Interreg
ITALIA-SLOVENIJA
UNIONE EUROPEA
EVROPSKA UNIJA
NANO-REGION
Progetto strategico co-finanziato dal Fondo europeo di sviluppo regionale
Strateški projekt sofinancira Evropski sklad za regionalni razvoj

PROOF-OF-CONCEPT EXPERIMENT REPORT

ANALYSIS OF NANOPARTICLES IN BEEHIVE AIR STORED IN PRESSURIZED CANS

Authors: [1]Egon Pavlica, [2]Mattia Fanetti, [3]Alessandro Esposito, [3]Alois Bonifacio, [3]Valter Sergo

1 - Laboratory for Organic Matter Physics, University of Nova Gorica

Vipavska 11c, 5270 Ajdovščina, Slovenija

2 - Materials Research Laboratory, University of Nova Gorica

Vipavska 11c, 5270 Ajdovščina, Slovenija

3 - Departimento of Engineering and Architecture, University of Trieste

Via Alfonso Valerio 6a, 34127 Trieste, Italy

Proof-of-Concept experiment details

Received sample: Three pressurized cans (200 ml) filled with beehive air.

Description by proposer: Three cans were filled with air, which was captured from a beehive during the active season of the year. The active season comprise spring and early summer months, when the bees are highly active by collecting honey and other biomaterials.

It was confirmed in the past by the chromatographic analysis that the air pumped directly from the beehive contains some aliphatic and aromatic aldehydes, alcohols, esters and even terpenes. In particular, some organic compounds were found to be pertinent to beehive's air, since they are not present in the outside air. The information provided by the proposer included the chromatograms with respective intensity charts of identified compounds as calculated by the mass spectrometer software.

Planned analysis:

1. Transfer the areosol and the particles in the air onto a substrate suitable for further analysis.
2. SEM/EDX investigation: Localization and identification of particles from the bee-hive air. If succesfull, the measure is repeated after 1-3 months to see potential aging effect.
3. RAMAN or other optical spectroscopy: Localization and identification of particles from the bee-hive air. If succesfull, the measure is repeated after 1-3 months to see potential aging effect.

Main aim of the proposal: Localization and identification of particles from the bee-hive air. Recognition of different molecules present in the air and quantification of their concentration. If succesfull, the measure is repeated after 1-3 months to see potential aging effect.

Results on SEM/EDX investigation

Preparation method

A pressurized can was connected with a tubing to a polycarbonate filter housing hosting a polycarbonate filter membrane with 0.3 µm pore size. In order to promote air passthrough, the outlet of filter housing was connected to a vacuum pump (~ -0.3 bar, 0.1 dm^3/s). The membrane was coated with a thin layer of Au by sputtering before particle deposition. Air was released through the filter for 30 seconds.

Results of SEM investigation:

Au-coated membrane was characterized with scanning electron microscopy before and after particle deposition. Imaging beam energy was 15 keV and secondary electron detector was used. The results of SEM imaging are presented in Figs. 1-6.

(x25) Before particle deposition **After particle deposition**

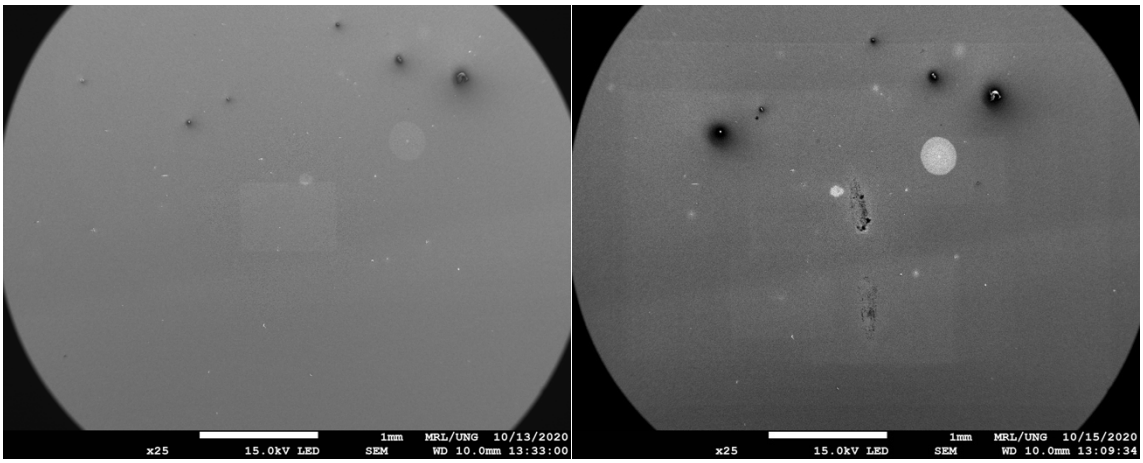

Figure 1: Scanning electron microscopy of Au coated membrane (left) before and (right) after particle deposition.

(x40) Before particle deposition **After particle deposition**

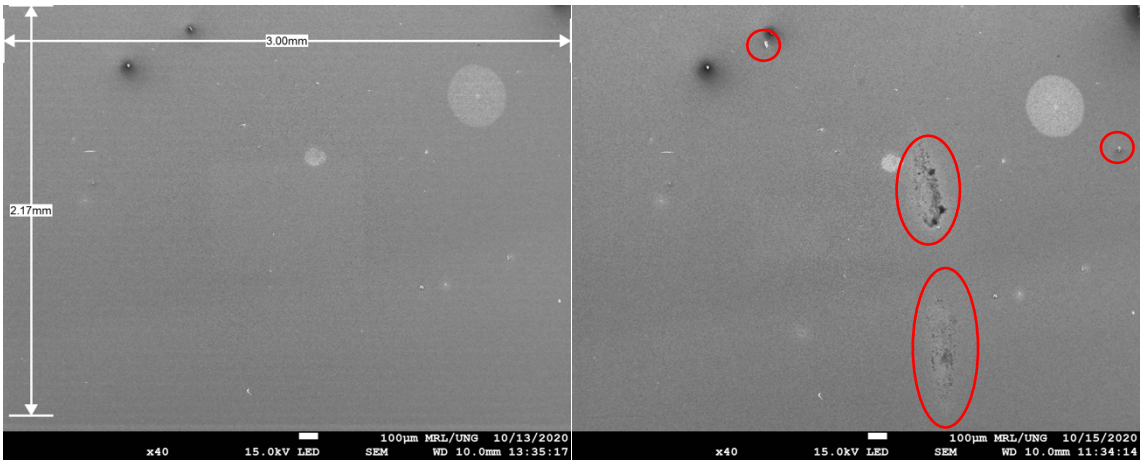

Figure 2: Scanning electron microscopy of Au coated membrane (left) before and (right) after particle deposition.

(x100) Before particle deposition **After particle deposition**

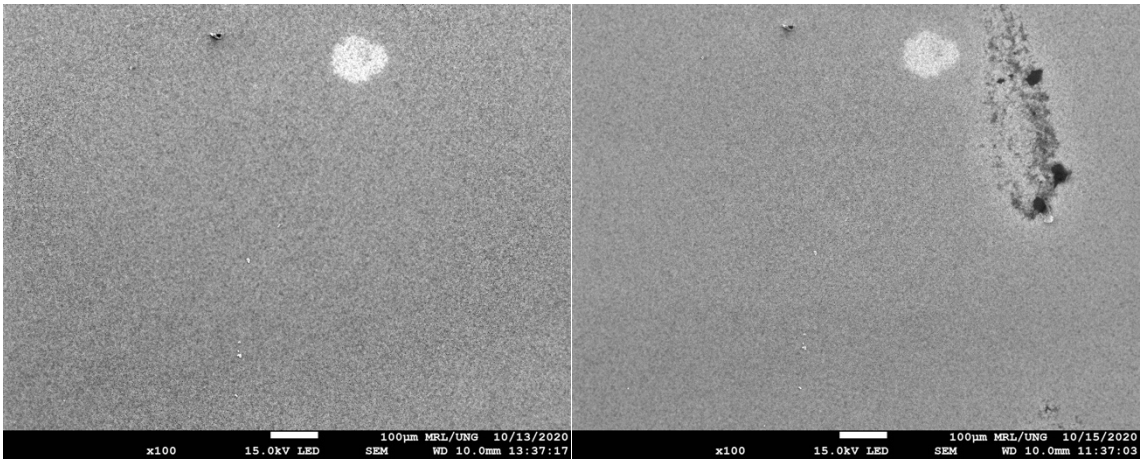

Figure 3: Scanning electron microscopy of Au coated membrane (left) before and (right) after particle deposition.

(x1k) Before particle deposition **(x2.5k) After particle deposition**

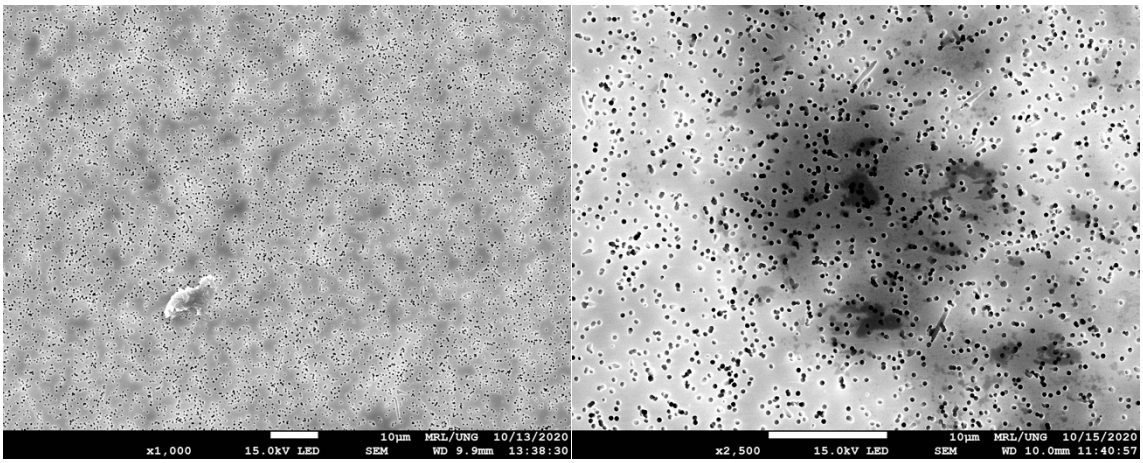

Figure 4: Scanning electron microscopy of Au coated membrane (left) before and (right) after particle deposition.

(x5k) Before particle deposition **(x12k) After particle deposition**

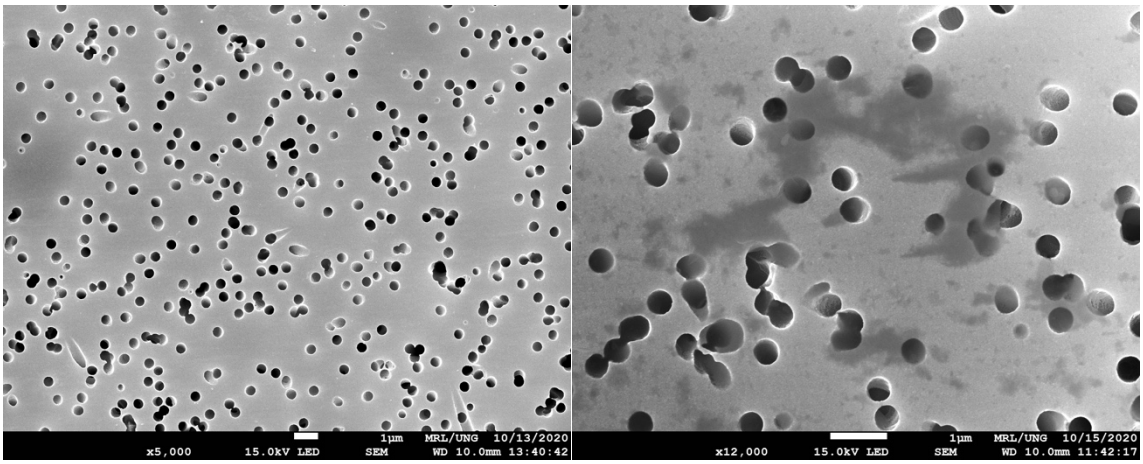

Figure 5: Scanning electron microscopy of Au coated membrane (left) before and (right) after particle deposition.

(x30k) Before particle deposition **(x37k) After particle deposition**

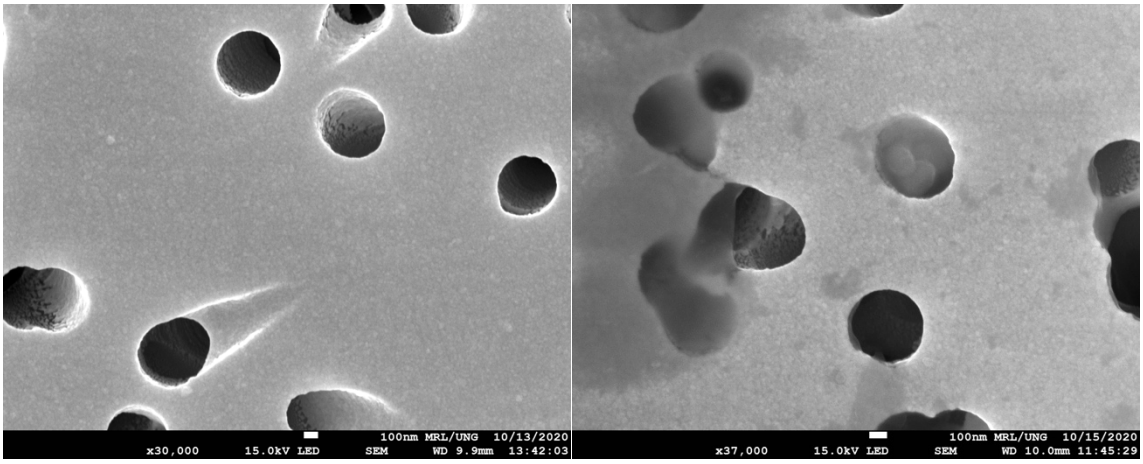

Figure 6: Scanning electron microscopy of Au coated membrane (left) before and (after) particle deposition.

Figs. 1-6 represent morphology of Au coated filtration membrane before and after particle deposition. Comparing the micrographs obtained before and after exposure to beehive air is used to identify deposited particles. Figure 1 represents membrane at the lowest magnification of 25x. We note that membrane had some structures already before deposition. These structures were ignored in the following analysis. After deposition, we observed the presence of two different features: solid particles, and large dark spots of spread material, probably agglomeration of smaller particles. The spot is zoomed in Figure 2 and Figure 3 for the sake of visibility. More, the difference is not limited to the dark spot. In Figure 4 and Figure 5 we present a close view of membrane surface and pores. Before deposition, the membrane surface exhibited a uniform contrast, suggesting that the surface is mainly Au. In fact, secondary electrons, which are used to imagine the surface, are sensitive to the presence even of a very thin layers at the surface. This is evident in the case after particle deposition. In some areas the micrograph of the membrane surface changed in contrast, exhibiting a variable surface emission of secondary electrons. This indicates the presence of a layer on the surface. In addition to observed patches, several particles were observed in pores of the membrane after deposition. Typical particles, clogged in pores, are presented in Figure 6. The size of clogged particles is similar to the size of pores.

In summary, the exposure of the membrane to the compressed beehive-air resulted in the deposition of material on top of Au surface, both in the form of solid particles and in the form of agglomerated material forming a continuous layer in some areas.

EDX analysis:

Energy-dispersive X-ray spectroscopy (EDX) characterization of Au coated membranes is presented in Figure 7-11. Beam energy was 15 keV, secondary electron detector was used for imaging. Based on the information provided by proposer, the beehive air carries aliphatic and aromatic aldehydes, alcohols, esters and terpenes. Similar results were observed by other researchers [1]. These organic compounds are mainly made of carbon, oxygen and hydrogen, which are lightweight materials, which exhibit relatively low X-ray fluorescence yield. In order to understand the elemental composition of adsorbed particles from beehive air, we performed EDX elemental mapping of different regions of the membrane filters as presented below.

Figure 7 represents elemental analysis of four different regions of bare Au surface prior to exposure to beehive air. Each of four histograms corresponds to different region. Each histogram represents distribution of X-ray energies, which correspond to characteristic fingerprints of different elements. Accordingly, peaks are labeled to identify which element they belong to. The analysis of four different regions demonstrate that membrane surface consists of mainly C (86%), O (8%), Au (5%). We observed also traces of Mg (0.1%). C and O belongs to membrane, while Au was deposited on top. Al peak results from the sample holder, which was used to mount membrane and is ignored in the following analysis.

Figure 8 represents elemental mapping of dark patches, which appeared on Au surface after exposure to beehive air. Compared to membrane before deposition, the overall sum of C is increased to 89%, while O and Au are reduced to 6% and 4.5%, respectively. This is the results of carbon-based compounds deposited on top of Au. Additional evidence is provided with elemental mapping of C and O. C and O maps exhibit increased signal intensity in the region correlated with the dark patch. Observed contrast is a demonstration that the C and O concentration varies. In contrast, maps of other elements are relatively uniform, indicating that either element concentration is negligible (low signal to noise ratio), either element concentration does not change across the surface.

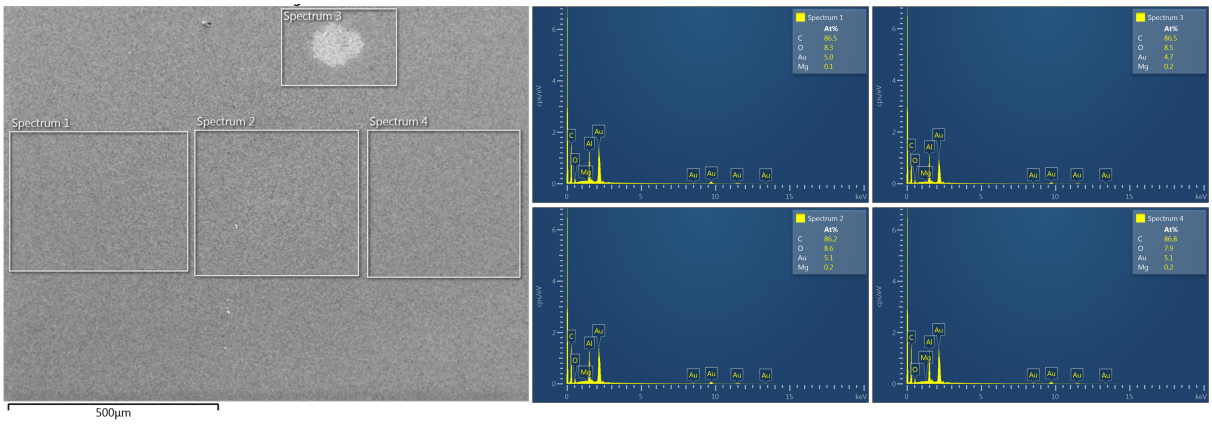

Figure 7: EDX based analysis of Au coated membrane before particle deposition. Analysed region corresponds to area presented in Figure 3.

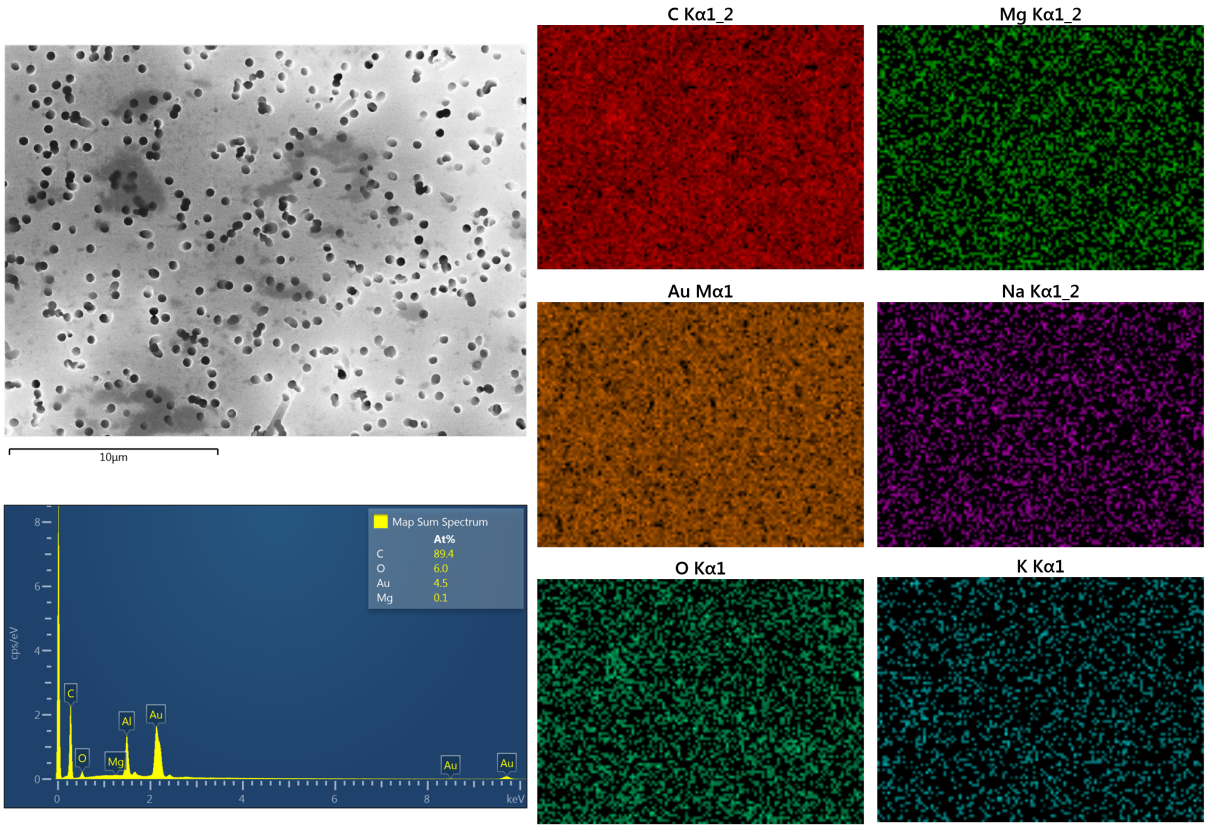

Figure 8: EDX based elemental mapping of particles deposited on Au coated membrane. Analysed region corresponds to area presented in Figure 5.

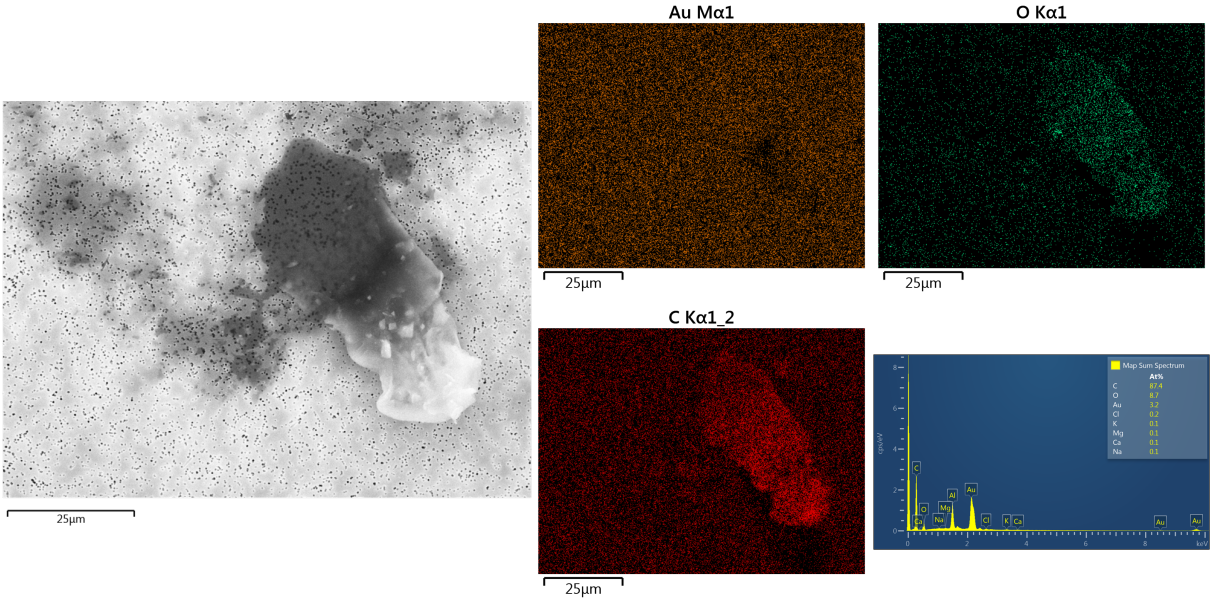

Figure 9: EDX based analysis of particles deposited on Au coated membrane. (Right) Elemental mapping of selected elements.

Figure 9 represents elemental map of relatively large particle, which appeared after exposure of membrane to beehive air. Particle is approximately 50 µm long and 25 µm wide. C and O maps exhibits high contrast, which is directly correlated to the shape of the particle. This confirms that the particle is C and O rich compound. In addition to C and O, other alkali elements were found in the observed particle.

Figure 10 represents elemental analysis of another particle, which appeared after exposure of membrane to beehive air. Particle's diameter is approximately 30 µm. Particle consists of relatively rich spectrum of elements. The highest contrast is in C and O maps, suggesting the particle is of organic origin. In addition, K and Cl maps indicate spots of relatively high intensity. These spots of K and Cl are spatially correlated. Therefore we believe that K and Cl form salt crystals, which are attached to the particle. A close view of N and Ca maps potentially correlate with the shape of the particle. Therefore, we speculate that particle compound contains also N and Ca. The later could be in the form of $CaCl_2$ salts, since its enhanced intensity correlates with Cl maps.

Figure 11 represents elemental analysis of a third particle, which appeared after exposure of membrane to beehive air. Particle seems to be a fold of larger flake. Elemental mapping similarly confirms that it is composed of C and O rich molecules. In addition, there is a few microns large grain attached to the particle. The grain is composed of Ca and O according to elemental mapping. In this particle K and Na are not detected.

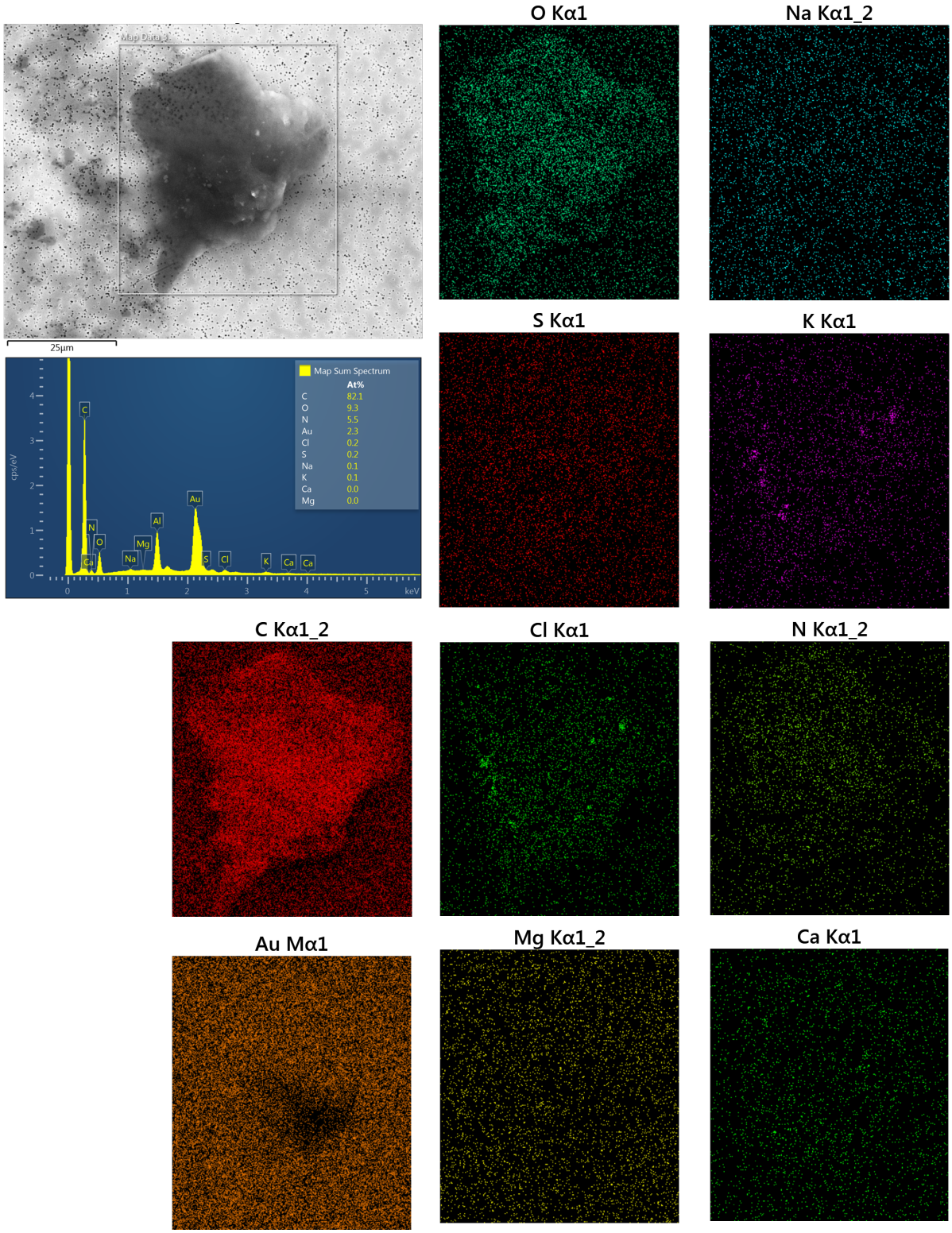

Figure 10: EDX based analysis of particles deposited on Au coated membrane. (Right) Elemental mapping of selected elements.

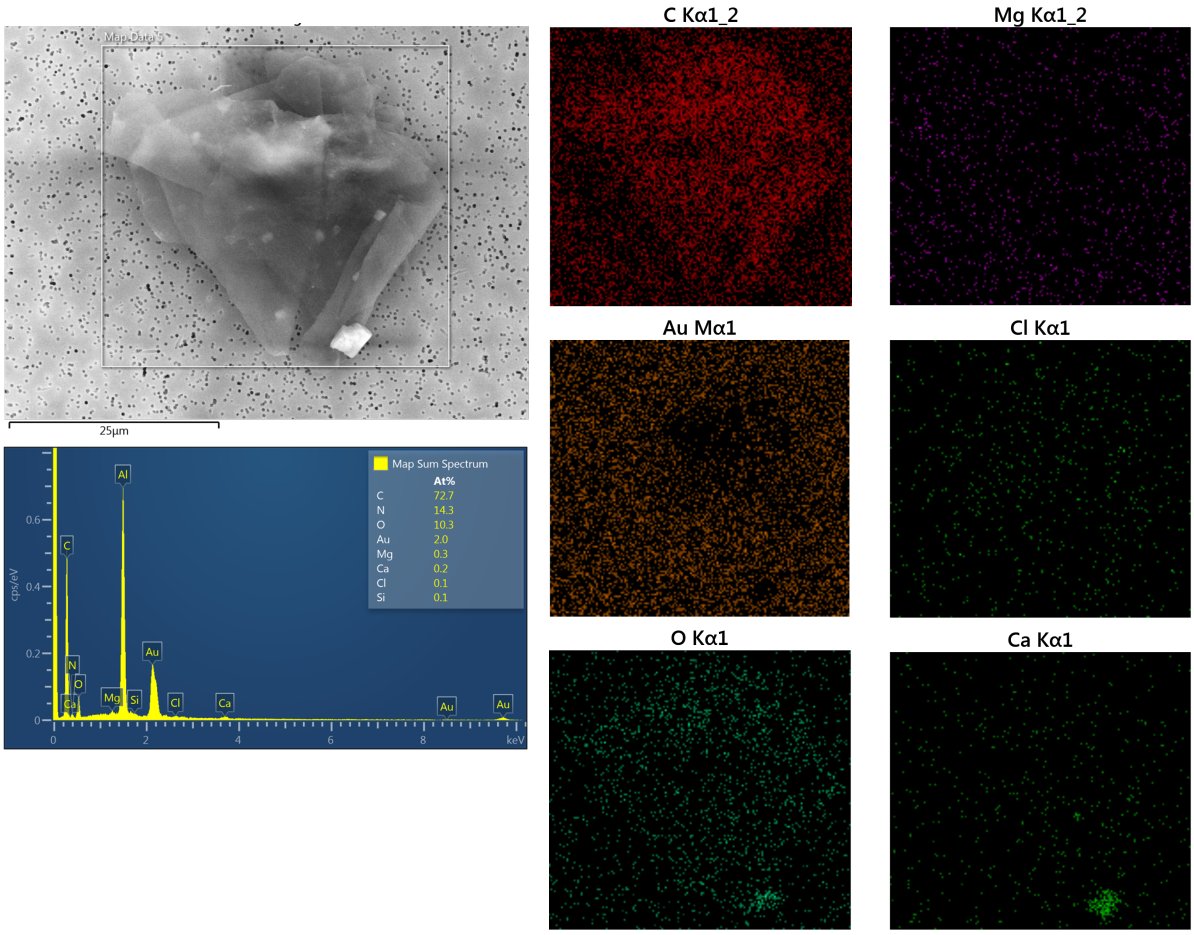

Figure 11: EDX based analysis of particles deposited on Au coated membrane. (Right) Elemental mapping of selected elements.

Results on SERS investigation

Surface-enhanced Raman spectroscopy was used to recognize different molecules, which were present in the cans of pressurized beehive air.

Equipment

Vacuum pump, bench centrifuge, tubing with a scree-filter edge, SERS dip-coated plasmonic paper, Centrifugal plasmonic paper stirp, Whatman filter paper, Silmeco SERS substrate, I-Raman Plus portable spectrometer (Laser at 785nm).

Preparation method

Three different samples were prepared in order to perform SERS analysis:

1) **Silmeco**: a Silmeco substrate has been measured as blank under SERS microscope to recorder the background signal. Silmeco has been treated with Beehive air, 30s flux directed on the top of the substrate by a p200 cut tip. Immediately after treatment the SERS signal was recorder. A drop of methanol successively was added to facilitate pillar collapse, and then re-measured.

2) **Centrifugal plasmonic paper (CSPP)**: a tubing with a scree filter support was installed on a vacuum pump, between the scree ending and the pump tubing on the filter support was placed a paper filter to adsorb Beehive components. After 30 second of vacuum flux exposure, the paper was cut into strip inserted into an Eppendorf tube and mixed with 150ul of silver colloids, then was centrifuged at 8000 rpm, for 10 minutes. the CPP strip was left to dry for 20 minutes and analysed. A clean centrifugal plasmonic strip was used to collect the background signal.

3) **SERS dip-coated plasmonic paper (pl paper)**: A SERS substrate was left to dry for 20 minutes. The same fluxing procedure of point 2 was used. To reduce the flux outside the substrate a paper filter sheet was cut to the same dimension of filter inside the scree ending part and placed into the filter with a window to leave exposed the SERS active surface.

Results of SERS investigation:

Results of SERS analysis are presented in Figure 12. Four measurements of three different samples are vertically shifted for the sake of clarity. Background is plotted with blue curve. Raman signal is plotted with red curve. By comparing the background and the Raman signal we conclude that there is no difference upon the exposure to beehive air. Probably the concentration of adsorbed particles is below the limit of detection of SERS method.

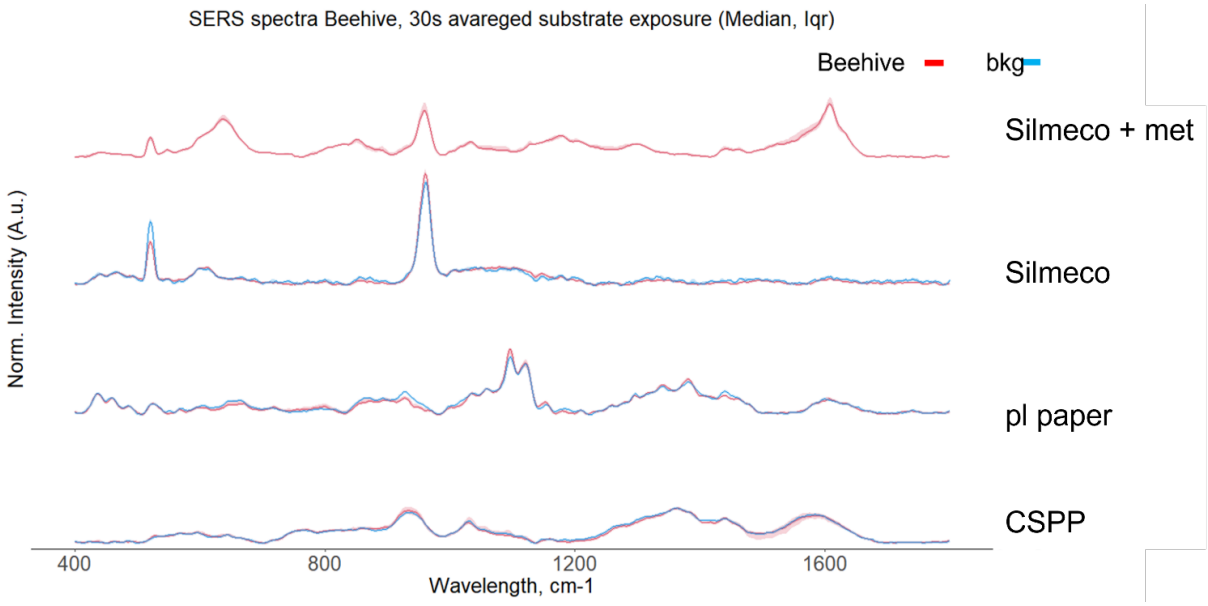

Figure 12: Over imposed SERS spectra of Beehive (red) versus background (blue), in different conditions: "CSPP" - centrifugal silver plasmonic paper; "Silmeco" - silver Silmeco substrate; "pl paper" - silver plasmonic paper; "Silmeco + met" - methanol washed beehive treated Silmeco substrate.

Summary of results

- We obtained micrographs of C and O rich compounds adsorbed on Au coated filter membranes after filtering pressurized beehive air from cans.

- Elemental mapping confirmed that adsorbed particles are C and O rich, presumably of organic origin.

- Elemental maps revealed presence of grains of alkali elements on adsorbed solid particles.

- Adsorbed amount of particles/molecules from a single pressurized can is below the limit of detection of the surface enhanced Raman spectroscopy, which was used to recognize adsorbed organic compounds.

Concluding remarks

By SEM it is possible to detect some particles coming from the beehive air. By EDX these particles displayed mostly C and O, sometimes also K, Ca and Cl, which makes reasonable to indicate these particles as made of organic compounds. Further information on the organic molecules species are not accessible with SEM/EDX.

Beside the particle detection, however, we notice that the number of the deposited particles is quite low. For this reason, SEM/EDX analysis seems not the best solution to quantitatively assess the particle nature and amount, and as a consequence also the possible effect of aging.

A non-spatially resolved technique, but potentially capable to identify the organic molecule species and quantify them, has been tried, i.e. surface enhanced Raman spectroscopy (SERS). However, no signal has been detected after deposition, most probably because the amount of organic molecules sticking on the substrate is too low. It is possible that an exposition to beehive air flow for a much longer time (longer than what is possible with compressed air cans) can provide some detectable signal.

In conclusion, even if SEM/EDX can provide some information on the presence and the morphology of particles from the beehive air, both SEM/EDX and Raman spectroscopy are not effective in recognizing the molecular species and in quantitatively assess their concentration for beehive air stored in pressurized cans.

References

(1) Abd El-Wahed, A. A.; Farag, M. A.; Eraqi, W. A.; Mersal, G. A. M.; Zhao, C.; Khalifa, S. A. M.; El-Seedi, H. R. Unravelling the Beehive Air Volatiles Profile as Analysed via Solid-Phase Microextraction (SPME) and Chemometrics. *Journal of King Saud University - Science* **2021**, *33* (5), 101449. https://doi.org/10.1016/j.jksus.2021.101449.

This report has been written by Egon Pavlica and Mattia Fanetti (Ajdovščina, 2 November 2021)