

1 Direct Evidences of Metal Inorganic Traces into  
2 Cigarettes

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26 **Highlights**

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35 **GRAPHICAL ABSTRACT**

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46 *Keywords.* Cigarette, tobacco, heat-not-burn, smoke, paper, filter, contaminant, heavy  
47 metal, particle, electron microscopy, chemical analysis.

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51 ABSTRACT

52 Today, environmental health research of adverse hybrid materials diffused by cigarette  
53 represents a new challenge for identifying new health risks directly related to the  
54 specific micro-sized materials in terms of their morpho-chemical features. Distinctive  
55 assumptions about the origin, the evolution? growth, and the functionalization of toxic  
56 elusive particles have been proposed by scientific research to attend the relevant  
57 toxicological aspects of observable behaviors. Therefore, direct morpho-chemical  
58 observations of the toxic hybrid particles are the most important factor for showing their  
59 adverse effects. Here, we report how metal inorganic particles, identified in three  
60 micrometric regions of the cigarette, evolve in their chemical size distributions into a  
61 self-assembled agglomerates ranging from ultrafine powder to large micrometric  
62 complex before and after smoking. Detailed morpho-chemical investigation on these  
63 metal inorganic materials interacting with cigarette components, quantified in situ  
64 through electron microscopy techniques, has been performed for one traditional and two  
65 heat-not-burn cigarettes of three different brands. The experimental informations  
66 gathered allowed us to figure out the evolution of the particles from the early stage  
67 (before smoking) to the final (after smoking) assembled in hazardous large  
68 agglomerates chemically manipulated and delivered by particles heat carrier, the  
69 smoke. In particular, our work shows the dual role of the adverse smoke, generated by  
70 burning and heating processes, capable of growing multi-elemental macro-aggregates  
71 and of transporting the toxic pollutants, thereby the diffusing of contaminants in the  
72 natural environment is independent from the safety engineered features adopted by  
73 tobacco company. The reported results represent a valuable background toward the full  
74 comprehension of evolution of the toxic materials into cigarettes responsible of altering  
75 and destroying the already contaminated nature, especially for human health.

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79 **1. Introduction**

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81 **Contributo Clinico**

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83 Sheet paper wrapping tobacco is engineered to be a porous system, consisting  
84 of a thin squeezed cellulose fibers arranged in micro-mesh structures filled by additive  
85 compounds. The amount and size of the fillers are presumably engineered in order to  
86 provide appropriate porosity useful for exchanging air through the sheet paper.[1] By  
87 tailoring the chemistry and amount of the filler and burn contents, the air flow passing  
88 through the paper should be aimed to convert the carbon monoxide production in less  
89 toxic carbon dioxide, this believing expedient of less toxicity task by catalytic oxidation  
90 has been widely studied.[2, 3, 4] In most cases, addition of a water-soluble salt (sodium  
91 bicarbonate, potassium bicarbonate or ammonium carbonate) to cigarette paper has  
92 been identified in order to increase the diffusion capacity to decrease carbon monoxide  
93 in cigarette smoke. [5] Unfortunately, the possibility of appearance of chronic diseases,  
94 the persisting inhalation of higher concentrations of CO<sub>2</sub> can affect respiratory function  
95 in mucosal irritation, contributing to myocardial ischemia and causing excitation followed  
96 by high risk for clinical depression.[6] In the additive substances context, different heavy  
97 metals compounds, entrapped in the sheet paper, have been involved for catalytic  
98 conversions of carbon monoxide mediated by paper porosity, during puffing. The heavy  
99 metal oxides inorganic substance introduced in the paper sheet have been assumed in  
100 form of particles and/or ultrafine powders mainly constituted of magnesium, silicon,  
101 aluminium, chromium, iron, copper, nickel, zinc, and etc..[7, 8] Moreover, the metal  
102 inorganic particles doping the cigarette paper are also used for reducing odour, visibility  
103 and emission of the side-stream smoke (calcium carbonate, magnesium oxide,  
104 magnesium carbonate, sodium acetate, sodium citrate and etc..). The particles become  
105 smaller for reducing the odour due to the thermal degradation during puffing, whereas  
106 other additive particles encapsulate the smoke for reducing both visibility and emission  
107 using potassium succinate, potassium citrate and magnesium carbonate. [9, 10, 11]  
108 The recent development in the manufacturing cigarette related to the pathologies  
109 cannot be analyzed only by considering the contaminants in the sheet paper. The

110 explanation has to sought also in the other components of the cigarette. The  
111 fundamental role of the filter in the cigarette is still ambiguous since it is not clear how  
112 much the filter can remove the toxic contaminants carried by gaseous smoke.  
113 Furthermore, an immediate question arises of whether the same filter may contain  
114 undesirable contaminants introduced by manufacturing processes, by which the same  
115 contaminants could be probably removed from the filter by heating smoke to be  
116 unfortunately inhaled. Actually, the alone sheet paper cannot be aimed to the significant  
117 conversion of the carbon monoxide and of other smoking targets. Therefore, the desired  
118 catalytic conversions have been also supported by adding metal inorganic compounds  
119 within the cellulose acetate fibre of the filter.[12] The dramatic effect on the natural  
120 environmental of the smoked filter is well know that cigarette butt is a plastic litter an  
121 hazardous pollutant for the human habitat. The major critical effect on the nature health  
122 (embracing human life) is the continue and increase of releasing heavy metals  
123 entrapped into the cigarette butts, creating a dangerous densely reservoir able to  
124 disperse free particulate of lead, cadmium, mercury and arsenic, etc..[13]. The  
125 uncontrolled free particulate is mainly constituted of chemical complex particles  
126 identified in nanometric size and more by dynamic light scattering and cp mas nmr  
127 spectroscopy techniques. [14, 15] Such exposure to the hazardous heavy metals has  
128 been classified of responsible as a human carcinogen.[16] Basically, microparticles and  
129 nanoparticles in ultrafine powder might be released into natural environment causing  
130 adverse changes and into human respiratory, cardiovascular, nervous, and reproductive  
131 systems. The adverse effects of the particles and particulate matter have well reported  
132 by Mulay et. al.. [17] Indeed, particle size is a critical determinant for macrophages and  
133 other phagocytes, these are the first cells to engulf particles for phagocytosis possible  
134 for microparticles of a fewest microns in size and easy for nanoparticles. Therefore, the  
135 micrometric pollutants need accurately characterize in terms of size-shape and of  
136 chemical reactive oxygen species since agglomerations of particles increasing their  
137 dimension easily overloading phagolysosomes. This root easily conducts to the  
138 metabolic storage diseases killing the cells followed by cangerogenesis effect. [18]  
139 Recently, the adverse effect of filter butts on the natural environmental have been  
140 investigated in order to convert the smoked filter in harmless tools for supplying different

141 industrial sectors [19, 20]. Therefore, this useful sustainable chemistry research need  
142 more knowledge on chemistry and size of the elusive particles contaminating the  
143 smoked cigarette especially for the filter or butt.

144 Herein, the morpho-chemical investigations of commercial cigarettes of three different  
145 brands have been focused on the evolution growth of micro-aggregate and microparticle  
146 linked to the effects of smoking traditional (**T**-cigarette) and two heat-not-burn cigarettes  
147 (**hnbA**- and **hnbB**-cigarettes). This work aims at pooling information from an  
148 appropriate morpho-chemical characterization useful for tracing the particles related  
149 their chemical contents to achieve desirable analyses of their evolution, studying three  
150 different regions of the cigarette before and after smoking. Morphological and chemical  
151 characterizations of three different regions of the cigarette illustrated in **Figure 1?** have  
152 been investigated in order to show quantitatively the presence of possible micrometric  
153 pollutants. This evaluation strategy provides a means to our understanding on finding  
154 the possible criteria of migration of the micrometric pollutants from the inner to outer of  
155 the cigarettes. To obtain insight into the self-assembly behavior of these hybrid  
156 materials at the microscopic scale, a combination of electron microscopy techniques of  
157 VP-SEM, EDS multi-mapping analysis, and image processing techniques [21] have  
158 been exploited in order to obtain all possible experimental information for exploring the  
159 following objectives: *i*) morphometric features of the porosity and micrometric particles  
160 assembled into a fibre packing network of sheet paper and their corresponding chemical  
161 microanalyses (**Figure 1 and 2**), *ii*) fiber porosity of unsmoked filters and the  
162 contaminants deposited on (**Figure 3**), *iii*) direct evidences of the particles in  
163 micrometric and ultrafine dimension identified on the fibre surface of smoked filter  
164 characterized by chemical spatial distribution imaging (**Figure 4**). *iv*) evolution growth of  
165 the metal inorganic contaminants in terms of changing size associated to their chemical  
166 elements on the two different regions of the unsmoked and smoked filters (**Figure 5**).  
167 Appropriate imaging analyses have been exploited to perform accurate quantitative  
168 measurements of size-shape and of chemical spatial distribution of the individual  
169 particles, paying attention to the statistical analysis. [22] Since the growth size-shape of  
170 the metal inorganic materials represents an important step in understanding their brutal  
171 chemical activities, appropriate observations on the evolved contaminants have

172 provided some considerations focusing on the adverse property of the smoke as a  
173 particles carrier of toxic elements. In fact, our morpho-chemical observations have been  
174 essential for showing the tremendous effect of the smoke capable of bringing high  
175 amount of particles to be self-assembled into a coexisting phases of a large  
176 agglomerate because of the persistence and accumulative of puffing. Finally, integrate  
177 the above scientific objectives in a synergistic manner have been essential for  
178 understanding the directed evidence of the unwanted assembly dependent on the  
179 chemical-size-shape contaminants and on the manufacturing engineering involved in  
180 the three different brands presented, here.

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## 182 **2. Experimental**

### 183 *2.1. Materials*

184 Traditional and two heat-not-burn commercial cigarettes from three common brands  
185 were purchased new and analysed before smoking. After smoking, the participants  
186 smoked cigarette packs by numero 5 traditional cigarette, numero 5 heat-not burn  
187 cigarette of type A, and numero 5 heat-not burn cigarette of type B. All users of cigarette  
188 were smokers. All participants signed an informed consent before taking the sample.  
189 The study design was conducted in accordance with the Declaration of Helsinki, and the  
190 protocol was approved by the Department of Public Health and Infectious Diseases,  
191 Sapienza University of Rome (No. ???? Prot. No. ?????).

### 192 *2.2. Preparations Samples*

193 All samples were observed and analyzed in their original state without conductive  
194 coating or any other supplementary substances used for conventional sample  
195 preparation in electron microscopy technique.

### 196 *2.3 Variable Pressure Scanning Electron Microscopy*

197 All components of the cigarette, paper and metallic sheets and fiber filters, were  
198 observed using a variable pressure scanning electron microscopy (VP-SEM, Hitachi  
199 SU-3500) supported by dual energy dispersive X-ray spectroscopy detectors (dEDS)

200 arranged in parallel configuration (Bruker, XFlash® 6|60) able to high sensitivity  
201 elemental analysis by their large active area of a 60 mm<sup>2</sup> each. The samples were  
202 directly settled onto a carbon planchet stub without conductive coating. [23, 24] By  
203 appropriate control of the chamber pressure, particular attention was paid to avoid crack  
204 formations of the content structures. [25] All samples were observed at an accelerating  
205 voltage depending on the features of the pressure used in the chamber to avoid  
206 radiation damage and drifting image, fatal for EDS multi-mapping analyses.  
207 Furthermore, the experimental approach focused on detecting as much information as  
208 possible, thereby the morpho-chemical investigation was concretized at low  
209 magnification in order to improve the statistical observation/analysis of the  
210 nanostructured microparticles. [26] Therefore, it should be noted that eventual metal  
211 inorganic contaminations could be detected at high magnification to show the existence  
212 of specie under microscopic scale, but our scientific research was focusing on  
213 hazardous particle up to micrometric scale. [27, 28, 29]

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### 215 **3. Results and Discussion**

216 Morphological and chemical characterizations of three different regions of the cigarette  
217 were investigated in their native states using low vacuum VP-SEM-dEDS (**Figure 1?**).  
218 By this investigation strategy, our intention is to reveal directly by imaging and tracking  
219 techniques the progressive evolution of the elusive metal inorganic particles on local  
220 micrometric regions because of the adverse effect of the smoke. The choice of these  
221 regions is based on the main origins of contaminations (because they participate  
222 directly to the chemical and physics mechanisms (thermal decomposition (pyrolysis,  
223 pyrosynthesis and combustion)) of burning and heating processes of the traditional and  
224 heat-not-burn cigarettes.), before and after smoking. **Region I** focused on the inner  
225 sheet wrapping tobacco were examined, as shown in **Figure 1** and **2**. A manufactured  
226 tobacco leaf laying on the paper sheet-**T** was observed (**Figure 1a**). In particular, the  
227 surface of the leaf evidences the dehydrated thickness walls of xylem vessels reinforced  
228 with cellulose and lignin polymer fibres, forming an annular and reticulate shape (orange  
229 arrowheads). Magnification of **Figure 1a** better shows squeezed and dried tubular



230 vessel possessing a thickness wall of about 3.50  $\mu\text{m}$  covered by bright particles of  
231 quasi-rectangular shape (red short dashed line, **Figure 1b**). The further presented  
232 investigation of the tobacco has been considered in order to reveal an eventual  
233 correlation with the origin of the contaminant particles. The morphometric dimension of  
234 the bright particles has been correlated with their chemical constituents by EDS spectra  
235 and with the chemical spatial distribution using EDS multi-elemental images. EDS multi-  
236 mapping was capable to identify micrometric and nanometric contaminants constituted  
237 of aluminium, magnesium and calcium silicates by investigating the peak intensity of the  
238 spectrum within a selected micrometric region of interest and observable by overlaying  
239 **Figures 1b-OAl, 1b-Si, 1b-Mg and 1b-Ca** (second letters are referred both to the  
240 chemical element and EDS mapping images). Metal inorganic silicates containing  
241 particles deposited on the leaf surfaces were presumably derived from trapping soil  
242 particles. [30]. Inorganic calcium carbonate is clearly visible by overlaying the C and Ca  
243 elements in **Figure 1b-CCa**, aggregating in micro and nanometric particles. Sodium  
244 chloride can be recognized by merging similar color shape or texture between **Figure**  
245 **1b-Na** and **1b-Cl**. The bright particles of about 3.0  $\mu\text{m}$  in size are shown by overlaying  
246 **Figure 1b-S** and **1b-K** providing a visual identification of potassium sulphate. The origin  
247 of this can be a supplement used for the best growth of the tobacco plants, belonging to  
248 the potassium fertilizer (phosphate, chloride, nitrate, etc..) responsible for tobacco plant  
249 contamination with hazardous implications for soil fertility and human health. [31] These  
250 fertilizers absorbed by tobacco leaves create one more of environmental hazard for  
251 smokers. The revealed potassium sulphate exerts a favourable influence upon the  
252 burning process, producing sulphuric acid a potentially hazardous element. **Figure 1b-P**  
253 shows a well distributed phosphorus contents of possible nanometric dimension. The  
254 content of P can be also associate to the presence of the most dangerous form known  
255 as of white phosphorus  $\text{P}_4$  or phosphoric acid  $\text{H}_2\text{PO}_4$  residuals used during the  
256 manufacturing processes. [32] The occurrence of different metal inorganic substances  
257 in tobacco cigarette have mainly be attributed from cultivation or production of tobacco  
258 leaf. [33] By tracing metal inorganic pollutants, the morpho-chemical investigations has  
259 been employed for paper sheet of traditional cigarette (**Figure 1c**). The morphometric  
260 analysis revealed squeezed natural cellulose fiber wide in average of  $22.08 \pm 1.79 \mu\text{m}$ ,

261 forming a porous network filled with micrometric compounds. Although, the interesting  
262 engineered pore-shaping is strictly dependent on the coexisting phase of compound  
263 materials within the fibre packing network. The adopted manufacturing strategy seems  
264 to be critical in favouring the conversion of the toxic monoxide gas through the smallest  
265 micrometric porosity, but many chemical oxidizing agents are involved because of the  
266 additive microcompounds. At this stage, even small micrometric fillers seem to have  
267 been engineered for healthy implication or for improving product performance, a new  
268 investigation on their chemical and spatial distributions is quite necessary.

269 The investigated region I exhibited heterogeneous chemical contaminants located on  
270 the cellulose fiber network, which are mainly constituted of metal inorganic silicate with  
271 micrometric and nanometric dimensions. Metal silicate of aluminum and magnesium are  
272 shown in **Figures 1c-OAl** and **-Mg** and the inorganic silicates of sodium and potassium  
273 are evident since the color texture of the **Figures 1c-Na** and **-K** follow the silicon of  
274 **Figure 1c-Si**. Micrometric and nanometric particles of iron in oxide state are visible in  
275 **Figure 1c-Fe**. Interestingly, a heavy metal contaminants of potential hazard have been  
276 detected in form of Uranium and Nickel probably originate from natural sediments in  
277 chemical form of nickel uranium oxide ( $\text{NiU}_3\text{O}_{10}$ ) (**Figure 1c-U** and **-Ni**) [34]. The platelet  
278 particle magnified in the Inset of **Figure 1c** have an area of  $14.95 \mu\text{m}^2$  with radius of  
279  $5.53 \mu\text{m}$ , overlapping another microplatelet of the same specie. Other two small U  
280 microparticles in different regions occurred with dimension of about  $3 \mu\text{m}$ . Calcium  
281 carbonate in **Figure 1c-CCa** is mainly located among the squeezed cellulosic fibres as  
282 main additive filler compound for reducing the carbon monoxide delivery. [35]  
283 Furthermore, small amount of sodium, phosphorus, sulphur, chlorine and potassium  
284 were identified. By considering similar color shape or texture of the multi-mapping  
285 images, chemical substances could be related to the presence of sodium magnesium  
286 carbonate, also known as "eitelite", [36] and the toxic sodium sulphate is a principal salt  
287 in proliferation of grown agriculture, which are harmful to human health [37]. Part of the  
288 metal inorganic specie detected on the sheet paper have been also observed on the  
289 tobacco leaf especially for metal inorganic silicates and inorganic fertilizer, thereby the  
290 manufacturing processes are serious capable of contamination different components of  
291 the cigarette.

292 By pooling informations across multiple data sources, appropriate magnifications were  
293 chosen, quantitative morphometric porosity of the manufactured paper network has  
294 been investigated on probed area of  $252 \times 189 \mu\text{m}^2$  (**Figures 1c**). A 2D contour maps of  
295 316 micrometric pores of the paper sheet-**T** were measured, (**Figure 1Sc-CM**). The  
296 analyzed pores have been plotted in a frequency profiles displayed beside the  
297 corresponding 2D filled pores map of the VP-SEM images (**Figures 1c-T**). The bin  
298 distributions were processed by Lorentzian fitting (red line) and the frequency  
299 distribution of the sheet paper was estimated to be centred around the mean value of  
300  $0.81 \pm 0.03 \mu\text{m}$ . Previously study of Eitzinger et al. on the pores size of cigarette papers  
301 indicated that large pores ranging from 2.5 to  $10 \mu\text{m}$  were sensitivity to the air  
302 permeability processes, while the fundamental diffusion capacity changed in smallest  
303 pore under  $1.0 \mu\text{m}$  radius. [38]. Our finding suggests that new manufactured product is  
304 mainly focusing on exchanging air through smallest pore of the cigarette papers based  
305 on diffusion capacity for reducing the carbon monoxide; while air permeability could be  
306 compensated by filter ventilation, as shown below. Quantification of the amount of  
307 space available for air flow has been evaluated in term of pore-shaping using two  
308 metrics: the surface area or porosity related to the circularity a measure of  
309 perimeter/area ratio (a factor shape ranging from 0 representing a more elongated rod  
310 to 1 representing a perfect circle).

311 Both brands of the heat-not-burn cigarettes have been manufactured with a sheet  
312 wrapped in a paper over-wrap (**Figure 2**). The inner layer of type **hnbA** in contact with  
313 tobacco shows smooth surface with bright wide stripes observable in **Figure 2a**. By  
314 magnifying the region of interested supported by EDS multimapping, it is evident that  
315 the sheet shows roughness wide stripes, having a metallic nature (**Figure 2b**). Similarly,  
316 the sheet of the heat-not-burn product of type **B** shows smooth surface in **Figure 2c**.  
317 Different micrometric impurities on the surface can be observed in the magnified image  
318 of **Figure 2d**. Aluminium is still the prevailing content, alloying with iron microparticles  
319 (**Figure 2d-OAI**). Micrometric silicate and calcium carbonate particles rise from the  
320 manufacturing processes, while presence of micrometric island of pure carbon raised  
321 form tobacco can be noticed in the mapping image (**Figure 2d-CCa**). In particular, a  
322 micrometric platelet with an area of  $40.80 \mu\text{m}^2$  and radius of  $9.79 \mu\text{m}$  is shown on the

323 metallic layer (blue arrowed ?da inserire?, **Figure 2d**). The chemical imaging analysis  
324 has recognized a heavy metal pollutant of iron-chromium oxide alloy, which might be  
325 originate from manufacturing production as stainless steel tool or from natural soil in  
326 which chromium may exist in two main oxidation states, Cr(III) and in hazardous nature  
327 of Cr(VI). [39] The external sheet paper-**hnbA** over wrapping the metal sheet-**hnbA**  
328 possesses similar packed micro-manufactured fibres filled with particles, as shown in  
329 **Figure 2e**. Herein, micrometric platelets having elongated shapes are well visible, one  
330 of them in the magnified Inset image. The morphometric analysis estimated an average  
331 area of  $205.22 \pm 81.67 \mu\text{m}^2$  and major radius of  $30.73 \pm 8.74 \mu\text{m}$ , while chemical spatial  
332 distribution analysis confirms the heavy metallic species characterized of alloying in  
333 form of aluminium-magnesium-iron-copper, as shown in **Figures 2e-OAl, -Mg, -Fe, and**  
334 **-Cu**. Further sodium contents following the color texture of the micro-platelets in **Figure**  
335 **2e-Na** can be considered part of the metallic alloy. The detected complex alloy toxic in  
336 its chemical structure and dimension might be originated during cigarette manufacturing  
337 and packing or storage processes. [40] Iron oxides and titanium dioxide of micrometric  
338 dimension are well dispersed in different area (**Figure 2e-Fe and -Ti**). Other metal  
339 inorganic nanostructured microparticles participate in different chemical phase:  
340 aluminium-magnesium-calcium-potassium silicate, calcium carbonate, and small  
341 amount sodium-phosphorous-sulphur-chlorine contents under micrometric dimension.  
342 Additionally, morpho-chemical investigation on manufacturing tobacco of the **hnbB**-  
343 cigarette has been performed showing densely packed tobacco in a stick shape that  
344 does not burn when used (**Figure 2g**). [41] By magnify the interested region in cyan dot  
345 line square, a micrometric particle of tick polygonal shape with area of  $323.50 \mu\text{m}^2$  and  
346 radius of  $23.23 \mu\text{m}$  can be observed in **Figure 2h**. The large microparticle chemically  
347 shows its metallic nature of aluminium, magnesium, chromium and iron elements. The  
348 heavy metal contaminant of iron-chromium oxide entrapped into the tobacco stick might  
349 represent a natural alloy covered by aluminium and manganese silicates, which may be  
350 originated from manufacturing processes. Moreover, its particular size-shape let us to  
351 consider the metal contaminant of natural origin compared to the detected iron-  
352 chromium oxide in **Figure 2d**, having a platelet-like shape perhaps a scattered stainless  
353 steel of cutting tool. The toxic elements of uranium and iron-chromium oxide detected

354 could originate from tobacco plants that might be contaminated by soils naturally or by  
355 contaminated water. [42] From a toxicological point of view, the burning and heating  
356 processes occurred in the cigarettes may responsible of inducing oxidation of Fe oxide  
357 bound Cr(III) with formation of hazardous Cr(VI) heating at more than 400°C with  
358 potential adverse health effects to human. [43] The remaining elements recognized by  
359 mapping image analysis are morphological and chemically similar to the tobacco  
360 detected into conventional cigarette, shown in **Figure 1b**. Yet from a toxicological  
361 perspective, tobacco leaves are contaminated with particles of aluminium-magnesium-  
362 calcium silicates that if inhaled provide more severe high health risk. [44] Fundamental,  
363 fertilizer such as potassium sulphate is still evident. Metal inorganic contaminants of  
364 aluminium and magnesium silicates and the inorganic presence of calcium carbonate,  
365 sodium chloride and phosphorus substances are clearly visible in the chemical images.  
366 To investigate how the metal inorganic contaminations might be migrated through T-  
367 and hnb-cigarettes, the filters of porous cellulose acetate fibres have been  
368 characterized before tracing nanostructured microparticles on their surface of the  
369 unsmoked and smoked fibre filters at tobacco and mouthpiece sides (**Figure 1, Region**  
370 **II and III**). The analyzed filters of the different brands, forming sponge-like materials,  
371 have similar morphology in shape at head filter side in contact with tobacco are shown  
372 in **Figure 3**. Three brands of fibre filters show Y shape fibres almost aligned with slightly  
373 difference of the cross-sectioned size. The evaluated Y dimensions of the single fibre  
374 have showed an increment of the surface area from traditional to heat-not-burn fibre  
375 filters. The increased dimension of the surface area might be related to the  
376 manufacturing purpose for increasing the amount of additive substances for enhancing  
377 the chemical puffing performances. In order to investigate a possible migration of the  
378 additive compounds through the filter, quantitative porous analyses among the cellulose  
379 fibres network have been performed on a probed area of 1263.16 x 947.37  $\mu\text{m}^2$  at low  
380 magnification for improving the statistical observation/analysis (**Figures 3a, 3c, and 3e**).  
381 A 2D contour maps of 174 (fibre filter-T), 74 (fibre filter-hnbA) and 62 (fibre filter-hnbB)  
382 micrometric pores were measured, respectively (**Figure 2S**). The analyzed pore-sizes  
383 have been plotted in a frequency profiles displayed beside the corresponding 2D filled  
384 pores map of the VP-SEM images (**Figures 3a-T and 3e-hnbB**). Traditional filter

385 exhibited pore size distribution with high frequencies of fitted profile ranging from 22 to  
386 208  $\mu\text{m}$ , whereas heat-not-burn filters showed wide spatial distribution of low  
387 frequencies but with more multi-peaks fitting of the histograms ranging from 20 to 373  
388  $\mu\text{m}$ . The pore size distributions, best-fitted to a Lorentzian convolution curve, seemed to  
389 exhibit difference among the pore distributions of the fibre filters. By gathering the main  
390 fitted peaks close in position, their relative variations were estimated to be centered  
391 around the average values of 26.12, 62.93, 109.40, 151.21, 187.73, 246.33 and 328.35  
392  $\mu\text{m}$  with incremental variation of  $46.91 \pm 6.97 \mu\text{m}$ . The estimated analysis provides  
393 quantitative informations on the ranging size of the particles that may be confined  
394 through the pore fibres. In this regard, the spatial shape of the pores is also a relevant  
395 information for understanding the limited access to the nanostructured microparticles to  
396 be caught by fiber filter network. The high porosity value had a shape factor centered at  
397 0.12, an evidence of pores shaping mainly with more elongated shape (rod-like) and low  
398 porosity at 0.50 circularity of an elongated oval shape. Similarly, fibre filter-**hnbA**  
399 showed high porosity of 7.60 % with implication of preferential porosity, shaping in a  
400 more-elongated rod like (0.09) and low amount of elongated quasi-oval shape (0.50) of  
401 1.01 % porosity. Formation of anisotropic pore structures in multi-shaping domains from  
402 more elongated rod to less elongated oval (0.1-0.7) can be assigned to the fibre filter-  
403 **hnbB**. By summarizing the mainly fitted peaks, we were able to gather the peaks at  
404 0.14 (5.40%), 0.26 (10.69%), 0.49 (3.36%) and 0.69 (2.33%) in terms of circularity and  
405 porosity. Moreover, the pores circularity showed a slight shift toward the circularity axis  
406 compared to the fibre filter-**hnbA**, providing an enlargement of the microscopy pore-  
407 shaping. The manufactured fibre filters of the **hnb**-cigarettes exhibited an increase in  
408 porosity/circularity compared to the traditional one probably to increase air ventilation  
409 since the inner sheet wrapping tobacco is a smooth metal sheet without pores. From the  
410 presented morphometric analyses, all fibre filters are capable of transporting high  
411 amount of air and large microparticles of variable micrometric sizes with preferential  
412 more elongated shapes. In particular, the filter of type **hnbB** might be host  
413 microparticles of high variability in size-shape. High porosity of the filter has been  
414 related to a fundamental intrinsic property of the manufactured cigarettes in order to  
415 reduce the toxicity of carbon monoxide by increasing the filter ventilation or cigarette

416 paper porosity. [45] This first evidence suggests that chemicals oxidizing agents and  
417 high amount of micrometric pollutants independently from their intrinsic size-shape  
418 properties could easily reach the human body via inhalation.

419 In this regard, it is interesting to establish the appearance of metal inorganic substances  
420 on the fiber filter before and after smoking, using ESDS multi-mapping analysis. The  
421 morpho-chemical analyses were employed at the same probed area of 252 by 189  $\mu\text{m}$ .  
422 Magnification of **Figure 3a** shows existence of different microparticles deposited among  
423 cellulose acetate fibres of the unsmoked traditional cigarette (**Figure 3b**). First evidence  
424 is the appearance of large microparticle possessing an estimated average area of  
425  $168.80 \mu\text{m}^2$  and radius of  $19.60 \mu\text{m}$ , and yielding a size allowed for a possible migration  
426 through the filter. The chemical analysis confirms the formation of a nanostructured  
427 alloy constituted of heavy metal elements of aluminium, silicon, iron, nickel, copper and  
428 more interesting are the intense appearances of the toxic osmium element in **Figure**  
429 **3b-Os**. The enhanced aluminium signal of the X-ray microanalysis let to suppose a  
430 mixed formation of osmium complex alloying mainly with aluminium and nickel in minor  
431 amount. Notably, whereas agglomerate of aluminium-magnesium-sodium-phosphorous  
432 silicates could be attributed to the manufacturing contaminants due to their standard  
433 presence found in this habitat. Moreover, a microparticle of  $7.90 \mu\text{m}$  in size of osmium  
434 complex can be also observed, but alloyed with copper content, as shown in **Figure 3b-**  
435 **Cu**. Silicates of aluminium-magnesium-titanium-calcium-sodium-phosphorous are also  
436 dispersed in small amount around the identified alloy microparticles. Further, titanium  
437 and iron token part in an oxide states. The main inorganic particles preferentially in  
438 nanometric dimensions are observable in small amount of different chemical phases of  
439 calcium carbonate, potassium sulphate, and sodium chloride. Filter of type **hnBA**  
440 possesses cellulose acetate fibers of roughness surface due to the high amount of  
441 micrometric compounds deposited on, shown in **Figure 3d**. EDS imaging analysis  
442 clearly show an increase of microparticles different in size and shape constituted mainly  
443 of silicates, as displayed in **Figure 3d-Si**. By analyzing the chemical spatial distribution,  
444 the silicates are based on aluminium-magnesium-titanium-calcium-sodium-phosphorous  
445 and a further titanium and iron oxides were detected in smallest micrometric  
446 dimensions. Inorganic aggregation already detected onto fiber filter of traditional

447 cigarette occurred in nanometric powder. The increasing in amount of additive  
448 microparticles onto cellulose acetate fibers is in agreement with the proposal  
449 hypothesis, regarding the increased surface area of the manufactured fibres of the heat-  
450 not-burn products. Fibre filter-**hnbB** product also exhibited microcompounds  
451 aggregating in metal inorganic particles on the cellulose acetate fibres mainly identified  
452 as aluminium-titanium-calcium silicates and calcium carbonate. Minor concentration of  
453 Na, P, S, and Cl elements were distributed uniformly in nanometric size, shown in the  
454 EDS mapping images of **Figure 3f**. Metal and inorganic contaminations have been  
455 identified on unsmoked filters with quite similar amount and species. The complexity  
456 single objects recognized on the fibres were estimated with dimensions ranging from  
457 2.05 to 9.50  $\mu\text{m}$ , forming a nanostructured microaggregates. Ultrafine potassium  
458 sulphides and sodium chloride were well distributed on the cellulose acetate fibres with  
459 under micron dimension on the fibres of type **hnb**. This inorganic chemical specie can  
460 be occurred due to the physical contact with tobacco, as detected by multi-chemical  
461 analyses of **Figure 1b** and **2h**. By quantitatively morphometric evaluation, iron oxides  
462 particles were prevailing in fibre filter-**T** with  $2.94\pm 0.57 \mu\text{m}$  in average dimension while  
463 ultrafine sub-micrometric particles were only detected in fibre filter-**hnbA**. Silicates  
464 formed a nanostructure microparticles with average dimension of  $3.18\pm 0.19 \mu\text{m}$  and of  
465 magnesium in ultrafine dimension (fibre filter-**T**),  $4.49\pm 0.47 \mu\text{m}$  and  $3.70\pm 0.31 \mu\text{m}$  (fibre  
466 filter-**hnbA**), and  $4.54\pm 0.99 \mu\text{m}$  and magnesium silicate was not detected (fibre filter-  
467 **hnbB**). Inorganic compounds are predominant in a coexisting multi-phase of calcium  
468 carbonate with  $3.09\pm 0.19 \mu\text{m}$  (fibre filter-**T**),  $3.92\pm 0.49 \mu\text{m}$  (fibre filter-**hnbA**), and  
469  $5.00\pm 0.46 \mu\text{m}$  (fibre filter-**hnbB**) dimensions; titanium dioxide with  $3.40\pm 0.65 \mu\text{m}$  (fibre  
470 filter-**T**),  $2.64\pm 0.37 \mu\text{m}$  (fibre filter-**hnbA**), and  $4.54\pm 0.58 \mu\text{m}$  (fibre filter-**hnbB**); and  
471 sodium-phosphorous-chlorine and potassium formed complexes of ultrafine powder  
472 under micrometric size. Hence, the critical impact of heavy metals (Iron-Nickel-Copper-  
473 Osmium) were mostly generated by traditional fibre filter probably because of the  
474 different manufacturing and packing processes compared to the fibre filter of type **hnb**.  
475 The detected heavy metal might be attributed to their catalytic property in conversion  
476 the carbon monoxide or unwanted contaminations originated from the assembly line of  
477 the factory.[46] Differently, the amount of the metal inorganic silicates were preferential



478 employed in the fibre filters of type **hnb**, including the additive substances of calcium  
479 carbonate and titanium oxide.

480 At this stage, however, even small changes in the burning and heating processes might  
481 be occurred after smoking, a new validation is necessary for expanding our  
482 understanding on the evolution features of the metal inorganic particles. First result was  
483 to observe decomposed cellulose acetate fibers of smoked cigarettes because of  
484 burning and heating processes, compromising also the porosity purpose in term of  
485 ventilation (Region II of **Figure 1**, **Figures 4a**, **-c**, and **-e**). Basically, the combustion of  
486 cellulose acetate fibers favorites the oxidative formation of a poisonous, colorless and  
487 odourless gas of the toxic carbon monoxide and further toxic oxidizing agents; which  
488 can be easily inhaled during puffing. [47, 48] Volatile products during thermal heating  
489 processes have also released solid particles on the melted fibers, coming from tobacco  
490 column (**Figures 4b**, **-d**, and **-f**). The observed micrometric object placed on the top of  
491 the fibers, not seen before, is a result of aggregating nanostructured particles because  
492 of the frequent puffs through rapid heating and high air-flow of the contents. Fibre filter-  
493 **T**, at tobacco side after smoking, exhibits micrometric globulars of  $31.07 \pm 5.98 \mu\text{m}$  in  
494 average in **Figure 4b**. The formed globular were mainly aggregating with phosphorous-  
495 sulphur-chlorine-potassium-calcium and silicates of aluminum and magnesium self-  
496 assembled together with less amount of iron, manganese, phosphorous, sulphur and  
497 chlorine (white arrowed???, **Figure 4b**). Metal contaminations of iron oxide were  
498 present at the surface in small nanostructured microparticles with average dimension of  
499  $2.79 \pm 0.21 \mu\text{m}$  and silicates of aluminum, magnesium, titanium with  $3.15 \pm 0.33 \mu\text{m}$  and  
500  $2.48 \pm 0.14 \mu\text{m}$ . Furthermore, heavy metal chromium and manganese contents well-  
501 dispersed on melted fiber network in ultrafine dimensions after smoking appeared. This  
502 is an evidence that particles subjected of heating process are volatile capable of  
503 traveling through the tobacco column also in heat-not-burn cigarettes. The metallic  
504 contents have been considered an intentional product critical for catalytic activity in  
505 conversion carbon monoxide to carbon dioxide dependent upon the particle dimension  
506 ranging from 3 nm to 5  $\mu\text{m}$  [49]. The first appearance of micrometric manganese  
507 particles in oxide state might be used for its catalytic property. [50] This represents a  
508 further advice of hazardous evidences since manganese has been detected in several

509 brain regions after smoke inhalation causing inflammatory changes. [51]. Different  
510 behavior of the dimension evolution has been noticed for inorganic compounds of  
511 phosphorous-sulphur-chlorine and potassium contents changed from ultrafine powder of  
512 sub-micron size in microparticles with an estimated average dimension of  $3.38\pm 0.36$   
513  $\mu\text{m}$ , after smoking. Their size increments are shown in **Figures 4b-Na, -P, -S, Cl, and -**  
514 **K**. On the contrary, the additive of calcium carbonate and titanium dioxide degraded in  
515 small microparticles with dimensions of  $3.14\pm 0.41$  and  $2.35\pm 0.21$   $\mu\text{m}$  compared to the  
516 unsmoked filter, performing their properly industrial task of catalysts/oxidants. [52]  
517 Therefore, the burning process have reduced their amount in a volatile component  
518 traveling in the mainstream smoke that are able to provoke irritation to the respiratory  
519 systems through short-term inhalation. [53, 54] Fibre filter of smoked **hnbA**-cigarette  
520 show an macrometric aggregate of an estimated area of  $7015.02$   $\mu\text{m}^2$  and radius of  
521  $126.04$   $\mu\text{m}$  (**Figure 4d**, region II of **Figure 1**). This macrometric object is an  
522 agglomerate of microparticles mainly composed by sodium-phosphorous-sulphur-  
523 chlorine-potassium and magnesium silicate in a coexisting phases of ultrafine  
524 dimension and micrometric particles of sulphur and potassium ( $3.80\pm 0.31$  and  
525  $3.68\pm 0.24$   $\mu\text{m}$ ) and calcium carbonate ( $3.58\pm 0.26$   $\mu\text{m}$ ). By changing the puffing process  
526 in heating without burning, metal oxide contaminants of magnesium, manganese and  
527 iron in oxide states occurred in ultrafine dimensions, while silicate of aluminum with  
528  $3.52\pm 0.20$   $\mu\text{m}$  in average. Similarly, to the traditional fibre filter, the additive titanium  
529 dioxide did not take part to the self-aggregating heating processes confined around the  
530 macrometric object, having dimension of  $2.95\pm 0.13$   $\mu\text{m}$  observable in **Figure 4d-Ti** and  
531 (**Figure 4b-Ti**). Smoked fibre filter-**hnbB** concretely exhibits melted cellulose acetate  
532 fibres (**Figure 4e**), which led to unwanted exposure of potentially oxidizing agents in  
533 form of volatile fractions capable of reaching the human respiratory system by  
534 inhalation. Similarly, macroaggregates occurred in highly variable sizes from  $810.40$  to  
535  $29980.02$   $\mu\text{m}^2$  of the surface area and radius from  $33.89$  to  $218.20$   $\mu\text{m}$ . The  
536 macroaggregate large of  $108.81$   $\mu\text{m}$  is mainly constituted by phosphorous-sulphur-  
537 potassium-calcium and magnesium silicates (**Figure 4f-K, -Ca, -Mg, and -Cl**). In  
538 particular, calcium, sulphur and potassium contents tended to self-aggregate not only in  
539 ultrafine dimensions, but also in micrometric particles with an average dimension of

540 2.93±0.25 µm. The corresponding ED multi-mapping images show presence of metal  
541 microparticles of iron with average dimension of 2.63±0.37 µm and aluminum and  
542 magnesium silicates of 2.96±0.28 µm, while manganese still appeared in sub-micron  
543 dimension as ultrafine powders. The titanium dioxide still confined outside the  
544 macrometric object in ultrafine powder and calcium carbonate with dimensions of  
545 2.89±0.26 µm. [55] It should be noticed an absence of titanium dioxide into the  
546 macroaggregates may perhaps be imputable to the charging and salt concentration  
547 behaviors of the macrometric object and not to its melting temperature (1843° C). [56]  
548 The surface and chemical characterizations of the cellulose acetate fibers at  
549 mouthpiece side were also investigated after smoking (region III, **Figure 1**). This  
550 strategy would involve the possible tracing of metal-inorganic contaminants located at  
551 the external side of the cigarettes in order to show their potential toxicity because of the  
552 persistence and accumulative exposures to the environmental context (waste products)  
553 and in the human health (chemical oxidizing agents). Fibre filter-**T** of smoked cigarette  
554 physically in contact with human mouth still shows the existence of macroaggregates  
555 with irregular shape in **Figure 4g** compared to the globular shape detected in **Figure 4b**  
556 The dimension of the macroaggregate was estimated, having an area of 7997.04 µm<sup>2</sup>  
557 and radius of 142.79 µm mainly constituted by potassium-calcium-chlorine-sulphate-  
558 phosphorous and metal inorganic silicates (**Figure 4h**). The difference in size-shape  
559 could be attributed to the accumulation effect and different temperatures reached at  
560 different sides of the smoked filter, wherein high temperature of the burning processes  
561 is capable of shaping the macroaggregate at tobacco side. Metal contaminations of iron  
562 oxide were still present in small nanostructured microparticles with average dimension  
563 of 2.64±0.33 µm and silicates of aluminum and magnesium with 2.85±0.35 and  
564 4.38±0.48 µm, respectively. Furthermore, metal chromium content well-dispersed on  
565 melted fiber network in ultrafine dimensions appeared and manganese were not  
566 detected at the chosen magnification perhaps because of their smallest nanometric  
567 dimensions. The inorganic species self-aggregated into the macroaggregate in ultrafine  
568 dimension and in microparticles of 3.81±0.39 µm (phosphorous-sulphur) and 6.44±0.94  
569 µm (chlorine-potassium) in size. The additive of calcium carbonate and titanium dioxide  
570 aggregated in microparticles of 6.48±0.94 and 3.04±0.51 µm in size. Notice, same

571 macroaggregates formed by similar chemical specie have been recognized on fibre filter  
572 of the traditional cigarette at tobacco side after smoking. Therefore, toxic large particles  
573 may easily travel through the filter of estimated high porosity (54.34 %) and multi-  
574 dimensional pores ranging from 20 to 373  $\mu\text{m}$ . The critical meaning of these results are  
575 not only the toxic contaminants capable of reaching the human respiratory system, but  
576 they can also be collected and transported by saliva in the human body, considering  
577 also the frequently contacts with mouth and fingers. In the case of the smoked **hnbA**-  
578 cigarette, the last component at mouthpiece side is a hollow tube constituted of a thick  
579 sheet paper wrapped in another paper over-wrap. **Figure 4i** shows the internal sheet  
580 paper in which symmetrical oval voids are visible, probably used as ventilation holes.  
581 **Figure 4j** magnified region close to the hole clearly shows micrometric particles filling  
582 the fibre packing network that it is compromised by heating process compared to the  
583 sheet papers of the unsmoked cigarette studied before. Microparticles of metal  
584 inorganic silicates are clear visible in the EDS multi-mapping images. The main  
585 contents were constituted of aluminium silicate with dimension of  $7.09\pm 1.00\ \mu\text{m}$ , while  
586 manganese and potassium silicate had a dimension of  $4.96\pm 0.94\ \mu\text{m}$  and of  $8.30\pm 1.60$   
587  $\mu\text{m}$ , respectively. Potassium contents detected in microparticles was aggregated with  
588 aluminum and silicon probably forming an microparticles of volatile potassium aluminum  
589 silicate (usually mica). The metal inorganic silicates seem to be increased in size and  
590 shape compared to the detected ones on the smoked fiber filter of region II (**Figure 4d**).  
591 Moreover, the chemical and dimensional increment is also visible by observing the  
592 multi-mapping images of the sheet paper-**hnbA** of **Figure 2e**. Metal oxide contaminant  
593 of iron in oxide state occurred in ultrafine dimensions in coexisting phase with  
594 microaggregates of  $4.64\pm 0.88\ \mu\text{m}$  (**Figure 4j-Fe**). Calcium carbonate and titanium  
595 dioxide exhibited microparticles with dimensions of  $4.59\pm 0.39$  and  $2.89\pm 0.31\ \mu\text{m}$ . The  
596 inorganic specie constituted by sodium-phosphorous-sulphur-chlorine showed  
597 coexisting phases in ultrafine powder. Differently, the mouthpiece filter of smoked  
598 **hnbB**-cigarette seems less contaminated compare to the others (**Figure 4k**). Bright  
599 microparticles of  $4.05\pm 0.26\ \mu\text{m}$  in average size were mainly constituted by sodium-  
600 phosphorous-sulphur-potassium coexisting with ultrafine powder (**Figure 4l**). Metal  
601 silicates of aluminum and magnesium were formed mainly of ultrafine powder.

602 Existence of inorganic silicates of sodium and potassium could be confirmed by  
603 microaggregation of silicon with dimension of  $2.87\pm 0.21$   $\mu\text{m}$  in size. Calcium carbonate  
604 and titanium dioxide aggregated in small microparticles of  $2.84\pm 0.27$  and  $3.10\pm 0.26$   $\mu\text{m}$   
605 in presence also of ultrafine powders. Calcium carbonate, titanium dioxide and silicates  
606 are the main metal inorganic contaminations traced everywhere into the cigarettes. The  
607 calcium carbonate microaggregate considered a non-toxic inorganic material used for  
608 tuning the burning rate of cigarette in order to reduce the carbon monoxide delivery.  
609 However, it is notable that nanometric calcium carbonate fillers, founded in amount of  
610 about 22% or more in 30-40 wt.%, should not be underestimated since may cause  
611 irritation to nose, throat and respiratory system, as reported by New Jersey Department  
612 of Health (US) considered a hazardous substance. Furthermore, a nano- $\text{CaCO}_3$   
613 exposure was significantly associated with pulmonary hypofunction. [57] The role of  
614 titanium element in the filter, aggregating chemically in titanium dioxide particles  
615 especially in **T**-cigarettes, has been considered as an additive tool for capturing the  
616 toxic tobacco-specific nitrosamine *via* chemical absorption and for decomposing the  
617 filter waste by photodegradation processes (e.g. UV radiations of sunlight activation)  
618 [58, 59]. But, no doubt, toxicity studies have been also shown that  $\text{TiO}_2$  nanoparticles  
619 may induce acute inflammations of the nose/throat/lung by repeating dose inhalations  
620 [60, 61]. Aluminum and silicate particles, revealed both into **T**- and **hnb**-cigarettes, have  
621 been employed for reducing carbon monoxide and for manufacturing process as casing  
622 materials in cigarettes. But these toxic contents in crystalline aggregations may be  
623 responsible for respiratory disease [62, 63]. Exposure in large amount of silicate dust  
624 induces silicosis causing chronic inflammatory to nose and upper respiratory tract. [64]  
625 After smoking, the metal inorganic compounds evolved by growing in dimensionally on  
626 the fibre filters at both side of **Region II** and **III**, forming both macro and  
627 microaggregates into coexisting multi-phases. In this regard, the size of the  
628 microparticles have been plotted related to their chemical constituents (**Figure 5**);  
629 wherein the particles size plotted with 1  $\mu\text{m}$  are only an indicative representation of  
630 belonging to the ultrafine size with submicron dimension (cyan area, ultrafine powder  
631 region). Furthermore, the presence of fewest micrometric particles on analyzed area of  
632  $47628 \mu\text{m}^2$  have been neglect to be considered part of the ultrafine powder region.

633 The metal inorganic contaminants located on the **region II** show a similar trend of the  
634 sizes in which the metal elements (Mg, Al, Si, Ti, and Fe) self-aggregating in  
635 nanostructured micrometric particles (**Figure 5a**). Instead, the chemical elements  
636 forming inorganic substances (Na, P, S, Cl and K) aggregate mainly in ultrafine powder.  
637 In particular, fiber filter-**T** shows slightly reduced size of the metal inorganic  
638 contaminants and only heavy metal of iron oxide in microscopic scale occurred  
639 compared to the **hnb**-cigarettes almost similar in size among them. These differences  
640 can be attributed to the different manufacturing and packing processes in which the  
641 tobacco company of the **hnb**-cigarettes increased the quantity and size of the additive  
642 substances (EDS-elemental mapping of **Figure 3**), providing more toxic events and  
643 exposures to the hazards and risks to humans and ecosystems during smoking. This  
644 engineered strategy of the **hnb** products could be attributed to the smooth surface  
645 without pores of the metal sheet wrapping tobacco; wherein the promoted heating  
646 ventilation in altering smoke by chemical oxidative reactions is only focused in the fibre  
647 filters differently for the traditional cigarette.

648 In the **region II**, chemical specie detected on filters of smoked filter takes notice of the  
649 same chemistry finding in unsmoked, but it should be emphasized that the migrated  
650 microcompounds have slight reduced their sizes for the metal elements (**Figure 5b**).  
651 Conversely, particles increase their size on micrometric scale for the elements  
652 constituting inorganic species (Na, P, S, Cl and K) especially for the smoked fibre filter-  
653 **T**; probably due to the burning processes and puffing intensity able to charge the smoke  
654 of more toxic contaminants, traveling toward the tobacco column. Indeed, the inorganic  
655 species revealed in the paper sheet and tobacco increased their size from ultrafine  
656 dimension to final grew in particle of micrometric dimension on the filter after smoking.  
657 On the other hand, the metal element contributions reduced in size on the fibre filters  
658 that should be attributed to the burning and heating temperatures since the heating  
659 temperature in the **hnb**-cigarettes might reach about 300° C, while the burning  
660 processes in the traditional cigarettes may reach a temperature of about 700° C. [65,  
661 66] Therefore, the combustion and degradation of the metal inorganic substances  
662 occurred in the cigarettes could be considered less for **hnb**-cigarettes. But it is relevant  
663 to consider that the amount of the carrier particles (smoke) increase in the tobacco

664 column of the **hnb**-cigarettes because of the absent of porosity in the metal sheet,  
665 thereby the amount of the carried toxic substances originated from the treated tobacco  
666 stick increase also in ultrafine dimensions. Additional evidences in increasing the  
667 amount of the inorganic specie are the revealed macro-objects. This experimental result  
668 represents a remarkable evidence that the persistence of the smoking is capable of  
669 accumulating substances in micrometric and ultrafine dimension to build up hazardous  
670 macro-agglomerates with time by heating flow. By evaluating the chemical-size of the  
671 metal elements (Mg, Al, Si, Ti, *Mn* and Fe) belonging to microaggregates after smoking,  
672 they also taken part to the thermal decomposition (pyrolysis, pyrosynthesis and  
673 combustion) with decreasing in size in particular for the additive magnesium, calcium  
674 and titanium. It is interesting to notice the appearance for the first time of manganese in  
675 ultrafine powder in all smoked filter cigarettes and chromium for traditional one, which is  
676 still an evidence that the migration processes of heavy metal particles in ultrafine  
677 dimension through the tobacco column occurred. Therefore, ultrafine powders of heavy  
678 metal contaminants may be carried easily by mainstream smoke including also passive  
679 diffusion for which has been also established acute adverse effect on respiratory  
680 function for active or passive smokers. [67] Actually, these toxic elements belonging to  
681 heavy metals can easily reach the pulmonary system for which has been detected in  
682 highest amount in smokers than those in nonsmokers. [68]

683 By comparing the particle contaminants with the smoked filter at mouthpiece side  
684 (**Figure 5c**), metal inorganic elements increased in size in particular for **T**-cigarettes in  
685 which the macro-objects unfortunately were still observed. The size contaminants of  
686 fiber filter-**hnbA** show an excessive increment, probably due to the different component  
687 analyzed of the cigarette of **Region III**. Indeed, the sheet paper-**hnbA** shows increased  
688 size of particle especially for the metal element attributable to the silicates contaminants  
689 already detected in the others sheet paper but with less amount (**Figure 1 and 2**). Yet  
690 from a toxicological perspective, aluminium silicates may accumulate in the lungs after  
691 smoking for seven to seventy-five pack years [69].The inorganic elements of the fiber  
692 filter-**hnbB** attributable to the effective toxic substance concretely increased in size. The  
693 heavy metal contaminants are strongly present in size for iron oxide in the traditional  
694 and **hnbA** fiber filters. This is a first direct evidence that particles subjected of burning

695 and heating processes are volatile in the cigarettes capable of traveling and aggregating  
696 through the fibre filter in both traditional and heat-not-burn cigarettes. These results  
697 concretely confirm that the smoke may be considered as a particles carrier of toxic  
698 elements.

699 The undesirable inorganic substances, well known for major contamination events and  
700 exposures, might be chemically associated to the formation of particulate phase  
701 consisting of volatile substances: calcium carbonate, sodium-sulphur-carbonate  
702 chloride, sodium-phosphorous-potassium sulphide, sodium phosphide and magnesium  
703 sodium salt. From toxicological perspectives, there is a large literature on adverse  
704 health effects of the toxic inorganic aggregate, in particular the vapour phase flow in the  
705 filter releasing volatile sulphuric compounds raising from manufacturing processes of  
706 the cellulose fibres. [70] Potassium sulphate and chloride are evident other organic  
707 particulate in cigarettes. [71] Breathing small amounts of these particulate inorganic  
708 agglomerate for short periods of time has adversely effects on human respiratory  
709 system by irritating eyes, nose, throat, lungs with sneezing, coughing and sore throat.  
710 [72]

711

#### 712 **4. Conclusion**

713 A deep look into the self-assembling behavior of nanostructured microparticles revealed  
714 in three different brands of cigarettes have been locally investigated in their morpho-  
715 chemical aspects at microscopic scale. The approach here used for investigating the  
716 metal inorganic microparticles provides a direct experimental evidences of their ability to  
717 migrate within the cigarette carried by gaseous mainstream smoke capable of creating  
718 microscopic building blocks of different size, shape, and composition. To get an insight  
719 into the self-assembly behavior of the microparticles, electron microscopy imaging  
720 techniques supported by appropriate imaging analyses have been exploited, paying  
721 attention to the high-sensitivity of the organic species to the electron beam.

722 As seen, by combining experimental electron microscopy techniques supported by  
723 simple imaging analyses, we were able to determine several morpho-chemical features:  
724 i) the morpho-chemical investigations on the paper sheet (**T-**, **hnbA-** and **hnbB-**  
725 cigarettes) have determinate the ability of the metal inorganic nanostructured



726 microparticles to be assembled into a fibre packing network. These compounds, as  
727 manufacturing fillers and burn additive contents, were mainly detected in chemistry and  
728 size-shape (metal inorganic silicates, chlorides, sulphides, salts, etc.). Unfortunately,  
729 high toxic heavy metals have been also detected on the paper or metallic sheet and into  
730 the tobacco, showing large microparticles constituted by Vanadium, Chromium, Iron,  
731 Nickel, Copper, and Uranium as hazardous ingredients for natural environment and  
732 human health (**Figure 1** and **2**); *ii*) Fibre filters of unsmoked cigarettes have been  
733 investigated in terms of morphometric porosity related to the direct evidence of metal  
734 inorganic contaminants observed. The quantitative porous analyses establish that the  
735 real aspect of occurred migration of the detected heavy metal microparticles (Iron-Nickel-  
736 Copper-Osmium), including metal inorganic silicate and more chemicals oxidizing  
737 agents. Although, these first evidences of pollutants strictly dependent from the  
738 manufacturing and packing processes allow some production flexibility in this regard, it  
739 may also prove the need of employing different manufacturing processes to protect the  
740 nature and human health (**Figure 3**); *iii*) for expanding the focus of the presented  
741 research, the smoked fibre filters were analyzed for tracing the metal inorganic  
742 contaminations. At tobacco side, heavy metal contaminations of manganese and  
743 chromium have migrated through the tobacco column to reach the burned fiber filter.  
744 The findings of this study show the hazardous role of the smoking, which behaves like a  
745 carrier not only for hazardous particles, but also for inorganic poisonous gas developed  
746 by the burned plastic fibres together with chemicals oxidizing agents at nanoscopic  
747 scale. These results are strongly supported by the evidence of macro-objects due to the  
748 persistence and accumulative of smoking capable of carrying high amount of particles  
749 to be self-assembled into a coexisting phases of a macrometric agglomerate (**Figure 4**).  
750 The formed macroaggregates on the smoked filter at mouthpiece side can be explained  
751 by considering that the manufactured filters are not an obstacle for the toxic volatile  
752 substances of large and variable sizes, as demonstrated by the porosity study  
753 presented here; *iv*) Interestingly, the evolution study on the particle size before and after  
754 smoking represent a concrete result of the potential hazardous of smoke and the  
755 unsuitable engineered filters produced by the tobacco company. This is well established  
756 by the experimental analyses, wherein particle size ranging from 2.5 to 7  $\mu\text{m}$  in size are

757 capable of traveling throughout the fibre filter in both traditional and heat-not-burn  
758 cigarettes (**Figure 5**). The direct evidence of macro-objects in coexisting multi-phases  
759 shows the unambiguous effect of the smoke capable of accumulating massive load of  
760 particles to form agglomerate of dimension ranging from 20 to 150  $\mu\text{m}$ . Therefore, these  
761 results represent a concrete evidence of the dangerous smoke as a particles carrier of  
762 toxic elements.

763 Finally, these results provide concrete informations for all scientific aspects in helping  
764 tobacco researchers to understand the mechanisms of smoke formation and useful not  
765 only for understanding the formation of any type of metal inorganic contaminants, but  
766 also for tailoring specific microfiber materials that represents a current challenging  
767 research in catching the elusive toxic microparticle in gaseous media to avoid the  
768 transport of toxic chemicals for the delicate natural environment consequently for the  
769 human respiratory tract.

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### 773 **Declaration of Competing Interest**

774 The authors declare that they have no known competing financial interests or personal  
775 relationships that could have appeared to influence the work reported in this paper.

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777

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- <sup>1</sup> Eitzinger, B., Gleinser, M., Bachmann, S. and Volgger, D. The Pore Size Distribution of Naturally Porous Cigarette Paper and its Relation to Permeability and Diffusion Capacity. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research* **26**, 7 (2015).
- <sup>2</sup> Shen, J., Li, J., Qiana, X., Ren, W., Fatehic, P. A review on engineering of cellulosic cigarette paper to reduce carbon monoxide delivery of cigarettes. *Carbohydrate Polymers* **10**, 769–775 (2014).
- <sup>3</sup> Royer, S. and Duprez D. Catalytic Oxidation of Carbon Monoxide over Transition Metal Oxides. *ChemCatChem* **3**, 24–65 (2011).
- <sup>4</sup> Zhu, X., Hu, B. & Jiang, Z. Cloud point extraction combined with graphite furnace atomic absorption spectrometry for the determination of chromium species and their distribution in cigarette and cigarette ash. *Intern. J. Environ. Anal. Chem.* **84**, 927–934 (2004).
- <sup>5</sup> Shen, J., Li, J., Qiana, X., Ren, W., Fatehic, P. A review on engineering of cellulosic cigarette paper to reduce carbon monoxide delivery of cigarettes. *Carbohydrate Polymers* **10**, 769–775 (2014).
- <sup>6</sup> U.S. Department of Health and Human Services. *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, (2010).
- <sup>7</sup> Zumbado, M., Luzardo, O. P., Rodríguez-Hernández, Á., Boada, L. D., Henríquez-Hernández, L. A. Differential exposure to 33 toxic elements through cigarette smoking, based on the type of tobacco and rolling paper used. *Environmental Research* **169**, 368–376 (2019).
- <sup>8</sup> Caruso, R. V., O'Connor, R. J., Stephens, W. E., Cummings, K. M., and Fong, G. T. Toxic Metal Concentrations in Cigarettes Obtained from U.S. Smokers in 2009: Results from the International Tobacco Control (ITC) United States Survey Cohort. *Int. J. Environ. Res. Public Health* **11**, 202-217 (2014).
- <sup>9</sup> Scientific Committee on Health, Environmental and Emerging Risks *SCHEER*. Opinion on Additives used in tobacco products (Tobacco additives II), (2016).
- <sup>10</sup> Connolly, G. N., Wayne, G. D., Lymperis, D., Doherty M. C. How cigarette additives are used to mask environmental tobacco smoke. *Tobacco Control* **9**, 283–291 (2000).
- <sup>11</sup> Luceri, F., Pieraccin, G., Moneti, G., and Dolara, P. Primary aromatic amines from side-stream cigarette smoke are common contaminants of indoor air. *Toxicology and Industrial Health.* **9**, 405-413 (1993).
- <sup>12</sup> Politi, S., Carcione, R., Tamburri, E., Matassa, R., Lavecchia, T., Angjellari, M. & Terranova, M. L. Graphene platelets from shungite rock modulate electropolymerization and charge storage mechanisms of soft-template synthesized polypyrrole-based nanocomposites. *Scientific Reports* **8**, 17045 (2018).
- <sup>13</sup> Marinello, S., Lolli, Gamberini, F. R., Rimini, B. A second life for cigarette butts? A review of recycling solutions. *Journal of Hazardous Materials* **384**, 121245 (2020).
- <sup>14</sup> Chevalier, Q., El Hadri, H., Petitjean, P., Bouhnik-Le Coz, M., Reynaud, S., Grassl, B., Gigault, J. Nano-litter from cigarette butts: Environmental implications and urgent consideration. *Chemosphere* **194**, 125e130 (2018).

- 
- <sup>15</sup> Bonanomi, G., Incerti, G., Cesarano, G., Gaglione, S. A., Lanzotti, V. Cigarette Butt Decomposition and Associated Chemical Changes Assessed by <sup>13</sup>C CPMAS NMR. *PLoS One*. **10**, e0117393 (2015).
- <sup>16</sup> Järup, L. Hazards of heavy metal contamination. *British Medical Bulletin* **68**, 167–182, (2003).
- <sup>17</sup> Mulay, S. R., Anders, H. J. Crystallopathies. *N Engl J Med* **374**, 2465-2476 (2016).
- <sup>18</sup> Mulay, S. R., Anders, H. J. Crystallopathies. *N Engl J Med* **374**, 2465-2476 (2016).
- <sup>19</sup> Marinello, S., Lolli, Gamberini, F. R., Rimini, B. A second life for cigarette butts? A review of recycling solutions. *Journal of Hazardous Materials* **384**, 121245 (2020).
- <sup>20</sup> Abu-Danso, E., Bagheri, A., Bhatnagar, A. Facile functionalization of cellulose from discarded cigarette butts for the removal of diclofenac pharmaceutical from water. *Carbohydrate Polymers* **219**, 46-55 (2019).
- <sup>21</sup> Bossù, M., Matassa, R., Relucenti, M., Iaculli, F., Salucci, A., Di Giorgio, G., Familiari, G., Polimeni, A., Di Carlo, S. Morpho-Chemical Observations of Human Deciduous Teeth Enamel in Response to Biomimetic Toothpastes Treatment. *Materials* **11**, 1803 (2020).
- <sup>22</sup> Matassa, R., Familiari, G., Battaglione, E., Sibilia, C., Leahu, G., Belardini, A., Venditti, I., Fontana, L., and Fratoddi I. Electron Microscopy Reveals Layered Architecture of Individual Gold Nanoparticles Self-Anchored by Fluorescence Monomers. *Nanoscale* **8**, 18161-18169, (2016).
- <sup>23</sup> Bearzotti, A., Papa, P., Macagnano, A., Zampetti, E., Venditti, I., Fioravanti, R., Fontana, L., Matassa, R., Familiari, G. and Fratoddi I. Environmental Hg vapours absorption and detection by using functionalized gold nanoparticles network. *Journal of Environmental Chemical Engineering* **6**, 4706-4713, (2018).
- <sup>24</sup> Delfini, A., Santoni, F., Bisegna, F., Piergentili, F., Pastore, R., Vricella, A., Albano, M., Familiari, G., Battaglione, E., Matassa, R., Marchetti M. Evaluation of atomic oxygen effects on nano-coated carbon-carbon structures for re-entry applications. *Acta Astronautica* **161**, 276-282 (2019).
- <sup>25</sup> Zoratto, N., Matassa, R., Montanari, E. *et al.* Glycerol as a green solvent for enhancing the formulation of dextran methacrylate and gellan-based semi-interpenetrating polymer networks. *J Mater Sci* **55**, 9562–9577 (2020).
- <sup>26</sup> Zanutto, A., Matassa, R., Saladino, M. L., Berrettoni, M., Giorgetti, M., Zamponi, S., Caponetti, E. Cobalt Hexacyanoferrate-Poly(MethylMethacrylate) Composite: Synthesis and Characterization. *Mat. Chem. Phys.* **120**, 118-122 (2010).
- <sup>27</sup> Toschi, F., Orlanducci, S., Guglielmotti, V., Cianchetta, I., Magni, C., Terranova, M. L., Pasquali, M., Tamburri, E., Matassa, R. Hybrid C-nanotubes/Si 3D nanostructures by one-step growth in a dual-plasma reactor. *Chem. Phys. Lett.* 539-540, 94-101, (2012).
- <sup>28</sup> Reina, G., Tamburri, E., Orlanducci, S., Gay, S., Matassa, R., Guglielmotti, V., Lavecchia, T., Terranova, M. L. Nanocarbon surfaces for biomedicine. *Biomatter*, **4**, e28537, (2014).
- <sup>29</sup> Orlanducci, S., Toschi, F., Guglielmotti, V., Cianchetta, I., Magni, C., Tamburri, E., Terranova, M. L., Matassa R. A viable and scalable route for the homo-growth of Si nanocones and Si/C nanostructures. *Cryst. Growth Des.* **12**, 4473-4478, (2012).
- <sup>30</sup> Halstead, M. M., Watson, C. H. and Pappas, R. S. Electron Microscopic Analysis of Surface Inorganic Substances on Oral and Combustible Tobacco Products. *J Anal Toxicol.* **39**, 698–701 (2015).

- 
- <sup>31</sup> Khan, S., Mulvaney, R., & Ellsworth, T. The potassium paradox: Implications for soil fertility, crop production and human health. *Renewable Agriculture and Food Systems*, **29**, 3-27 (2014).
- <sup>32</sup> Baz, S. S. Qualitative and Quantitative analysis of some imported cigarette samples locally used in Saudi Arabia by Scanning Electron Microscope and Energy Dispersive Spectroscopy *Journal of American Science* **11**, 121-128 (2015).
- <sup>33</sup> Talhout, R. Speciation of Metals and Metalloids in Tobacco and Tobacco Smoke - Implications for Health and Regulation. National Institute for Public Health and the Environment, Bilthoven, The Netherlands (2014).
- <sup>34</sup> Sowder, A. G., Bertsch, P. M. and Morris P. J. Partitioning and Availability of Uranium and Nickel in Contaminated Riparian Sediments. *J. Environ. Qual.* **32**, 885–898 (2003).
- <sup>35</sup> Shen, J., Li, J., Qiana, X., Ren, W., Fatehic, P. A review on engineering of cellulosic cigarette paper to reduce carbon monoxide delivery of cigarettes. *Carbohydrate Polymers* **10**, 769– 775 (2014).
- <sup>36</sup> Connolly, G.N., Wayne, G.D., Lymperis, D. Doherty, M. C. How cigarette additives are used to mask environmental tobacco smoke. *Tobacco Control* **9**, 283-291 (2000).
- <sup>37</sup> Pua, E. C., & Trevor, A. Differential Na<sub>2</sub>SO<sub>4</sub> tolerance in tobacco plants regenerated from Na<sub>2</sub>SO<sub>4</sub>-grown callus *Plant, Cell & Environment* **9**, 9-16 (1986).
- <sup>38</sup> Eitzinger, B., Gleinser, M., Bachmann, S. and Volgger, D. The Pore Size Distribution of Naturally Porous Cigarette Paper and its Relation to Permeability and Diffusion Capacity. *Beiträge zur Tabakforschung International/ Contributions to Tobacco Research* **26**, 7 (2015).
- <sup>39</sup> Halstead, M. M., Watson, C. H., Pappas, R.S. Electron Microscopic Analysis of Surface Inorganic Substances on Oral and Combustible Tobacco Products. *J Anal Toxicol.* **39**, 698-701 (2015).
- <sup>40</sup> Koutela, N., Fernández, E., Saru, M. L., Psillaki, E. A comprehensive study on the leaching of metals from heated tobacco sticks and cigarettes in water and natural waters. *Science of the Total Environment* **714**, 136700 (2020).
- <sup>41</sup> Schaller, J.P., Keller, D., Poget, L., et al. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. *Regul Toxicol Pharmacol.* **81**, S27-S47 (2016).
- <sup>42</sup> Zumbado, M., Luzardo, O. P., Rodríguez-Hernández, Á., Boada, L. D., Henríquez-Hernández, L. A. Differential exposure to 33 toxic elements through cigarette smoking, based on the type of tobacco and rolling paper used. *Environmental Research* **169**, 368–376 (2019).
- <sup>43</sup> Burton, E.D., Choppala, G., Karimian, N., Johnston, S. G. A new pathway for hexavalent chromium formation in soil: Fire-induced alteration of iron oxides. *Environ Pollut.* **247**, 618-625 (2019).
- <sup>44</sup> Halstead, M. M., Watson, C. H. and Pappas, R. S. Electron Microscopic Analysis of Surface Inorganic Substances on Oral and Combustible Tobacco Products. *J Anal Toxicol.* **39**, 698–701 (2015).
- <sup>45</sup> Kozłowski, L. T., O'Connor, R. J. Cigarette filter ventilation is a defective design because of misleading taste, bigger puffs, and blocked vents. *Tob Control.* **11**, i40-i50 (2002).
- <sup>46</sup> Chelucci, G., Baldino, S., Baratta, W. Recent advances in osmium-catalyzed hydrogenation and dehydrogenation reactions. *Acc Chem Res.* **48**, 363-379 (2015).

- 
- <sup>47</sup> Lucena, M. C., Alencar, A. E. V., Mazzeto, S. E., Soares, S. A. The effect of additives on the thermal degradation of cellulose acetate. *Polymer Degradation and Stability* **80**, 149–155 (2003).
- <sup>48</sup> Torikaiu, K., Uwano, Y., Nakamori, T., Tarora, W., Takahashi H. Study on tobacco components involved in the pyrolytic generation of selected smoke constituents. *Food and Chemical Toxicology* **43**, 559–568 (2005).
- <sup>49</sup> Masoudi Soltani, S., Yazdi, S.K. & Hosseini, S. Effects of pyrolysis conditions on the porous structure construction of mesoporous charred carbon from used cigarette filters. *Appl Nanosci* **4**, 551–569 (2014).
- <sup>50</sup> Lian, W., Ren, F., Liu, Q. *et al.* Evaluation of metal-doped manganese oxide octahedral molecular sieves in catalytic reduction of NO<sub>x</sub> from cigarette mainstream smoke. *J. Cent. South Univ. Technol.* **19**, 918–922 (2012).
- <sup>51</sup> Bakand, S., Hayes, A., Dechsakulthorn, F. Nanoparticles: a review of particle toxicology following inhalation exposure. *Inhal Toxicol.* **24**, 125-135 (2012).
- <sup>52</sup> Shen, J., Li, J., Qiana, X., Ren, W., Fatehic, P. A review on engineering of cellulosic cigarette paper to reduce carbon monoxide delivery of cigarettes. *Carbohydrate Polymers* **10**, 769– 775 (2014).
- <sup>53</sup> Brun, P., Kunz, A., Funke, M. Buried under gypsum powder - A rare respiratory complication. *Respir Med Case Rep.* **8**, 36-39 (2013).
- <sup>54</sup> Scientific Committee on Health, Environmental and Emerging Risks *SCHEER*. Opinion on Additives used in tobacco products (Tobacco additives II), (2016).
- <sup>55</sup> Krалева, E., Saladino, M. L., Matassa, R., Caponetti, E., Enzo, S. and Spojakina A. Phase formation in mixed TiO<sub>2</sub>-ZrO<sub>2</sub> oxides prepared by sol-gel method. *Journal of Structural Chemistry* **52**, 330-339 (2011).
- <sup>56</sup> Holmberg, J.P., Ahlberg, E., Bergenholtz, J., Hassellöv, M., Abbas, Z. Surface charge and interfacial potential of titanium dioxide nanoparticles: experimental and theoretical investigations. *J Colloid Interface Sci.* **407**, 168-176 (2013).
- <sup>57</sup> Li, G., Liang, L., Yang, J., *et al.* Pulmonary hypofunction due to calcium carbonate nanomaterial exposure in occupational workers: a cross-sectional study. *Nanotoxicology.* **12**, 571-585 (2018).
- <sup>58</sup> Deng, Q., Huang, C., Zhang, J., Xie, W., Xu, H., Wei, M. Selectively reduction of tobacco specific nitrosamines in cigarette smoke by use of nanostructural titanates. *Nanoscale* **5**, 5519-5523 (2013).
- <sup>59</sup> Slimen, H., Ochiai, T., Nakata, K., Murakami, T., Houas, A., Morito, Y. and Fujishima A. Photocatalytic Decomposition of Cigarette Smoke Using a TiO<sub>2</sub>-Impregnated Titanium Mesh Filter *Industrial & Engineering Chemistry Research* **51**, 587-590 (2012).
- <sup>60</sup> Scientific Committee on Health, Environmental and Emerging Risks *SCHEER*. Opinion on Additives used in tobacco products (Tobacco additives II), (2016).
- <sup>61</sup> Williams, M., Villarreal, A., Bozhilov, K., Lin, S., Talbot, P. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. *PLoS One.* **8**, e57987 (2013).
- <sup>62</sup> Farsalinos, K.E., Polosa, R. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review. *Ther Adv Drug Saf.* **5**, 67-86 (2014).
- <sup>63</sup> Williams, M., Villarreal, A., Bozhilov, K., Lin, S., Talbot, P. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. *PLoS One.* **8**, e57987 (2013).

- 
- <sup>64</sup> Brun, P., Kunz, A., Funke, M. Buried under gypsum powder - A rare respiratory complication. *Respir Med Case Rep.* **8**, 36-39 (2013).
- <sup>65</sup> Auer, R., Concha-Lozano, N., Jacot-Sadowski, I., Cornuz, J., Berthet, A. Heat-Not-Burn Tobacco Cigarettes: Smoke by Any Other Name. *JAMA Intern Med.* **177**, 1050-1052 (2017).
- <sup>66</sup> Masoudi Soltani, S., Yazdi, S.K. & Hosseini, S. Effects of pyrolysis conditions on the porous structure construction of mesoporous charred carbon from used cigarette filters. *Appl Nanosci* **4**, 551–569 (2014).
- <sup>67</sup> Kopa, P.N., Pawliczak, R. IQOS - a heat-not-burn (HnB) tobacco product - chemical composition and possible impact on oxidative stress and inflammatory response. A systematic review. *Toxicol Mech Methods.* **30**, 81-87 (2020).
- <sup>68</sup> Yu, Y. L., Zhuang, Y. T., Song, X. Y. Wang, J. H. Lyophilized carbon nanotubes/graphene oxide modified cigarette filter for the effective removal of cadmium and chromium from mainstream smoke. *Chemical Engineering Journal* **280**, 58–65 (2015).
- <sup>69</sup> Halstead, M. M., Watson, C. H. and Pappas, R. S. Electron Microscopic Analysis of Surface Inorganic Substances on Oral and Combustible Tobacco Products. *J Anal Toxicol.* **39**, 698–701 (2015).
- <sup>70</sup> Hoffmann, D., Hoffmann I. The changing cigarette, 1950-1995. *J Toxicol Environ Health.* **50**, 307-364 (1997).
- <sup>71</sup> Pappas, R. S., Halstead, M. M. and Watson, C. H. Electron Microscopic Analysis of Silicate and Calcium Particles in Cigarette Smoke Tar. *Int J Respir Pulm Med.* **3**, 3:039 (2016).
- <sup>72</sup> Baz, S. S. Qualitative and Quantitative analysis of some imported cigarette samples locally used in Saudi Arabia by Scanning Electron Microscope and Energy Dispersive Spectroscopy. *Journal of American Science* **11**, 121-128 (2015).