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Indoor Burning of Arabian Incense Generates Ultrafine-Rich Particles with Strong Oxidative Potential

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Abstract. Arabian incense (Bakhoor) burning is a widely practiced fragrancing and ceremonial activity, yet how the Bakhoor composition controls particle emissions and oxidative potential remains poorly constrained, especially under repeated use in low-ventilation settings. Here we characterized emissions from Bakhoor burning in a controlled chamber using a charcoal-assisted heating configuration representative of common practice and quantified aerosol oxidative potential using complementary acellular dithiothreitol (DTT) activity and a macrophage-based intracellular oxidative-stress response, with smoldering sidestream cigarette smoke as a benchmark source. Normalized by the initial Bakhoor mass per burn, Bakhoor burning produced particle mass and number emission rates of 670-1690 μg min^{-1} g^{-1} and (6-7) × 10^{11} particles min^{-1} g^{-1}, respectively. Ultrafine particles contributed 70-75% of the total particle number, and their emission rates substantially exceeded those from sidestream cigarette smoke. Across Bakhoor materials, emission magnitude followed a nonlinear power-law relationship with the loading of the hexane-soluble fraction, indicating that this fraction is an important control on particle production. In the acellular assay, the particle mass-normalized DTT consumption rate (OP_{DTTm}) was approximately 32 pmol min^{-1} μg^{-1}, modestly lower than that of cigarette smoke particles, whereas Bakhoor burning particles elicited stronger intracellular oxidative-stress responses. Ozone aging increased oxidative potential for both sources, and the acellular and cellular responses remained evident after aging equivalent to days of indoor exposure. Overall, Bakhoor burning represents a previously underrecognized source of ultrafine aerosol with substantial oxidative potential.

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1. Introduction

Global exposure assessments indicate that people spend approximately 60-90% of their time indoors (Sadoun et al., 2016; Pillarisetti et al., 2022), making indoor environments an important setting for air pollutant exposure. In arid regions such as the Middle East, this percentage can approach 100% (Sadoun et al., 2016), driven by extended periods of extreme heat and limited outdoor comfort. Despite this high exposure potential, indoor air quality has historically received less scientific and regulatory attention than outdoor pollution (Cincinelli and Martellini, 2017). Indoor air is shaped by both outdoor infiltration and indoor activities, with combustion-related processes often contributing substantially to particle exposure (Gligorovski and Abbatt, 2018). While major indoor combustion sources such as tobacco smoke and household solid-fuel use have been widely studied (Wang et al., 2018a), other routine combustion-based fragrancing and ceremonial practices remain comparatively under-characterized as particle sources.

A prominent example is the burning of Arabian incense (Bakhoor), a widely practiced fragrancing and ceremonial activity in the Middle East and other regions, in which a mixture of perfumed wood chips soaked with additives is heated on charcoal. In addition to the aromatic wood chips, Bakhoor materials often contain scented oils, resins, and botanical additives such as herbs and flowers (Wahab and Mostafa, 2007), forming a chemically complex mixture that can generate condensable precursors upon thermal degradation. Because Bakhoor is typically heated on smoldering charcoal, both the Bakhoor material and the ignition source can contribute to particle formation. Bakhoor burning commonly occurs indoors, often in enclosed spaces with limited ventilation, and is embedded in daily routines as well as religious and social gatherings. In Jazan, Saudi Arabia, 98% of households report regularly burning Bakhoor indoors (Jareebi et al., 2024). Frequent incense use has also been reported in Gulf regions (Yeatts et al., 2012), while household incense burning is likewise prevalent in parts of Asia (Pan et al., 2014; Zhao et al., 2025). This widespread use indicates that combustion-based fragrancing and ceremonial practices are not only regionally important, but also relevant to a broader class of indoor combustion sources. For such sources, quantitative descriptors such as source strength, particle size distribution, and particle reactivity are important for understanding emission characteristics and how they vary with source composition and burning conditions (Kuye and Kumar, 2023). Ultrafine particles are often of particular interest because of their importance to particle number emissions and their potential sensitivity to source and combustion conditions (Shen et al., 2017).

A limited number of studies have provided preliminary, largely qualitative evidence of potential concern related to Bakhoor burning. Elsayed et al. (2016) reported that raw, unburned Bakhoor materials contain a chemically complex mixture of organic constituents, including nitrogen-containing and other additive-related species. However, the composition of particles emitted during Bakhoor burning can differ substantially from the raw-material profile because thermochemical processing during heating and pyrolysis can transform the formulation and generate new condensable products that partition to the particle phase. Dalibalta et al. (2015) provided preliminary indications of potential concern in Bakhoor smoke by noting the presence of compounds classified as carcinogenic, toxic, and/or respiratory irritants; however, the evidence was not linked to size-resolved emissions or dose-relevant particle metrics. Alarifi et al. (2004) further reported structural changes in pneumocytes

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following repeated animal exposure to Bakhoor smoke. Taken together, these studies suggest that Bakhoor smoke warrants further quantitative evaluation using particle-based metrics relevant to inhalation exposure.

Here, we characterize particle emissions and oxidative responses of Bakhoor burning in a controlled chamber, using smoldering sidestream cigarette smoke as a benchmark indoor combustion source (Chen and Zhao, 2024). Real-time particle number and mass concentrations and size distributions were measured using a scanning mobility particle sizer, and size-resolved emission factors were quantified. Particular attention was given to ultrafine particles (UFPs, <100 nm) because of their high respiratory deposition efficiency and potential relevance to systemic health effects (Oberdörster et al., 2005). Control experiments were performed to quantify the respective contributions of the Bakhoor material and the ignition charcoal to emitted particle size distributions and emission factors. Oxidative responses were evaluated using two complementary approaches commonly applied in atmospheric and exposure research (Seo et al., 2025). These included acellular oxidative potential quantified by the dithiothreitol (DTT) assay and cellular responses assessed via intracellular oxidative-stress response and cell viability in alveolar macrophages. To probe potential chemical drivers, particle extracts were analyzed offline using high-performance liquid chromatography coupled with high-resolution mass spectrometry, and compositional metrics were examined in relation to DTT activity. Changes in chemical composition and associated oxidative responses of Bakhoor burning particles were also evaluated following ozone exposure as an additional probe of post-emission processing. This work aims to better constrain the size-resolved emissions, source contributions, and oxidative responses of particles emitted from Bakhoor burning.

2. Methods

2.1 Generation and collection of particles from Bakhoor burning and sidestream cigarette smoke

All burning experiments were conducted inside a custom-built acrylic chamber (50 × 50 × 50 cm). Three commercially available Bakhoor products were purchased from local markets in Saudi Arabia, selected based on consumer popularity. For each experiment, 1 g of Bakhoor was placed onto a smoldering charcoal briquette, consistent with common household practice. The charcoal was a commercially available quick-lighting type typically used for Bakhoor burning. Charcoal-only burns were conducted under the same chamber configuration to quantify emissions attributable to the ignition source. As a benchmark, smoldering sidestream cigarette smoke was generated in the same chamber to provide a stable and continuous particle stream. Although this approach differs from puffing-based protocols, it improves reproducibility and has been used in prior emission studies (Ott et al., 2021; Wu et al., 2012). Once Bakhoor was placed on the smoldering charcoal, visible smoke generation began immediately and typically persisted for approximately 20 min. The chamber was operated under a steady-state flow-through mode with purified air supplied at 20 L min^{-1}. Size-resolved particle number concentrations were continuously measured using a Scanning Electrical Mobility Spectrometer (SEMS; Model 2100, Brechtel Manufacturing Inc.) over a mobility diameter range of 10–500 nm. Larger particles (500–2500 nm) were quantified using an optical particle counter (OPC; Brechtel Manufacturing Inc.). Prior to each experiment, background particle concentrations in the chamber were reduced to <1 particle cm^{-3}. Particle number size distributions from the SEMS and OPC were converted to mass concentrations assuming

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an effective particle density of 1 g cm^{-3}. In addition to real-time measurements, aerosol samples were collected on 47 mm quartz and PTFE filters, each at approximately 6 L min^{-1} for offline chemical analysis. Quartz filters were pre-baked at 550 °C for 12 h to minimize background organic contamination.

2.2 Emission rate calculations

Particle mass emission rates (E, μg min^{-1}) were quantified using a size-resolved material-balance approach (Wang and Chan, 2023; Jiang et al., 2021), where particle losses were treated separately for each size bin. The chamber was continuously mixed using two internal fans, and emission rates were therefore derived using a well-mixed flow-through chamber mass-balance framework. For analysis, particles were grouped into the following diameter bins: 10-100, 100-200, 200-300, 300-400, 400-500, 500-1000, 1000-1500, 1500-2000, and 2000-2500 nm.

For each size bin *i*, the time-dependent mass balance in the well-mixed chamber is

$$V\frac{dC_i}{dt} = -F \times C_i + E_i - k_i \times V \times C_i, \quad \text{(Eq. 1)}$$

where V is the volume of the chamber (m^3), C_i is the mass concentration of particles in size bin i (μg m^{-3}), F is the outlet airflow rate (m^3 min^{-1}), E_i is the particle emission rate in bin i (μg min^{-1}), and k_i is the loss rate constant for particles in bin i (min^{-1}). Particle losses were assumed to follow first-order decay, consistent with previous chamber-based emission studies (Wang and Chan, 2023). Following each Bakhoor burning event, particle concentrations exhibited near-exponential decay, which was used to estimate k_i by fitting the decay curves, following the approach described by Jiang et al. (2021). Emission rates were then integrated over the full burning period and summed across all size bins to obtain the total particulate emission rate.

2.3 Ozone aging of particle samples

To simulate oxidative aging, PM-loaded filters were placed in an in-line filter holder and exposed to a continuous flow of ozone diluted in zero air. Ozone was generated by passing zero air through a mercury lamp ozone generator (Model 610, Jelight Inc., USA), and its concentration was continuously monitored at the outlet of the filter holder using an ozone analyzer (Model 106-L, 2B Technologies Inc.). Two ozone levels were applied, approximately 200 ppb and 1500 ppb, for 30 min. These exposures correspond to approximately 20 h and 150 h of equivalent indoor ozone exposure, respectively, assuming a representative indoor ozone level of 5 ppb (Nazaroff and Weschler, 2022). This accelerated protocol was selected for practicality and reproducibility and to isolate ozone-driven processing under controlled conditions. Although filter-bound PM could, in principle, be exposed to lower ozone concentrations for longer periods, multi-day treatments increase the likelihood of uncontrolled changes that are not directly attributable to ozone, including slow volatilization of semi-volatile constituents, continued dark aging, and time-dependent particle-filter interactions. Using elevated ozone over short durations therefore enables consistent, well-defined oxidant doses while minimizing these confounding processes.

2.4 Molecular characterization of organics in Bakhoor-burning particles

To analyze the molecular composition of organic species in Bakhoor-burning particles, filter samples were extracted in acetonitrile via 30-minute sonication, followed by filtration through a 0.22 μm PTFE syringe filter. Acetonitrile was selected over methanol to minimize solvent-induced artifacts, such as the methanolysis of conjugated carbonyl species (Chen et al.,

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2022). The extracts were analyzed using ultra-high-performance liquid chromatography coupled with high-resolution Orbitrap mass spectrometry (UHPLC-HRMS; Orbitrap ID-X, Thermo Fisher Scientific Inc.), equipped with an electrospray ionization (ESI) source operated in positive ion mode. Chromatographic separation was performed on an Acquity HSS T3 column (1.8 µm, 2.1 mm × 100 mm; Waters Corp.) under conditions optimized for semi-polar organic compounds (Wang et al., 2021; Go et al., 2022). The liquid chromatography gradient and flow parameters were configured to improve separation efficiency and minimize matrix effects, following established protocols for analyzing dissolved organic matter and aerosol extracts (Go et al., 2022; Liang et al., 2024; Patriarca et al., 2018). Data acquisition and molecular formula assignment were conducted using Compound Discoverer software (Thermo Fisher Scientific Inc.), with elemental compositions determined from exact m/z values and isotopic patterns. This analytical workflow enabled untargeted molecular profiling of the complex organic mixtures emitted from Bakhoor burning.

2.5 Acellular dithiothreitol (DTT) assay

The oxidative potential of particulate matter is a key indicator of its capacity to generate reactive oxygen species (ROS) and induce oxidative stress (Jiang et al., 2019). Among acellular assays, the DTT assay is widely used as a proxy for particle redox activity and quantification of the rate of DTT consumption by redox-active species that catalyze electron-transfer reactions (Cho et al., 2005). The experimental protocol followed established methods reported previously (Gao et al., 2017; Fang et al., 2017; Lyu et al., 2018; Cho et al., 2005). Briefly, filter samples were extracted in deionized water by 30 minutes of sonication. Given the potential for radical formation during sonication, a comparison between sonication and vortex shaking was conducted. The results showed no significant difference in DTT consumption, indicating that sonication did not introduce measurable artifacts. For the measurement of water-soluble oxidative potential ($OP^{WS\text{-}DTT}$), the extract was filtered and 0.7 mL of the filtrate was mixed with 0.1 mL of 1 mM DTT solution and 0.2 mL of potassium phosphate buffer (0.5 M, pH 7.4). The mixture was incubated at 37 °C in a thermostatic shaker with continuous agitation. At designated time points (0, 5, 10, 15, 25, and 35 minutes), 0.1 mL aliquots were withdrawn and quenched by adding trichloroacetic acid (TCA). The residual DTT in each aliquot was then reacted with Tris buffer (0.4 M containing 20 mM EDTA) and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The reaction yields a yellow chromophore that absorbs at 412 nm, which was quantified using a microplate reader. To determine the total oxidative potential ($OP^{Total\text{-}DTT}$), the same procedure was used, except the extract was not filtered, and the filter itself remained in the reaction mixture. This ensured that DTT-active species associated with insoluble particles suspended in the extract or still attached to the filter surface were also included in the measurement (Fang et al., 2017; Gao et al., 2017). To minimize photo-degradation of DTT and DTNB, all procedures were performed under low-light conditions by minimizing workspace lighting and covering vials with aluminum foil when not in use.

2.6 Cellular macrophage assays

Alveolar macrophages represent the first line of defense in the respiratory system and are widely used to assess oxidative and toxicological responses to PM exposure, particularly through intracellular ROS generation (Liu et al., 2023; Liu et al., 2020b; Liu et al., 2020a; Tuet et al., 2019). In this study, murine alveolar macrophages (MH-S, ATCC CRL-2019) were cultured in RPMI-1640 medium (ATCC) supplemented with 10% fetal bovine serum (FBS; VWR), 1% penicillin–streptomycin (Pen-

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Strep; VWR), and 50 μM β-mercaptoethanol (BME; Sigma-Aldrich). Cells were maintained at 37 °C in a humidified 5% CO_2 incubator. Prior to ROS measurements, 96-well plates were pre-coated with 10% FBS in phosphate-buffered saline (PBS) to promote cell adherence. MH-S cells were seeded at a density of 2×10^4 cells per well and incubated with the ROS-sensitive fluorescent probe carboxy-H_2DCFDA (Molecular Probes, C-400). To introduce particulate exposure, PTFE filters loaded with either fresh or ozone-aged Bakhoor burning particles were directly immersed in the culture medium. A 3-minute vortex mixing step was applied to enhance dispersion of PM into the media. Cells were exposed for 24 hours, after which the filters were removed and intracellular oxidative-stress responses were measured using a microplate reader (excitation/emission: 485/525 nm). All treatments were conducted in triplicate using independent filter samples. Blank controls (media only, no PM) were used to normalize fluorescence values.

A mitochondrial dehydrogenase (MTT) assay was conducted to evaluate cell viability following 24-hour exposure to PM. After exposure, the culture medium in each well was replaced with 100 μL of fresh medium, and 10 μL of MTT solution (5 mg mL^{-1}) was added. Cells were incubated for an additional 4 hours at 37 °C to facilitate the formation of purple formazan crystals. Following this, 100 μL of a solubilization solution consisting of 10% SDS in 0.01 M HCl was added to each well, and incubation was continued for a further 15–18 hours under the same conditions. The absorbance at 570 nm was then measured using a microplate reader to quantify formazan formation, which serves as an indicator of mitochondrial enzymatic activity and, consequently, cell viability. A viability calibration curve was established by preparing mixtures of live and heat-inactivated (autoclaved) cells at defined ratios, treating them with the same MTT procedure, and recording their absorbance at 570 nm to relate optical density to cell survival.

3. Results and discussion

3.1 Emission characteristics of Bakhoor burning particles

Figure 1A-B show the size distributions of particle number and mass for emissions from Bakhoor burning, with smoldering sidestream cigarette smoke included as a comparative benchmark. Because Bakhoor was heated on a smoldering charcoal briquette, the measured distributions reflect combined contributions from the Bakhoor material and the ignition source. A charcoal-only control is therefore included to isolate charcoal-derived emissions. In the number distribution (Figure 1A), charcoal-only burning produced a weak nucleation-mode signal largely confined to the ultrafine range, whereas Bakhoor burned on charcoal yielded substantially higher concentrations and a bimodal profile, with a dominant ultrafine mode near 30 nm and a secondary mode around 160 nm. The ultrafine-mode peak was approximately 1.4 times the secondary peak, indicating strong enrichment of ultrafine particles (UFPs, <100 nm) by number. Sidestream cigarette smoke, by comparison, exhibited a unimodal number distribution centered at approximately 110 nm under the protocol used here, with a substantially lower UFP contribution. In the mass distribution (Figure 1B), Bakhoor burning produced a unimodal profile centered at approximately 350 nm, indicating that emitted mass was dominated by accumulation-mode particles. Time-resolved measurements further show that particle concentrations rose within seconds after ignition, peaked at approximately 5 min, and then decayed (Figure 1C).

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Figure 1D summarizes emission rates normalized to the initial Bakhoor mass per burn and to the mass of cigarette tobacco filler consumed in the sidestream benchmark. On this basis, Bakhoor burning produced particle mass emission rates of 670-1690 μg min^{-1} g^{-1}, substantially higher than the sidestream cigarette benchmark (~250 μg min^{-1} g^{-1}). Because the cigarette benchmark was generated without active puffing, the resulting emission factors provide a conservative reference relative to active smoking conditions reported in the literature (Hu et al., 2023). In the context of typical indoor sources, these mass emission rates place Bakhoor burning toward the upper end of reported emission rates for activities including tobacco and incense burning, moxa, candles, and non-combustion sources such as cooking and e-cigarettes (117–3367 μg min^{-1}), while recognizing that reported rates can vary with experimental configuration and normalization (Hu et al., 2023; Chuang et al., 2013; Jetter et al., 2002; Tian et al., 2021). Unlike conventional incense burning, however, Bakhoor combines a charcoal ignition source with a chemically complex mixture of perfumed wood and additives. Number-based emission rates for Bakhoor reached (6-7)×10^{11} particles min^{-1} g^{-1}, with UFPs accounting for 70–75% of total number, compared with ~47% for the cigarette benchmark. Taken together, these results identify Bakhoor burning as a high-intensity indoor particle source that is strongly enriched in UFPs by number while also producing substantial fine-particle mass emissions.

3.2 The potential drivers of particle formation during Bakhoor burning

Figure 2A shows that charcoal-only burning produces a short-lived nucleation-mode signal immediately after ignition that decays rapidly, whereas Bakhoor burning sustains elevated particle concentrations and extends to larger particle diameters. This contrast indicates that particle formation is not controlled by the ignition source alone, but depends strongly on the Bakhoor material itself. To identify the Bakhoor components responsible for this behavior, Bakhoor materials were operationally separated into the hexane-soluble fraction (HSF) and the wood residue. The HSF is a solvent-defined operational fraction containing hexane-soluble organic material, which may include fragrance oils, resins, and related additives. The residue represents the nonextractable wood matrix. Burning the corresponding wood residues yielded nearly identical particle mass emission rates across the three Bakhoor types, whereas the original Bakhoor materials varied by approximately 2.5-fold in mass emission rates (Figure 2B). This contrast indicates that the observed differences among commercial products are primarily driven by the HSF rather than by the base wood substrate under the conditions used here. To verify that solvent extraction did not alter the wood matrix and independently affect emissions, we conducted a reconstitution experiment in which the HSF was recombined with the wood residue at the appropriate mass fractions. The reconstituted material reproduced particle mass emission rates similar to those of the original samples (Figure S3), further indicating that the observed differences are driven primarily by the presence or absence of the HSF rather than by extraction-induced changes to the wood substrate.

Across the original Bakhoor samples, the HSF loading (9-22% by mass) was strongly associated with particle mass emissions (Figure 2B), with Type 2 exhibiting both the highest HSF loading and the highest mass emission rate. Perturbation experiments that partially reduced the HSF (tissue wiping) and increased it by recombining the HSF with the wood residue collapsed onto a common relationship between emission rate and HSF loading across all Bakhoor types. This relationship supports HSF loading as the primary control on emission magnitude, even though the composition of the HSF varied slightly among samples (Figure S4). This dependence was well described by a power-law relationship ($\beta \approx 2.1$; Figure 2B),

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demonstrating a nonlinear response in which emissions increase more steeply as the HSF increases. Adding HSF to reach approximately 10 wt % of the reconstructed material increased mass emission rates by ~1.7-fold, whereas increasing the HSF content to approximately 20 wt % increased emissions by ~4.3-fold. This pattern suggests a threshold-like increase in effective particle formation efficiency, where incremental increases in available precursors result in disproportionate increases in particle production (Zhang et al., 2010).

Thermogravimetric analysis (TGA) was used to assess whether the observed HSF dependence could be primarily explained by simple evaporation of intact additives. Across the three Bakhoor types, thermograms were similar and showed only minor mass loss (approximately 5%) below 100-120 °C (Figure S5), whereas most mass loss occurred between approximately 150 and 350 °C. Thermocouple measurements showed that the charcoal temperature reached approximately 300 °C within 15 s of sustained ignition in our setup (Figure S6), suggesting that Bakhoor materials are quickly driven into a higher-temperature regime rather than remaining in a low-temperature evaporation window. The reported onset of wood pyrolysis (approximately 180 °C) refers primarily to the lignocellulosic wood substrate (Escalante et al., 2022). In contrast, the HSF consists of nonstructural, solvent-extractable organics introduced during product formulation, which may volatilize and thermally transform over overlapping temperature windows during heating on charcoal. Taken together, these results suggest that HSF-linked particle formation is not solely explained by low-temperature evaporation and likely involves thermochemical processing during heating that generates condensable products, as illustrated in Figure 2C. Because TGA does not provide a mass balance for emitted particles, it is used here to bracket plausible precursor-generation regimes rather than to quantify particle yields.

3.3 Oxidative potential of particles from Bakhoor burning

To evaluate the oxidative potential of particles emitted from Bakhoor burning, we applied the dithiothreitol (DTT) assay. We report the particle mass-normalized DTT consumption rate (OP_{DTTm}, pmol min^{-1} μg^{-1}) for both filtered aqueous extracts and total (unfiltered) aqueous suspensions to gauge the relative contributions of water-soluble versus suspension-associated components. Fresh Bakhoor extracts exhibited an average OP_{DTTm} of approximately 32 pmol min^{-1} μg^{-1} (Figure 3A). This magnitude lies within reported ranges for laboratory-generated particles from indoor combustion sources (8-95 pmol min^{-1} μg^{-1}; Hu et al., 2023; Zhang et al., 2024) and for raw wood/biomass materials such as pine (35-117 pmol min^{-1} μg^{-1}; Zhang et al., 2024), and is comparable to values reported for ambient PM and biomass-burning organic aerosol fractions. Notably, the OP_{DTTm} of Bakhoor burning particles exceeds the typical range for wildfire-influenced PM (2-16 pmol min^{-1} μg^{-1}; Fang et al., 2023), reaching values near the upper end of the wildfire range (around 32 pmol min^{-1} μg^{-1}; Isenor et al., 2025). Under the same assay conditions, cigarette smoke particles showed a higher OP_{DTTm} of approximately 50 pmol min^{-1} μg^{-1}, about 40% higher than Bakhoor burning particles. To assess the contribution of insoluble components, OP_{DTTm} was also measured for total particle suspensions (Fang et al., 2023; Charrier and Anastasio, 2012; Brehmer et al., 2019). Only a modest enhancement (approximately 10%) was observed relative to filtered extracts (Figure 3A), suggesting that DTT-reactive species are captured predominantly in the aqueous phase under the extraction conditions used here.

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Oxidative aging can further shape exposure-relevant redox properties because indoor oxidants can transform particle composition and, in turn, DTT consumption (Wong et al., 2019; Wang et al., 2023). Given the limited penetration of shortwave UV radiation indoors, we focus here on ozone-driven aging (Wang et al., 2018a; Yuan et al., 2025). Residential air exchange rates typically range from 0.05 to 1 h^{-1}(Zhao and Liu, 2020; Salthammer, 2011), corresponding to ventilation-based particle residence times of approximately 1-20 h. Moreover, the high indoor surface-to-volume ratio promotes rapid particle deposition to surfaces (Abbatt and Wang, 2020), creating reservoirs that are not removed by ventilation and can persist for days; these deposits can continue to undergo chemical processing and may contribute to exposure through subsequent resuspension or disturbance of indoor surfaces. After an ozone dose equivalent to approximately 150 h at an indoor ozone level of 5 ppb (Figure 3B), OP_{DTTm} for water extracts increased by approximately 15% for both Bakhoor and cigarette smoke particles. This increase suggests the net formation of more DTT-active oxygenated products during ozonolysis, consistent with a rise in the carbon oxidation state (OSc) derived from mass spectrometric analysis (Figures S7 and S8). Across fresh and O_3-aged samples, the median OP_{DTTm} of Bakhoor water extracts fell within the broad range reported for laboratory combustion-derived PM and overlapped values reported for field PM, although many field studies report total OP_{DTTm}, which is typically higher than the water-soluble fraction. Prior work by Wong et al. (2019) reported an initial increase in OP_{DTTm} (~20%) during the early stages of ozonolysis of biomass-burning particles, followed by a decrease toward baseline after extended aging (up to 70 h), suggesting that some DTT-active products can be transient. Quinone-like redox-cycling species, which catalyze ROS formation, have been proposed as plausible contributors to the observed changes in oxidative potential (Xiong et al., 2017). These observations motivate identifying the chemical drivers that govern DTT consumption in Bakhoor smoke.

3.4 Potential chemical contributors to DTT activity

To examine which particle-phase constituents may underlie the observed DTT response, we analyzed extracts of Bakhoor burning and cigarette smoke particles using UHPLC-HRMS. Both sources showed broad distributions of detected features spanning $m/z \approx$ 100-400 (Figure S9), consistent with LC/ESI-MS characterizations of biomass-burning organic aerosol and combustion-derived organic aerosol (Smith et al., 2009). Despite comparable m/z envelopes, the assigned elemental-composition classes differed markedly between the two sources. Bakhoor burning particles were dominated by CHO formulas (~65% of assigned species), whereas cigarette smoke particles were relatively less oxygenated and strongly enriched in nitrogen-containing formulas (CHN+CHON≈84%) (Figure S9). Since elemental class alone provides limited insight into structural motifs relevant to redox cycling, we also computed the modified aromaticity index (AI_{mod}, see Text S1 for details) as a proxy for unsaturation and aromatic character and classified formulas as aromatic ($AI_{mod} \geq 0.5$), olefinic ($0 < AI_{mod} < 0.5$), or aliphatic ($AI_{mod} = 0$) (Koch and Dittmar, 2006; Song et al., 2021; Song et al., 2019).

We then related OP_{DTTm} to molecular-formula-derived metrics (AI_{mod}, O:C, N:C) across fresh and ozone-aged samples from both sources (Figure 4). Across the full dataset, aromatic-like formulas ($AI_{mod} \geq 0.5$) exhibited the strongest positive association with OP_{DTTm} (R^2>0.5). The strongest correlations were observed for moderately oxygenated formulas (O:C ≈ 0.05-0.15), indicating that the DTT response likely tracks most closely with unsaturated/aromatic-like formulas and is consistent with the O:C range often associated with redox-active aromatic products, including quinone-like structures (Charrier and

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Anastasio, 2012). In contrast, highly oxidized formulas (e.g., acid-rich CHO species at higher O:C) and aliphatic-like formulas ($AI_{mod} = 0$) showed weaker associations with OP_{DTTm} (Shultz et al., 2011; Park et al., 2006). These interpretations are based on molecular formulas and bulk metrics. Definitive assignment to specific compound classes (e.g., individual quinones) requires targeted identification and standards.

Nitrogen-containing aromatic-like formulas also exhibited positive associations with OP_{DTTm}, with the strongest relationships observed at N:C≈0.10-0.25. Because the strongest correlations overall occurred for aromatic-like formulas with high AI_{mod}, this pattern suggests that nitrogen substitution within unsaturated/aromatic-like structures may mark an additional set of formulas associated with DTT activity at the molecular-formula level. The intrinsic DTT reactivity of individual nitrogen-containing organics remains less well constrained than that of quinone-type species, but prior work has linked nitroaromatics and N-heterocycles to enhanced DTT responses in complex mixtures (Lai et al., 2025). Nitrogen functionalities may also influence redox cycling indirectly, for example by modulating electron density or stabilizing redox intermediates in mixed organic matrices (Dou et al., 2015). Consistent with these associations, Bakhoor-burning particles were dominated by CHO formulas and only a minority met the aromatic-like criterion ($AI_{mod} \geq 0.5$; Figure S11), whereas a substantially larger fraction of cigarette-derived CHN/CHON formulas were aromatic-like (Figure S11), corresponding to the higher OP_{DTTm} observed for cigarette smoke particles.

Finally, since dissolved transition metals can contribute to DTT activity directly and/or via interactions with organics (Jiang et al., 2019; Wang et al., 2018b), we conducted metal-chelation tests using Chelex resin. OP_{DTTm} changed negligibly after chelation (Figure S12), indicating a minimal contribution from dissolved metals and suggesting that OP_{DTTm} in both sources is dominated by organic species.

3.5 Cellular oxidative-stress responses induced by Bakhoor burning particles

To further assess cellular oxidative-stress responses to Bakhoor burning particles, we quantified intracellular fluorescence signals in alveolar macrophages using a DCFH-based assay and reported responses as fold changes relative to untreated controls. This cellular endpoint complements the acellular DTT assay by capturing integrated intracellular oxidant signaling under particle exposure. As described in the Methods, five particle dose levels were tested for each sample, dose-response relationships were fitted using the Hill equation, and the area under the fitted dose-response curve (AUC) was used as an integrative metric to quantify the overall oxidative response (Figure 5A) (Tuet et al., 2016).

Bakhoor burning particles elicited stronger DCFH fluorescence responses than sidestream cigarette smoke particles across the tested dose range (Figure 5B). The AUC metric ranged from 0.51 to 0.62 for Bakhoor samples, higher than 0.41 for cigarette smoke. The magnitude of the response induced by Bakhoor burning particles was comparable to that reported for naphthalene-derived secondary organic aerosol, a well-established oxidative-stress inducer in cellular assays (Tuet et al., 2017). At higher particle doses, the fluorescence response approached a plateau (Figure 5A), consistent with saturation of the assay response and/or the onset of cytotoxicity, as supported by the accompanying MTT viability results (Figure S13).

Notably, these cellular oxidative-stress responses differed from the acellular DTT results, for which cigarette smoke particles exhibited 47-100% higher OP_{DTTm} than Bakhoor burning particles. Such divergence is expected because DTT and

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DCFH-based cellular readouts probe different chemical and biological processes and can be weakly correlated for complex mixtures (Kim et al., 2014; Tuet et al., 2016; Seo et al., 2025). Similar assay-dependent discrepancies have been reported previously; for example, Tuet et al. (2016) observed weak or absent correlations between DTT activity and DCFH-based cellular oxidative-stress responses for some wintertime ambient PM samples. Mechanistically, DTT primarily reflects the electron-transfer capacity of extractable species in a simplified buffer system, whereas the cellular assay integrates particle delivery and uptake, intracellular bioactivation, depletion of redox buffers, and activation of oxidant-generating pathways (Kim et al., 2014). Collectively, these results underscore that DTT alone may not capture the full spectrum of oxidative-stress potential for indoor combustion aerosols.

Although mechanistic attribution of cellular signaling to specific molecules is inherently challenging, the compositional information provides plausible hypotheses for why Bakhoor burning particles produced stronger cellular oxidative-stress responses. Bakhoor extracts were enriched in oxygenated CHO formulas (Figures S9 and S10), consistent with a larger contribution from oxygenated organics, some of which may contain electrophilic carbonyl functionality. Electrophilic aldehydes and related carbonyls are well known to form covalent adducts with cellular nucleophiles, particularly cysteine thiols in glutathione and proteins, thereby depleting intracellular thiol buffering capacity and perturbing redox homeostasis in ways that can amplify downstream oxidant signaling (Fritz and Petersen, 2013; Lopachin and Gavin, 2014). AI_{mod} classification further places a substantial fraction of Bakhoor CHO formulas in the olefinic and non-aromatic unsaturated regimes (Figure S11). These less-condensed CHO structures may be more conformationally flexible than condensed aromatic frameworks and may interact differently with lipid phases, potentially affecting cellular delivery and subsequent bioactivation (Liu et al., 2011). By contrast, cigarette smoke particles were enriched in nitrogen-containing formulas (CHN/CHON, Figure S11) yet produced a lower net DCFH response under the exposure conditions used here, suggesting that the dominant chemical drivers of OP_{DTTm} and cellular oxidative-stress responses differ across sources.

4. Conclusions and atmospheric implications

This study demonstrates that Bakhoor burning is a potent indoor source of fine particles, particularly with a substantial ultrafine fraction. Across the three products tested here, Bakhoor burning produced particle mass and number emission rates of 670-1690 μg min^{-1} g^{-1} and (6-7) × 10^{11} particles min^{-1} g^{-1}, respectively, with ultrafine particles accounting for 70-75 % of total particle number. On a mass-normalized basis, these emission rates were approximately 4.3-fold higher than those of the sidestream cigarette benchmark. Together with the observed size distributions, these results indicate that routine Bakhoor use can generate exposure-relevant particle burdens comparable to, and in some use scenarios exceeding, those from more widely recognized indoor combustion sources. Because respiratory deposition varies strongly with particle diameter and differs between the tracheobronchial (Tb) and alveolar (Al) regions (Figures 6A and 6B), lung-deposited surface area (LDSA; see Text S2) was estimated as an inhalation-relevant dose metric that has been shown to track inflammatory and cytotoxic responses across particle types more consistently than particle mass alone (Hofmann, 2011; Oh et al., 2023). Using the mean emission factors measured here together with size-resolved regional deposition fractions from Hofmann (2011), the Bakhoor-

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to-cigarette LDSA ratio exhibits strong size dependence and reaches its highest values in the ultrafine range (Figure 6B). Because particles at intermediate diameters dominate the emitted size distribution and the integrated surface-area dose (Figure S14), the LDSA ratio integrated over the measured size range is approximately 4 for both the Tb and Al regions (Figure 6C). For a reported Bakhoor use rate of 0.4-2.9 g d^{-1} (Bu-Olayan and Thomas, 2021), this scaling suggests that the daily inhalation-relevant dose from Bakhoor burning may be comparable to that from multiple cigarettes of smoldering sidestream smoke under the benchmark conditions used here, although the real-world equivalence will vary with room volume, ventilation, and burning practice.

Across the samples tested here, the combination of acellular OP_{DTTm} and macrophage DCFH responses shows that Bakhoor burning particles exhibit substantial oxidative potential and cellular oxidative-stress responses. Relative to sidestream cigarette smoke particles, these responses differ between assays on a per-mass basis and persist after ozone aging over multi-day equivalent exposures. Fresh Bakhoor extracts showed an average OP_{DTTm} of approximately 32 pmol min^{-1} μg^{-1}, ozone aging increased OP_{DTTm} by about 15 % under the conditions used here, and the DCFH-based AUC values for Bakhoor samples ranged from 0.51 to 0.62, compared with 0.41 for the cigarette benchmark. These results suggest that the oxidative properties of Bakhoor-burning particles can remain relevant after post-emission aging, rather than being limited to freshly emitted particles alone. This may be particularly important in indoor environments with limited ventilation and repeated daily use, where particle accumulation and continued chemical processing by indoor oxidants and surface interactions may extend the relevance of these responses beyond the point of emission. Such conditions are especially relevant in hot-arid regions such as the Middle East, where indoor spaces are frequently cooled using air-conditioning systems and windows may remain closed; in many settings with limited outdoor-air supply, air-exchange rates can be low (for example, <0.5 h^{-1}) (Indraganti et al., 2016; Diapouli et al., 2013). The accompanying reduction in cell viability further suggests that these oxidative responses may be associated with measurable biological effects under the in vitro exposure conditions tested here. Relating these in vitro exposure conditions to human lung dose, however, will require dedicated dosimetry and exposure modeling and should be addressed in future work.

The control and perturbation experiments further identify the hexane-soluble fraction (HSF) as an important leverage point for particle formation during Bakhoor burning. Operational treatments that reduced the HSF, including solvent extraction to generate wood residue and a simple tissue-wiping step to partially remove surface-associated HSF, lowered particle mass emissions. However, these perturbations yielded slightly higher particle mass-normalized OP_{DTTm} relative to untreated material (Figure S15), indicating that emission reductions do not necessarily lead to a reduction in oxidative potential on a mass basis. Nevertheless, when OP_{DTTm} is combined with the corresponding emission factors to estimate emission-weighted total DTT consumption, the net oxidative burden decreases for the HSF-reduced treatments (Figure S15). These results indicate that the HSF strongly influences both emission magnitude and oxidative properties. Because HSF loading varies across products, lowering HSF loading may provide a practical pathway to reduce emitted particle mass and, consequently, exposure, although hazard-relevant endpoints should be evaluated in parallel rather than inferred from emissions alone. At the user level, wiping Bakhoor pieces prior to burning may partially remove surface-associated HSF and reduce emissions, although the effectiveness

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of this measure will likely vary across products and user practice and should be quantified under realistic operating conditions. Electric Bakhoor burners may also represent a useful alternative by eliminating charcoal-assisted combustion and potentially reducing combustion-related particle formation, but their net effects on emissions and toxicity require evaluation under realistic use conditions before broad recommendations can be made. More broadly, these results identify source composition and burning configuration as important controls on particle emissions from Bakhoor burning, while highlighting the additional role of ventilation in shaping real-use exposure.

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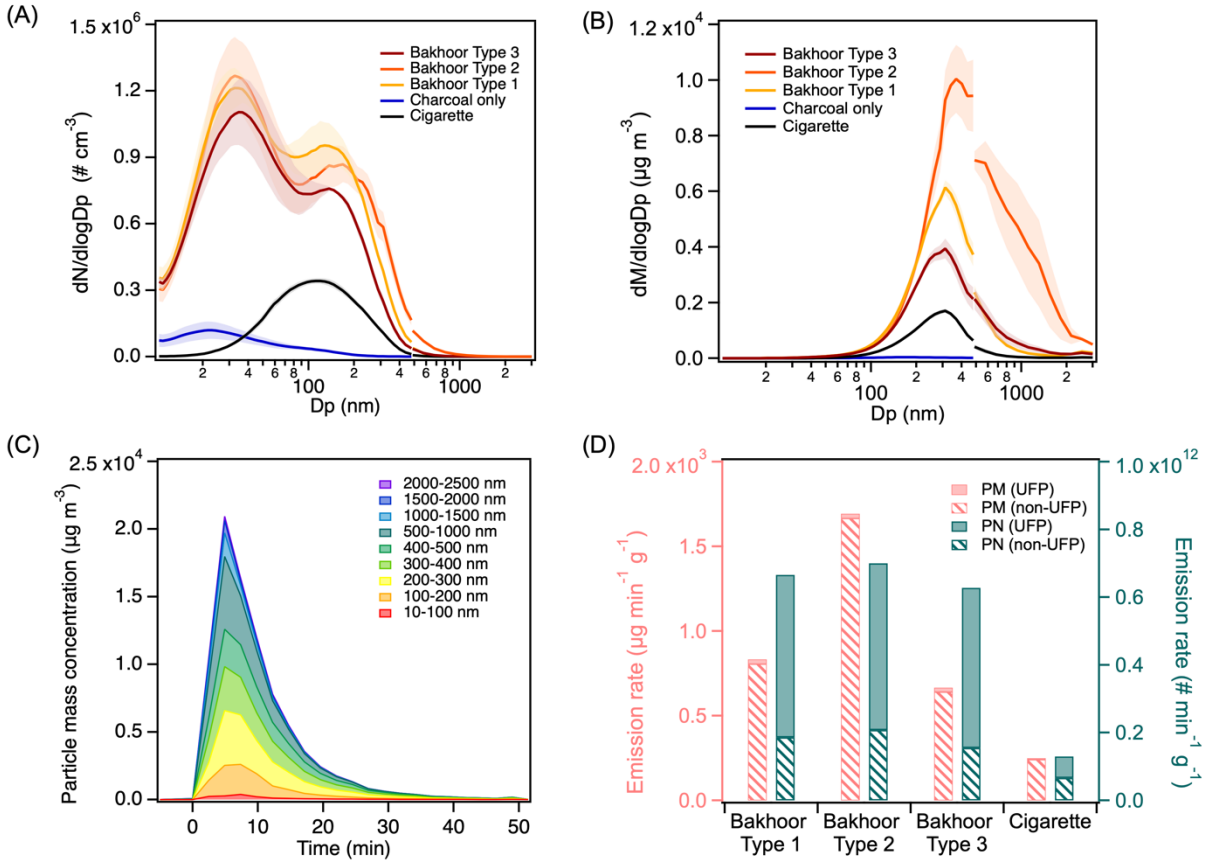

412 **Figure 1.** (A) Number and (B) mass size distributions measured in the chamber during Bakhoor burning on charcoal (three
413 products) and smoldering sidestream cigarette smoke, with a charcoal-only control; distributions are shown without size-
414 dependent loss correction. (C) Average particle mass concentration as a function of time following Bakhoor burning, resolved
415 by particle size ranges and shown without loss correction. (D) Particle emission rates, with mass (left axis) and number (right
416 axis) normalized to the initial Bakhoor mass per burn and to the mass of cigarette tobacco filler, respectively, reported
417 separately for ultrafine and non-ultrafine size fractions and corrected for size-dependent losses. The apparent discontinuity
418 near 500 nm in panels A and B reflects the use of two instruments: particles below 500 nm were measured by the SEMS,
419 whereas particles above 500 nm were measured by the OPC.
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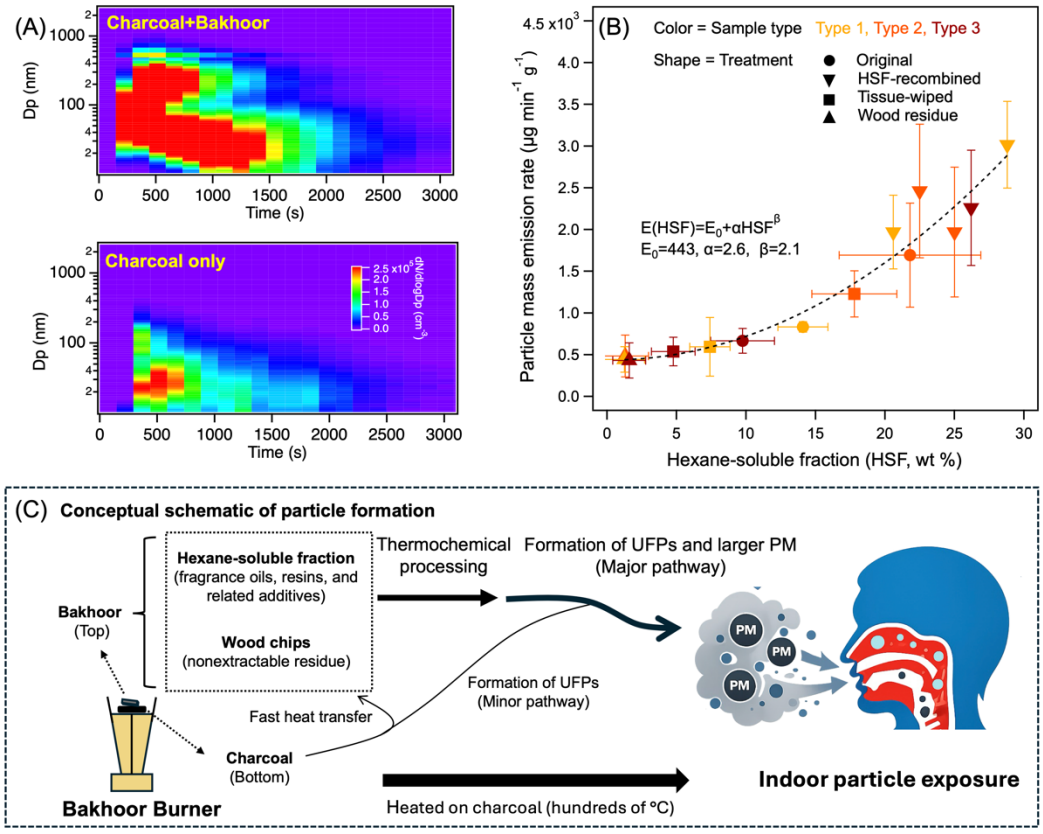

Figure 2. (A) Time-resolved particle number size distributions ($dN/dlogD_p$) measured during Bakhoor burning (with charcoal used for ignition) and during a charcoal-only control, shown as contour maps of mobility diameter (D_p) versus time. (B) Particle mass emission rate as a function of the loading of the hexane-soluble fraction (HSF, wt %) in Bakhoor materials. Colors denote Bakhoor type, and symbols denote treatment (original, HSF-recombined, tissue-wiped, and wood residue). The dashed curve shows an empirical fit, $E(HSF) = E_0 + \alpha HSF^{\beta}$ (parameters shown). (C) Conceptual schematic linking Bakhoor composition and heating on charcoal to fine particle formation and indoor particle exposure.

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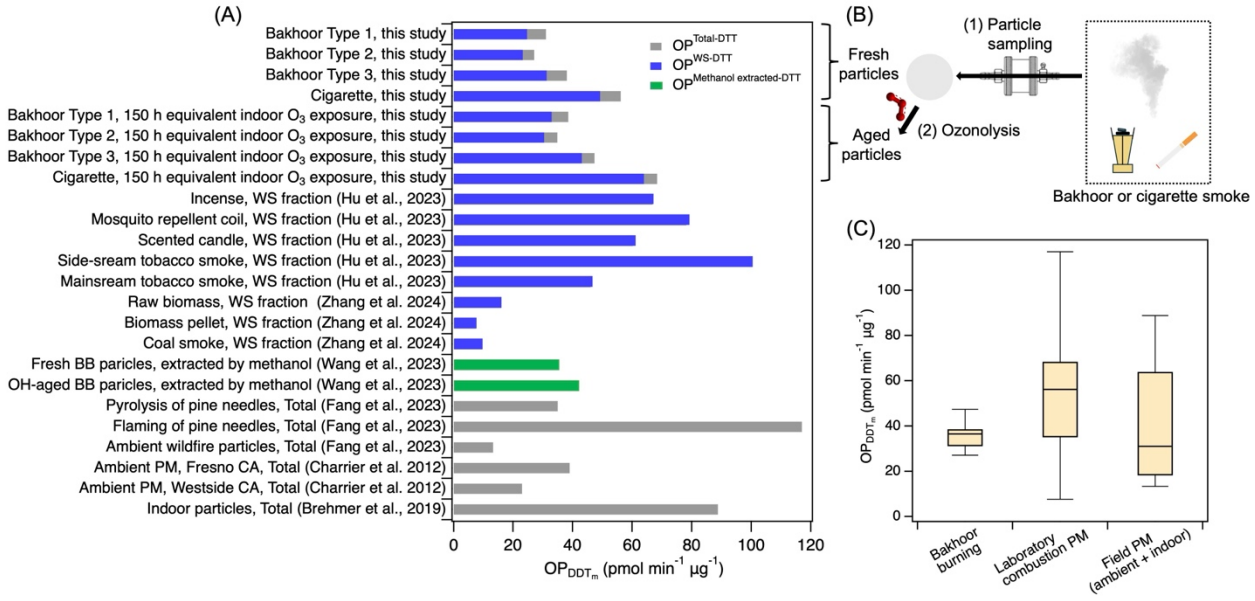

Figure 3. (A) The mass-normalized DTT consumption rate (OP_{DTTm}) for Bakhoor burning and sidestream cigarette smoke particles in this study (fresh and ozone-aged), shown alongside literature values for other laboratory combustion aerosols and field PM. (B) Schematic of particle collection on filters and subsequent ozonolysis of PM-loaded filters prior to DTT analysis. (C) Box plot summarizing OP_{DTTm} grouped as Bakhoor burning, other laboratory combustion PM and field PM.

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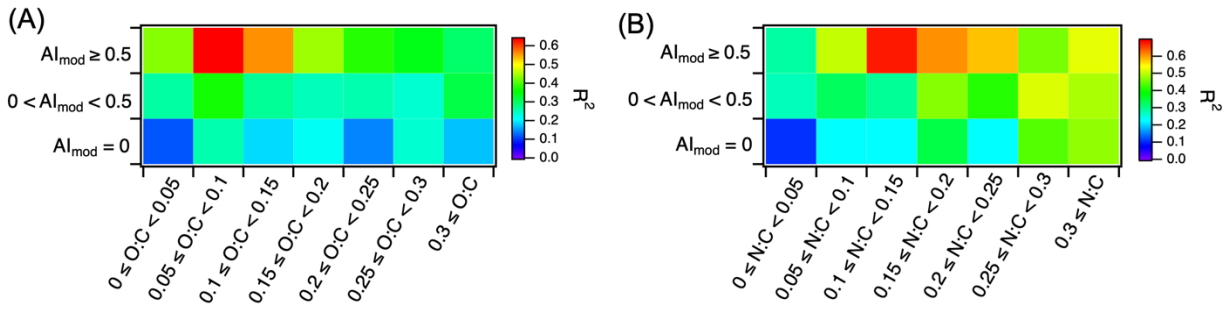

Figure 4. Heat maps of the coefficient of determination (R^2) from linear regressions relating the mass-normalized DTT consumption rate (OP_{DTTm}) to the summed UHPLC–HRMS signal intensity of molecular-formula bins grouped by modified aromaticity index (AI_{mod}) and (A) oxygen-to-carbon (O:C) or (B) nitrogen-to-carbon (N:C) ratio, across fresh and ozone-aged Bakhoor burning and cigarette smoke samples.

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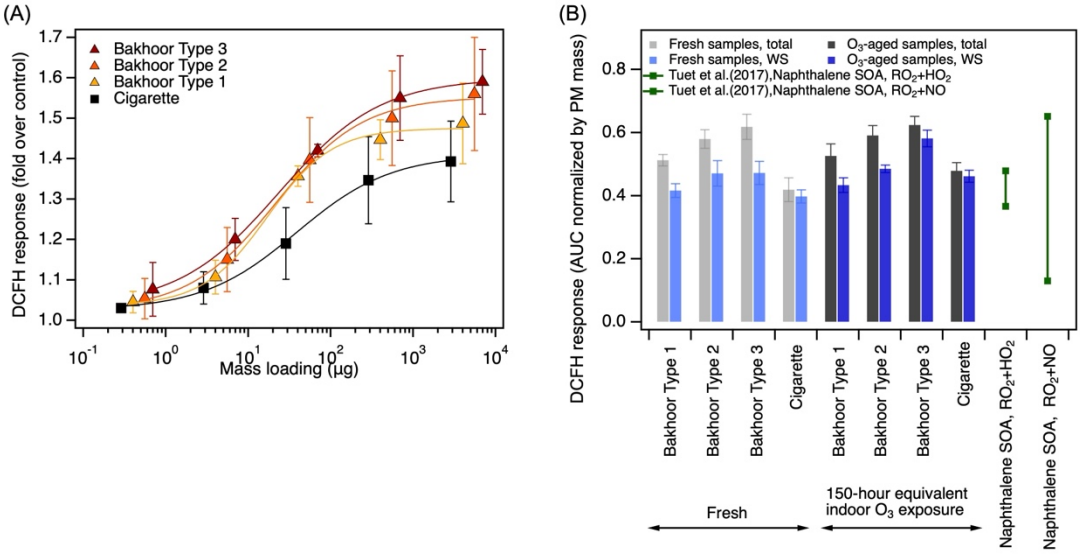

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461 **Figure 5.** Intracellular oxidative-stress responses (DCFH assay) induced by particles from three Bakhoor types and sidestream
462 cigarette smoke. (A) Dose–response curves of DCFH fluorescence (reported as fold change relative to untreated controls) as
463 a function of particle mass loading; solid lines denote Hill-equation fits. (B) Integrated responses quantified as the area under
464 the fitted dose–response curve (AUC), normalized by particle mass, for fresh and O_3-aged samples and for total particle
465 suspensions and water-soluble (WS) extracts. Reference values for naphthalene SOA are from Tuet et al. (2017)

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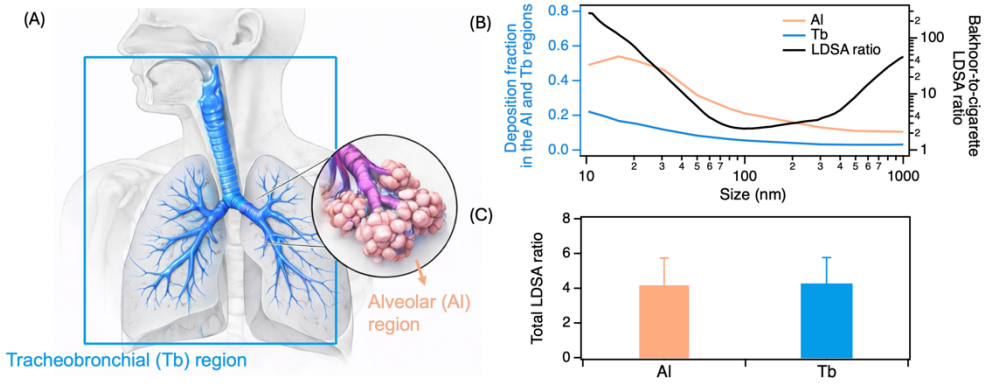

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477 **Figure 6.** (A) Schematic of the human respiratory tract highlighting the tracheobronchial (Tb) and alveolar (Al) regions used
478 for deposition calculations. (B) Size-resolved regional deposition fractions for the Tb and Al regions (left axis) and the
479 corresponding size-resolved ratio of lung-deposited surface area (LDSA) for Bakhoor burning particles relative to sidestream
480 cigarette smoke particles (right axis). (C) Bakhoor-to-cigarette LDSA ratio integrated over the measured particle size range
481 for the Tb and Al regions; error bars denote ±1 standard deviation (1σ).

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Data availability. All datasets used in this study, as well as the data products generated during the analysis, are available from the corresponding author upon request. These data are not publicly archived because they are maintained as multiple processed and analysis-specific files rather than as a repository-ready dataset.

Author contributions. CKC and LZ designed the experiment; LZ and ZL conducted the experiments; LZ, ZL and WX performed the data interpretation; LZ, ZL, WX, RJH and CKC wrote the paper. All authors contributed to the paper with useful scientific discussions or comments.

Competing interests. The contact author has declared that neither they nor their co-authors have any competing interests.

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