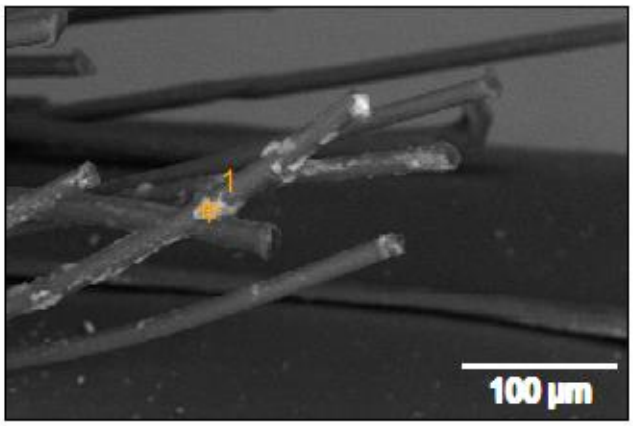


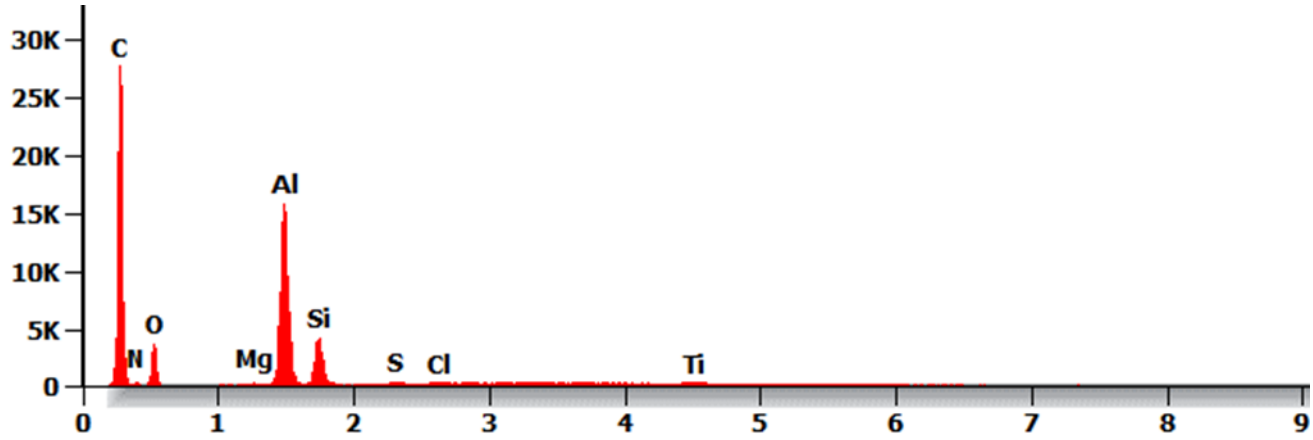
Analysis 6 of Table III. The image shows an area of the sample (magnification 100x).



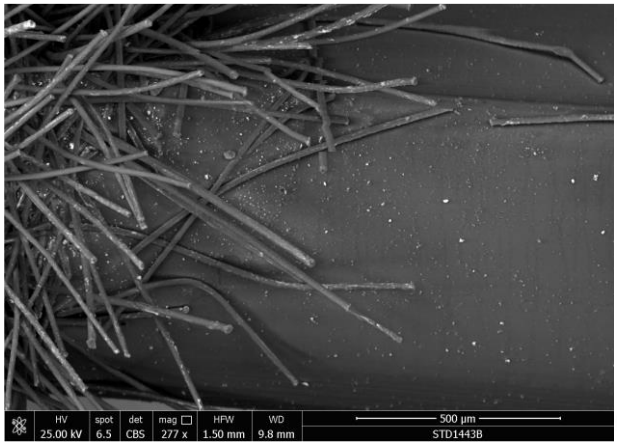
Analysis 7 of Table III. The image shows an area of the sample (magnification 251x).

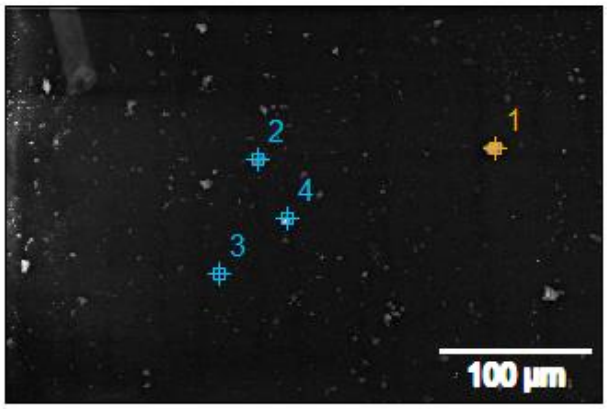


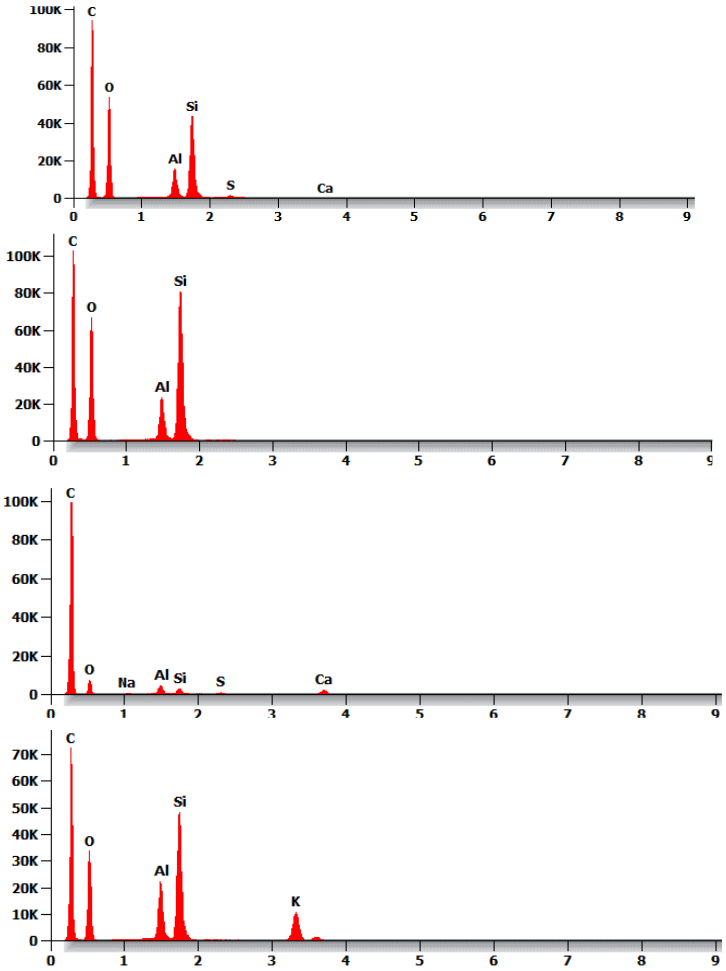




Analysis 8 of Table III. The image shows an area of the sample (magnification 1013x) where a particle is visible. The EDS analysis shows that it is composed of Carbon, Aluminium, Silicon, Oxygen, Sulfur, Nitrogen, Titanium, Chlorine and Magnesium. Similar composition is present in all the fibres analysed.







Analysis 9 of Table III. The image shows an area of the sample (magnification 227x) where four particles are visible. The EDS analysis shows that the first is composed of Carbon, Silicon, Oxygen, and Aluminium; the second of Carbon, Oxygen, Silicon, Aluminium, Sulfur and Calcium; the third of Carbon, Oxygen, Aluminium, Silicon, Calcium, Sulfur and Sodium; the fourth of Carbon, Silicon, Oxygen, Aluminium and Potassium.

6.Conclusions

The analyses carried out on the three specimens of swabs showed that their morphologies and their chemical compositions look similar. The optical microscopic investigation performed reveals that some fibres are already broken inside the package.

The devices are composed of a plastic cylindric core to which longitudinal fibres are attached.

It seems that the samples are mainly composed of what is probably polyamide fibres (nylon) (Carbon, Oxygen, Nitrogen), mixed or coated with Aluminium, Silicon, Sulfur, Chlorine, Titanium. The coating does not look homogenous. The EDS analyses reveal that some spurious particulate matter appears to be incorporated in the structure. Some debris, probably dust that has been incorporated during the manufacturing process, are present on the fibres.

Particles composed of Calcium or, occasionally, Gold are present on the fibres. This can testify to an insufficiently clean production. It must be concluded that, though the medical device is sterilized (but we could not ascertain the degree of sterility), is, in a way, dirty because polluted by foreign bodies.

It is reasonable to suppose that those pollutants can be transferred to the mucosa of those who have been subject to the sampling, and the same phenomenon can occur due to the not-homogenous coating and the broken fibres themselves. If sampling is done with excessive energy, the mucosa can be damaged, and bleeding, occasionally lasting a few days, is a common symptom. If dirt and fragments of the broken fibres are left on a damaged tissue, they can induce a granuloma and injuries that can remain chronic. Fibrosis of the tissue is possible, with an alteration of the voice as an immediately noticeable consequence, as is the case with a cold.

Given the purpose for which this medical device is manufactured, the question arises whether some confounding effect can be achieved from the identified contamination when the biological material taken with the use of that swab is introduced into the instrument used for PCR analysis. The accuracy of the operations carried out in the instrument could be affected by an interaction such as to invalidate the result (for example, the issue of a false positive).

These devices must not be chewed, but producers give no indications or warning on the subject. In any case, at least children must not be allowed to chew them. In that case, further fragmentation

can occur, and broken fibres can be ingested with all the obvious consequences of the phenomenon.

To our knowledge, there is no study regarding the physiological elimination of those foreign bodies either through the faeces or through any other modality.

N.B. The Author of the present report is an expert of Biomaterials and biocompatibility. She is a Fellow of the Societies of Biomaterials and Engineering.

7. Bibliography

References refer to the methodology adopted for present investigation:

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2. A.M. Gatti, S. Montanari “Nanopathology: The health impact of nanoparticles” Ed. Pan Stanford 2008, 1-212.
3. A.M. Gatti, S. Montanari, Case studies in nanotoxicology and particle toxicology, Elsevier, USA, 2015, 1-198.
4. A.M. Gatti, S. Montanari “Advances in Nanopathology.” Ed. Pan Stanford 2021, 1-190.