

The Y-Chromosome STRs Profile of Louis XVII (1785-1795?)

G rard Lucotte¹, Christian Cr pin², Thierry Thomasset³

¹Institut d'Anthropologie Mol culaire, 75005 Paris, France

²15 B^d de la Pierre Blanche, 11000 Carcassone, France

³Service d'Analyse Physico-Chimique, UTC, 50201, Compi gne, France

Abstract: We have studied by SEM-EDX some hairs from a lock of hair of the King of France Louis XVI and Queen Marie-Antoinette 's children. Among these hairs, the blond hair number three is covered with skin-cells and dandruffs. The mtDNA haplogroup of dandruffs of this hair is that of Marie-Antoinette ; the Y-STRs profile of this hair is that of Louis XVI, previously known. Consequently this hair is one of Louis XVII, one of Louis XVI's son.

Keywords: Y-Chromosome STRs Profile, Louis XVII, King Louis XVI 's Paternity

Louis-Charles (born in 1785) is the second son of the King of France Louis XVI (1754-1793) and of Queen Marie-Antoinette (1755-1793). Louis-Charles was proclaimed Louis XVII, King of France, immediately after his father's death. Previously (Lucotte *et al.* , 2015) we have compared mitochondrial DNA (mtDNA) sequence, obtained from Louis XVII 's authentic hairs, with that of Marie-Antoinette ; but, until now, we were not able to obtain Louis XVII 's Y-STRs (Short Tandem Repeats DNA markers of the Y-chromosome) profile. In the present study we find – on another hair material – Louis XVII 's Y-STRs profile and we compare it to that of Louis XVI, which we recently published (Lucotte *et al.* , 2016).

Materials and Methods

Materials

On June 26 2011, the de Baudreuil brothers displayed two objects coming from their inheritance at the Rouillac office (Cheverny, France) (**Figure 1**) : **i** : a gold ring of Queen Marie-Antoinette, surrounded by her hairs ; **ii** : a folded letter, with inscriptions. The ring was kept in a case, where we can see the following inscription : "Cheveux de la Reine Marie Antoinette"" (Hairs of Queen Marie Antoinette) , "Bague donn e par la Reine en 1792   la M^{ise} de Soucy n e de Makau Sous-Gouvernante des Enfants de France" (Ring given by the Queen in 1792 to the Marquise de Soucy born de Makau sub-governess of the Children of France). Ren e-Suzanne-Marie Louise of Mackau (1758-1841) was sub-governess of the children of France in 1780.

Laure-Ad la de-Louise de Fitte de Soucy married in 1863 Emile-Alexandre-Louis de Baudreuil, and the de Baudreuil brothers are, today, the descendants of this couple. Laure-Ad la de-Louise de Fitte de Soucy

was a daughter of Philippe-Babolin de Fitte de Soucy (1807-1843), himself a son of Louis-Xavier de Fitte (1775-1840) Marquis de Soucy. Louis-Xavier de Fitte was the elder son of Fran ois-Louis de Fitte (1751-1793) and Ren e-Suzanne-Marie-Louise de Mackau.

At the surface of the folded letter (**Figure 2**) we can see the following inscription : "Cheveu des Augustes enfants de France" (Hairs from the Auguste children of France). In the unfolded paper of the letter is a lock of hair, tied up with a green thread.

Microscopy techniques.

The hairs and other objects were examined in confocal stereoscopic micrography.

Electronic microscopy was realized by Scanning Electron Microscopy (SEM) with the Philips XL30 model (environmental version). GSE and BSE procedures were used, the last one to detect heavy elements. The blond hair number 3 was examined with another electron microscope (SEM-2), the FEI model Quanta 250f FEG apparatus ; that was the LFD procedure that was used.

Elemental analyses for each object were realized on SEM-1 by X-Ray Microfluorescence (XRMF), this microscope being equipped with a Bruker AXS Energy Dispersive X-ray (EDX) ; the system of analysis is PGT (Spirit Model, of Princeton Gamma Technology). Each elemental analysis is given in the form of a spectrum, with kiloelectrons / Volts (keV) on the abscissa and elemental peak heights in ordinates.

This article is published under the terms of the Creative Commons Attribution License 4.0

Author(s) retain the copyright of this article. Publication rights with Alkhaer Publications.

Published at: <http://www.ijsciences.com/pub/issue/2019-02/>

DOI: 10.18483/ijSci.1899; Online ISSN: 2305-3925; Print ISSN: 2410-4477



G rard Lucotte (Correspondence)

+06 98 82 92 61

DNA Techniques

Genomic DNA was extracted (Lucotte, 2010) from the blond hair number 3 using a standard method (0.5M EDTA, sarcosyl 20% and proteinase K 10mg/ml) and purified using a commercial kit (NucleoSpin⁺ Kit ; Macherey-Nagel, Duren, Germany) in accordance with the manufacturer's instructions.

From this extracted genomic DNA, we obtain the mt-DNA sequence intervals for HVR1 and HVR2 (Hypervariable Regions 1 and 2) by PCR (Polymerase Chain Reaction) with primers F15971 and R16410 and with primers L15 and H484, respectively. PCR products were purified from agarose gel. Both strands of the amplified mt-DNA fragments removed were directly sequenced (Big Dye Terminator Cycle sequencing kit ; Applied Biosystems).

From the extracted genomic DNA, we amplified 16 Y-STRs by using the AmpFirst Identifier PCR amplification kit (Amp FIRSTY filer[™], Applied Biosystems), according to the instructions given by the Company ; this amplification kit is specially adapted to the study of ancient DNA.

RESULTS

1. The paper of the letter.

The paper corner located in the upper right part of the unfolded

letter was cut off for analysis. Examined in optical microscopy (**Figure 3**), it is of green colour. Some part of it, examined by SEM (in GSE), at magnification of x100, shows that it is made up of an intertwine of intact vegetal fibers.

The global spectrum of this part shows elevated peaks (of carbon and oxygen) corresponding to an organic matter. **Figure 4** shows the SEM photograph of some portion of such a fiber ; it corresponds to a textile fiber of linen (a cylindrical fiber, of about 20 µm of thickness, of which the smooth surface shows regularly spaced characteristic nodes). Elemental analysis of this fiber shows a spectrum similar to that of the global spectrum of the paper.

This paper is a "papier-chiffon", a sort of luxurious paper that was used in the past for the writing of the official and fiduciary documents. Presence of sulphur, potassium and aluminium in spectras suggest that alun (a sulphate double of potassium hydrated), of chemical formula : $KAl(SO_4)_2 \cdot 12H_2O$ was used as a mordant to stain the paper with some green organic colouring.

2. The ink of the paper inscription.

Figure 5 shows enlarged views (both in optical and electronic microscopy) of the upper part of the terminal letter s of the written word "des". Spectrum of the white matter (seen in BSE) located at this point (**Figure 6**) shows that the corresponding bright particles are sulphur and iron-rich.

This indicates that the ink is a "ferro-gallic ink", iron sulphate particles added darkening again the blackness of the organic matter constituting the ink. This sort of ferro-gallic ink was commonly used in Europe (from the Middle-Ages to the end of the 18th Century, and even after).

3. The green thread around the lock of hair.

Figure 7 shows an enlarged optical view of the lock of hair. There are two sorts of hair colouring : blond hairs and red hairs. Both these sorts of hair are rolled up (and tied) with a green thread.

Figure 8 shows different aspects of the fibers constituting such a thread. Green in colour the fibers are long and cylindrical, with a regular thickness. The lower SEM photograph of the figure shows one fiber extremity, with a bi-triangular end characteristic of a silk fiber. **Figure 9** shows some portion of such a fiber (of 16µm of thickness), which is smooth. The corresponding spectrum shows a main peak of carbon, a little peak of oxygen, and a very little peak of sulphur (this last organic element being characteristic of silk).

But they are also aluminium and sulphur little peaks in the spectrum. Like for the paper, the green colour of the silk fibers is due to an organic green pigment linked to the silk by the alun mordant.

4. The hairs of the lock.

Three hairs of each colour were taken from the lock (**Figure 10**). The following figures summarize results we obtained concerning the longest hair for each category.

Figure 11 shows both extremities of red hair number 1 ; they correspond to a hair that was cut off (direction of the point scales of each row permitting to orientate the hair). SEM-1 photograph of **Figure 12** shows the middle part of the hair, where the thickness is of 65.5 µm. On this photograph we can see that the average distance between the scale row is about 5 µm, a characteristic of human hair (the hair surface is covered with mineral particles). The spectrum shows the peaks characteristics of hair composition : two peaks (of organic matter) corresponding to carbon and oxygen, a very little peak of nitrogen, and a consistent peak of sulphur (disulphur bridges of the keratin) ; there are chlorine

and sodium, corresponding to the salt (ClNa) of sweat. An example of a mineral deposit on the hair surface is shown on **Figure 13** ; its spectrum is that of an alumina-silicate iron-rich (a clay) particle. A total number of thirty-two of such particles are deposited on the hair surface. Photograph of **Figure 14** shows another example of an other sort of mineral deposit on the hair surface ; its spectrum shows a relatively elevated main peak of calcium (so it is that of calcite, or another form of calcium carbonate). We found a total number of eleven such particles on the hair surface. **Figure 15** shows an example of an *Acari* body portion adhering to the hair surface (four of such shells –or shell fragments – were found, stuck to the scales). The hair surface is multi-parasited, because we observe on its surface a clutch of some sort of Arthropod. We have evidence that this sort of hairs were treated with iron sulphate (**Figure 16**), a commonly used phytosanitary product of chemical formula FeSO₄. We have also observed at least four samples of *Cyanophytaes* (**Figure 17**) parasiting the hair surface.

Figure 18 shows both the cut extremities of blond hair number 1. SEM-1 photograph and spectrum of **Figure 19** show that this hair is, by aspect and composition, a typical human hair. Its diameter in the middle part is of 56.2 µm. Exploration of its surface

shows only two clay particles and one calcium carbonate particle (we do not find any iron sulphate product). **Figure 20** shows an adhering skin-cell, of the same chemical composition than that of hair scales (but differing for lower relative sulphur content and by the presence of phosphorous). An example of a micel of soap is shown on the SEM photograph of **Figure 21** (its composition is mainly of carbon and oxygen) ; we found six samples of such a product on the hair surface, this attesting that this hair was intensively washed, soaped and cleaned. Photograph of **Figure 22** shows an example of a set of metallic particles (of silver sulfure, with traces of copper) that is loaded on the hair surface (four sets of such a product were found). This attests that the hair was finely silver-decorated after washing. **Figure 23** shows the unique example found of a set of at least three particles (with angular outlines) located on the hair surface. As these structures are highly-resistant to an intense (at the maximal power of the SEM apparatus) X-ray fine beam and that their chemical compositions are mainly of carbon, they are probably diamond micro-splinters particles.

So, the two hairs are cut off hairs ; both are similar in aspect and chemical composition, that are characteristic of human hairs. **Table 1** shows the main points by which they differ.

Table 1 : Main differences between the two hairs.

	Red hair	Blond hair
Thickness	65.5 µm	56.2 µm
Deposits on the scales surface	numerous	few
Numbers of clay particles	32	2
Numbers of calcium carbonate particles	11	1
Number of skin-cells	none	1
Numbers of Acari shields	4	none
Number of insect clutch	1	none
Numbers of Cyanophytaes	4	none
Numbers of FeSO₄ particles	6	none
Numbers of soap micels	none	6
Numbers of metallic particles of silver sulfure	none	4
Diamond micro-splinters set	none	3

The two sorts of hairs entangled in the lock differ by their preparations : while blond hair was carefully soaped and washed and after that decorated with some precious materials, red hair is more “native” – because more covered by clay and calcium carbonate earth minerals – and constitutes a dirty matter ; that hair is covered by arthropod remains and infected by some *Cyanophytaes* (iron sulphate was used to treat it). These two sorts of hairs (unwashed red hairs and washed and decorated blond hairs) were further entangled in the hair lock we observe.

The following figures show skin-cells and dandruffs found on the blond hair number 3 surface. This hair, previously studied, has been shown especially rich with these formations ; so, a special procedure (on SEM-2, in LFD, 2400x) was used to analyse it more precisely.

Figure 24 shows the first skin-cells found ; of a relatively great size (about 30 µm of maximal length), these cells are flattened and longitudinally folded. Their compositions are about the same to those of hair scales, but with phosphorus and a

relatively lower proportion of sulphur. **Figure 25** shows the first dandruff found ; similar in composition to that of skin-cells, it has a smaller size, a bulging surface and well delimited contours. **Figure 26** shows the second dandruff observed, and **Figure 27** the aspects of the two following dandruffs found. The full-length exploration of the blond hair number 3 surface allows us to detect about thirty dandruffs on it.

Contrary to skin-cells, dandruffs are multi-nucleated cells that contain appreciable amounts of nuclear and cytoplasmic DNA (Lucotte and Bouin Wilkinson, 2014). It is the reason why we have extracted genomic DNA from the whole blond hair number 3.

5. DNA of the blond hair number 3.

We obtained about 39ng of genomic DNA from the blond hair number 3. The detection procedure of the mtDNA mutations was replicated twice. **Table 2** summarizes results we obtain.

Table 2 : Detection of mtDNA mutations.

	Present study	Lucotte et al., 2015
HVR1 sequence	no mutation	no mutation
HVR2 sequence	152C, 194T, 263G, 315.1C	152C, 194T, 263G, 315.1C

There is no mutation in the HVR1 sequence, and four mutations (152C, 194T, 263G and 315.1C) in the HVR2 sequence ; these mutations are the same as those previously found (Lucotte et al., 2015) in the other authentic hairs of Louis XVII. This combination (sometimes improperly called “the Habsbourg haplotype”) was initially found (Jehaes *et al.* , 2001) in some present time descendants of Marie-Antoinette (notably for the present day living Queen Anna of Romania).

We have very recently (Lucotte, *in preparation*) established (for Marie-Antoinette’s authentic hairs, kept in a medallion) that the corresponding mtDNA haplogroup is truly the one that belongs to Queen Marie-Antoinette. Consultation of recent data-banks has shown that this mtDNA haplogroup is rare (it was found in less than twenty subjects in the world).

The detection procedure of the Y-STRs profile was also replicated twice. **Table 3** summarizes results we obtain concerning this profile.

Table 3. Detection of allele values for fifteen Y-STRs.

Y-STRs	Allele values	
	Present study	Lucotte et al., 2016
DYS19	14	14
DYS385.a	11	11
DYS385.b	14	14
DYS389.I	14	14
DYS389.b	16	16
DYS390	23	23
DYS391	11	11
DYS392	13	13
DYS393	13	13
DYS438	12	12
Y-GATA-A4	13	13
DYS448	19	19
DYS456	16	16
DYS458	18	18
Y-GATA-C4	23	?
Y-GATA-H4	12	12

Allele values are identical for fourteen Y-STRs. That confirms that Louis XVII is truly Louis XVI’s son.

DISCUSSION AND CONCLUSION

We worked on a lock of hair kept in a letter which, together with a ring entangled with Marie-Antoinette’s hairs, were given at that time to the Marquise de Soucy (born de Makau). The letter was passed on by inheritance to the Soucy today

descendants. SEM-EDX analyses of the letter paper have shown that it is a special sort of “papier-chiffon”, constituted mainly of linen fibers ; the ink of the inscription on the paper was a special sort of ferro-gallic ink. Both these materials are compatible with those of a letter written in the 18th Century.

The lock of hair kept in the letter is surrounded and tied by a precious green silk threads. There are two sorts of hairs in the lock concerning colour : red hair and blond hair. This indicates that the lock of hair is constituted by the hair of two different subjects.

The two sorts of hair differ also by the diameters : a thickness of 65.5 μm for a red hair (red hair number 1 being taken as an example), and of 56.2 μm for a blond hair (blond hair number 1 being taken as an example). The main diameter of a normal adult hair is about 80 μm (Deedrick and Koch, 2014), and dessication (as for hairs studied here) is responsible of a limited reduction only of this thickness. A hair thickness of 65.5 μm (corresponding to the normal increase of the hair diameter during growth) is suggestive of that of a teenager, and a hair thickness of 56.2 μm of that of a younger subject.

The hair colour differentiation, by itself, must also be taken in consideration : we know that the hair colour of the young Louis-Charles was blond, while his elder sister Marie-Thérèse (1778-1851) had red hair, or more exactly (just like her mother , Marie-Antoine) “blond-cendré”.

The two sorts of hair-coloring are also important to consider. We think that these two colorations correspond to two subjects only (the inscription, in the plural, “Cheveux des Augustes enfants de France” indicates that they were only two), whose hair is in the lock : these two subjects are Louis-Charles and Marie-Thérèse. The first Dauphin Louis-Joseph (born in 1781) died in 1789, and the last child Sophie (born in 1786) died one year after. As Marie-Antoinette had given to Renée de Soucy the ring (and the hairs) in 1792, the two surviving children were alive at that time.

We established that, unlike red hairs, blond hairs were carefully prepared (washed, soaped and decorated) ; these two sorts of hairs were, secondarily, intertwined in the lock. This is an ancient usual process of hair embracing, concerning persons who are affectively or/and familiarly tightly linked ; for example (Lucotte et al. , 2016), we have observed that Louis XVI’s hairs and those of his wife

Marie-Antoinette are intertwined this way in the lock of hair studied.

We have observed that blond hair number 3 is particularly charged with skin-cells and dandruffs, tightly linked to its surface. A sufficient quantity of DNA was extracted from it ; the corresponding mtDNA mutations detected correspond to a particular mtDNA haplogroup that is the same than that obtained from another Louis XVII ‘s authentic hair (Lucotte *et al.*, 2015), and that of Marie-Antoinette.

The Y-STRs profile obtained is strictly identical to that of Louis XVI (Lucotte *et al.* , 2016). This establishes that Louis XVI is the biological father of Louis XVII.

Numerous rumours, since the beginning, doubted about Louis XVI paternity of his children. We demonstrate here that Louis-Charles (Louis XVII) is truly Louis XVI’s son.

Acknowledgements

We thank the de Baudreuil brothers who permitted us to study the lock of hair, and Arnaud de Genlis for his complete genealogy of the de Soucy family. This study is included in our research program of genetic investigations developed at the Institute of Molecular Anthropology (Paris) concerning royal families in France.

References

1. D.W. Deedrick and S.L. Koch (2014). Microscopy of hair, Part 1 : A practical guide and manual for human hairs. *Forensic Science Communications*, 6 : 1-45.
2. E. Jehaes, H. Pfeiffer, K. Toprak, R. Decorte, B. Brinkmann and J.J. Cassiman (2001). Mitochondrial DNA analysis of the putative heart of Louis XVII, son of Louis XVI and Marie-Antoinette. *European Journal of Human Genetics*, 9 : 185-190.
3. G. Lucotte (2010). A rare variant of the mtDNA HVS1 sequence in the hairs of Napoléon’s family. *Investigative Genetics*, 1 (7) : 1-6.
4. G. Lucotte and A. Bouin Wilkinson (2014). An autosomal STR profile of Napoléon the First. *Open Journal of Genetics*, 4 : 292-299.
5. G. Lucotte, T. Thomasset and C. Crépin (2015). The mitochondrial DNA mitotype of Louis XVII (1785-1795?). *International Journal of Sciences*, 4 (11) : 10-18.
6. G. Lucotte, T. Thomasset and S. Wen (2016). The DNA Y-STRs profile of Louis XVI (1754-1793). *International Journal of Sciences*, 5 (04) : 68-93.

Figure 1 : **A** : The ring of Marie-Antoinette. **B** : The ring into its case. **C** : The folded letter.



Figure 2 : *Above* : The folded letter, with inscriptions. *Below* : The letter, unfolded, containing hairs and the green thread.

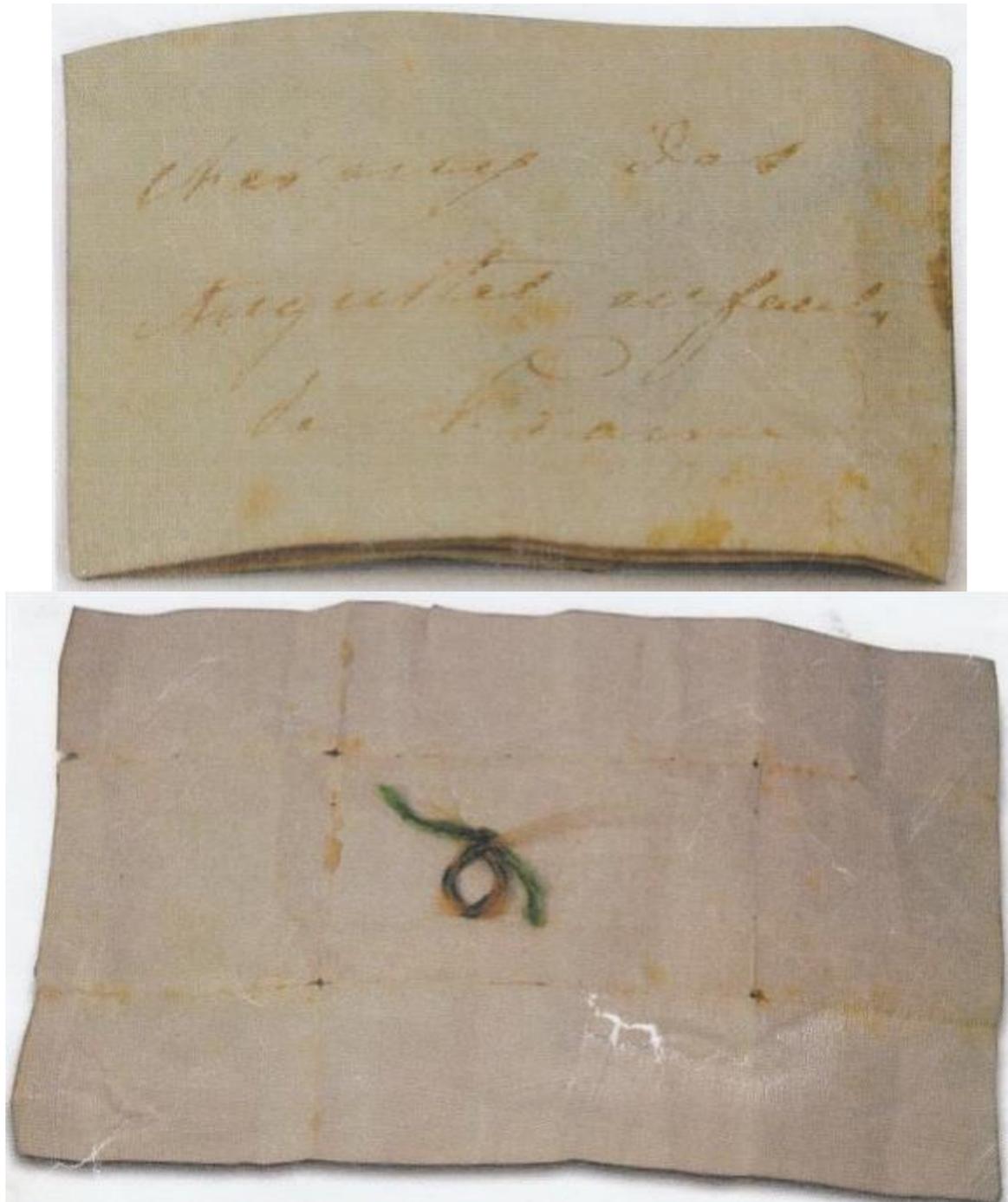


Figure 3 : *Above photograph* : Optical view (5x) of the paper corner. *Below photograph* : SEM-1 photograph (in GSE, 100x) of the paper. *Below* : Global spectrum of the paper. C : carbon ; O : oxygen ; Mg : magnesium ; Al : aluminium ; S : sulphur ; K : potassium ; Ca (two peaks) : calcium.

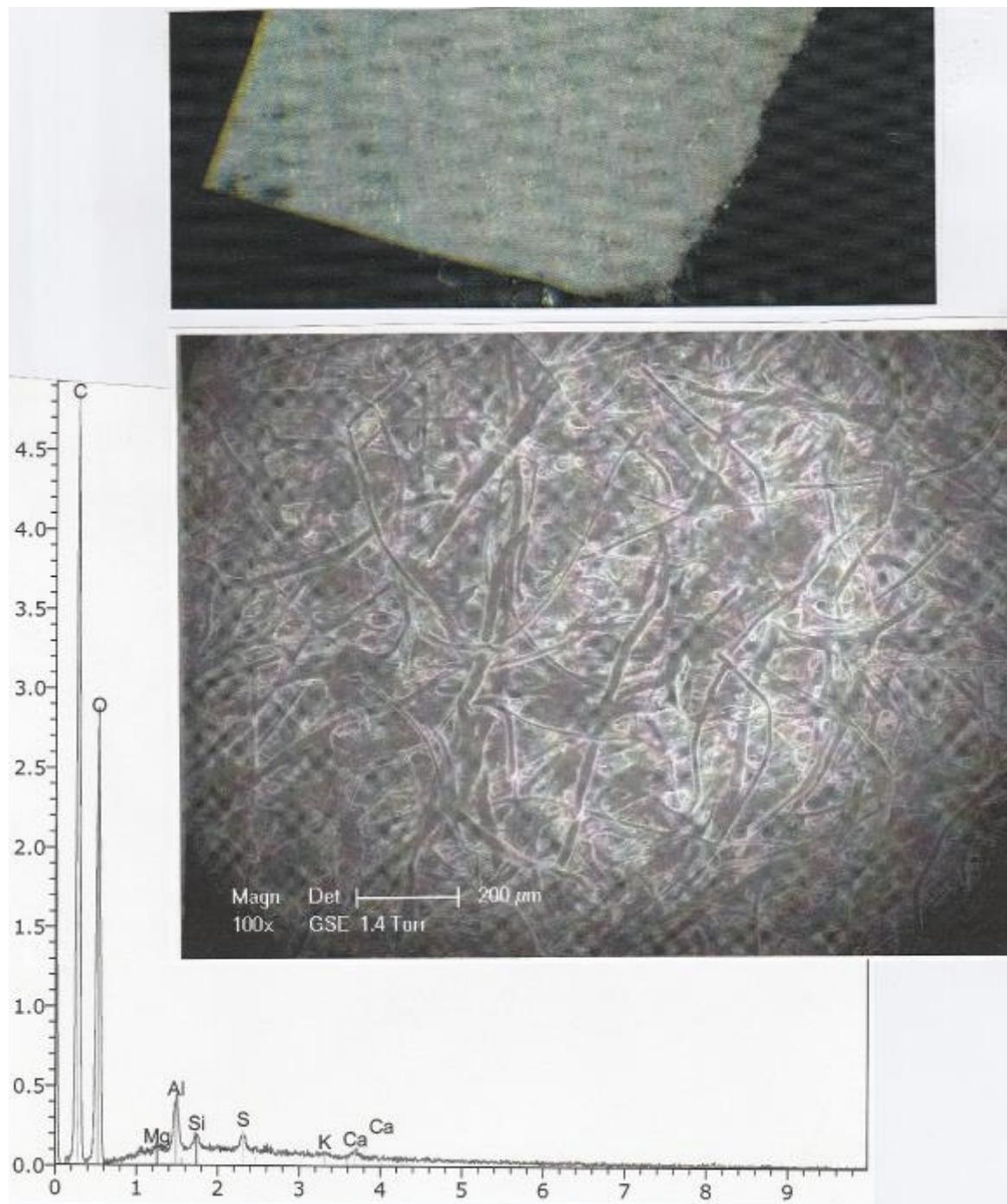


Figure 4 : Fibers of the paper. *Above* : SEM-1 photograph (in GSE, 1200x) showing a paper fiber (N : nodes) ; the black dot on it indicates the location where elemental analysis is realized . *Below* : Spectrum at the black dot.

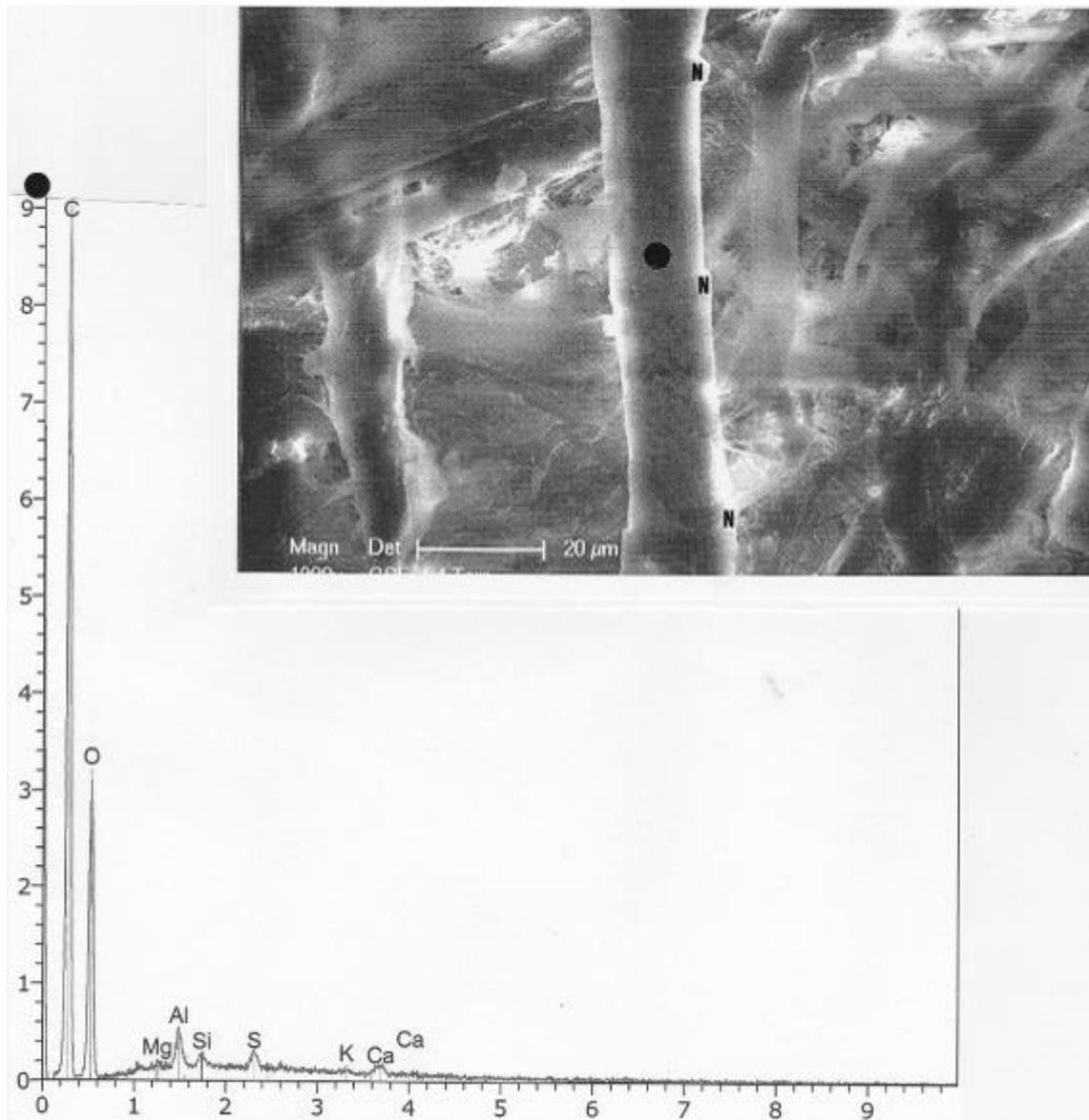


Figure 5 : The ink. *Above* : Optical view (50x) of the s' point (M : plastic marker). *Below* : SEM-1 photograph (in GSE, 100x) of the same paper region.

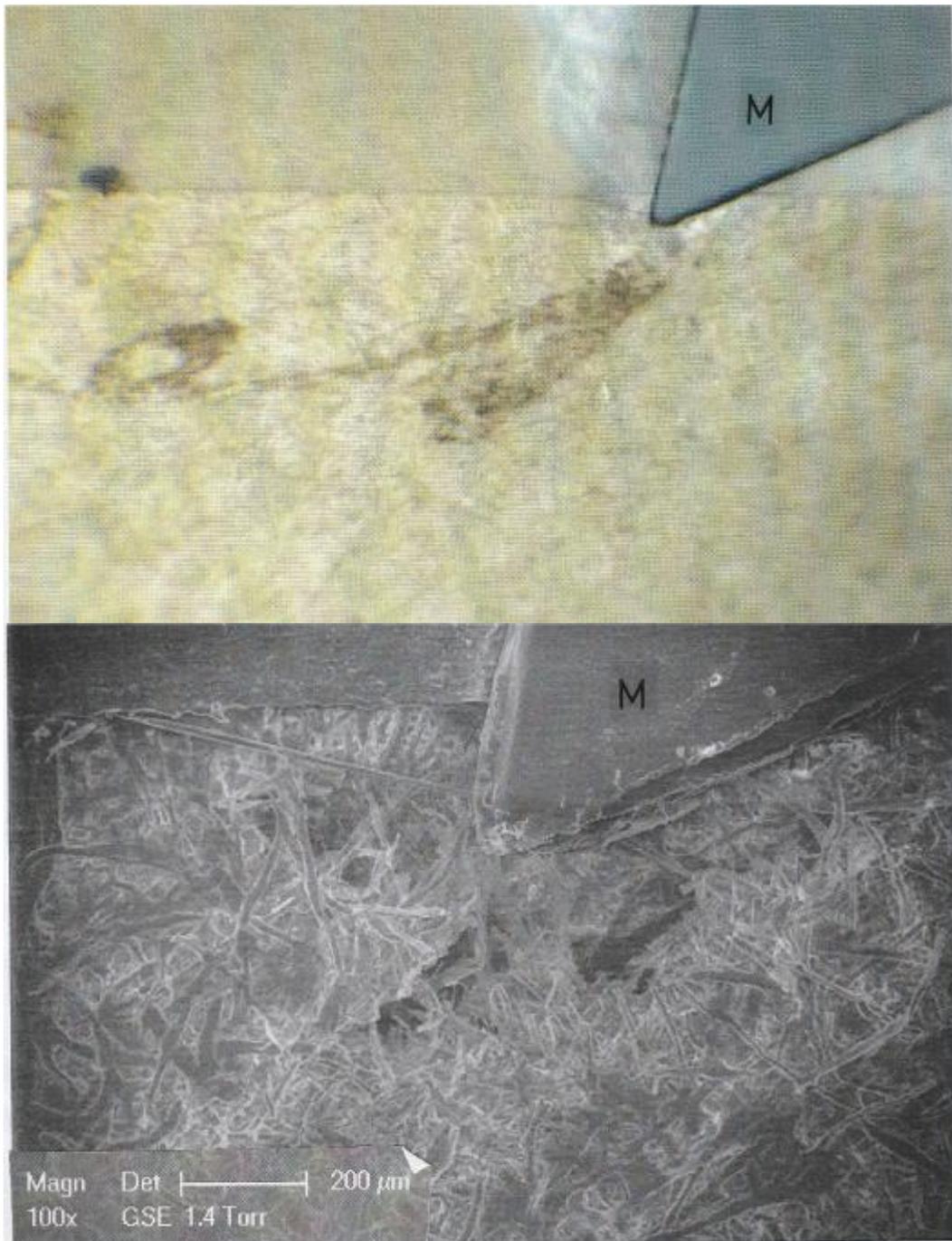


Figure 6 : Above : SEM-1 photograph (in BSE, 400x) centred on the same region. Below : Spectrum at the black dot. Fe (three peaks) : iron ; Na : sodium ; Si : silicium.

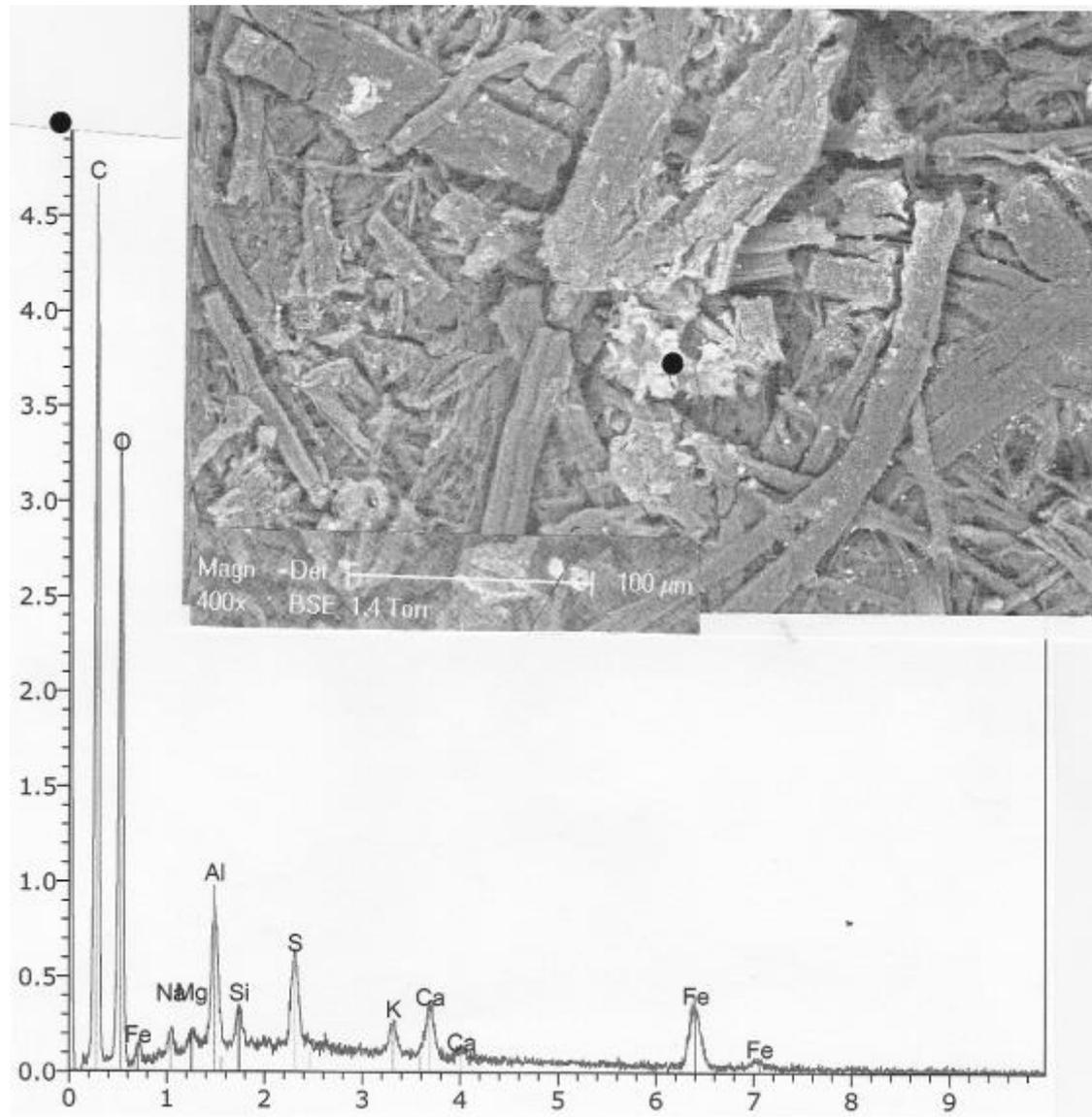


Figure 7 : The lock of hairs. Optical view (75x) of the lock. G : the various portions of the green thread (N : the thread node) ; RH : red hairs ; BH : blond hair.

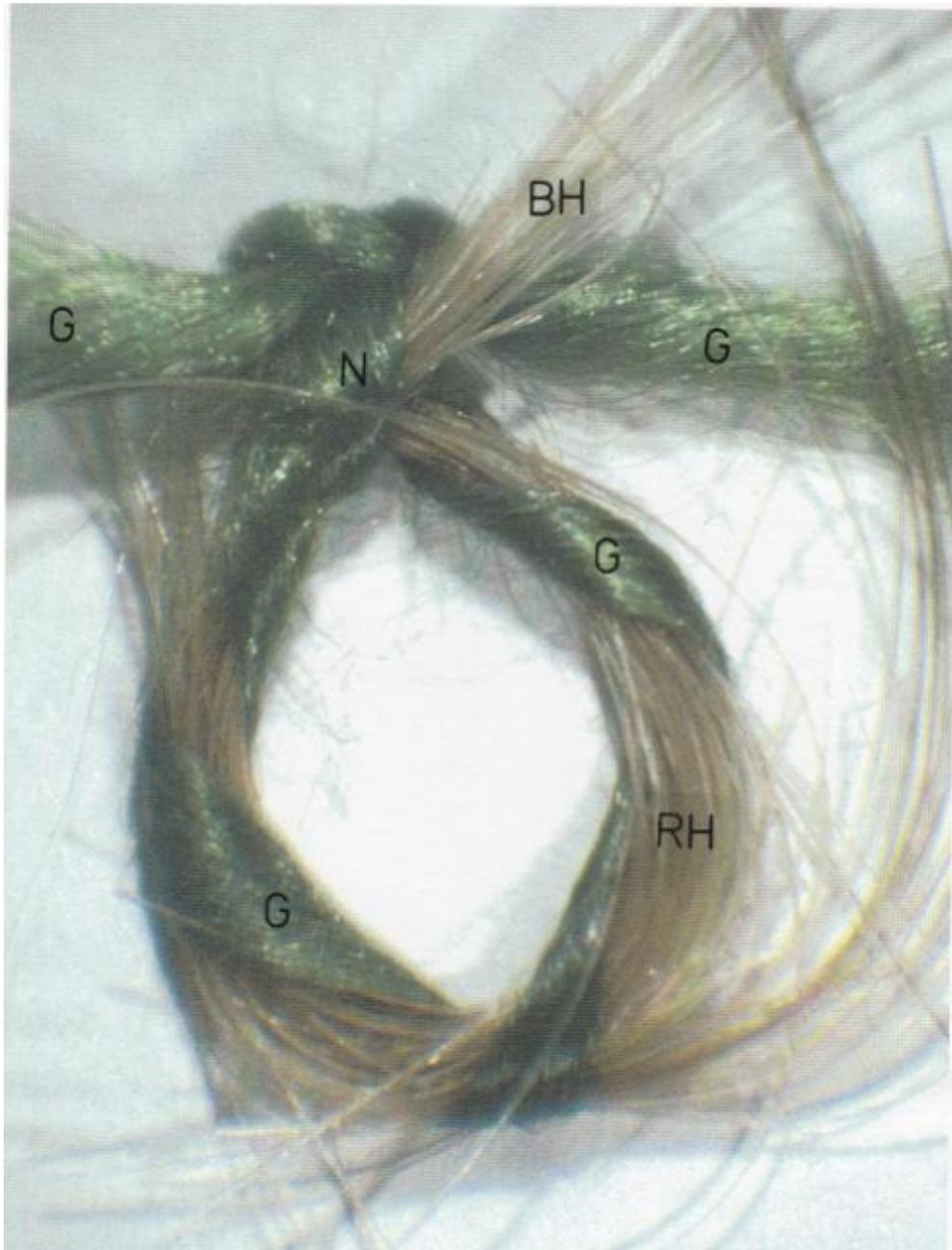


Figure 8 : The green thread. **A** : Optical view (110x) of the thread fibers. **B** : SEM-1 photograph (in GSE, 100x) of the fibers. **C** : SEM-1 photograph (in GSE, 6000x) of a fiber extremity.

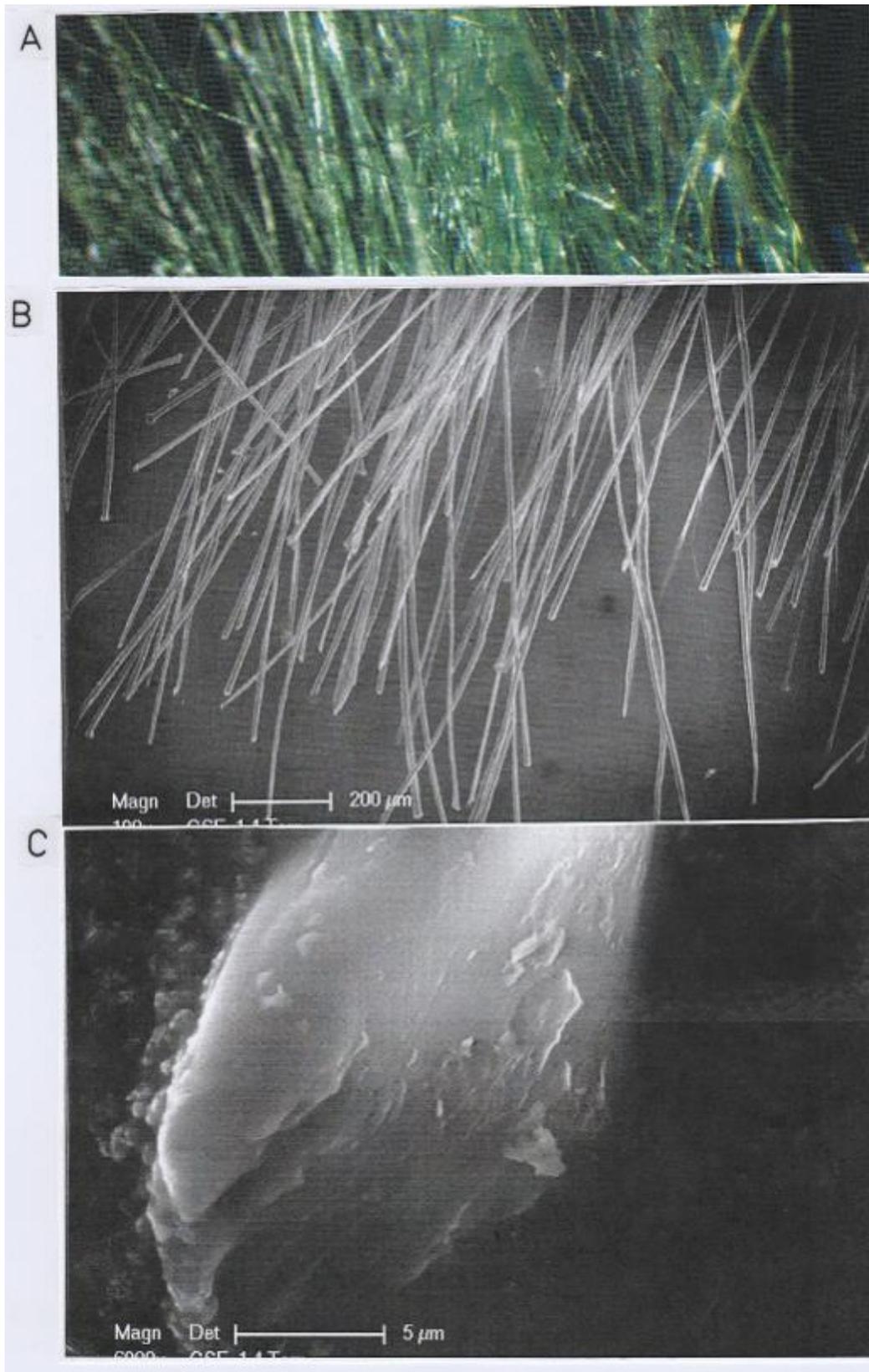


Figure 9 : Three fiber portions. *Above* : SEM-1 photograph (in GSE, 3000x) of the fiber portions (dimension is in μm). *Below* : Spectrum at the black dot (N : nitrogen).

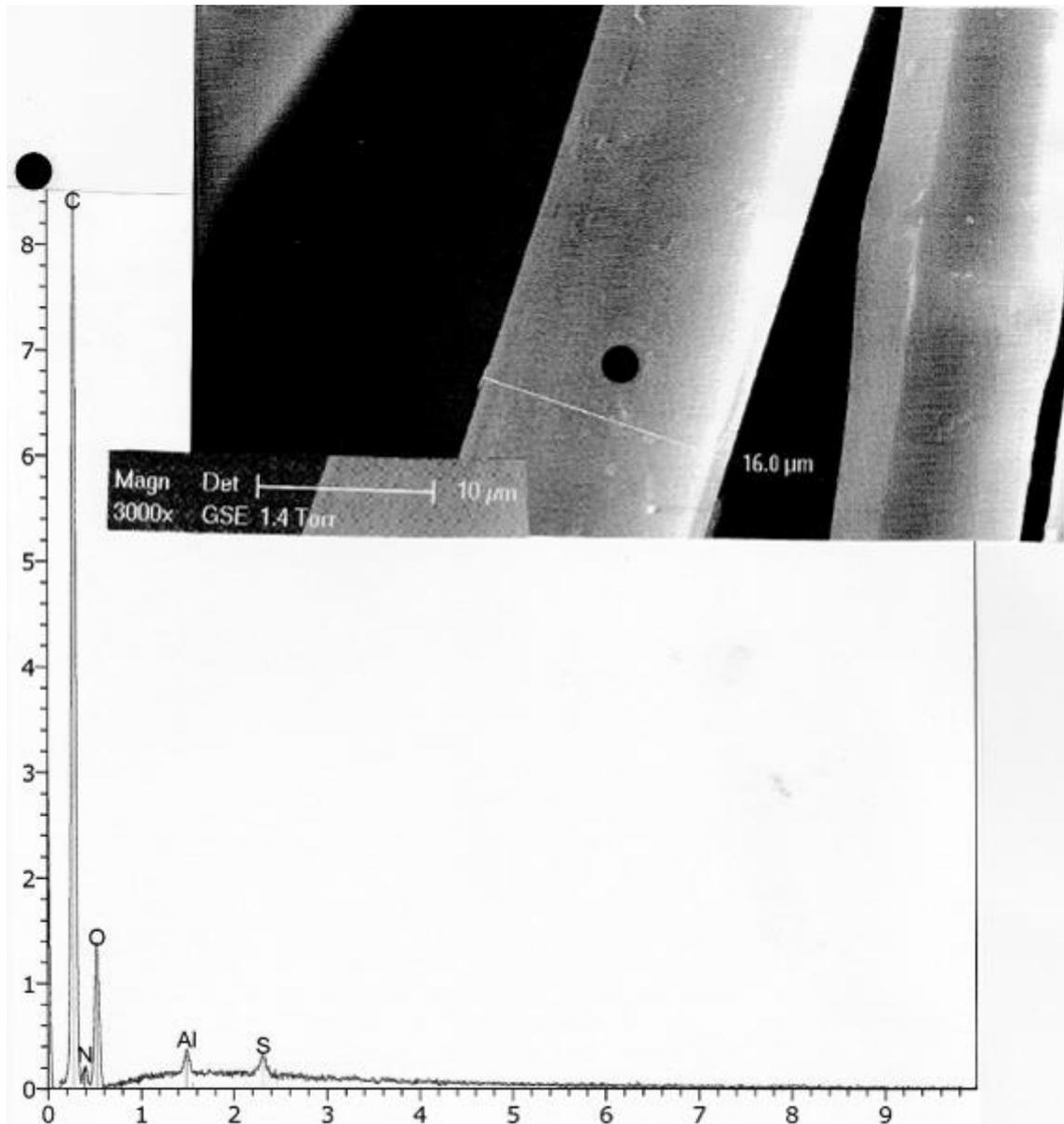


Figure 10 : The studied hairs. *Above* : Optical view of three (1-3) red hairs. *Below* : Optical view of three (1-3) blond hairs (the second one being tripartite).

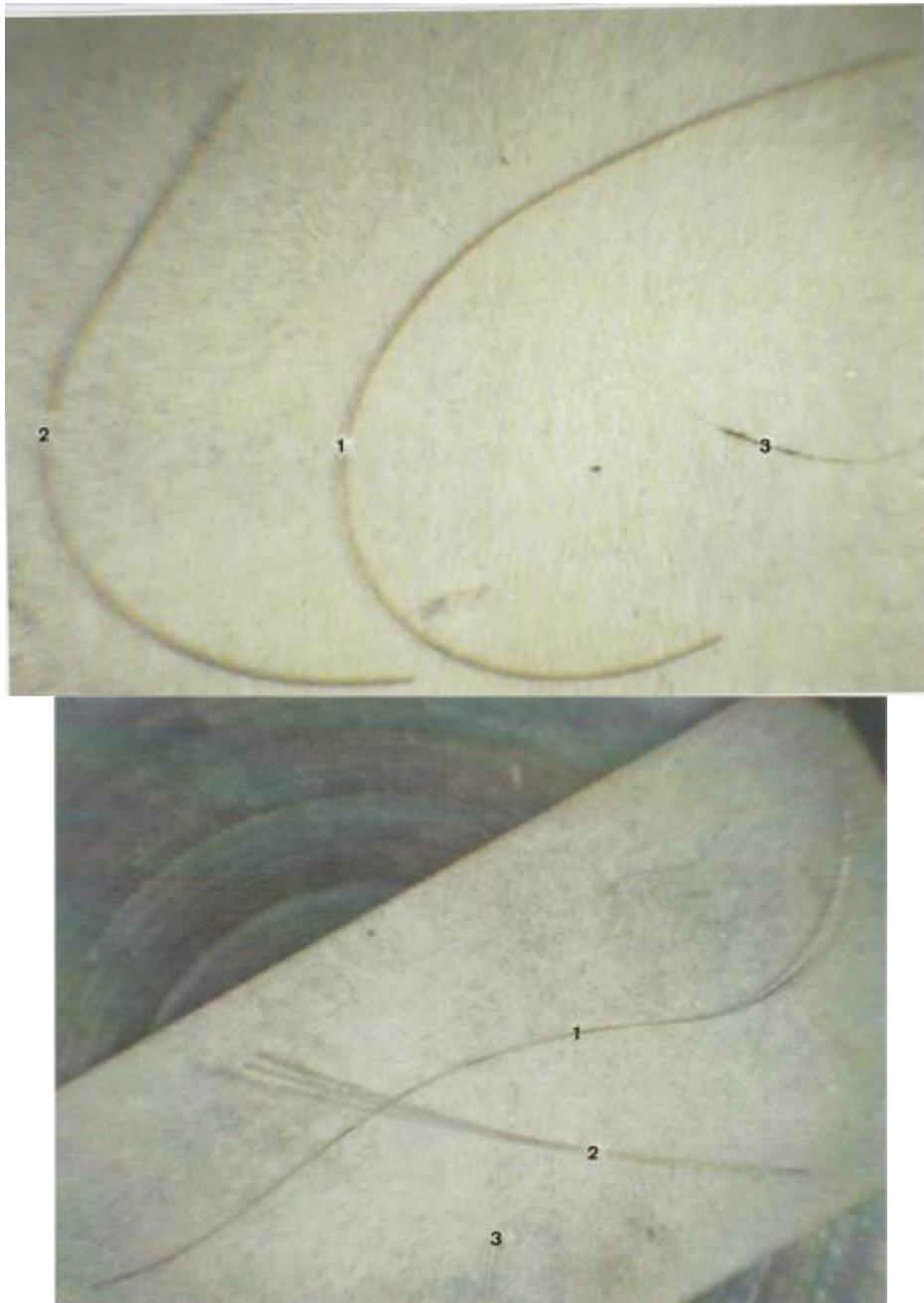


Figure 11 : SEM-1 photograph (in GSE) of the right (1500x) end (above) and of the left (1000x) end (below) of the red hair number 1.

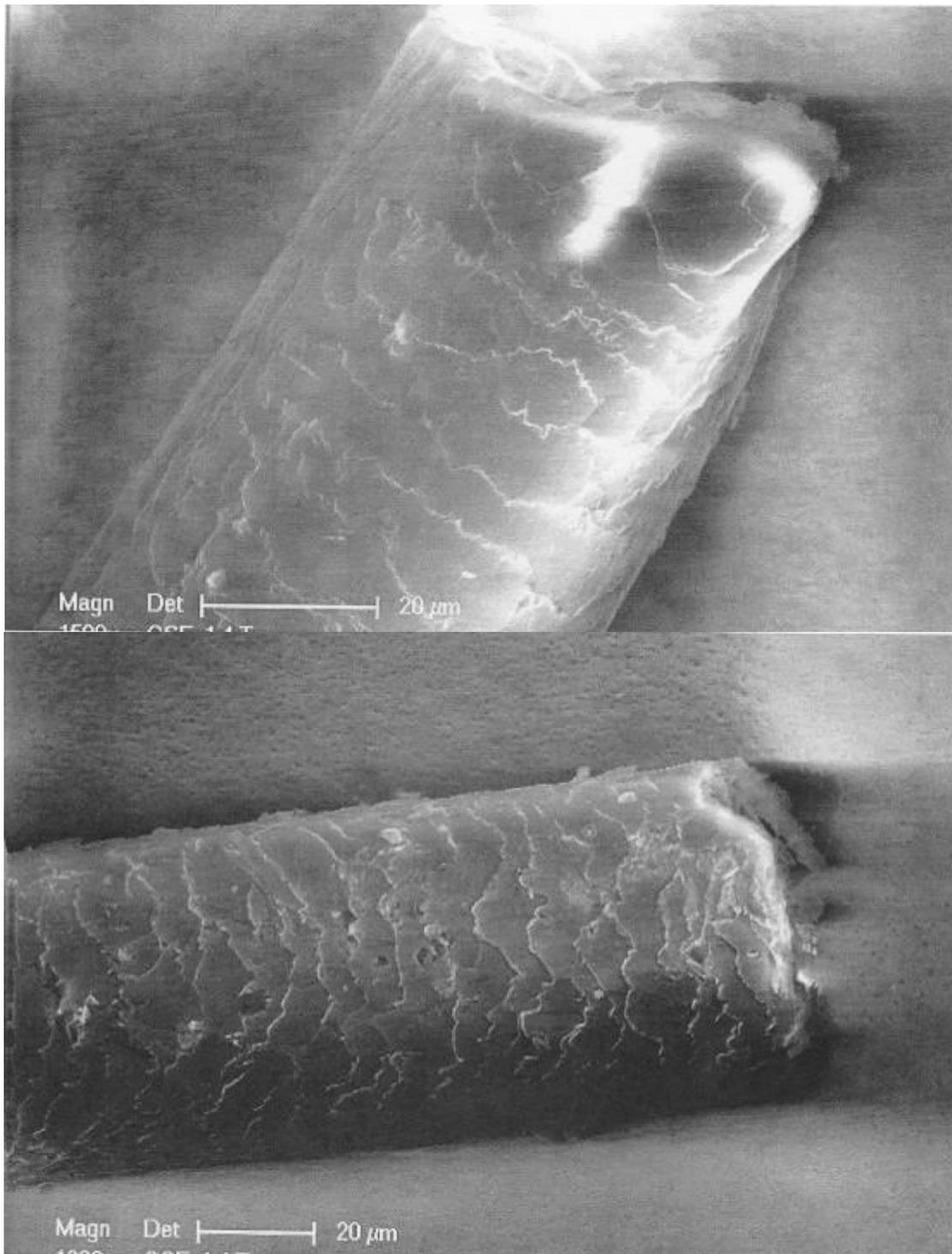


Figure 12 : *Above* : SEM-1 photograph (in GSE, 1200x) of a portion of the hair corresponding to its middle part (dimension in μm). *Below* : Spectrum at the black dot. C : carbon ; N : nitrogen ; O : oxygen ; Na : sodium ; S (two peaks) : sulphur ; Cl : chlorine ; K : potassium ; Ca (two peaks) : calcium.

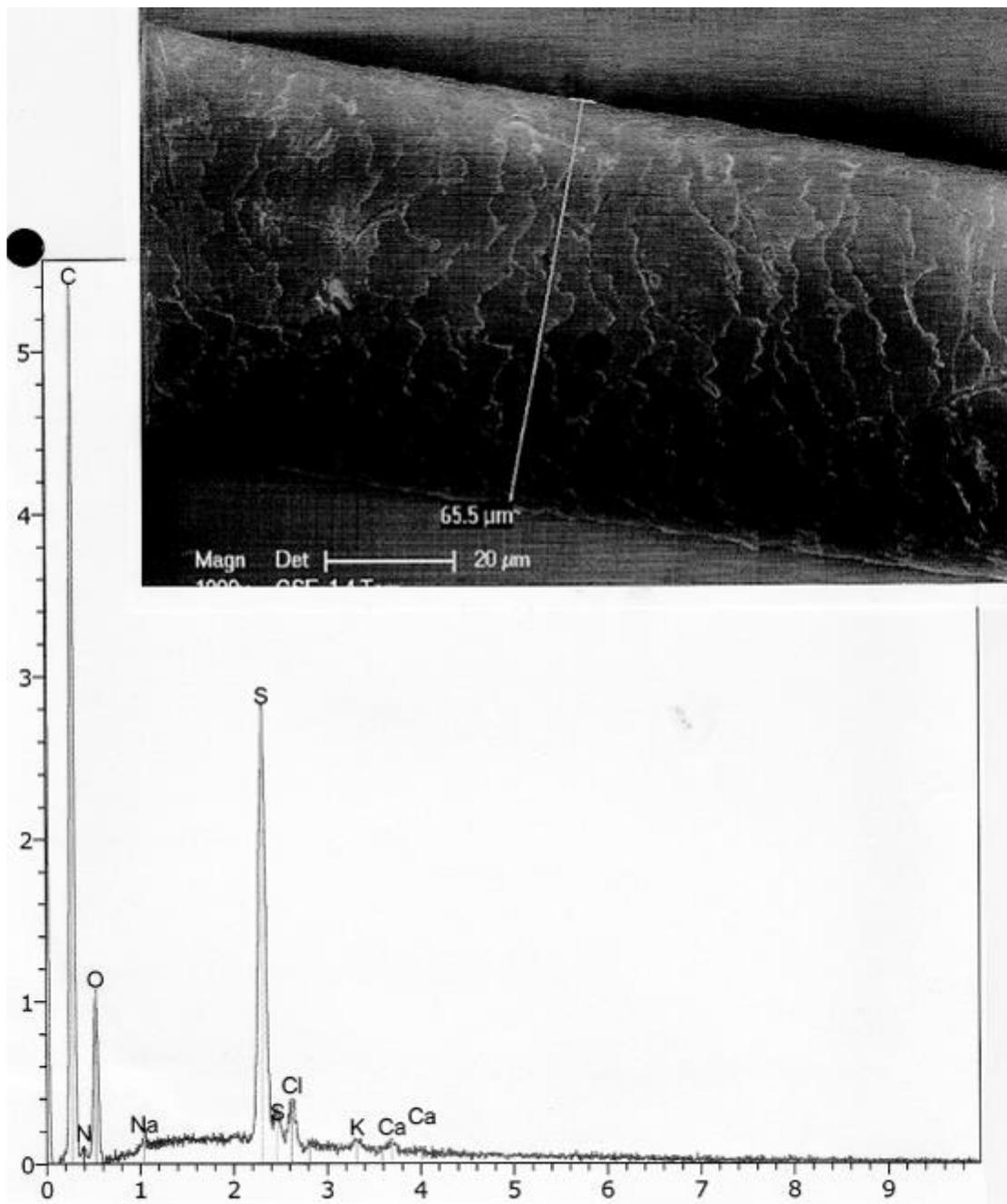


Figure 13 : *Above* : SEM-1 photograph (in GSE, 3000x) of the hair portion showing a mineral particle. *Below* : Spectrum of the particle. C : carbon ; N : nitrogen ; O : oxygen ; Fe (three peaks) : iron ; Mg : magnesium ; Al : aluminium ; Si : silicium ; S (two peaks) : sulphur ; Cl : chlorine ; K : potassium ; Ca (two peaks) : calcium.

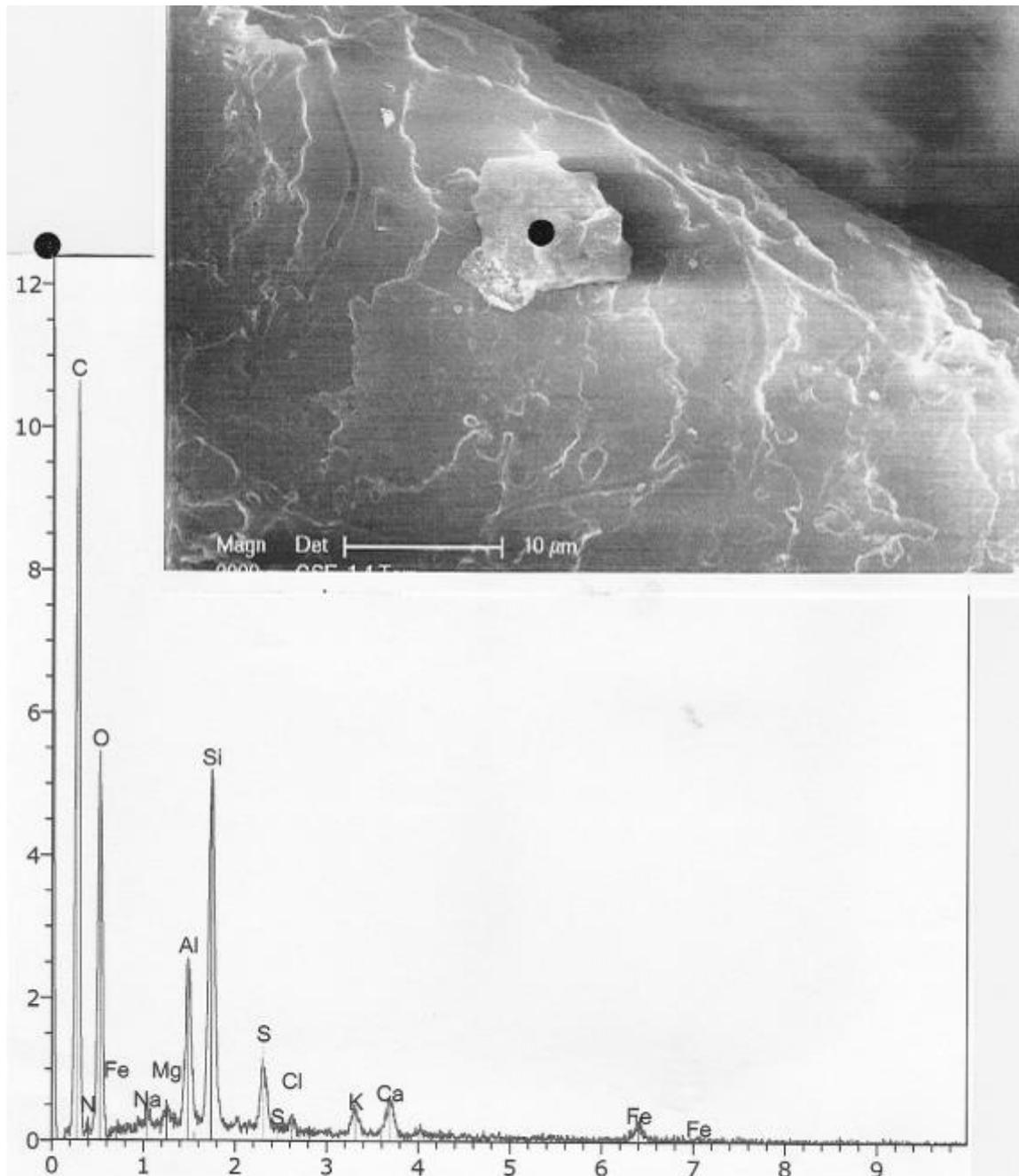


Figure 14 : *Above* : SEM-1 photograph (in GSE, 1500x) of another hair portion showing another sort of mineral particle. *Below* : Spectrum of the particle. C : carbon ; O : oxygen ; Na : sodium ; S (two peaks) : sulphur ; Cl : chlorine ; Ca (two peaks) : calcium.

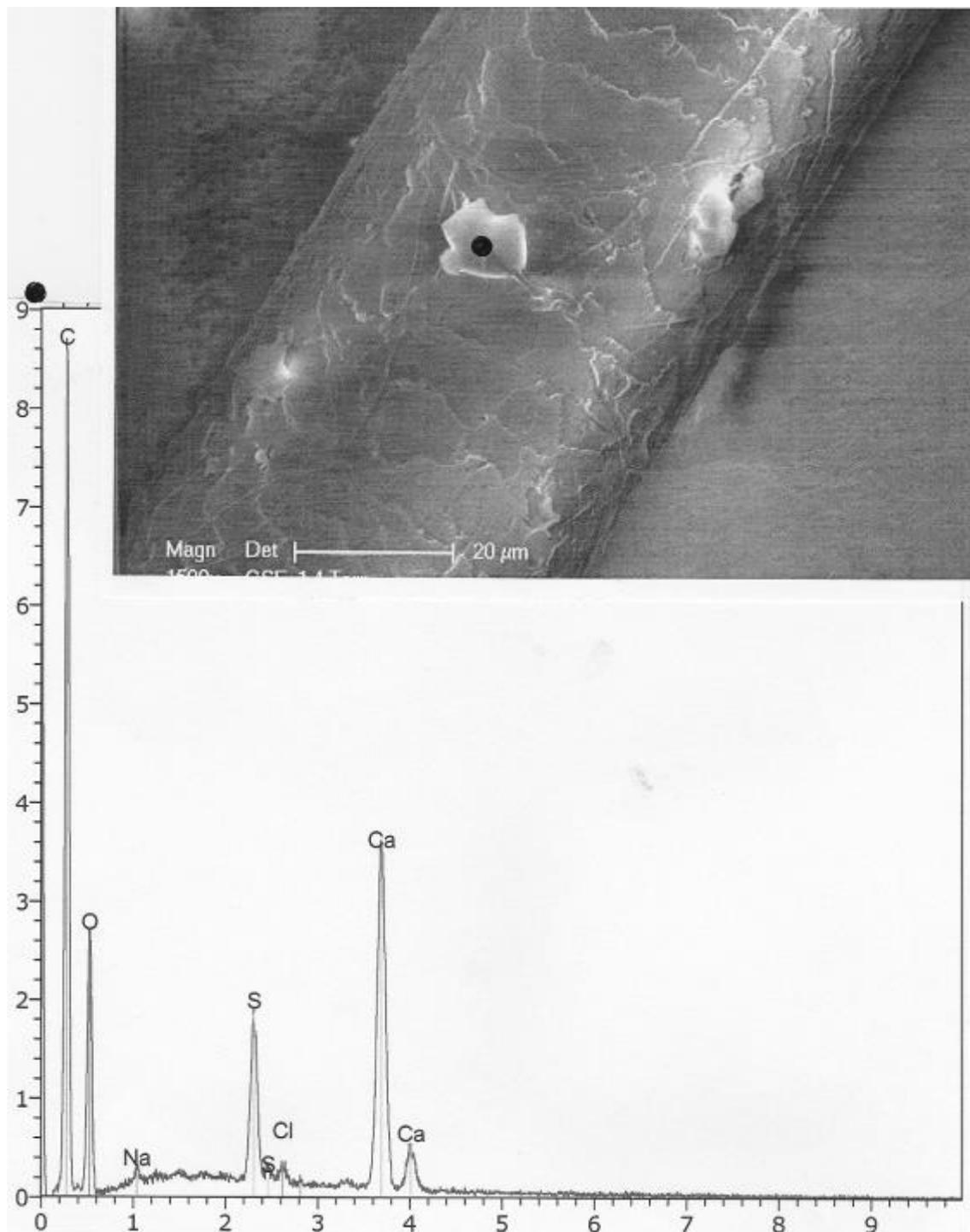


Figure 15 : *Above* : SEM-1 photograph (in BSE, 2000x) of an Acari. *Below* : SEM-1 photograph (in BSE, 2000x) of an insect clutch.

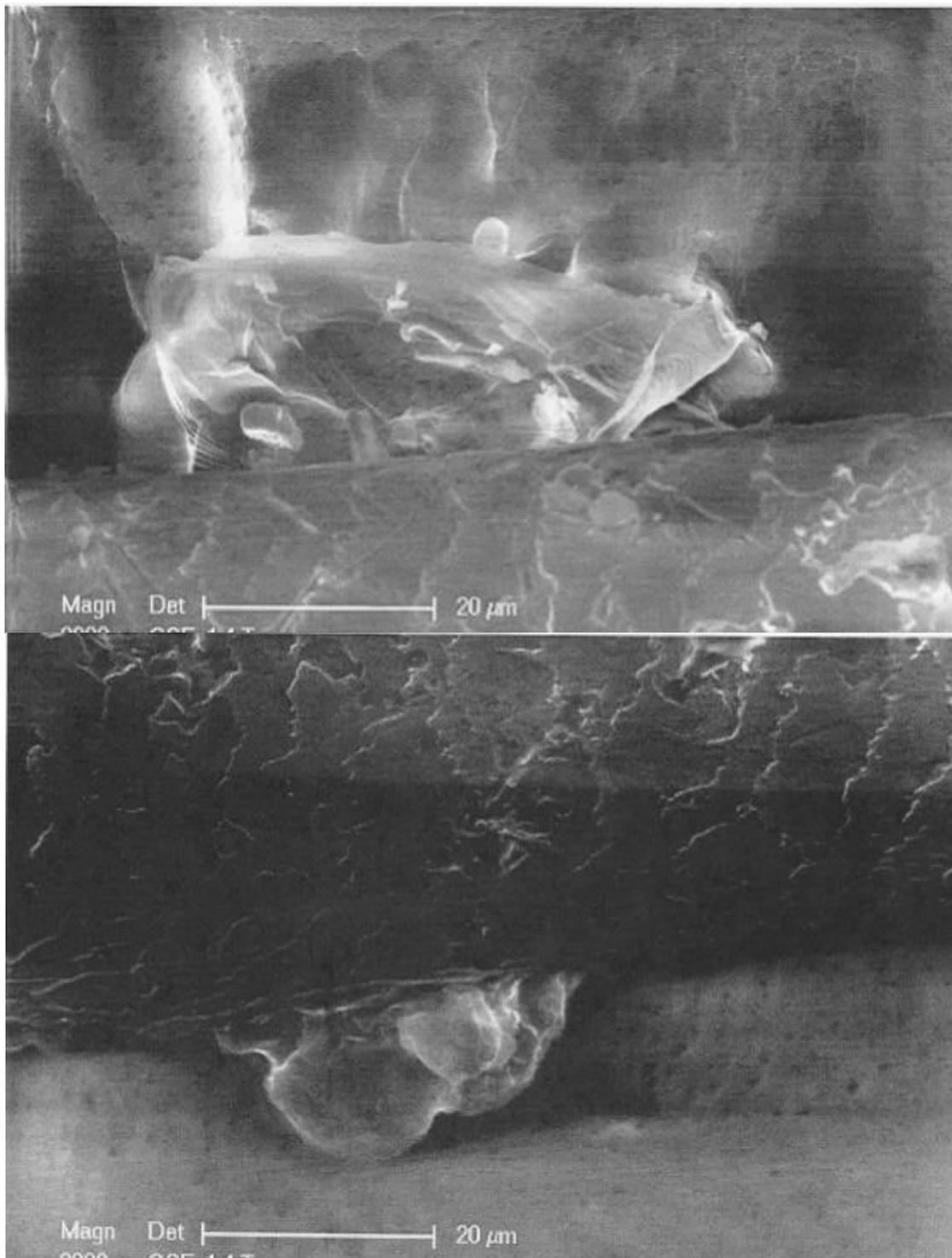


Figure 16 : *Above* : SEM-1 photograph (in GSE, 1500x) of an hair portion showing a rounded particle deposited on it. *Below* : Spectrum of the particle. C : carbon ; O : oxygen ; Fe (three peaks) : iron ; Na : sodium ; S (two peaks) : sulphur.

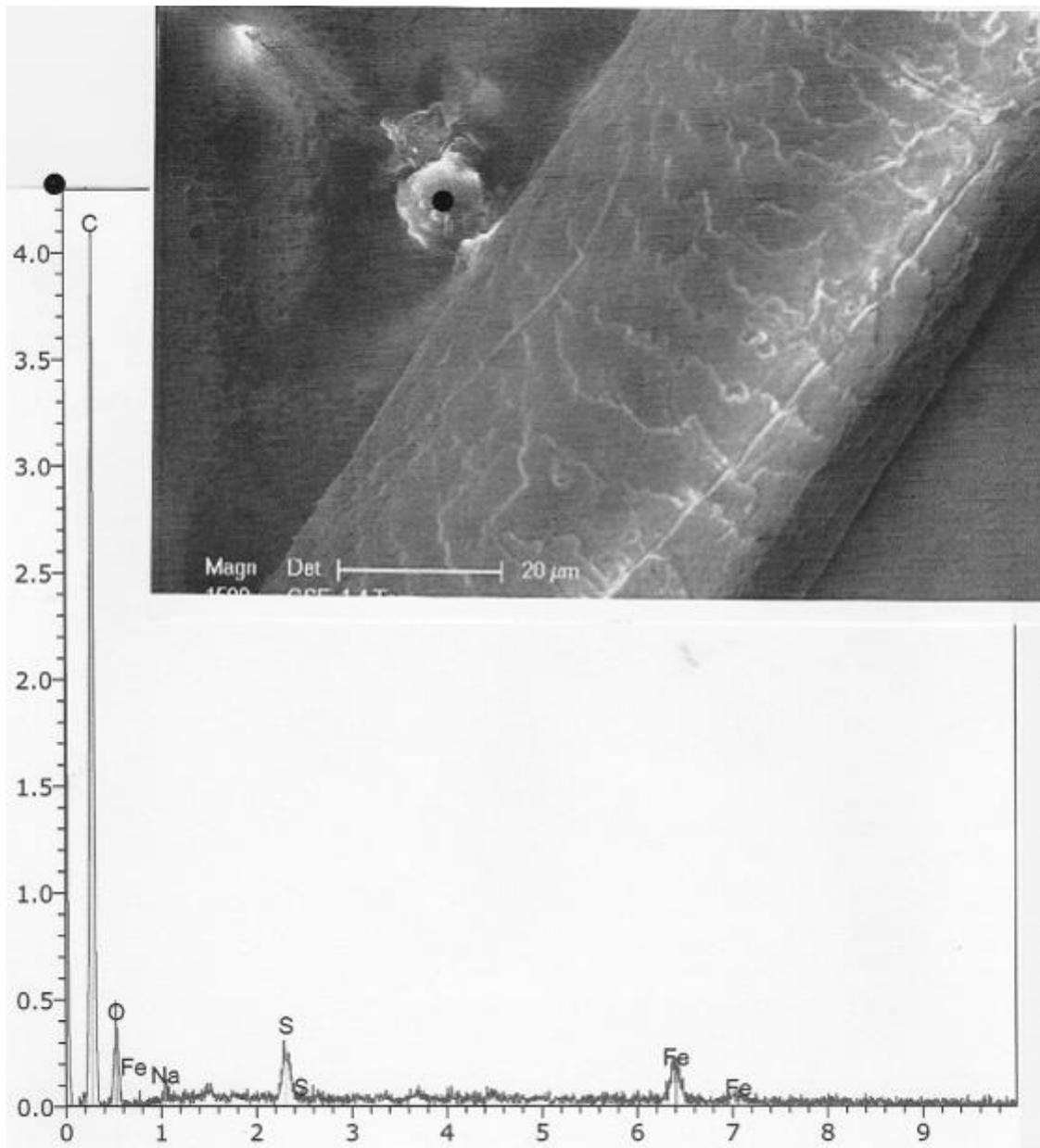


Figure 17. Above : SEM-1 photograph (in GSE, 1500x) of coiled filaments of a *Cyanophyceae*, loaded on the hair surface. Below : Multi-elements spectrum of the *Cyanophyceae*.

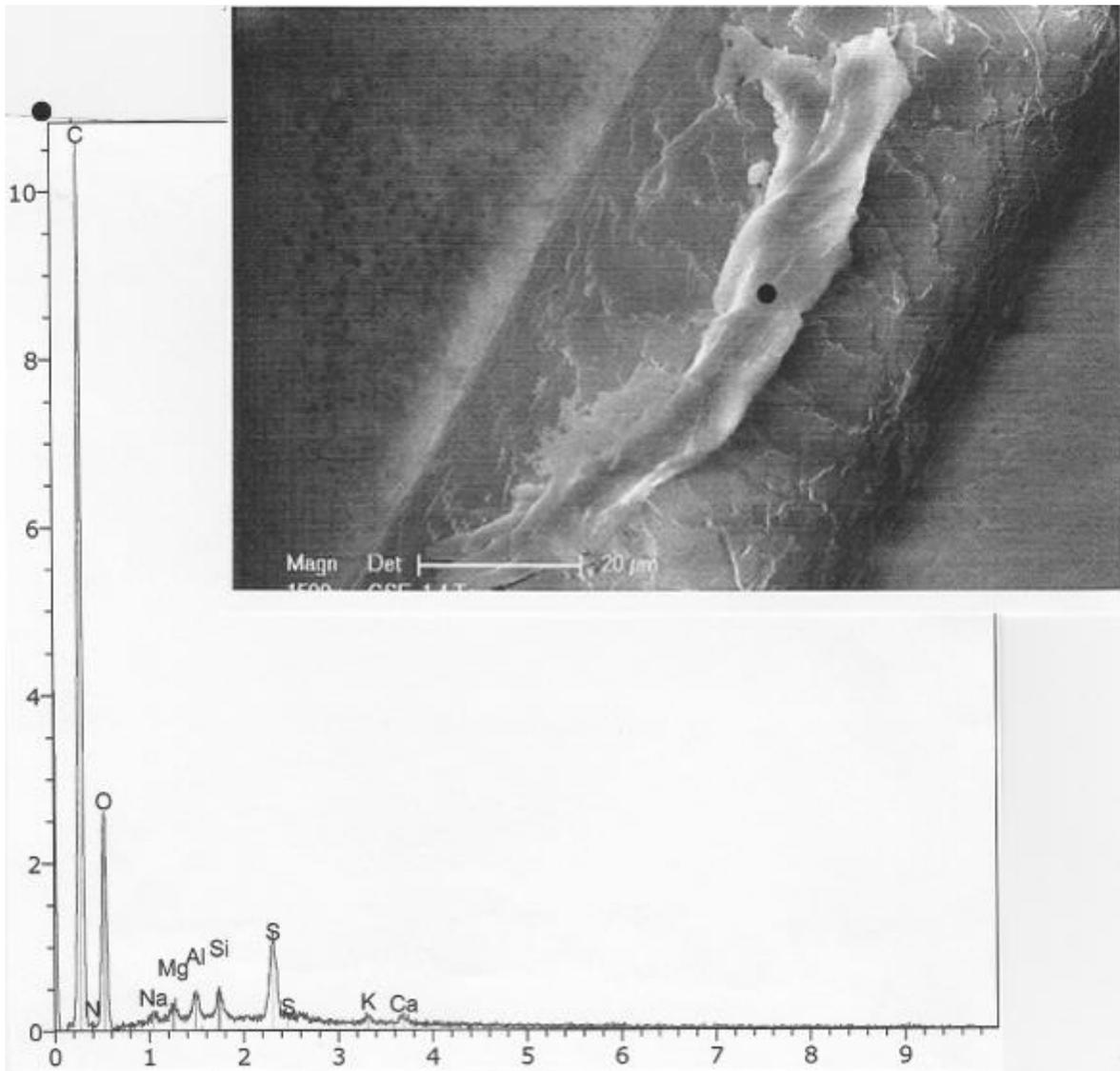


Figure 18 : SEM-1 photograph (in GSE) of the right (1200x) end (above) and of the left (1000x) end (below) of the blond hair number 1.

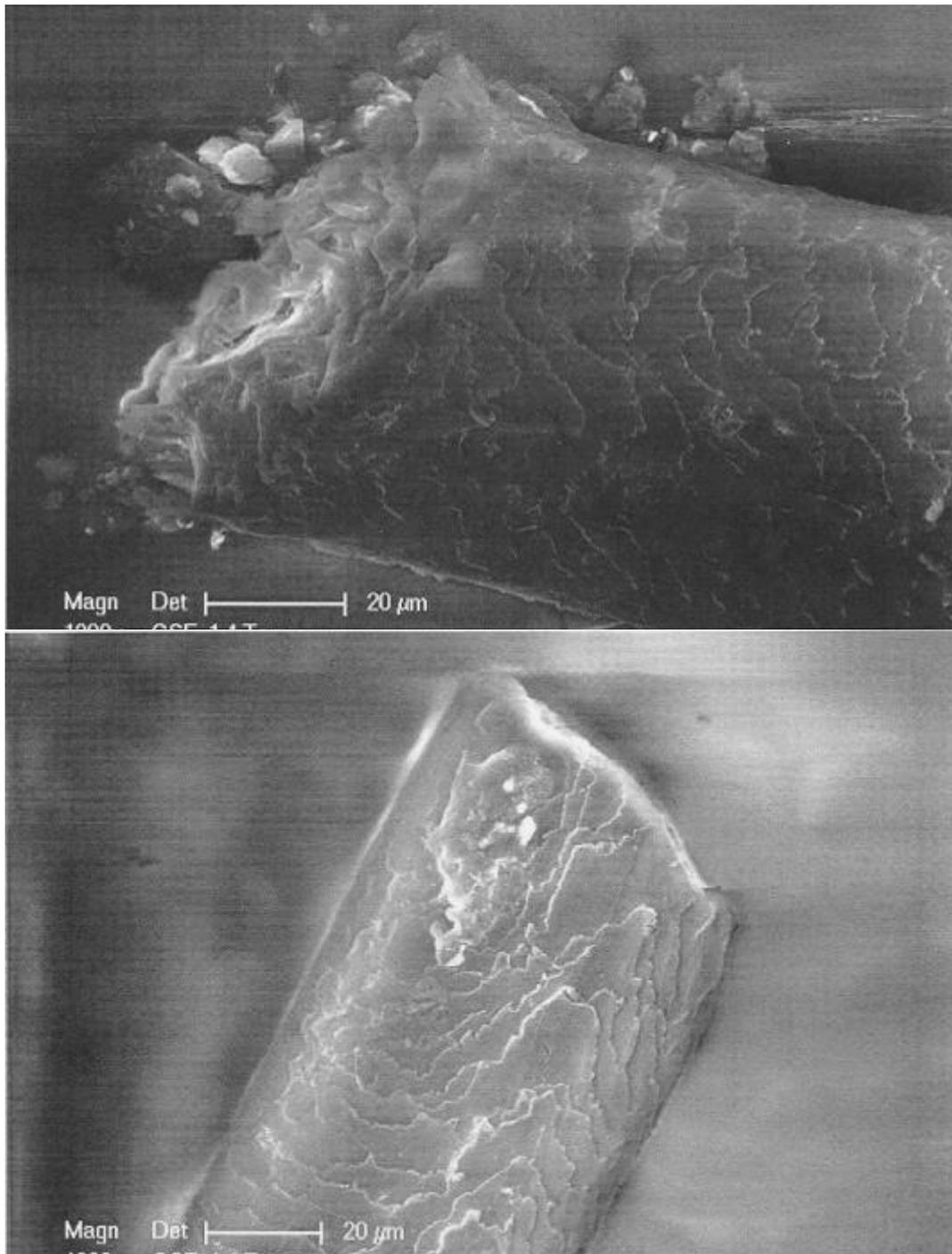


Figure 19 : *Above* : SEM-1 photograph (in GSE, 1500x) of a portion of the hair corresponding to its middle part (dimension in μm). *Below* : Spectrum at the black dot.

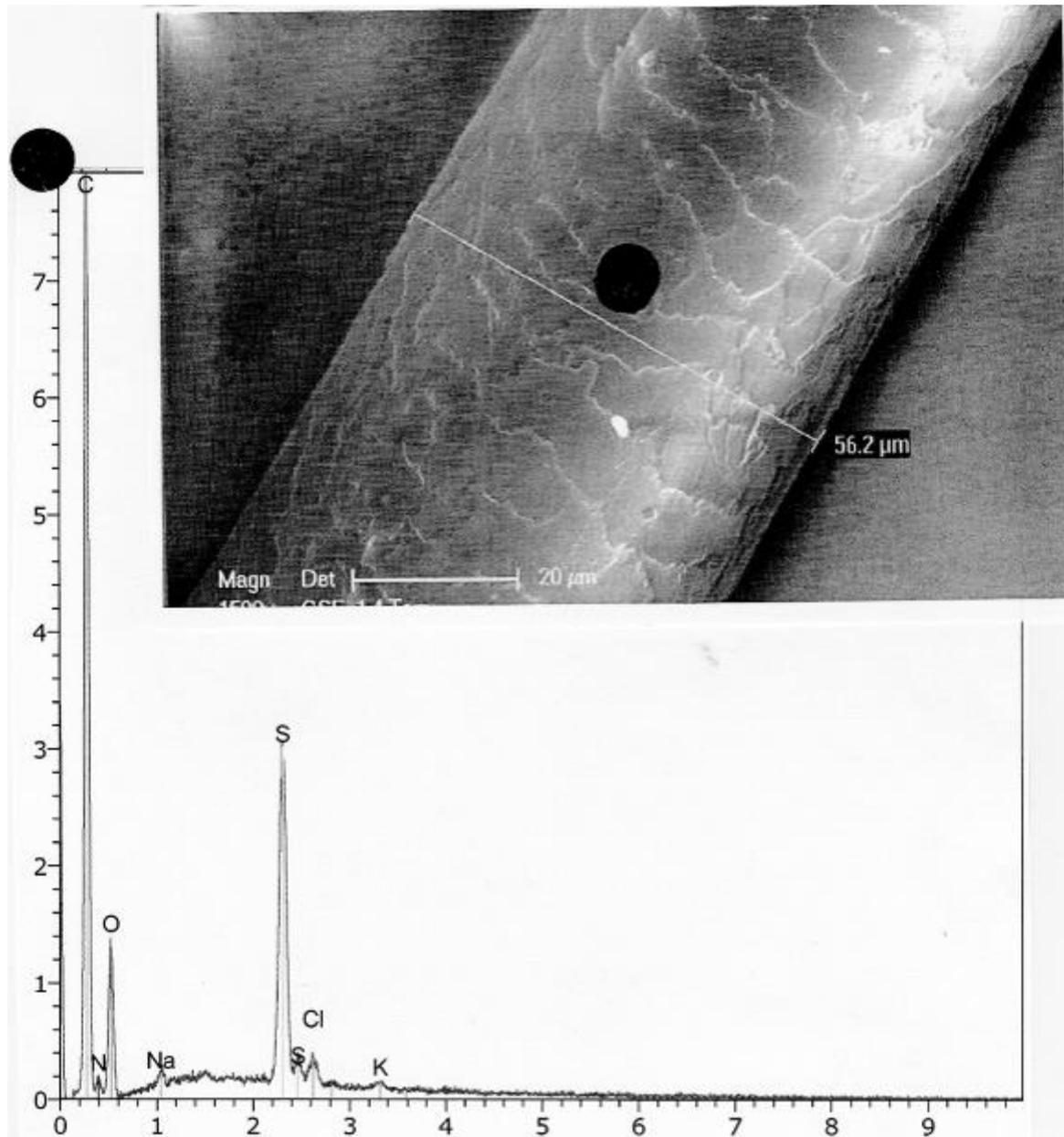


Figure 20 : *Above* : SEM-1 photograph (in GSE, 2000x) of a skin-cell, adhering to the hair surface. *Below* : Spectrum at the black dot. C : carbon ; N : nitrogen ; O : oxygen ; Na : sodium ; P : phosphorous ; S (two peaks) : sulphur ; Cl : chlorine ; K : potassium ; Ca (two peaks) : calcium.

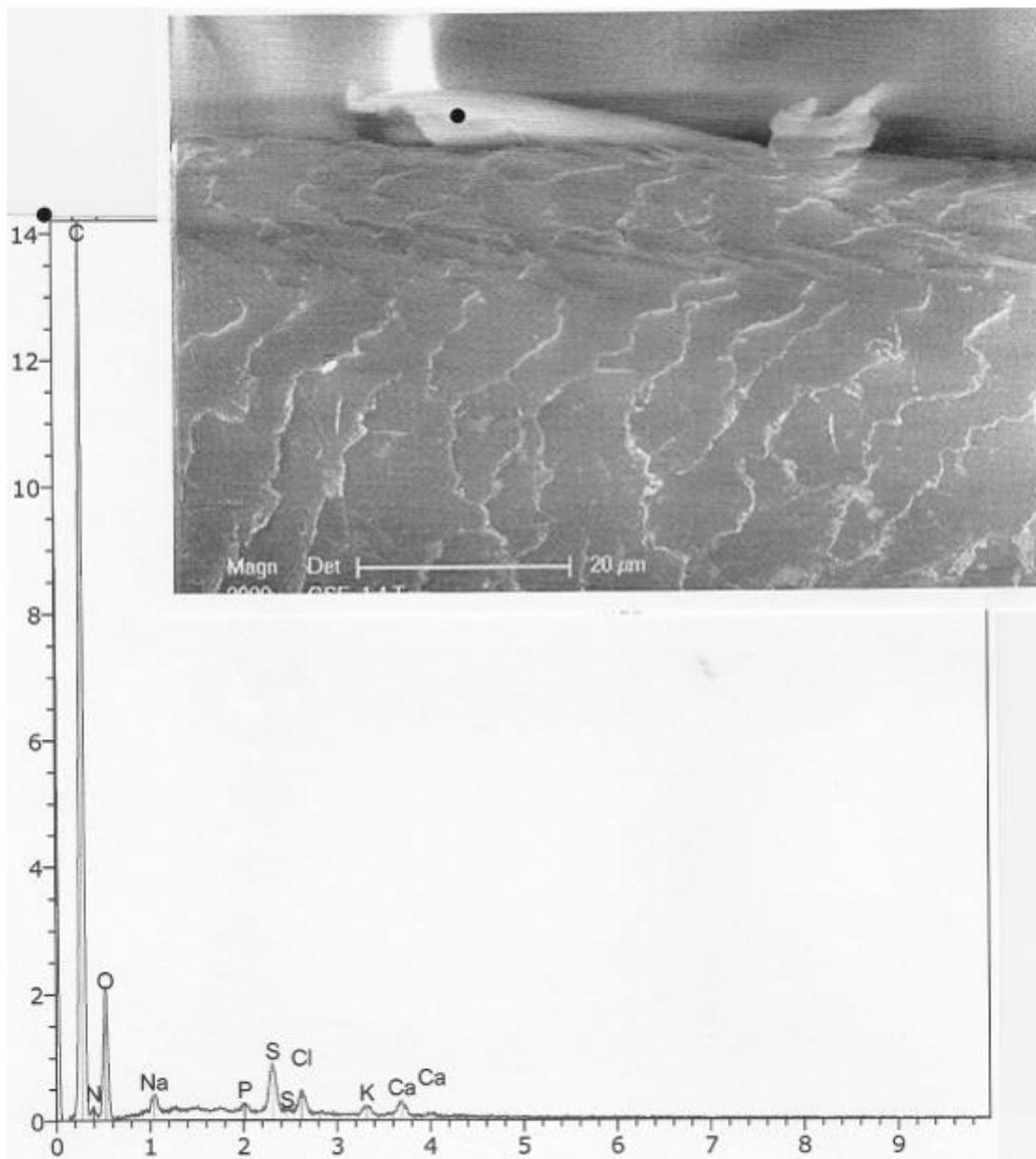


Figure 21 : *Above* : SEM-1 photograph (in GSE, 1500x) of a micel located on the hair surface. *Below* : Spectrum at the black dot.

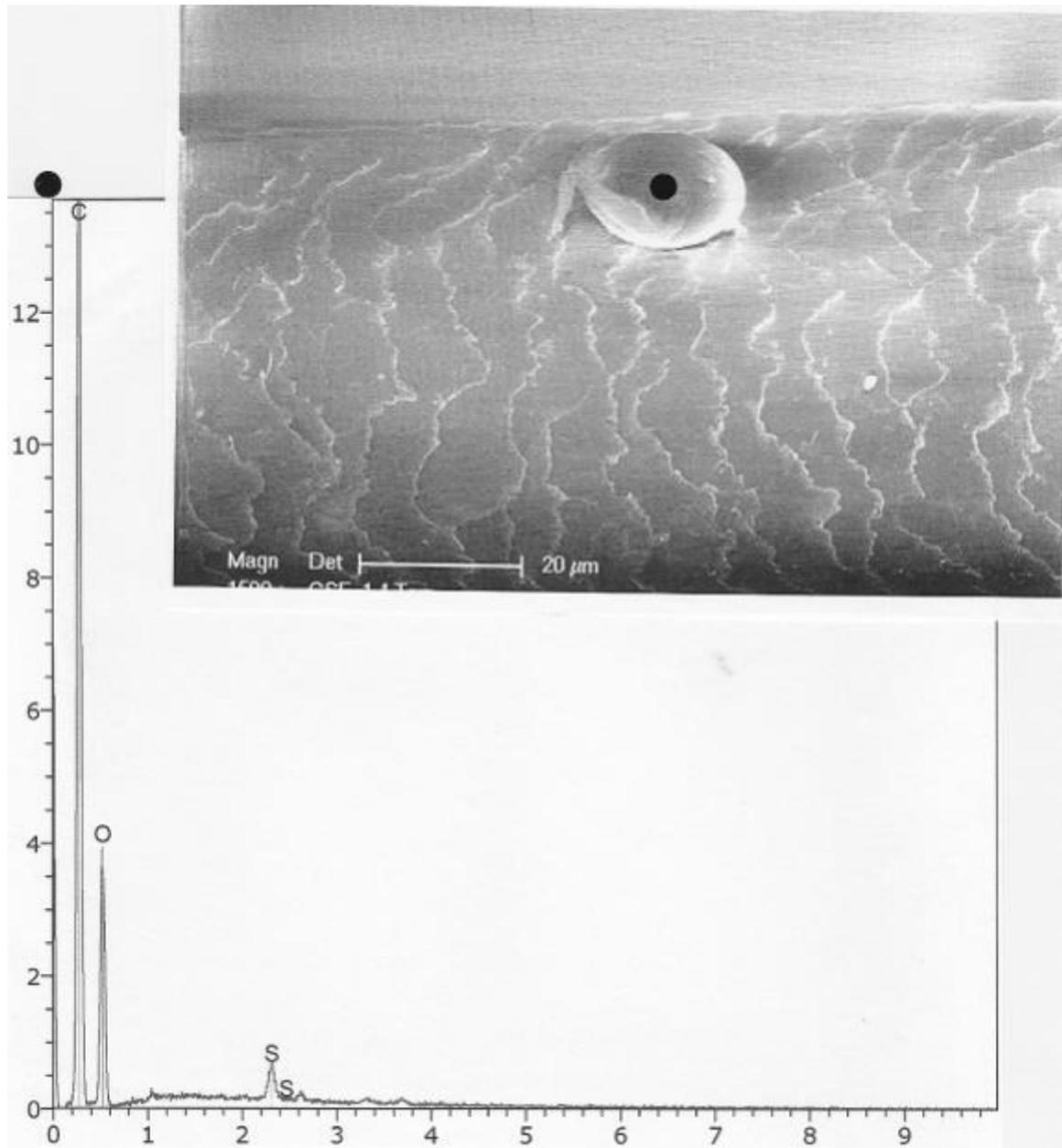


Figure 22 : *Above* : SEM-1 photograph (in GSE, 4000x) showing metallic particles located on the hair surface. *Below* : Spectrum at the black dot. C : carbon ; N : nitrogen ; O : oxygen ; Cu (three peaks) : copper ; S (two peaks) : sulphur ; Cl : chlorine ; Ag (three peaks) : copper ; S (two peaks) : sulphur ; Cl : chlorine ; Ag (three peaks) : silver ; Ca (two peaks) : calcium.

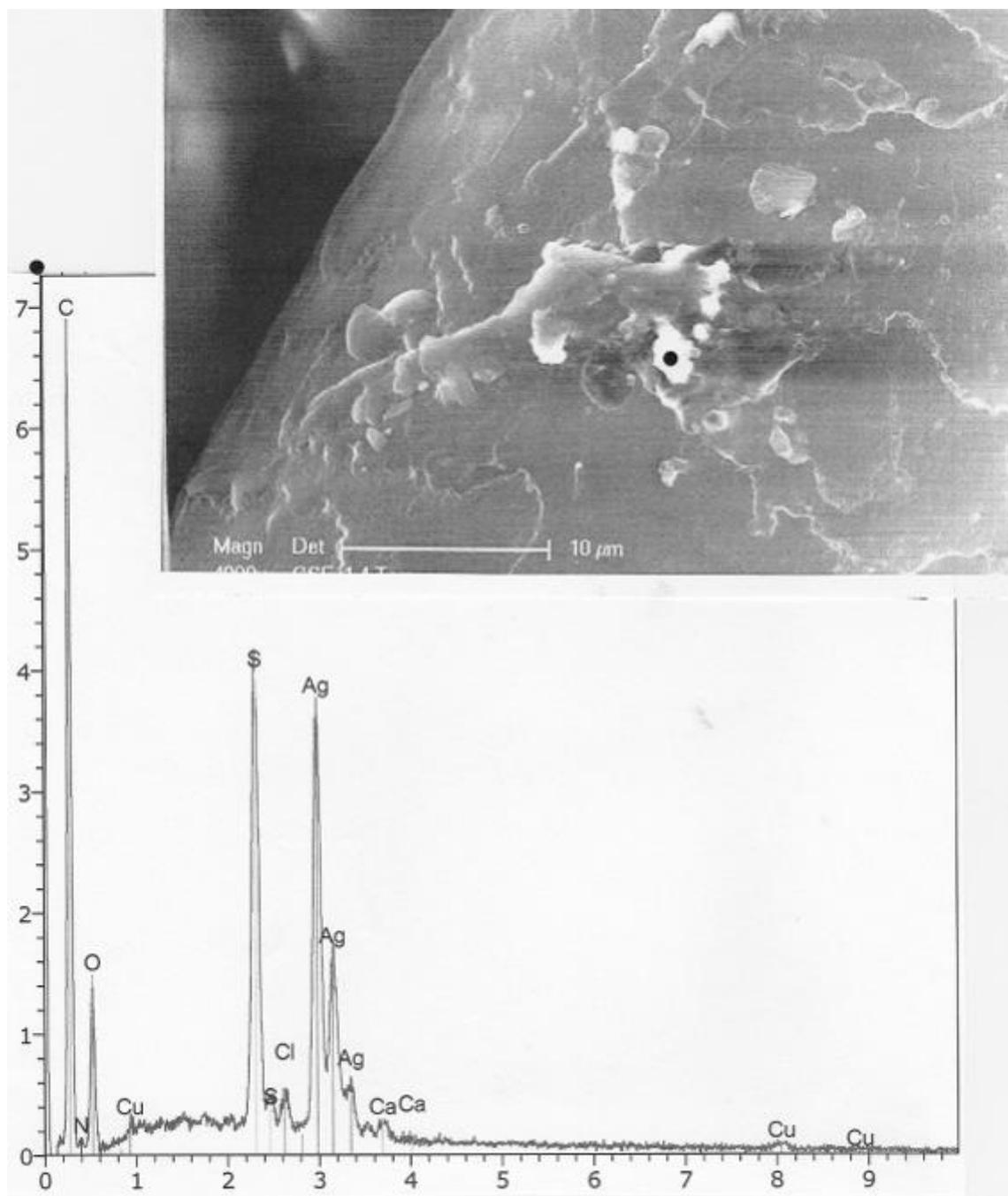


Figure 23 : *Above* : SEM-1 photograph (in GSE, 2000x) of a set of particles located on the hair surface. *Below* : Spectrum at the black dot.

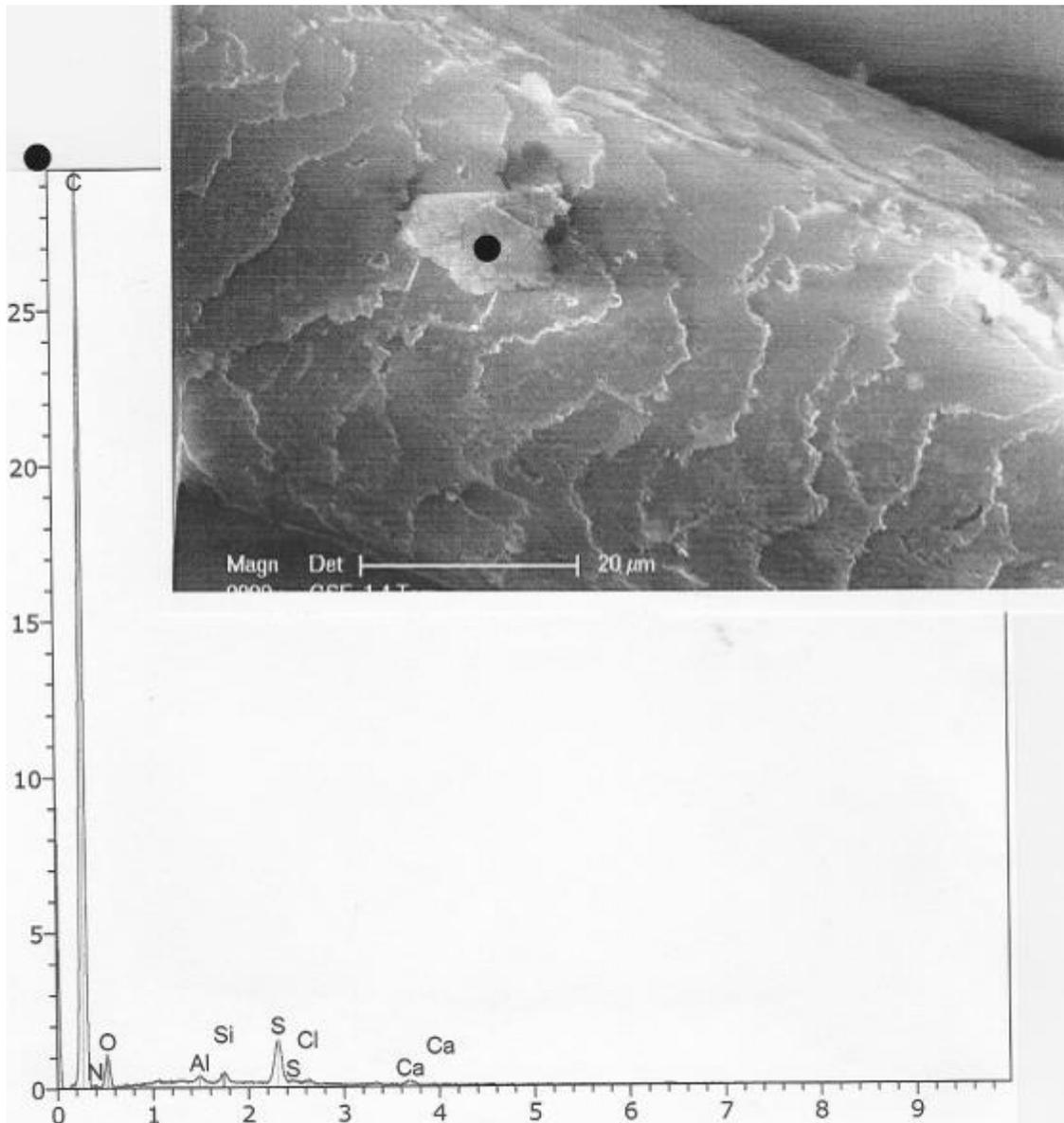


Figure 24 : Above : SEM-2 photograph (in LFD, 2400x) of a skin-cells (F : folds). Below : Spectrum at +.

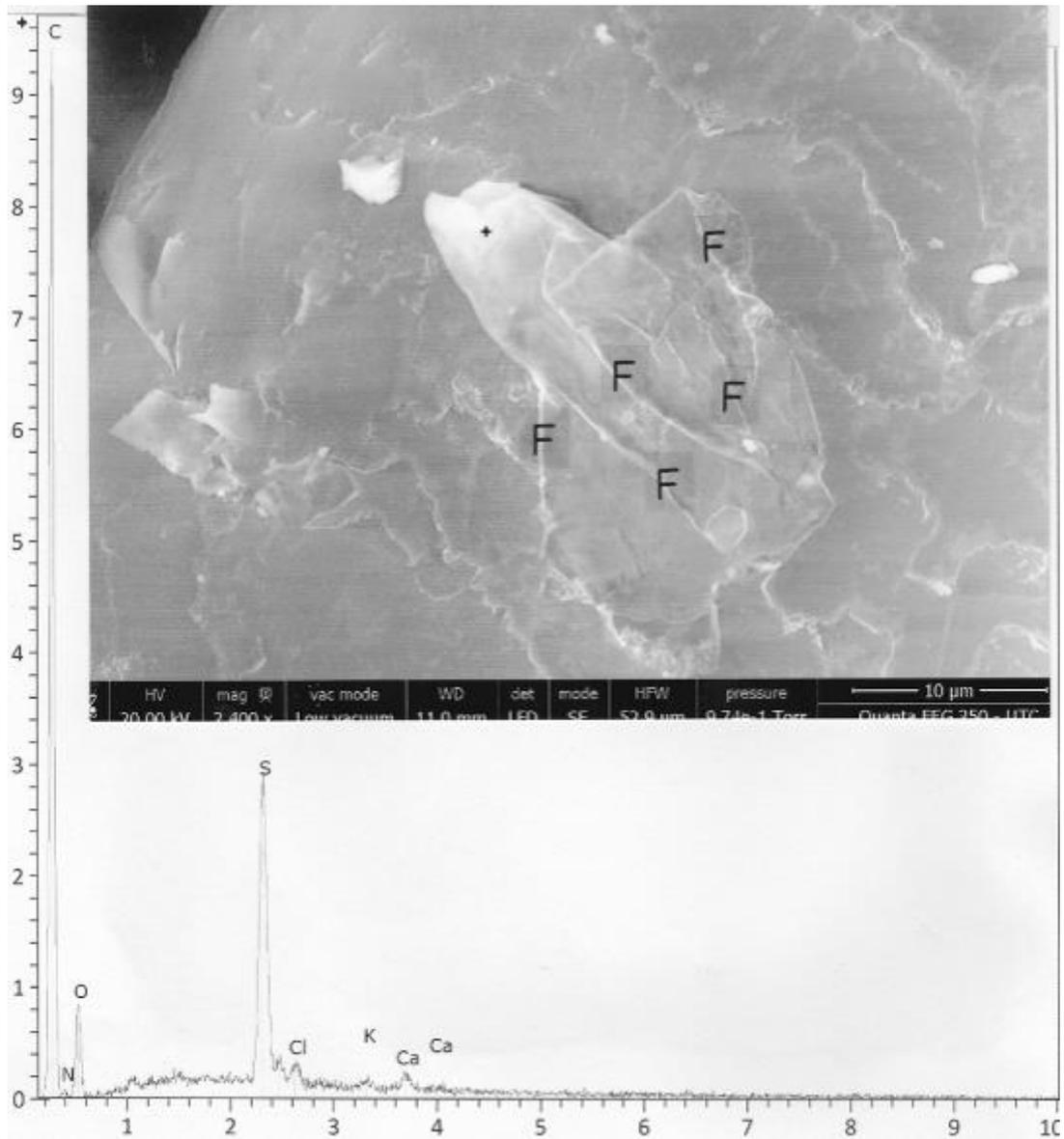


Figure 25 : *Above* : SEM-2 photograph (in LFD, 2400x) of the first (1) dandruff observed. *Below* : Spectrum at 1.

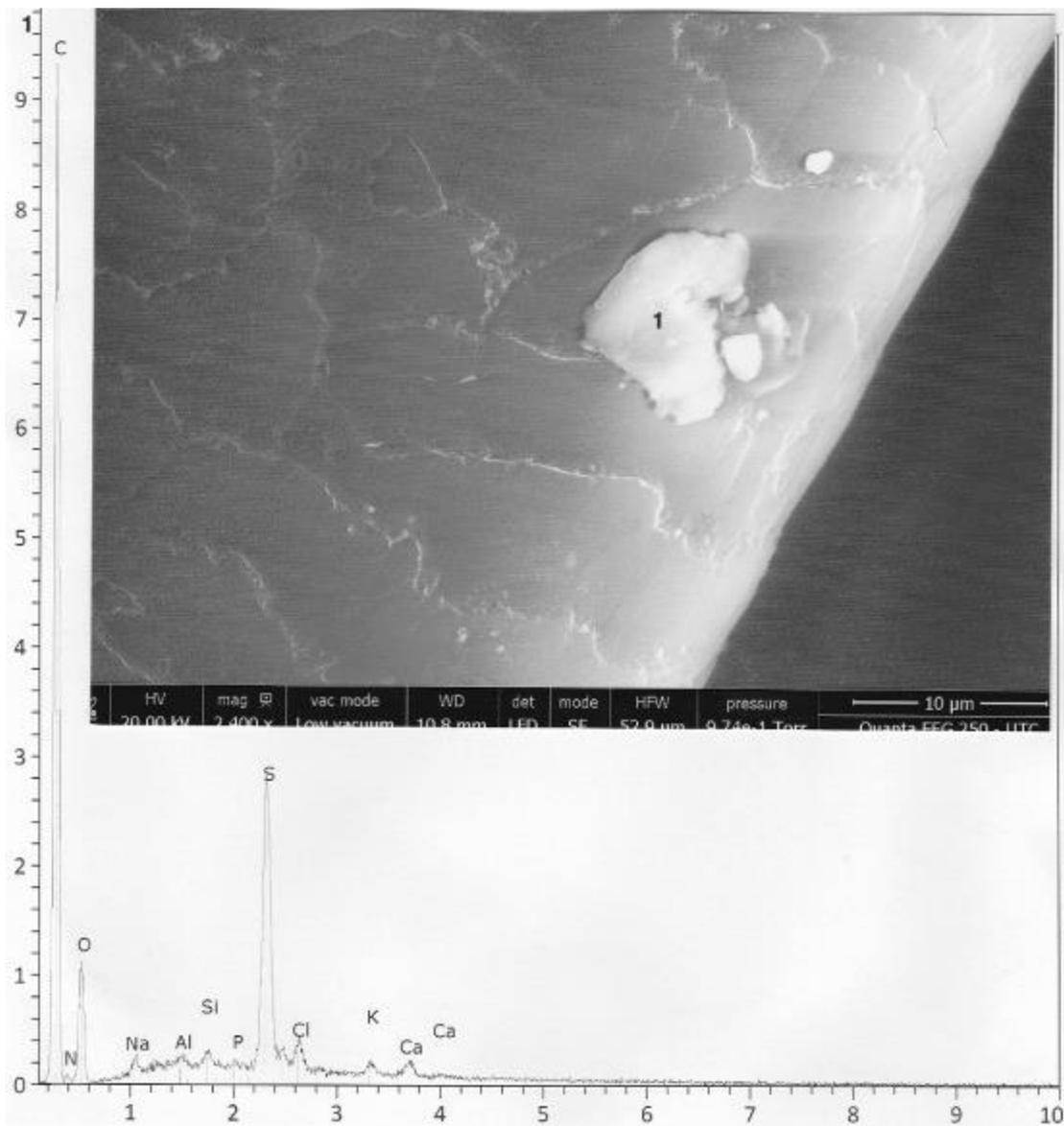


Figure 26 : *Above* : SEM-2 photograph (in LFD, 2400x) of the second (2) dandruff observed. *Below* : Spectrum at 2.

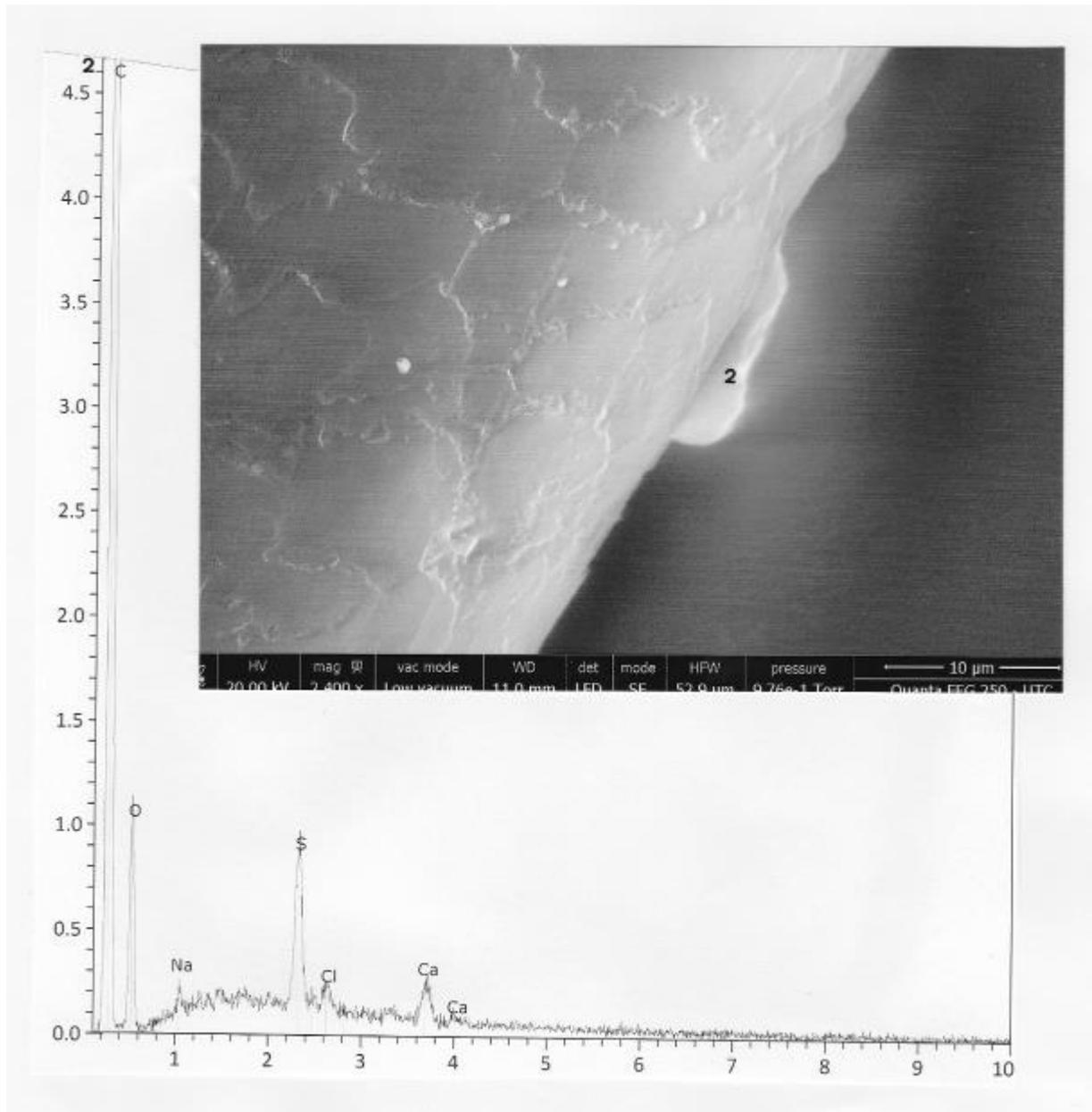


Figure 27 : SEM-2 photographs (in LFD, 2400x) of the third (3) and fourth (4) dandruffs observed.

