SAQN Awards End of Project Report

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Project Title	
Using high-resolution techniques to assess the potential health and climate impacts of biomass burning aerosol	
Project Team	
Name	Role (PI / Co-I)
Dr. Sanghamitra Mukhopadhyay (SM)	**PI**
Dr. Sri Hapsari Budisulistiorini (SHB)	**Co-I/Project Lead**
Dr. Chang Guo (CG)	**Co-I**
Dr. Andy Ward (AW)	**Co-I**
Proposed activities (copy from your project proposal)	

1. ***Chemical analysis (UoY and STFC; 10 weeks)***

   1.1. Samples preparation (UoY; 1 week). We will analyse PM samples collected during wildfires. We will contact the SAQN mentors to get access to such samples in the coming months. The samples will then be analysed using the Q Exactive Orbitrap mass spectrometer. Samples will be prepared for spectroscopy and biological analyses.

   1.2. Offline spectroscopy (STFC; 3 weeks). We will do Raman and IR spectroscopy and X-ray diffractions on prepared samples.

   1.3. Neutron spectroscopy (STFC; 4 weeks). We will apply beam time to do neutron spectroscopy for these particulates to understand their structure and dynamics. These spectroscopic measurements will use four samples and associated exposed bronchial epithelial cells. Sixteen days of beam time will be sought for LET, TOSCA, IRIS and OSIRIS beamlines. As ISIS

is under maintenance, the first application will go out in December, and associated beamtime will be available from spring to summer in 2022.

1.4. Aerosol trapping spectroscopy (STFC; 2 weeks). We will use Ultrasonic nebulisation to re-aerosolise the samples from the dispersions used in LCMS studies with the aim of determining the refractive index and stability of the aerosol.

2. Cellular toxicological effects (PHE; 12 weeks)

2.1. Human bronchial epithelial cell culture system (e.g. BEASE-2B cells) will be used for BBA exposure.

2.2. Established toxicity test (e.g. LDH assay) will be carried out to monitor the toxic impact imparted on dose escalation of the BBA samples.

2.3. Oxidative stress changes after BBA exposure, e.g. change in the expression of selected genes related to oxidative stress.

2.4. Biological sample collections (cells -/+ BBA exposure) for neutron spectroscopy.

3. Data finalisation and publication preparation (2 weeks)

Please report on the activities completed in the project

1. Chemical analysis (UoY and STFC)

1.1. Samples preparation and chemical analysis at UoY.
Samples of ambient PM2.5 during wildfire and non-wildfire were prepared at UoY and shipped for analysis at STFC and PHE. A blank was also prepared for analysis along with the samples (Figure 1).
Samples preparation involved adding 10 mL of methanol onto the filters containing ambient PM2.5. The solution was then filtered, dried, and dissolved in 1 mL of 50:50 (v/v) Water:Methanol for analysis using High-Performance Liquid Chromatography coupled to negative ion electrospray ionisation and Orbitrap Mass Spectrometry (HPLC-ESI(-)-Orbitrap MS). For analysis using UV-Vis spectroscopy, the extracts were dissolved in 1 mL of methanol.

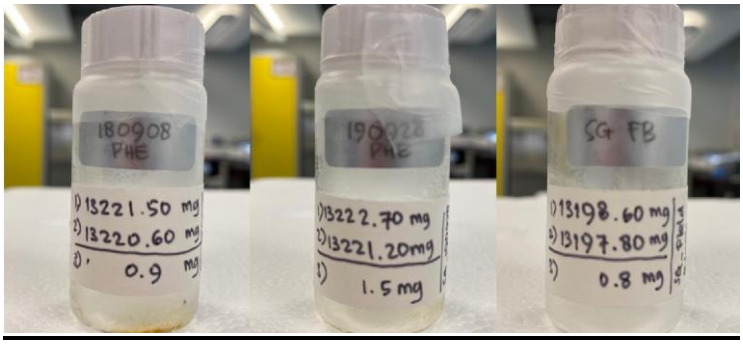

Figure 1. Biomass burning aerosol samples. From left to right: non-wildfire, wildfire, and field blank.

Chemical analysis using HPLC-ESI(-)-Orbitrap MS resulted in hundreds of species characterised from the ambient samples. In Figure 2, the characterised organic species are grouped into oxidised organic species (CHO), organic species with NO3 group (CHNO), organic species with NO3 and SO4 functional groups (CHNOS), organic species with SO4 functional group that hydrophilic (CHOSw) and less-hydrophilic (CHOSm), and other species.
Figure 2A shows a larger number of organic species measured during wildfires. This translated to a higher relative abundance for wildfire samples across different species except for the CHOSw species (Figure 2B). Considering the total abundance of all species, each sample shows different features. Contributions of CHO, CHOSw, and CHOSm are higher for the non-wildfire sample, whereas CHNO and CHNOS are larger in the wildfire sample (Figure 2C).

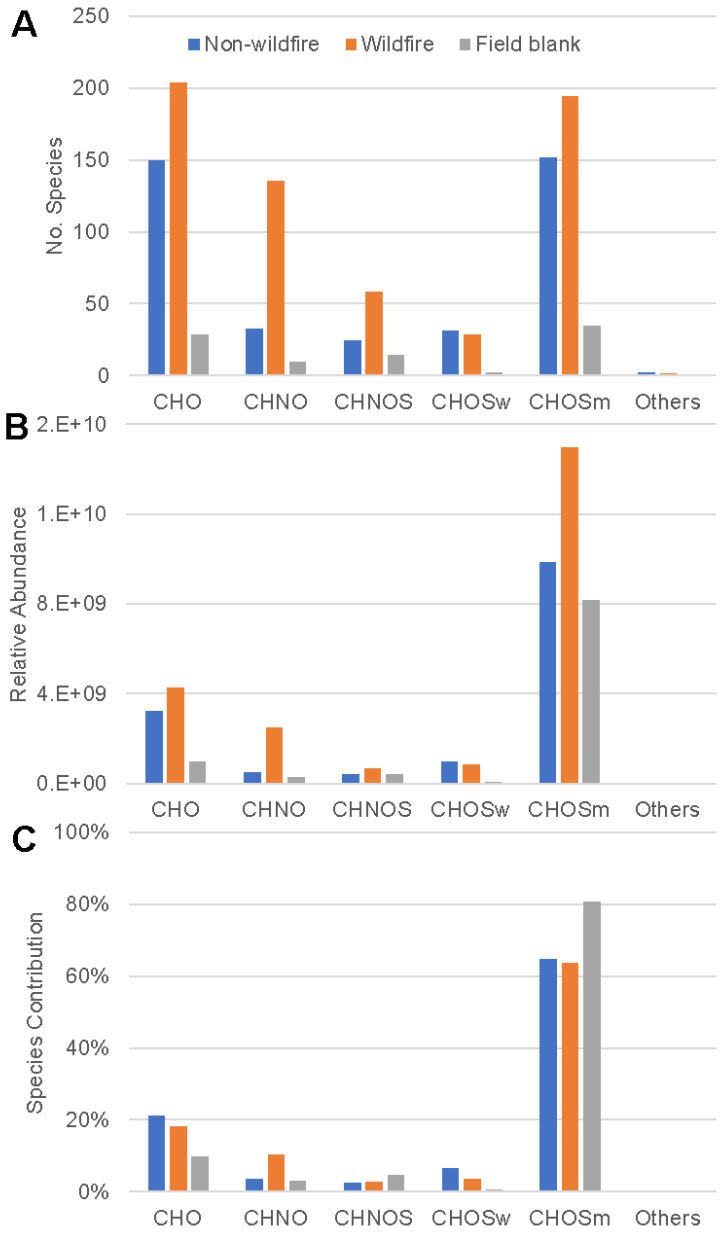

Figure 2. Organic species are characterised in the non-wildfire, wildfire, and field blank samples. (A) The number of compounds, (B) total relative abundance, and (C) contribution of each species group.

The higher contribution of CHNO and CHNOS species in the wildfire sample influenced

the total light absorption. Figure 3 reveals that the wildfire sample absorbed more light in the visible and ultraviolet range than the non-wildfire sample.

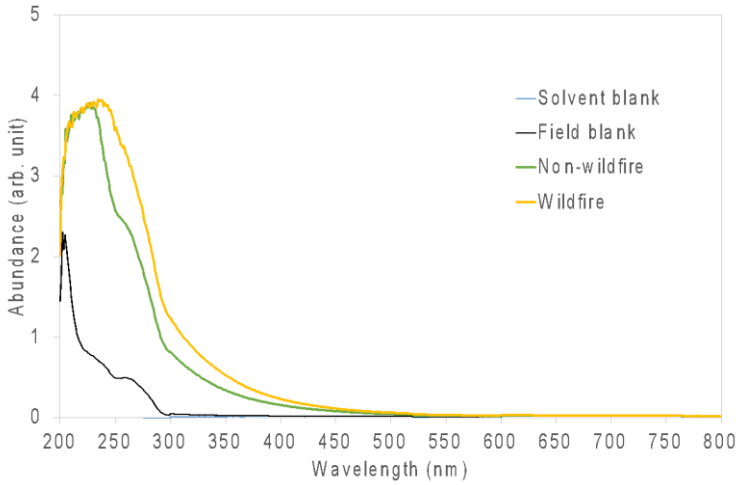

Figure 3. Light-absorbing characteristics of non-wildfire and wildfire samples.

1.2. Offline spectroscopy at STFC.

We planned to do offline spectroscopy using Raman and Fourier Transfer Infrared (FTIR) spectroscopy techniques.

- The Raman spectroscopy analysis did not give any results. This suggests the samples contain species that are not scattering light.
- The XRD analysis was not working due to the high solvent-to-sample ratio.
- The FTIR analysis successfully differentiated the sample from wildfire and no-wildfire periods. Also, this suggests the samples are dominated by chromophores that absorb light.

Fourier transform infrared (FTIR) spectroscopy has been taken in the attenuated total reflection (ATR) mode. ATR offers a very short path length of the sample's IR light, making this technique useful for highly absorbing materials such as aqueous solutions and long-chain polymers. FTIR spectra were recorded at room temperature with a Bruker Vertex 70 Fourier transform infrared spectrometer using a Bruker Diamond single reflection total internal reflection (ATR) accessory at ISIS Neutron and Muon Source, STFC. To record these spectra, we used 4 cm^{-1} resolution and 256 scans. The spectra were corrected for the wavelength-dependent path length (ATR corrections) using the Bruker software.

Three samples were measured in the form of liquid. These are biomass samples with wildfire haze, non-wildfire haze and a blank. The result is shown in Figure 4.

The observed peaks are assigned using available assignments of IR spectra of common organic compounds [1-2]. In both spectra of biomass haze, the strongest peak appeared around 1350-1500 cm^{-1}, which are assigned to N=O, S=O stretching and C-H scissoring bending modes. The region 1500-1700 cm^{-1} is wider and stronger in the biomass sample, with wildfire haze showing an increasing presence of nitro-aromatic compounds in the wildfire haze sample. Although weak, the presence of long-chain hydrocarbon compounds can also be interpreted in the wildfire haze sample. The absence of OH- stretching mode in the wildfire haze sample is not understood. The shape of the peak near 1350-1500 cm^{-1} has changed in the wildfire haze sample. However, this region is composed of many peaks, which are also there in the non-wildfire haze. Therefore, the individual contributions

of different compounds in this region are not well defined. Therefore, more experiments have to be done to establish these assignments.

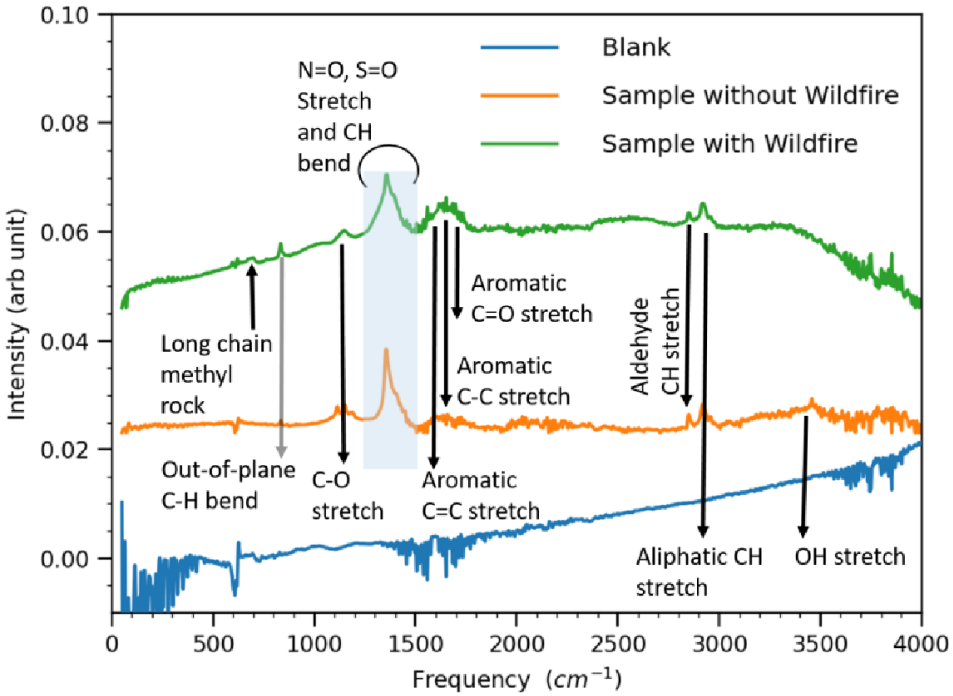

Figure 4. FTIR spectra of three samples (green) biomass with wildfire haze (orange) biomass without wildfire haze (blue) blank.

1.3. Neutron spectroscopy (STFC)

We applied to get access to use the near and intermediate-range order diffractometer (NIMROD) and inelastic neutron scattering spectrometer (TOSCA-**RB2220268**). Both proposals were unsuccessful due small amount of available samples.

1.4. Aerosol trapping spectroscopy (STFC)

The aerosol trapping spectroscopy did not get access time at CLF (**RB22230014**).

2. Cellular toxicological effects (PHE)

2.1. Biomass exposure.

BEAS-2B was originally established as an immortalised but non-tumorigenic epithelial cell line from the human bronchial epithelium. Because of general recognition for its bronchial epithelial origin, the BEAS-2B cell line has been widely used as an in vitro cell model in a large variety of toxicity assessments for inhalable contaminants.

BEASE-2B cells were exposed to biomass and controls (wildfire, non-wildfire, field blank) at different concentrations (0.001 – 0.1 mg/mL) for different exposure durations (1 hr, 4 hrs and 24 hrs).

2.2. The biological toxicity effects of biomass in the BEASE-2B cells.

The lactate dehydrogenase (LDH) assay was used to assess cytotoxicity (Figure 5). The LDH assay measures the LDH released in the cell culture medium due to the membrane permeability disruption. Results based on this study indicated that only the highest tested

exposure dose (0.1 mg/mL) of wildfire biomass could induce some toxicological effects post 24 hours of exposure, whereas all other exposure conditions didn't induce a significant increase.

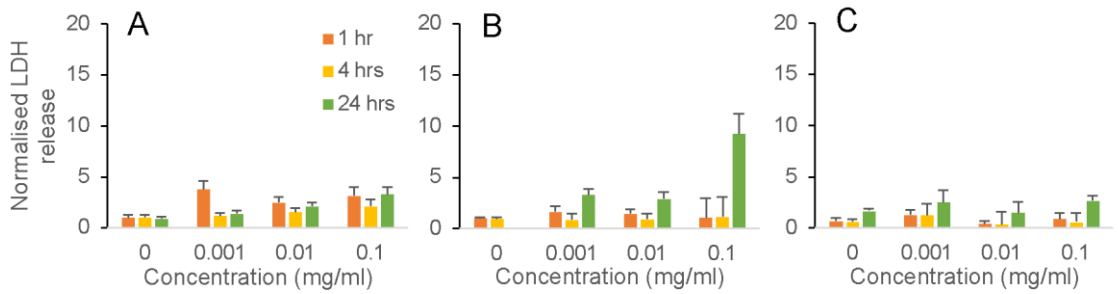

Figure 5. Biological toxicity analysis of aerosols from (A) non-wildfire aerosols, (B) wildfire aerosols, and (C) filter blank.

2.3 Oxidative stress induced by biomass exposure in the BEASE-2B cells.

The expression of selected genes was checked by qPCR (Figure 6). HMOX1 and NQO1 are biomarkers for oxidative stress. HMOX1 is an inducible isoform of heme oxygenase whose transcription is triggered by a wide variety of stressors, indicating general oxidative stress. The NQO1 is a conserved target gene of NRF2 and can serve to monitor the activity of the NRF2 pathway. For both genes, expression was increased when exposed to wildfire biomass samples at 0.1 mg/mL post 24 hours exposure.

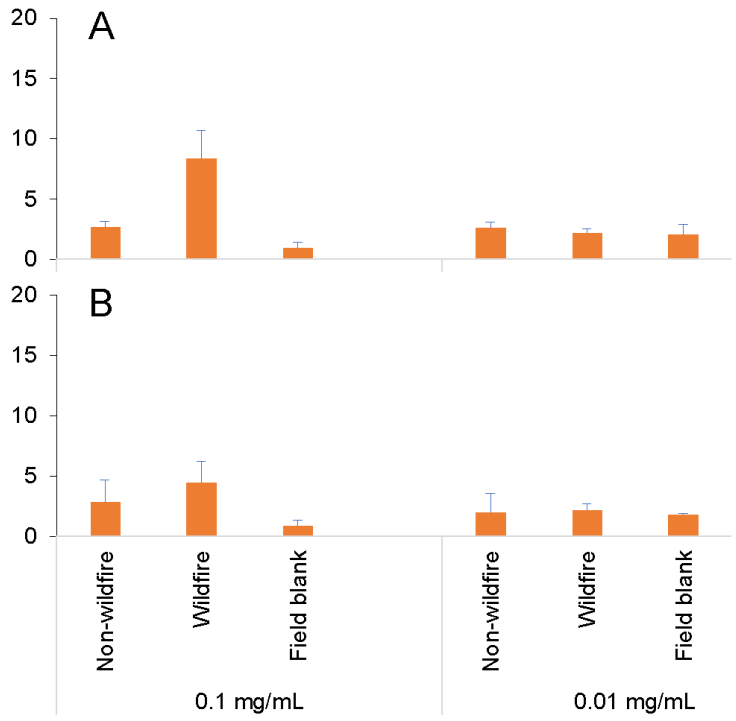

Figure 6. Oxidative stress changes are shown by (A) HMOX1 and (B) NQO1 genes expression.

The above biological analysis indicates that the cellular toxicity of biomass is dose- and time-dependent.

References

[1] IR Spectrum Table (https://www.niu.edu/clas/chembio/research/analytical-lab/ftir/ir-frequencies-table.shtml)
[2] D. Zhang et al., RSC Adv., **7**, 6849 (2017)

What are the next steps for this research? Will you be applying for further funding? What will you need to continue researching this topic?

This project demonstrated the scope of analysis for ambient PM2.5 influenced by wildfire. The FTIR complements the high-resolution mass spectrometry analysis for elucidating the chemical composition of ambient PM2.5. The toxicological analysis can be extended further by exposing BBA on cells before performing neutron spectroscopy.

Results from this analysis will be used to develop a proposal for NERC Independent Research Fellowship for **SHB**.

Please outline the role of STFC in this project

STFC's roles in the project include funding the project, providing networking opportunities online and in-person, and advising in proposal writing. STFC has provided facilities for FTIR, Raman and XRD. STFC also has provided assistance in writing beam time proposals. Unfortunately, due to the small volume of the sample and strong interference from the solvent, some of these experiments did not work as expected.

Please list a brief list of all outputs and impacts below. These may include papers, articles or blogs, presentations at events or conferences, meetings about future plans for the research. Please include links wherever possible

Our project was significantly delayed due to the inability to access the STFC facility through the access time call. However, we managed to do some experiments in between access times. The results of the experiment will be finalised in March-April 2023.

Were there any unexpected outcomes as part of the project?

The unexpected outcome is the high interference of solvent to sample in the XRD analysis.

Due to the limited amount of biomass samples, limited analysis could be performed on biological endpoints.

What are your plans to share the outcomes of this research with others? (Give details of any future meetings, conferences, papers or other dissemination planned)

This project will be presented at the Clean Air Networks Conference in Birmingham on 5-6 July 2023 and the European Aerosol Conference in Malaga, Spain on 3-8 September 2023. Moreover, the results will be written into a paper on wildfire particle biological impacts for the International Journal of Molecular Science.

Project Impact: What is the most significant output/impact from this project?

The most significant impact is a collaboration between the scientists and a better

understanding of analytical capabilities from different institutions.