Direct Evidences of Metal Inorganic Traces into Cigarettes

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Highlights

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GRAPHICAL ABSTRACT

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ABSTRACT

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

1. Introduction

Contributo Clinico

Sheet paper wrapping tobacco is engineered to be a porous system, consisting of a thin squeezed cellulose fibers arranged in micro-mesh structures filled by additive compounds. The amount and size of the fillers are presumably engineered in order to provide appropriate porosity useful for exchanging air through the sheet paper.[1] By tailoring the chemistry and amount of the filler and burn contents, the air flow passing through the paper should be aimed to convert the carbon monoxide production in less toxic carbon dioxide, this believing expedient of less toxicity task by catalytic oxidation has been widely studied.[2, 3, 4] In most cases, addition of a water-soluble salt (sodium bicarbonate, potassium bicarbonate or ammonium carbonate) to cigarette paper has been identified in order to increase the diffusion capacity to decrease carbon monoxide in cigarette smoke. [5] Unfortunately, the possibility of appearance of chronic diseases, the persisting inhalation of higher concentrations of CO_2 can affect respiratory function in mucosal irritation, contributing to myocardial ischemia and causing excitation followed by high risk for clinical depression.[6] In the additive substances context, different heavy metals compounds, entrapped in the sheet paper, have been involved for catalytic conversions of carbon monoxide mediated by paper porosity, during puffing. The heavy metal oxides inorganic substance introduced in the paper sheet have been assumed in form of particles and/or ultrafine powders mainly constituted of magnesium, silicon, aluminium, chromium, iron, copper, nickel, zinc, and etc..[7, 8] Moreover, the metal inorganic particles doping the cigarette paper are also used for reducing odour, visibility and emission of the side-stream smoke (calcium carbonate, magnesium oxide, magnesium carbonate, sodium acetate, sodium citrate and etc..). The particles become smaller for reducing the odour due to the thermal degradation during puffing, whereas other additive particles encapsulate the smoke for reducing both visibility and emission using potassium succinate, potassium citrate and magnesium carbonate. [9, 10, 11]

The recent development in the manufacturing cigarette related to the pathologies cannot be analyzed only by considering the contaminants in the sheet paper. The explanation has to sought also in the other components of the cigarette. The fundamental role of the filter in the cigarette is still ambiguous since it is not clear how much the filter can remove the toxic contaminants carried by gaseous smoke. Furthermore, an immediate question arises of whether the same filter may contain undesirable contaminants introduced by manufacturing processes, by which the same contaminants could be probably removed from the filter by heating smoke to be unfortunately inhaled. Actually, the alone sheet paper cannot be aimed to the significant conversion of the carbon monoxide and of other smoking targets. Therefore, the desired catalytic conversions have been also supported by adding metal inorganic compounds within the cellulose acetate fibre of the filter.[12] The dramatic effect on the natural environmental of the smoked filter is well know that cigarette butt is a plastic litter an hazardous pollutant for the human habitat. The major critical effect on the nature health (embracing human life) is the continue and increase of releasing heavy metals entrapped into the cigarette butts, creating a dangerous densely reservoir able to disperse free particulate of lead, cadmium, mercury and arsenic, etc..[13]. The uncontrolled free particulate is mainly constituted of chemical complex particles identified in nanometric size and more by dynamic light scattering and cp mas nmr spectroscopy techniques. [14, 15] Such exposure to the hazardous heavy metals has been classified of responsible as a human carcinogen.[16] Basically, microparticles and nanoparticles in ultrafine powder might be released into natural environment causing adverse changes and into human respiratory, cardiovascular, nervous, and reproductive systems. The adverse effects of the particles and particulate matter have well reported by Mulay et. al.. [17] Indeed, particle size is a critical determinant for macrophages and other phagocytes, these are the first cells to engulf particles for phagocytosis possible for microparticles of a fewest microns in size and easy for nanoparticles. Therefore, the micrometric pollutants need accurately characterize in terms of size-shape and of chemical reactive oxygen species since agglomerations of particles increasing their dimension easily overloading phagolysosomes. This root easily conducts to the metabolic storage diseases killing the cells followed by cangerogenis effect. [18] Recently, the adverse effect of filter butts on the natural environmental have been investigated in order to convert the smoked filter in harmless tools for suppling different industrial sectors [19, 20]. Therefore, this useful sustainable chemistry research need more knowledge on chemistry and size of the elusive particles contaminating the smoked cigarette especially for the filter or butt.

Herein, the morpho-chemical investigations of commercial cigarettes of three different brands have been focused on the evolution growth of micro-aggregate and microparticle linked to the effects of smoking traditional (**T**-cigarette) and two heat-not-burn cigarettes (**hnbA**- and **hnbB**-cigarettes). This work aims at pooling information from an appropriate morpho-chemical characterization useful for tracing the particles related their chemical contents to achieve desirable analyses of their evolution, studying three different regions of the cigarette before and after smoking. Morphological and chemical characterizations of three different regions of the cigarette illustrated in **Figure 1?** have been investigated in order to show quantitatively the presence of possible micrometric pollutants. This evaluation strategy provides a means to our understanding on finding the possible criteria of migration of the micrometric pollutants from the inner to outer of the cigarettes. To obtain insight into the self-assembly behavior of these hybrid materials at the microscopic scale, a combination of electron microscopy techniques of VP-SEM, EDS multi-mapping analysis, and image processing techniques [21] have been exploited in order to obtain all possible experimental information for exploring the following objectives: *i*) morphometric features of the porosity and micrometric particles assembled into a fibre packing network of sheet paper and their corresponding chemical microanalyses (**Figure 1 and 2**), *ii*) fiber porosity of unsmoked filters and the contaminants deposited on (**Figure 3**), *iii*) direct evidences of the particles in micrometric and ultrafine dimension identified on the fibre surface of smoked filter characterized by chemical spatial distribution imaging (**Figure 4**). *iv*) evolution growth of the metal inorganic contaminants in terms of changing size associated to their chemical elements on the two different regions of the unsmoked and smoked filters (**Figure 5**). Appropriate imaging analyses have been exploited to perform accurate quantitative measurements of size-shape and of chemical spatial distribution of the individual particles, paying attention to the statistical analysis. [22] Since the growth size-shape of the metal inorganic materials represents an important step in understanding their brutal chemical activities, appropriate observations on the evolved contaminants have provided some considerations focusing on the adverse property of the smoke as a particles carrier of toxic elements. In fact, our morpho-chemical observations have been essential for showing the tremendous effect of the smoke capable of bringing high amount of particles to be self-assembled into a coexisting phases of a large agglomerate because of the persistence and accumulative of puffing. Finally, integrate the above scientific objectives in a synergistic manner have been essential for understanding the directed evidence of the unwanted assembly dependent on the chemical-size-shape contaminants and on the manufacturing engineering involved in the three different brands presented, here.

2. Experimental

2.1. Materials

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

2.2. Preparations Samples

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

2.3 Variable Pressure Scanning Electron Microscopy

All components of the cigarette, paper and metallic sheets and fiber filters, were observed using a variable pressure scanning electron microscopy (VP-SEM, Hitachi SU-3500) supported by dual energy dispersive X-ray spectroscopy detectors (dEDS) arranged in parallel configuration (Bruker, XFlash® 6|60) able to high sensitivity elemental analysis by their large active area of a 60 mm^2 each. The samples were directly settled onto a carbon planchet stub without conductive coating. [23, 24] By appropriate control of the chamber pressure, particular attention was paid to avoid crack formations of the content structures. [25] All samples were observed at an accelerating voltage depending on the features of the pressure used in the chamber to avoid radiation damage and drifting image, fatal for EDS multi-mapping analyses. Furthermore, the experimental approach focused on detecting as much information as possible, thereby the morpho-chemical investigation was concretized at low magnification in order to improve the statistical observation/analysis of the nanostructured microparticles. [26] Therefore, it should be noted that eventual metal inorganic contaminations could be detected at high magnification to show the existence of specie under microscopic scale, but our scientific research was focusing on hazardous particle up to micrometric scale. [27, 28, 29]

3. Results and Discussion

Morphological and chemical characterizations of three different regions of the cigarette were investigated in their native states using low vacuum VP-SEM-dEDS (**Figure 1?)**. By this investigation strategy, our intention is to reveal directly by imaging and tracking techniques the progressive evolution of the elusive metal inorganic particles on local micrometric regions because of the adverse effect of the smoke. The choice of these regions is based on the main origins of contaminations (because they participate directly to the chemical and physics mechanisms (thermal decomposition (pyrolysis, pyrosynthesis and combustion)) of burning and heating processes of the traditional and heat-not-burn cigarettes.), before and after smoking. **Region I** focused on the inner sheet wrapping tobacco were examined, as shown in **Figure 1** and **2**. A manufactured tobacco leaf laying on the paper sheet-**T** was observed (**Figure 1a)**. In particular, the surface of the leaf evidences the dehydrated thickness walls of xylem vessels reinforced with cellulose and lignin polymer fibres, forming an annular and reticulate shape (orange arrowheads). Magnification of **Figure 1a** better shows squeezed and dried tubular vessel possessing a thickness wall of about 3.50 μm covered by bright particles of quasi-rectangular shape (red short dashed line, **Figure 1b**). The further presented investigation of the tobacco has been considered in order to reveal an eventual correlation with the origin of the contaminant particles. The morphometric dimension of the bright particles has been correlated with their chemical constituents by EDS spectra and with the chemical spatial distribution using EDS multi-elemental images. EDS multi-mapping was capable to identify micrometric and nanometric contaminants constituted of aluminium, magnesium and calcium silicates by investigating the peak intensity of the spectrum within a selected micrometric region of interest and observable by overlaying **Figures 1b-OAl, 1b-Si**, **1b-Mg** and **1b-Ca** (second letters are referred both to the chemical element and EDS mapping images). Metal inorganic silicates containing particles deposited on the leaf surfaces were presumably derived from trapping soil particles. [30]. Inorganic calcium carbonate is clearly visible by overlaying the C and Ca elements in **Figure 1b-CCa,** aggregating in micro and nanometric particles. Sodium chloride can be recognized by merging similar color shape or texture between **Figure 1b-Na** and **1b-Cl**. The bright particles of about 3.0 μm in size are shown by overlaying **Figure 1b-S** and **1b-K** providing a visual identification of potassium sulphate. The origin of this can be a supplement used for the best growth of the tobacco plants, belonging to the potassium fertilizer (phosphate, chloride, nitrate, etc..) responsible for tobacco plant contamination with hazardous implications for soil fertility and human health. [31] These fertilizers absorbed by tobacco leaves create one more of environmental hazard for smokers. The revealed potassium sulphate exerts a favourable influence upon the burning process, producing sulphuric acid a potentially hazardous element. **Figure 1b-P** shows a well distributed phosphorus contents of possible nanometric dimension. The content of P can be also associate to the presence of the most dangerous form known as of white phosphorus P_4 or phosphoric acid H_2PO_4 residuals used during the manufacturing processes. [32] The occurrence of different metal inorganic substances in tobacco cigarette have mainly be attributed from cultivation or production of tobacco leaf. [33] By tracing metal inorganic pollutants, the morpho-chemical investigations has been employed for paper sheet of traditional cigarette (**Figure 1c**). The morphometric analysis revealed squeezed natural cellulose fiber wide in average of 22.08±1.79 μm, forming a porous network filled with micrometric compounds. Although, the interesting engineered pore-shaping is strictly dependent on the coexisting phase of compound materials within the fibre packing network. The adopted manufacturing strategy seems to be critical in favouring the conversion of the toxic monoxide gas through the smallest micrometric porosity, but many chemical oxidizing agents are involved because of the additive microcompounds. At this stage, even small micrometric fillers seem to have been engineered for healthy implication or for improving product performance, a new investigation on their chemical and spatial distributions is quite necessary.

The investigated region **I** exhibited heterogeneous chemical contaminants located on the cellulose fiber network, which are mainly constituted of metal inorganic silicate with micrometric and nanometric dimensions. Metal silicate of aluminum and magnesium are shown in **Figures 1c-OAl** and **–Mg** and the inorganic silicates of sodium and potassium are evident since the color texture of the **Figures 1c-Na** and **–K** follow the silicon of **Figure 1c-Si**. Micrometric and nanometric particles of iron in oxide state are visible in **Figure 1c-Fe**. Interestingly, a heavy metal contaminants of potential hazard have been detected in form of Uranium and Nickel probably originate from natural sediments in chemical form of nickel uranium oxide (NiU_3O_{10}) (**Figure 1c-U** and **-Ni**) [34]. The platelet particle magnified in the Inset of **Figure 1c** have an area of 14.95 μm^2 with radius of 5.53 µm, overlapping another microplatelet of the same specie. Other two small U microparticles in different regions occurred with dimension of about 3 µm. Calcium carbonate in **Figure 1c-CCa** is mainly located among the squeezed cellulosic fibres as main additive filler compound for reducing the carbon monoxide delivery. [35] Furthermore, small amount of sodium, phosphorus, sulphur, chlorine and potassium were identified. By considering similar color shape or texture of the multi-mapping images, chemical substances could be related to the presence of sodium magnesium carbonate, also known as “eitelite”, [36] and the toxic sodium sulphate is a principal salt in proliferation of grown agriculture, which are harmful to human health [37]. Part of the metal inorganic specie detected on the sheet paper have been also observed on the tobacco leaf especially for metal inorganic silicates and inorganic fertilizer, thereby the manufacturing processes are serious capable of contamination different components of the cigarette.

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

Both brands of the heat-not-burn cigarettes have been manufactured with a sheet wrapped in a paper over-wrap (**Figure 2**). The inner layer of type **hnbA** in contact with tobacco shows smooth surface with bright wide stripes observable in **Figure 2a**. By magnifying the region of interested supported by EDS multimapping, it is evident that the sheet shows roughness wide stripes, having a metallic nature (**Figure 2b**). Similarly, the sheet of the heat-not-burn product of type **B** shows smooth surface in **Figure 2c**. Different micrometric impurities on the surface can be observed in the magnified image of **Figure 2d**. Aluminium is still the prevailing content, alloying with iron microparticles (**Figure 2d-OAl**). Micrometric silicate and calcium carbonate particles rise from the manufacturing processes, while presence of micrometric island of pure carbon raised form tobacco can be noticed in the mapping image (**Figure 2d-CCa**). In particular, a micrometric platelet with an area of 40.80 μm^2 and radius of 9.79 μm is shown on the metallic layer (blue arrowed ?da inserire?, **Figure 2d**). The chemical imaging analysis has recognized a heavy metal pollutant of iron-chromium oxide alloy, which might be originate from manufacturing production as stainless steel tool or from natural soil in which chromium may exist in two main oxidation states, Cr(III) and in hazardous nature of Cr(VI). [39] The external sheet paper-**hnbA** over wrapping the metal sheet-**hnbA** possesses similar packed micro-manufactured fibres filled with particles, as shown in **Figure 2e**. Herein, micrometric platelets having elongated shapes are well visible, one of them in the magnified Inset image. The morphometric analysis estimated an average area of 205.22 ± 81.67 μm^2 and major radius of 30.73 ± 8.74 μm, while chemical spatial distribution analysis confirms the heavy metallic species characterized of alloying in form of aluminium-magnesium-iron-copper, as shown in **Figures 2e-OAl**, **-Mg**, **-Fe**, and **-Cu**. Further sodium contents following the color texture of the micro-platelets in **Figure 2e-Na** can be considered part of the metallic alloy. The detected complex alloy toxic in its chemical structure and dimension might be originated during cigarette manufacturing and packing or storage processes. [40] Iron oxides and titanium dioxide of micrometric dimension are well dispersed in different area (**Figure 2e-Fe** and **-Ti**). Other metal inorganic nanostructured microparticles participate in different chemical phase: aluminium-magnesium-calcium-potassium silicate, calcium carbonate, and small amount sodium-phosphorous-sulphur-chlorine contents under micrometric dimension.

Additionally, morpho-chemical investigation on manufacturing tobacco of the **hnbB**-cigarette has been performed showing densely packed tobacco in a stick shape that does not burn when used (**Figure 2g**). [41] By magnify the interested region in cyan dot line square, a micrometric particle of tick polygonal shape with area of 323.50 μm^2 and radius of 23.23 μm can be observed in **Figure 2h**. The large microparticle chemically shows its metallic nature of aluminium, magnesium, chromium and iron elements. The heavy metal contaminant of iron-chromium oxide entrapped into the tobacco stick might represent a natural alloy covered by aluminium and manganese silicates, which may be originated from manufacturing processes. Moreover, its particular size-shape let us to consider the metal contaminant of natural origin compared to the detected iron-chromium oxide in **Figure 2d**, having a platelet-like shape perhaps a scattered stainless steel of cutting tool. The toxic elements of uranium and iron-chromium oxide detected could originate from tobacco plants that might be contaminated by soils naturally or by contaminated water. [42] From a toxicological point of view, the burning and heating processes occurred in the cigarettes may responsible of inducing oxidation of Fe oxide bound Cr(III) with formation of hazardous Cr(VI) heating at more than 400°C with potential adverse health effects to human. [43] The remaining elements recognized by mapping image analysis are morphological and chemically similar to the tobacco detected into conventional cigarette, shown in **Figure 1b**. Yet from a toxicological perspective, tobacco leaves are contaminated with particles of aluminium-magnesium-calcium silicates that if inhaled provide more severe high health risk. [44] Fundamental, fertilizer suck as potassium sulphate is still evident. Metal inorganic contaminants of aluminium and magnesium silicates and the inorganic presence of calcium carbonate, sodium chloride and phosphorus substances are clearly visible in the chemical images.

To investigate how the metal inorganic contaminations might be migrated through **T**- and **hnb**-cigarettes, the filters of porous cellulose acetate fibres have been characterized before tracing nanostructured microparticles on their surface of the unsmoked and smoked fibre filters at tobacco and mouthpiece sides (**Figure 1**, **Region II** and **III**). The analyzed filters of the different brands, forming sponge-like materials, have similar morphology in shape at head filter side in contact with tobacco are shown in **Figure 3**. Three brands of fibre filters show Y shape fibres almost aligned with slightly difference of the cross-sectioned size. The evaluated Y dimensions of the single fibre have showed an increment of the surface area from traditional to heat-not-burn fibre filters. The increased dimension of the surface area might be related to the manufacturing purpose for increasing the amount of additive substances for enhancing the chemical puffing performances. In order to investigate a possible migration of the additive compounds through the filter, quantitative porous analyses among the cellulose fibres network have been performed on a probed area of 1263.16 x 947.37 μm^2 at low magnification for improving the statistical observation/analysis (**Figures 3a, 3c, and 3e**). A 2D contour maps of 174 (fibre filter-**T**), 74 (fibre filter-**hnbA**) and 62 (fibre filter-**hnbB**) micrometric pores were measured, respectively (**Figure 2S**). The analyzed pore-sizes have been plotted in a frequency profiles displayed beside the corresponding 2D filled pores map of the VP-SEM images (**Figures 3a-T and 3e-hnbB**). Traditional filter exhibited pore size distribution with high frequencies of fitted profile ranging from 22 to 208 µm, whereas heat-not-burn filters showed wide spatial distribution of low frequencies but with more multi-peaks fitting of the histograms ranging from 20 to 373 µm. The pore size distributions, best-fitted to a Lorentzian convolution curve, seemed to exhibit difference among the pore distributions of the fibre filters. By gathering the main fitted peaks close in position, their relative variations were estimated to be centered around the average values of 26.12, 62.93, 109.40, 151.21, 187.73, 246.33 and 328.35 µm with incremental variation of 46.91±6.97 µm. The estimated analysis provides quantitative informations on the ranging size of the particles that may be confined through the pore fibres. In this regard, the spatial shape of the pores is also a relevant information for understanding the limited access to the nanostructured microparticles to be caught by fiber filter network. The high porosity value had a shape factor centered at 0.12, an evidence of pores shaping mainly with more elongated shape (rod-like) and low porosity at 0.50 circularity of an elongated oval shape. Similarly, fibre filter-**hnbA** showed high porosity of 7.60 % with implication of preferential porosity, shaping in a more-elongated rod like (0.09) and low amount of elongated quasi-oval shape (0.50) of 1.01 % porosity. Formation of anisotropic pore structures in multi-shaping domains from more elongated rod to less elongated oval (0.1-0.7) can be assigned to the fibre filter-**hnbB**. By summarizing the mainly fitted peaks, we were able to gather the peaks at 0.14 (5.40%), 0.26 (10.69%), 0.49 (3.36%) and 0.69 (2.33%) in terms of circularity and porosity. Moreover, the pores circularity showed a slight shift toward the circularity axis compared to the fibre filter-**hnbA**, providing an enlargement of the microscopy pore-shaping. The manufactured fibre filters of the **hnb**-cigarettes exhibited an increase in porosity/circularity compared to the traditional one probably to increase air ventilation since the inner sheet wrapping tobacco is a smooth metal sheet without pores. From the presented morphometric analyses, all fibre filters are capable of transporting high amount of air and large microparticles of variable micrometric sizes with preferential more elongated shapes. In particular, the filter of type **hnbB** might be host microparticles of high variability in size-shape. High porosity of the filter has been related to a fundamental intrinsic property of the manufactured cigarettes in order to reduce the toxicity of carbon monoxide by increasing the filter ventilation or cigarette paper porosity. [45] This first evidence suggests that chemicals oxidizing agents and high amount of micrometric pollutants independently from their intrinsic size-shape properties could easily reach the human body via inhalation.

In this regard, it is interesting to establish the appearance of metal inorganic substances on the fiber filter before and after smoking, using ESDS multi-mapping analysis. The morpho-chemical analyses were employed at the same probed area of 252 by 189 µm. Magnification of **Figure 3a** shows existence of different microparticles deposited among cellulose acetate fibres of the unsmoked traditional cigarette (**Figure 3b**). First evidence is the appearance of large microparticle possessing an estimated average area of 168.80 µm^2 and radius of 19.60 µm, and yielding a size allowed for a possible migration through the filter. The chemical analysis confirms the formation of a nanostructured alloy constituted of heavy metal elements of aluminium, silicon, iron, nickel, copper and more interesting are the intense appearances of the toxic osmium element in **Figure 3b-Os**. The enhanced aluminium signal of the X-ray microanalysis let to suppose a mixed formation of osmium complex alloying mainly with aluminium and nickel in minor amount. Notably, whereas agglomerate of aluminium-magnesium-sodium-phosphorous silicates could be attributed to the manufacturing contaminants due to their standard presence found in this habitat. Moreover, a microparticle of 7.90 µm in size of osmium complex can be also observed, but alloyed with copper content, as shown in **Figure 3b-Cu**. Silicates of aluminium-magnesium-titanium-calcium-sodium-phosphorous are also dispersed in small amount around the identified alloy microparticles. Further, titanium and iron token part in an oxide states. The main inorganic particles preferentially in nanometric dimensions are observable in small amount of different chemical phases of calcium carbonate, potassium sulphate, and sodium chloride. Filter of type **hnbA** possesses cellulose acetate fibers of roughness surface due to the high amount of micrometric compounds deposited on, shown in **Figure 3d**. EDS imaging analysis clearly show an increase of microparticles different in size and shape constituted mainly of silicates, as displayed in **Figure 3d-Si**. By analyzing the chemical spatial distribution, the silicates are based on aluminium-magnesium-titanium-calcium-sodium-phosphorous and a further titanium and iron oxides were detected in smallest micrometric dimensions. Inorganic aggregation already detected onto fiber filter of traditional cigarette occurred in nanometric powder. The increasing in amount of additive microparticles onto cellulose acetate fibers is in agreement with the proposal hypothesis, regarding the increased surface area of the manufactured fibres of the heat-not-burn products. Fibre filter-**hnbB** product also exhibited microcompounds aggregating in metal inorganic particles on the cellulose acetate fibres manly identified as aluminium-titanium-calcium silicates and calcium carbonate. Minor concentration of Na, P, S, and Cl elements were distributed uniformly in nanometric size, shown in the EDS mapping images of **Figure 3f**. Metal and inorganic contaminations have been identified on unsmoked filters with quite similar amount and species. The complexity single objects recognized on the fibres were estimated with dimensions ranging from 2.05 to 9.50 µm, forming a nanostructured microaggregates. Ultrafine potassium sulphides and sodium chloride were well distributed on the cellulose acetate fibres with under micron dimension on the fibres of type **hnb**. This inorganic chemical specie can be occurred due to the physical contact with tobacco, as detected by multi-chemical analyses of **Figure 1b** and **2h**. By quantitatively morphometric evaluation, iron oxides particles were prevailing in fibre filter-**T** with 2.94±0.57 µm in average dimension while ultrafine sub-micrometric particles were only detected in fibre filter-**hnbA**. Silicates formed a nanostructure microparticles with average dimension of 3.18±0.19 µm and of magnesium in ultrafine dimension (fibre filter-**T**), 4.49±0.47 µm and 3.70±0.31 µm (fibre filter-**hnbA**), and 4.54±0.99 µm and magnesium silicate was not detected (fibre filter-**hnbB**). Inorganic compounds are predominant in a coexisting multi-phase of calcium carbonate with 3.09±0.19 µm (fibre filter-**T**), 3.92±0.49 µm (fibre filter-**hnbA**), and 5.00±0.46 µm (fibre filter-**hnbB**) dimensions; titanium dioxide with 3.40±0.65 µm (fibre filter-**T**), 2.64±0.37 µm (fibre filter-**hnbA**), and 4.54±0.58 µm (fibre filter-**hnbB**); and sodium-phosphorous-chlorine and potassium formed complexes of ultrafine powder under micrometric size. Hence, the critical impact of heavy metals (Iron-Nickel-Copper-Osmium) were mostly generated by traditional fibre filter probably because of the different manufacturing and packing processes compared to the fibre filter of type **hnb**. The detected heavy metal might be attributed to their catalytic property in conversion the carbon monoxide or unwanted contaminations originated from the assembly line of the factory.[46] Differently, the amount of the metal inorganic silicates were preferential employed in the fibre filters of type **hnb**, including the additive substances of calcium carbonate and titanium oxide.

At this stage, however, even small changes in the burning and heating processes might be occurred after smoking, a new validation is necessary for expanding our understanding on the evolution features of the metal inorganic particles. First result was to observe decomposed cellulose acetate fibers of smoked cigarettes because of burning and heating processes, compromising also the porosity purpose in term of ventilation (Region **II** of **Figure 1**, **Figures 4a**, **-c**, and **-e**). Basically, the combustion of cellulose acetate fibers favorites the oxidative formation of a poisonous, colorless and odourless gas of the toxic carbon monoxide and further toxic oxidizing agents; which can be easily inhaled during puffing. [47, 48] Volatile products during thermal heating processes have also released solid particles on the melted fibers, coming from tobacco column (**Figures 4b**, **-d**, and **-f**). The observed micrometric object placed on the top of the fibers, not seen before, is a result of aggregating nanostructured particles because of the frequent puffs through rapid heating and high air-flow of the contents. Fibre filter-**T**, at tobacco side after smoking, exhibits micrometric globulars of 31.07±5.98 μm in average in **Figure 4b**. The formed globular were mainly aggregating with phosphorous-sulphur-chlorine-potassium-calcium and silicates of aluminum and magnesium self-assembled together with less amount of iron, manganese, phosphorous, sulphur and chlorine (white arrowed???, **Figure 4b**). Metal contaminations of iron oxide were present at the surface in small nanostructured microparticles with average dimension of 2.79±0.21 μm and silicates of aluminum, magnesium, titanium with 3.15±0.33 μm and 2.48±0.14 μm. Furthermore, heavy metal chromium and manganese contents well-dispersed on melted fiber network in ultrafine dimensions after smoking appeared. This is an evidence that particles subjected of heating process are volatile capable of traveling through the tobacco column also in heat-not-burn cigarettes. The metallic contents have been considered an intentional product critical for catalytic activity in conversion carbon monoxide to carbon dioxide dependent upon the particle dimension ranging from 3 nm to 5 μm [49]. The first appearance of micrometric manganese particles in oxide state might be used for its catalytic property. [50] This represents a further advice of hazardous evidences since manganese has been detected in several brain regions after smoke inhalation causing inflammatory changes. [51]. Different behavior of the dimension evolution has been noticed for inorganic compounds of phosphorous-sulphur-chlorine and potassium contents changed from ultrafine powder of sub-micron size in microparticles with an estimated average dimension of 3.38±0.36 µm, after smoking. Their size increments are shown in **Figures 4b-Na**, **-P**, **-S**, **Cl**, and **–K**. On the contrary, the additive of calcium carbonate and titanium dioxide degraded in small microparticles with dimensions of 3.14±0.41 and 2.35±0.21 µm compared to the unsmoked filter, performing their properly industrial task of catalysts/oxidants. [52] Therefore, the burning process have reduced their amount in a volatile component traveling in the mainstream smoke that are able to provoke irritation to the respiratory systems through short-term inhalation. [53, 54] Fibre filter of smoked **hnbA**-cigarette show an macrometric aggregate of an estimated area of 7015.02 µm^{2} and radius of 126.04 µm (**Figure 4d**, region II of **Figure 1**). This macrometric object is an agglomerate of microparticles mainly composed by sodium-phosphorous-sulphur-chlorine-potassium and magnesium silicate in a coexisting phases of ultrafine dimension and micrometric particles of sulphur and potassium (3.80±0.31 and 3.68±0.24 µm) and calcium carbonate (3.58±0.26 µm). By changing the puffing process in heating without burning, metal oxide contaminants of magnesium, manganese and iron in oxide states occurred in ultrafine dimensions, while silicate of aluminum with 3.52±0.20 µm in average. Similarly, to the traditional fibre filter, the additive titanium dioxide did not take part to the self-aggregating heating processes confined around the macrometric object, having dimension of 2.95±0.13 µm observable in **Figure 4d-Ti** and (**Figure 4b-Ti**). Smoked fibre filter-**hnbB** concretely exhibits melted cellulose acetate fibres (**Figure 4e**), which led to unwanted exposure of potentially oxidizing agents in form of volatile fractions capable of reaching the human respiratory system by inhalation. Similarly, macroaggregates occurred in highly variable sizes from 810.40 to 29980.02 µm^{2} of the surface area and radius from 33.89 to 218.20 µm. The macroaggregate large of 108.81 µm is mainly constituted by phosphorous-sulphur-potassium-calcium and magnesium silicates (**Figure 4f-K**, **-Ca**, **-Mg**, and **-Cl**). In particular, calcium, sulphur and potassium contents tended to self-aggregate not only in ultrafine dimensions, but also in micrometric particles with an average dimension of

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

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

Existence of inorganic silicates of sodium and potassium could be confirmed by microaggregation of silicon with dimension of 2.87±0.21 μm in size. Calcium carbonate and titanium dioxide aggregated in small microparticles of 2.84±0.27 and 3.10±0.26 μm in presence also of ultrafine powders. Calcium carbonate, titanium dioxide and silicates are the main metal inorganic contaminations traced everywhere into the cigarettes. The calcium carbonate microaggregate considered a non-toxic inorganic material used for tuning the burning rate of cigarette in order to reduce the carbon monoxide delivery. However, it is notable that nanometric calcium carbonate fillers, founded in amount of about 22% or more in 30-40 wt.%, should not be underestimated since may cause irritation to nose, throat and respiratory system, as reported by New Jersey Department of Health (US) considered a hazardous substance. Furthermore, a nano-$CaCO_3$ exposure was significantly associated with pulmonary hypofunction. [57] The role of titanium element in the filter, aggregating chemically in titanium dioxide particles especially in **T**-cigarettes, has been considered as an additive tool for capturing the toxic tobacco-specific nitrosamine *via* chemical absorption and for decomposing the filter waste by photodegradation processes (e.g. UV radiations of sunlight activation) [58, 59]. But, no doubt, toxicity studies have been also shown that TiO2 nanoparticles may induce acute inflammations of the nose/throat/lung by repeating dose inhalations [60, 61]. Aluminum and silicate particles, revealed both into **T**- and **hnb**-cigarettes, have been employed for reducing carbon monoxide and for manufacturing process as casing materials in cigarettes. But these toxic contents in crystalline aggregations may be responsible for respiratory disease [62, 63]. Exposure in large amount of silicate dust induces silicosis causing chronic inflammatory to nose and upper respiratory tract. [64]

After smoking, the metal inorganic compounds evolved by growing in dimensionally on the fibre filters at both side of **Region II** and **III**, forming both macro and microaggregates into coexisting multi-phases. In this regard, the size of the microparticles have been plotted related to their chemical constituents (**Figure 5**); wherein the particles size plotted with 1 μm are only an indicative representation of belonging to the ultrafine size with submicron dimension (cyan area, ultrafine powder region). Furthermore, the presence of fewest micrometric particles on analyzed area of 47628 μm^2 have been neglect to be considered part of the ultrafine powder region.

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

In the **region II**, chemical specie detected on filters of smoked filter takes notice of the same chemistry finding in unsmoked, but it should be emphasized that the migrated microcompounds have slight reduced their sizes for the metal elements (**Figure 5b**). Conversely, particles increase their size on micrometric scale for the elements constituting inorganic species (Na, P, S, Cl and K) especially for the smoked fibre filter-**T**; probably due to the burning processes and puffing intensity able to charge the smoke of more toxic contaminants, traveling toward the tobacco column. Indeed, the inorganic species revealed in the paper sheet and tobacco increased their size from ultrafine dimension to final grew in particle of micrometric dimension on the filter after smoking. On the other hand, the metal element contributions reduced in size on the fibre filters that should be attributed to the burning and heating temperatures since the heating temperature in the **hnb**-cigarettes might reach about 300° C, while the burning processes in the traditional cigarettes may reach a temperature of about 700° C. [65, 66] Therefore, the combustion and degradation of the metal inorganic substances occurred in the cigarettes could be considered less for **hnb**-cigarettes. But it is relevant to consider that the amount of the carrier particles (smoke) increase in the tobacco column of the **hnb**-cigarettes because of the absent of porosity in the metal sheet, thereby the amount of the carried toxic substances originated from the treated tobacco stick increase also in ultrafine dimensions. Additional evidences in increasing the amount of the inorganic specie are the revealed macro-objects. This experimental result represents a remarkable evidence that the persistence of the smoking is capable of accumulating substances in micrometric and ultrafine dimension to build up hazardous macro-agglomerates with time by heating flow. By evaluating the chemical-size of the metal elements (Mg, Al, Si, Ti, *Mn* and Fe) belonging to microaggregates after smoking, they also taken part to the thermal decomposition (pyrolysis, pyrosynthesis and combustion) with decreasing in size in particular for the additive magnesium, calcium and titanium. It is interesting to notice the appearance for the first time of manganese in ultrafine powder in all smoked filter cigarettes and chromium for traditional one, which is still an evidence that the migration processes of heavy metal particles in ultrafine dimension through the tobacco column occurred. Therefore, ultrafine powders of heavy metal contaminants may be carried easily by mainstream smoke including also passive diffusion for which has been also established acute adverse effect on respiratory function for active or passive smokers. [67] Actually, these toxic elements belonging to heavy metals can easily reach the pulmonary system for which has been detected in highest amount in smokers than those in nonsmokers. [68]

By comparing the particle contaminants with the smoked filter at mouthpiece side (**Figure 5c**), metal inorganic elements increased in size in particular for **T**-cigarettes in which the macro-objects unfortunately were still observed. The size contaminants of fiber filter-**hnbA** show an excessive increment, probably due to the different component analyzed of the cigarette of **Region III**. Indeed, the sheet paper-**hnbA** shows increased size of particle especially for the metal element attributable to the silicates contaminants already detected in the others sheet paper but with less amount (**Figure 1** and **2**). Yet from a toxicological perspective, aluminium silicates may accumulate in the lungs after smoking for seven to seventy-five pack years [69].The inorganic elements of the fiber filter-**hnbB** attributable to the effective toxic substance concretely increased in size. The heavy metal contaminants are strongly present in size for iron oxide in the traditional and **hnbA** fiber filters. This is a first direct evidence that particles subjected of burning and heating processes are volatile in the cigarettes capable of traveling and aggregating through the fibre filter in both traditional and heat-not-burn cigarettes. These results concretely confirm that the smoke may be considered as a particles carrier of toxic elements.

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

4. Conclusion

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

As seen, by combining experimental electron microscopy techniques supported by simple imaging analyses, we were able to determine several morpho-chemical features: *i*) the morpho-chemical investigations on the paper sheet (**T**-, **hnbA**- and **hnbB**-cigarettes) have determinate the ability of the metal inorganic nanostructured microparticles to be assembled into a fibre packing network. These compounds, as manufacturing fillers and burn additive contents, were mainly detected in chemistry and size-shape (metal inorganic silicates, chlorides, sulphides, salts, etc.). Unfortunately, high toxic heavy metals have been also detected on the paper or metallic sheet and into the tobacco, showing large microparticles constituted by Vanadium, Chromium, Iron, Nickel, Copper, and Uranium as hazardous ingredients for natural environment and human health (**Figure 1** and **2**); *ii*) Fibre filters of unsmoked cigarettes have been investigated in terms of morphometric porosity related to the direct evidence of metal inorganic contaminants observed. The quantitative porous analyses establish that the real aspect of occured migration of the detected heavy metal microparticles (Iron-Nickel-Copper-Osmium), including metal inorganic silicate and more chemicals oxidizing agents. Although, these first evidences of pollutants strictly dependent from the manufacturing and packing processes allow some production flexibility in this regard, it may also prove the need of employing different manufacturing processes to protect the nature and human health (**Figure 3**); *iii*) for expanding the focus of the presented research, the smoked fibre filters were analyzed for tracing the metal inorganic contaminations. At tobacco side, heavy metal contaminations of manganese and chromium have migrated through the tobacco column to reach the burned fiber filter. The findings of this study show the hazardous role of the smoking, which behaves like a carrier not only for hazardous particles, but also for inorganic poisonous gas developed by the burned plastic fibres together with chemicals oxidizing agents at nanoscopic scale. These results are strongly supported by the evidence of macro-objects due to the persistence and accumulative of smoking capable of careering high amount of particles to be self-assembled into a coexisting phases of a macrometric agglomerate (**Figure 4**). The formed macroaggregates on the smoked filter at mouthpiece side can be explained by considering that the manufactured filters are not an obstacle for the toxic volatile substances of large and variable sizes, as demonstrated by the porosity study presented here; *iv*) Interestingly, the evolution study on the particle size before and after smoking represent a concrete result of the potential hazardous of smoke and the unsuitable engineered filters produced by the tobacco company. This is well established by the experimental analyses, wherein particle size ranging from 2.5 to 7 µm in size are capable of traveling throughout the fibre filter in both traditional and heat-not-burn cigarettes (**Figure 5**). The direct evidence of macro-objects in coexisting multi-phases shows the unambiguous effect of the smoke capable of accumulating massive load of particles to form agglomerate of dimension ranging from 20 to 150 µm. Therefore, these results represent a concrete evidence of the dangerous smoke as a particles carrier of toxic elements.

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

Declaration of Competing Interest

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

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