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Oxidative potential of fine particulate matter emitted from traditional and improved biomass cookstoves†

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Exposure to PM_{2.5} emitted from traditional biomass cookstoves is a significant health risk for nearly one-third of the global population. Improved cookstoves aim to reduce pollutant emissions, but there is limited evidence of whether PM_{2.5} toxicity is also reduced. Using the dithiothreitol (DTT) assay to measure the potential for PM_{2.5} chemical components to induce oxidative stress through antioxidant depletion and/or oxidant generation, we characterized the mass- and volume-normalized DTT activity of PM_{2.5} emitted from a traditional three-stone fire cookstove and three improved cookstoves burning wood or charcoal fuels. Although improved cookstoves typically yield lower PM_{2.5} mass concentrations compared to traditional three-stone cookstove, exposure to DTT active PM_{2.5} is not always reduced due to increases in mass-normalized DTT activity. A notable decrease in DTT active PM_{2.5} exposure (by 67%) was only observed for a forced-draft improved cookstove burning wood, where low PM_{2.5} mass concentration offsets the increased mass-normalized DTT activity. Additionally, elemental carbon and water-soluble organic matter were identified as key predictors of volume-normalized DTT activity. Compared to wood, the use of charcoal led to a 61–86% reduction in exposure to DTT active PM_{2.5}, owing to both lower PM_{2.5} mass concentration and mass-normalized DTT activity. This further supports a proposed strategy whereby biomass fuel treatment can potentially reduce household exposure to toxic PM_{2.5}. Collectively, our findings emphasized the need to consider not only the mass concentration but also the toxic properties of PM_{2.5} when evaluating the health impacts of cookstoves and fuels.

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Environmental significance

Household PM_{2.5} pollution from traditional biomass cookstoves is a leading global health risk. Although improved cookstoves aim to reduce PM_{2.5} concentration, increased toxic potency of chemical components can offset these benefits, potentially leading to increased exposure to toxic PM_{2.5}. In this study, we evaluated the mass concentration, chemical composition, and intrinsic toxicity of PM_{2.5} emitted from various cookstove/fuel combinations. We found that only certain improved cookstoves or the use of charcoal fuels can significantly reduce exposure to toxic PM_{2.5}. We emphasize the importance of assessing the toxic properties of PM_{2.5} in addition to mass concentration when evaluating the impacts of cookstove design and fuel treatment on air quality and human health.

1 Introduction

Approximately 2.4 billion people living in low- and middle-income countries rely on the combustion of solid biomass fuels, such as wood and charcoal, for daily heating and cooking needs.¹ The inefficient combustion of these fuels in traditional cookstoves emits harmful gaseous pollutants and fine particulate matter (PM_{2.5}) that are associated with multiple adverse

human health outcomes, including acute and chronic pulmonary cardiovascular effects.² Moreover, exposure to household air pollution, including those from solid-fuel combustion, is estimated to cause 4.3 million annual pre-mature deaths worldwide.³ This health burden disproportionately affects women and children who spend more time near cooking activities and consequently have increased cookstove pollutant exposure.⁴

One key mitigation strategy to reduce pollutant exposure involves the development and deployment of biomass cookstoves with improved combustion efficiency and reduced pollutant emissions.⁵ These improved cookstove are designed to enhance thermal insulation and optimize airflow for efficient fuel combustion.⁶ Despite diverse improved cookstove designs and configurations (*e.g.*, natural *vs.* forced draft; rocket *vs.*

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gasifier), laboratory and field studies have shown that improved cookstoves typically yield reductions in $PM_{2.5}$ emission compared to traditional stoves when burning solid biomass fuels.^{7–17} However, a reduction in $PM_{2.5}$ mass concentration may not correspond to a reduction in adverse health impacts if the intrinsic toxicity or potency (*i.e.*, toxicity per mass) of $PM_{2.5}$ is increased. Given the complexity of cookstove $PM_{2.5}$, which is a mixture of chemical components at varying concentrations,^{15–18} each with potentially differing toxicities, it is critical to characterize the toxic properties of $PM_{2.5}$ emitted from biomass cookstoves. This will allow for a comprehensive evaluation of their health impacts and potential health benefits of improved cookstove technologies.

While a growing number of studies have characterized the concentration of carcinogenic polycyclic aromatic hydrocarbons (PAH) in cookstove $PM_{2.5}$,^{16,19–22} other toxicity metrics of $PM_{2.5}$ emitted from improved biomass cookstoves remain less explored. Early studies have shown that for wood combustion, mutagenic potency,²³ and inflammation levels²⁴ were reduced for $PM_{2.5}$ emitted from forced- and natural-draft improved cookstoves compared to a traditional three-stone stove. Fuel type also influences cookstove $PM_{2.5}$ toxicity. In particular, cells exposed to $PM_{2.5}$ emitted from improved cookstoves that used briquettes and charcoal processed from wood showed lower concentrations of an inflammation biomarker compared to $PM_{2.5}$ emitted from the combustion of the unprocessed wood fuel.²⁵ A recent study by Champion *et al.* reported significantly lower mutagenic potency (up to two orders of magnitude) for $PM_{2.5}$ emitted from three different forced-drafted improved cookstoves burning two types of wood pellets compared to traditional stoves burning cut hardwood.²⁶ These research efforts collectively suggest that the toxicity of cookstove $PM_{2.5}$ depends on both the type of cookstove and fuel used. Further characterization of $PM_{2.5}$ toxicity for additional cookstoves and fuel types is warranted, to explore the generality of previously reported toxicity and $PM_{2.5}$ mass reduction for improved cookstoves.

Previous studies have established connections between cookstove $PM_{2.5}$ and toxicological/biological endpoints such as mutagenicity, carcinogenicity, and inflammation. However, the current understanding of cookstove $PM_{2.5}$ toxicity can be expanded by evaluating its oxidative potential (OP)—the capacity of PM to induce oxidative stress, which is a cellular condition of antioxidant–oxidant imbalance. Moreover, oxidative stress is considered a central mechanism responsible for many adverse health effects associated with $PM_{2.5}$ exposure.^{27,28} Studies have also indicated that OP is a health relevant metric for acute $PM_{2.5}$ health effects.^{29–31} A recent study examined the OP of $PM_{2.5}$ emitted from the combustion of coal and various biomass fuels in common household cookstoves used in Northwest China, where the combustion of biomass in different stoves influenced the amount of oxidant formed by $PM_{2.5}$.³² As such, characterizing the OP of $PM_{2.5}$ emitted from different cookstoves burning different fuel types can further contribute to the assessment of their potential health risks.

This study aims to address the aforementioned knowledge gaps by characterizing the OP of $PM_{2.5}$ emitted from the combustion of two biomass fuel types (wood and charcoal) in both

traditional and improved cookstoves (a total of 12 cookstove–fuel combinations). Of particular interest are three key objectives: (1) determining whether the transition from a traditional stove to improved cookstoves leads to a reduction in exposure to toxic $PM_{2.5}$; (2) examining the impact of wood and charcoal fuels on exposure to toxic $PM_{2.5}$; and (3) identifying the cookstove $PM_{2.5}$ chemical components that are associated with OP.

2 Methods

2.1 Burn events: biomass cookstove and fuel types

The tested cookstoves span a range of biomass burning cookstove technology (pictures of stoves and additional information are shown and described in Fig. S1 and Section S1†); a “minimally tended” traditional three-stone fire cookstove (TSF); two natural-draft rocket elbow improved cookstoves produced by EcoZoom (models: Dura and Versa); and a forced-draft gasifier improved cookstove produced by African Clean Energy (ACE; model: One). The following biomass fuel types were used for each cookstove (more information on fuel types is provided in Section S2†): split dry hardwood, charcoal lumps, and charcoal briquettes. Each stove and fuel combination were tested in triplicates (total of 36 burn events). All combustion experiments were conducted in a constant displacement exhaust hood ($\sim 2.8 \text{ m}^3 \text{ min}^{-1}$). Manufacture ignition instructions for the ACE cookstove include lighting two kerosene-soaked ceramic blocks ($3 \times 2 \times 2 \text{ cm}$). For consistency, this ignition protocol was employed for all cookstove–fuel combinations.

The collection of PM (described below) only took place during the low power ‘simmer phase’, where water was maintained within $\sim 3^\circ \text{C}$ below its boiling temperature, which is inline with the temperature criteria of the standardized water boiling test protocol.³³ The simmer phase simulates long cooking methods (*e.g.*, cooking legumes) that are common throughout the world.³³ PM was only collected during the simmering phase to prevent the contribution of the ignition material on the collected sample and that the long duration of this cooking period likely represents the longest exposure duration. A small aluminum pot containing 2.5 L of water was used for all experiments. Additional information regarding stove operation, such as fuel consumption, is presented in Table S1.†

2.2 $PM_{2.5}$ sample collection, OC/EC, and inorganic ion analysis

The emitted PM was sampled through a $PM_{2.5}$ cyclone inlet (URG, 2000-30EH) located $\sim 4 \text{ cm}$ above the cookstove (but below the top of the pot to avoid sampling of water vapour) at a constant flow rate (18 slpm) controlled by a mass-flow controller (Alicat Scientific) for 30 to 120 minutes to ensure the collection of sufficient $PM_{2.5}$ mass for the DTT assay. Integrated $PM_{2.5}$ samples were collected *via* two parallel 47 mm-diameter filter holders, each containing a front and back quartz filter (prebaked Tissuquartz, Pall Laboratory). The back filter was used to account for any positive artifact due to the adsorption of semi-volatile gases from biomass combustion;^{34,35}



all reported data were corrected for this artifact. The collected PM_{2.5} mass was determined gravimetrically with a microbalance (Satorius BCE1241-1S or Mettler Toledo XS105) and stored at -20 °C until analysis.

The filters were divided into portions for the determination of various chemical components. A portion of the filter (1–1.5 cm² punch) was analyzed for organic carbon (OC) and elemental carbon (EC) using an OC/EC analyzer by Concord Analytical Services Ltd (Concord, Canada) and Sunset Laboratory (North Carolina, USA) following the IMPROVED-A thermal/optical method.³⁶ OC was converted to organic matter (OM) using conversion factors of 1.5 and 1.2 for hardwood and charcoal, respectively.³⁷ A separate 1.5 cm² filter punch was used to determine water-soluble inorganic ions; the detailed ion chromatography (IC) analysis procedure is described in Section S3.† In brief, an IC system (940 Professional IC Vario, Metrohm) was used to measure the filtered sample extracts for anions (F⁻, Cl⁻, NO₂⁻, Br⁻, NO₃⁻, SO₄²⁻, and PO₄³⁻) and cations (Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, NH₄⁺) using A Supp 5 column (150 × 4.0 mm, Metrohm) and C4 column (150 × 4.0 mm, Metrohm), respectively. Quality assurance and quality control (QA/QC) procedures for the OC, EC, and inorganic ion data are described in Section S4.†

The combined mass of OM, EC, and measured inorganic ion mass was compared to the gravimetrically determined PM_{2.5} mass in a mass balance analysis (Fig. S2†). The analysis indicated the presence of an unknown component in PM_{2.5} (*i.e.*, not EC, OM, or measured inorganic ions), where the reconstructed mass from EC, OM, and inorganic ions represented, on average, 85% and 73% of the gravimetrically determined PM_{2.5} mass concentration for wood and charcoal fuels, respectively. We refer to the difference between the reconstructed OM + EC + measured inorganic mass and gravimetrically determined mass as the unknown component of PM_{2.5}.

2.3 DTT activity

The DTT assay is a commonly used acellular technique, in part owing to its ease of operation and rapid measurements, to quantify the potential of PM_{2.5} components to induce oxidative stress by monitoring the rate by which DTT decays due to the presence of reactive PM_{2.5} components.³⁸ DTT activities of the water-soluble (WS) and total (*i.e.*, water-soluble and -insoluble) components were measured using a modified protocol outlined in Wong *et al.*,³⁹ and Gao *et al.*,^{40,41} the detailed DTT assay procedure is described in Section S5.† Briefly, the WS-DTT activity represents the sample extract that was filtered (0.45 μm PTFE syringe filter, VWR) to remove insoluble material, while the total-DTT activity represents the unfiltered aliquot, with the PM_{2.5} filter punch remaining in solution throughout the reaction with DTT. We note that Gao *et al.* have compared the performance of various methods to characterize total-DTT activity and determined the approach taken by the current study (unfiltered aliquot with the PM_{2.5} filter punch remaining in solution) to be the most effective and precise method.⁴¹ The decay rate of DTT due to reaction with PM_{2.5} components in pH 7.4 phosphate buffer and at 37 °C was determined by

monitoring the concentration of unreacted DTT using UV-Vis absorption spectroscopy. The WS- and total-DTT activities for the back filter were also determined and accounted for; this correction was, on average, 17% of the uncorrected DTT activities. Using the corrected WS- and total-DTT activities and following the procedure of Fang *et al.*,⁴² the corrected DTT activities were normalized by the volume of air, to represent the volume-normalized WS- and total-DTT activity of PM_{2.5} (OP_{vol}^{WS-DTT} and OP_{vol}^{Total-DTT}; nmol min⁻¹ m⁻³). The difference between OP_{vol}^{WS-DTT} and OP_{vol}^{Total-DTT} represents volume-normalized water-insoluble (WI) DTT activity (OP_{vol}^{WI-DTT}).^{41,43–45} We note that this approach assumes WS- and WI-DTT activities are additive and does not account for potential (synergistic or antagonistic) interactions between the WS and WI components, which remain unknown and warrant future investigation. For intrinsic (potency) DTT activity, the corrected total-DTT activities were normalized by the PM_{2.5} mass on the filter punch (OP_{mass}^{Total-DTT}, pmol min⁻¹ μg⁻¹). We did not determine the mass-normalized WS- or WI-DTT activities as the mass concentrations of WS and WI PM_{2.5} components were not determined.

2.4 Data analysis

Analysis was conducted from different perspectives to examine the complex interactions between cookstove design and fuel types⁴⁶ on PM_{2.5} DTT activity: comparisons of different cookstoves for the same fuel type (*e.g.*, wood combustion in traditional *vs.* improved cookstoves) and different fuel types for the same cookstove type (*e.g.*, wood *vs.* charcoal combustion in a traditional stove). Following the approach of Champion *et al.*,¹⁸ two-way Wilcoxon rank-sum tests were used in evaluating statistical significance of comparisons. This statistical test was selected due to the limited number of burn events per stove/fuel combination and non-normal distribution of some datasets as indicated by Shapiro–Wilk test. Statistical significance was defined as $p \leq 0.05$.

To identify the component(s) that influence mass-normalized OP, Spearman rank correlations between OP_{mass}^{Total-DTT} and the mass fraction of measured chemical components were conducted. Since the mass concentrations of WS and WI components were not characterized, partial least squares regression (PLSR) models were developed to assess the influence of measured chemical components on OP_{vol}^{WS-DTT} and OP_{vol}^{WI-DTT}. Additionally, univariate correlations (*e.g.*, Spearman or Pearson correlations) of volume-normalized DTT activity are susceptible to multicollinearity effects as strong correlations of PM_{2.5} mass concentrations to chemical components can make assessments of individual chemical component's contribution to volume-normalized DTT challenging.^{47,48} PLSR can account for multicollinearity effects⁴⁹ and it is well suited for the identification of which predictors (chemical components) are best associated with the response (DTT activity). A OP_{vol}^{Total-DTT} PLSR model was also developed to compare with the Spearman correlation analysis.

To build the PLSR models, the input data were scaled and mean-normalized to remove effects related to differences in



data magnitude. A 10-fold cross-validation approach was used, and the optimal number of components (latent variables) was selected when the root mean squared error of prediction (RMSEP) was minimized. The number of components selected for each PLSR model and evaluation of model robustness using various metrics are described in Section S6 and Table S2.† PLSR models were developed using R (version 4.3.1) implemented in R Studio (+524), using packages pls and plsVarSel.⁵⁰

3 Results and discussion

All figures presented in the main text are pooled by broad fuel category (*i.e.*, wood and charcoal), with results for each stove/fuel combination shown in Section S7 (Fig. S3, S4, and S5).†

3.1 PM_{2.5} mass concentration

Out of all cookstove–fuel combinations tested, the PM_{2.5} mass concentration (Fig. 1a) was the highest for TSF burning wood at $41.8 \pm 25.6 \text{ mg m}^{-3}$ (mean \pm SD). For wood combustion, improved cookstoves led to reduced mean PM_{2.5} compared to

TSF: by 27–41% for natural-draft stoves (Dura and Versa) and 89% for forced-draft stove (ACE). The reduction in PM_{2.5} for improved cookstoves can be attributed to more complete fuel combustion as a result of enhanced airflow and heat loss reduction.⁵¹ ACE demonstrated a greater reduction than the two natural draft cookstoves due to the ability of forced-draft cookstoves to maintain a constant air flow, thereby enhancing combustion efficiency and combustion temperature which in turn results in reduced pollution emissions.^{52,53} While only the reduction in PM_{2.5} for ACE was statistically significant compared to TSF, the percentage reductions for improved cookstoves are consistent with real-world measurements. For example, a transition from traditional biomass cookstoves to Dura led to 46.4–51.1% reduction in household/personal PM_{2.5} emission^{54,55} and transition to ACE resulted in a ~25% reduction.¹⁵

For charcoal fuel combustion, improved cookstoves did not lead to statistically significant reductions in mean PM_{2.5} compared to TSF, as TSF exhibited substantial variability. This variability is potentially due to the non-uniform charcoal fuel structure that results in variable airflow in the less controlled fire of a traditional stove.¹² However, significant differences were observed among all improved stoves burning charcoal, with ACE demonstrating a lower mean PM_{2.5} by 43–77% compared to natural draft improved stoves. The results for both wood and charcoal combustion align with previous research, where improved cookstoves generally emit less PM_{2.5} compared to TSF, and amongst improved cookstoves, forced-draft stove emit the lowest PM_{2.5}.^{12–14,16,17}

Not all stoves resulted in significantly different PM_{2.5} from the change in fuel type. Mean PM_{2.5} for Versa was similar for wood and charcoal. While mean PM_{2.5} from charcoal combustion increased by 53% for ACE and reduced by 65% for TSF, these changes were not statistically significant. Only Dura exhibited a significant 51% reduction for charcoal fuel compared to wood. Of the limited number of previous studies that compared charcoal and wood combustion using the same stove, lower PM_{2.5} emissions for charcoal fuels by 7–39% were observed for a traditional stove,⁵⁶ 19–83% for a clean (semi-gasifier) stove,²⁵ and ~36% for a forced-draft gasifier stove.¹⁷ This reduction in PM_{2.5} from charcoal combustion is attributed to lower volatile content of charcoal fuels,^{16,25,56} as volatile matter is removed during the carbonization process in charcoal production.⁵⁶ Collectively, results from previous work and the current study suggest that compared to wood fuels, charcoal combustion generally results in lower PM_{2.5}. However, the magnitude of this reduction varies significantly due to differences in stove technologies, which highlights the complex interaction of fuel and stove on PM_{2.5} emission.⁴⁶

3.2 PM_{2.5} chemical composition

Variations in the chemical composition of PM_{2.5} were observed across the different stove–fuel combinations tested (Fig. 1b). For all tested stove–fuel combinations, TSF burning wood resulted in PM_{2.5} with the highest mean OM mass fraction (0.86 \pm 0.13), with some contribution from EC (0.07 \pm 0.05) and

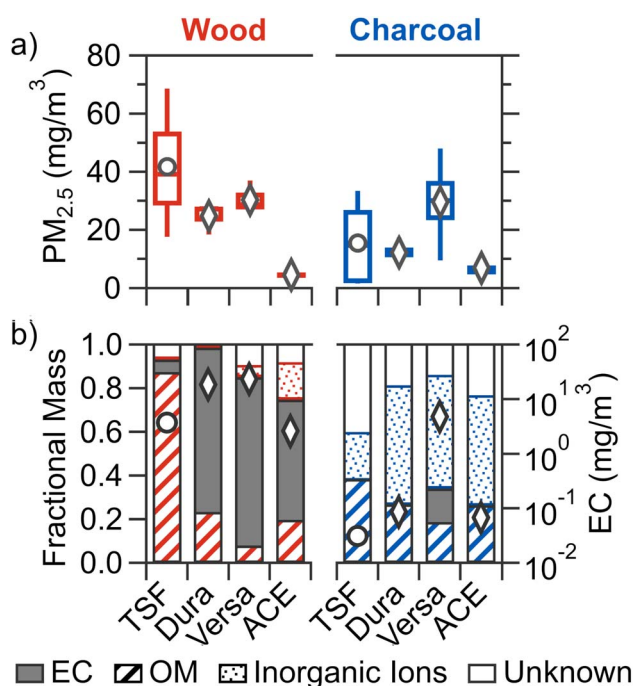


Fig. 1 For wood (red) and charcoal (blue) fuel combustion using each stove type: (a) box and whisker plots of PM_{2.5} mass concentration. The horizontal line within the box indicates the median value; the dark grey circle and diamond markers are the means for traditional and improved cookstoves, respectively; the lower and upper box boundaries represent the 25th and 75th percentiles; and the whiskers indicate the 5th and 95th percentiles. (b) Mean fractional mass contribution of OM (striped bar), EC (dark grey bar), inorganic ions (dotted bars), and unknown component (colourless bar). Note that while the mass contribution of EC cannot be easily seen in this figure for charcoal combustion, the mean EC mass concentrations (circle and diamond markers for traditional and improved cookstoves, respectively) are also shown to highlight the low, but non-zero concentrations.



unknown component (0.06 ± 0.06), as well as negligible contribution from inorganic ions (0.01 ± 0.01). This is consistent with previous laboratory and field studies of traditional stoves burning wood fuels, where the organic carbon constitutes 0.40–0.94 of the total carbon mass of $PM_{2.5}$.^{14–17,57}

For wood fuel combustion, compared to TSF, all improved cookstoves showed significantly higher mean fractional contributions by EC, ranging from 0.56 to 0.78, which aligns with previous studies.^{14–17} The higher contribution by EC is due to the higher combustion temperatures in insulated improved cookstoves favours EC formation, whereas the lower combustion temperature in a non-insulated traditional stove favour OM formation.⁵⁸

For charcoal combustion, the combined mass fraction of EC and OM to $PM_{2.5}$ ranges from 0.26–0.38 across all cookstoves. In particular, with the exception of Versa burning charcoal briquettes, low elemental carbon-to-total carbon ratios (EC:TC ratios are shown in Fig. S3†) ranging from 0.01 to 0.07 indicated the carbon is predominately organic with comparatively low EC, consistent with previous studies of charcoal combustion.^{16,17,57} No significant differences in fractional contribution of EC, OM, and non-carbon were observed across most cookstoves except for lower contribution of inorganic ions for TSF and higher EC contribution for Versa, which is further discussed in Section S7.†

Amongst each cookstove burning different fuel types, wood fuel resulted in significantly higher fractional contributions of EC compared to charcoal fuels for all cookstoves except for Versa. The lower contribution of EC (and higher OM) by charcoal fuels can be attributed to two reasons: (1) lower EC emissions for charcoal fuels as combustion occurs primarily in heterogeneous matter (*i.e.*, the surface of charcoal), in contrast to the gas-phase combustion of wood fuels that can result in PM-forming products;^{16,57} and (2) higher moisture content for wood fuel, which can result in lower combustion efficiency that favours OM formation in gas-phase combustion.^{58,59} In addition to the low EC observed with charcoal combustion, all stoves resulted in $PM_{2.5}$ that was predominately non-carbon (combined inorganic ion and unknown fraction ranges from 0.64–0.75), whereas wood combustion resulted in $PM_{2.5}$ that was mostly carbonaceous (combined inorganic ion and unknown fraction ranging from 0.07 to 0.28). For charcoal combustion, with exception to TSF, the non-carbon fraction is dominated by inorganic ions (ranging from 0.50 to 0.55) of which potassium and phosphate are typically the main inorganic ions (Fig. S5†). Previous studies have also reported reduced contribution of carbonaceous material to $PM_{2.5}$ and greater inorganic ion emissions for charcoal combustion compared to wood.^{16,56,60} The unknown fraction can be composed of metals such as aluminum, copper, and iron that have been detected in cookstove $PM_{2.5}$.⁵⁶ Both the current study and existing literature suggest that a significant portion of $PM_{2.5}$ from charcoal fuel combustion may not be carbonaceous.

3.3 Mass-normalized total-DTT activity

The mass-normalized total-DTT activity ($OP_{\text{mass}}^{\text{Total-DTT}}$; *i.e.*, total-DTT activity per mass of $PM_{2.5}$) was characterized to examine

whether different stove–fuel combinations resulted in $PM_{2.5}$ of varying intrinsic toxicities. Shown in Fig. 2a, for all cookstove–fuel combinations tested, mean values of $OP_{\text{mass}}^{\text{Total-DTT}}$ ranged from $(0.2 \text{ to } 1.4) \times 10^2 \text{ pmol min}^{-1} \mu\text{g}^{-1}$. These are comparable to biomass burning PM [$(0.17\text{--}1.50) \times 10^2 \text{ pmol min}^{-1} \mu\text{g}^{-1}$]^{61–63} and to $PM_{2.5}$ measured in the kitchens of rural households in the Tibetan plateau that predominately use biomass fuels for cooking [$(7.92\text{--}8.89) \times 10^1 \text{ pmol min}^{-1} \mu\text{g}^{-1}$] as reported by Brehmer *et al.*⁶⁴

For wood fuel, the mean $OP_{\text{mass}}^{\text{Total-DTT}}$ for improved cookstoves increased by 40–210% [$(0.6\text{--}1.4) \times 10^2 \text{ pmol min}^{-1} \mu\text{g}^{-1}$] relative to that of TSF ($4.4 \times 10^1 \text{ pmol min}^{-1} \mu\text{g}^{-1}$). However, only $OP_{\text{mass}}^{\text{Total-DTT}}$ for ACE and Dura was statistically higher than that of TSF, indicating that an equivalent exposure of $PM_{2.5}$ mass from these two improved stoves will result in a faster decay of a model antioxidant (DTT) compared to TSF. Mutlu *et al.* previously reported that $PM_{2.5}$ from a forced-draft cookstove burning wood was



Fig. 2 Box and whisker plots of (a) mass-normalized total-DTT activity ($OP_{\text{mass}}^{\text{Total-DTT}}$), (b) air volume-normalized total-DTT activity ($OP_{\text{vol}}^{\text{Total-DTT}}$), and (c) fraction of water-soluble (WS) to total volume-normalized DTT activity for wood (red) and charcoal (blue) fuel combustion in each stove type. The horizontal line within the box indicates median values; the lower and upper box boundaries represent the 25th and 75th percentiles; the whiskers indicate the 5th and 95th percentiles, the dark grey circle and diamond markers indicates the mean for traditional cookstove and improved cookstoves, respectively. The grey cross markers indicate outliers.



the most mutagenic on a per mass basis compared to emissions from a three-stone and a natural-draft cookstove.²³ Among improved cookstoves, differences in intrinsic toxicity were observed, with ACE having the highest mean $OP_{\text{mass}}^{\text{Total-DTT}}$ $[(1.4 \pm 0.4) \times 10^2 \text{ pmol min}^{-1} \mu\text{g}^{-1}]$, followed by Dura $[(9.8 \pm 3.4) \times 10^1 \text{ pmol min}^{-1} \mu\text{g}^{-1}]$, then Versa $[(6.2 \pm 1.0) \times 10^1 \text{ pmol min}^{-1} \mu\text{g}^{-1}]$; however only the differences between ACE and Versa are statistically significant. In contrast to wood fuel, a narrow range of $OP_{\text{mass}}^{\text{Total-DTT}}$ $[(2.0\text{--}5.9) \times 10^1 \text{ pmol min}^{-1} \mu\text{g}^{-1}]$ was observed for charcoal fuel combustion in all tested cookstoves, and no statistically significant differences were found between any two cookstoves burning charcoal, despite statistically different $PM_{2.5}$ mass among improved cookstoves. These contrasting effects further highlight the role of cookstove technology and fuel type in the intrinsic toxicity for $PM_{2.5}$ emitted.

Considering each cookstove burning different fuel types, no statistically significant differences were observed for TSF and Dura, whereas the use of charcoal fuel led to a statistically significant reduction of $OP_{\text{mass}}^{\text{Total-DTT}}$ by 68–76% for Versa and ACE. Notably, for Versa, the use of charcoal fuel compared to wood fuel yielded no reduction in $PM_{2.5}$ yet a 68% decrease in $OP_{\text{mass}}^{\text{Total-DTT}}$. In contrast, for ACE, there was a 53% increase in $PM_{2.5}$ but a 76% decrease in $OP_{\text{mass}}^{\text{Total-DTT}}$. These contrasting effects further indicate that changes in $PM_{2.5}$ mass do not necessarily correlate with the intrinsic toxicity of $PM_{2.5}$. In fact, these two metrics ($PM_{2.5}$ and $OP_{\text{mass}}^{\text{Total-DTT}}$), when used independently, do not accurately represent the exposure to toxic components in $PM_{2.5}$, as exposure to higher $PM_{2.5}$ does not necessarily indicate exposure to a greater mass of toxic $PM_{2.5}$ components. Indeed, to evaluate whether changes in the type of cookstove and fuel used will lead to a reduction in toxic $PM_{2.5}$ exposure, both $PM_{2.5}$ mass concentration and intrinsic toxicity need to be considered.

3.4 Volume-normalized DTT activity

To evaluate the potential adverse health risks associated with exposure to toxic chemicals in $PM_{2.5}$ emitted from biomass cookstoves, the total-DTT activity was normalized by the volume of air sampled ($OP_{\text{vol}}^{\text{Total-DTT}}$; $\text{nmol min}^{-1} \text{m}^{-3}$). This metric better represents the overall exposure to toxic $PM_{2.5}$ compared to $OP_{\text{mass}}^{\text{Total-DTT}}$ because in addition to $OP_{\text{mass}}^{\text{Total-DTT}}$, it also considers $PM_{2.5}$ mass concentration in the air. The $OP_{\text{vol}}^{\text{Total-DTT}}$ of $PM_{2.5}$ emitted by different cookstoves burning wood and charcoal fuels are shown on Fig. 2b. Here, the $OP_{\text{vol}}^{\text{Total-DTT}}$ represents contribution from both water-soluble and -insoluble components.

The mean $OP_{\text{vol}}^{\text{Total-DTT}}$ for all tested cookstoves burning wood and charcoal fuels ranges from $(0.2\text{--}2.3) \times 10^3 \text{ nmol min}^{-1} \text{m}^{-3}$. These $OP_{\text{vol}}^{\text{Total-DTT}}$ values are remarkably high compared to typical literature $OP_{\text{vol}}^{\text{Total-DTT}}$ values. Only one other study has reported high $OP_{\text{vol}}^{\text{Total-DTT}}$ values of $(0.089\text{--}3.500) \times 10^3 \text{ nmol min}^{-1} \text{m}^{-3}$ for $PM_{2.5}$ collected close to garbage burning piles ($\sim 0.5 \text{ m}$ above) in urban India.⁶⁵ In contrast, for the aforementioned study by Brehmer *et al.*, $OP_{\text{vol}}^{\text{Total-DTT}}$ for $PM_{2.5}$ collected over a 48 hours period in the kitchens of rural households in the Tibetan plateau that cook with biomass span

$(0.86\text{--}1.10) \times 10^1 \text{ nmol min}^{-1} \text{m}^{-3}$.⁶⁴ The much lower $OP_{\text{vol}}^{\text{Total-DTT}}$ values reported by Brehmer *et al.* can be attributable to indoor dilution and that cooking activities were unlikely to occur throughout the entire 48 hours sampling period. Given that $OP_{\text{mass}}^{\text{Total-DTT}}$ values of the current study are comparable to those reported by Brehmer *et al.*, the higher $OP_{\text{vol}}^{\text{Total-DTT}}$ values are a result of sampling relatively undiluted $PM_{2.5}$ emissions. Hence, the $OP_{\text{vol}}^{\text{Total-DTT}}$ values reported in this study represent the worst-case exposure scenario for a person who remains close to the cookstove when cooking (*i.e.*, when tending to the stove). Nonetheless, we note that comparisons of $OP_{\text{vol}}^{\text{Total-DTT}}$ values under identical sampling conditions provide insights into the impacts of cookstove technology and fuel type.

Considering wood fuel alone, mean $OP_{\text{vol}}^{\text{Total-DTT}}$ for both natural-draft stoves (Dura and Versa) were 6–33% higher than that of TSF $[(1.7 \pm 1.0) \times 10^3 \text{ nmol min}^{-1} \text{m}^{-3}]$, but not statistically so. The forced-draft stove (ACE) exhibited the lowest $OP_{\text{vol}}^{\text{Total-DTT}}$ $[(5.7 \pm 1.2) \times 10^2 \text{ nmol min}^{-1} \text{m}^{-3}]$, which was a statistically significant reduction of 67–75% from the three other stoves. Despite $PM_{2.5}$ from ACE-wood being the most DTT active per mass, the $OP_{\text{vol}}^{\text{Total-DTT}}$ was the lowest among cookstoves burning wood burning, which is owed to the low $PM_{2.5}$ mass emitted. These results indicate that not all improved cookstoves burning wood result in reduced exposure to DTT active $PM_{2.5}$ compared to TSF. For charcoal fuels, Versa had the highest mean $OP_{\text{vol}}^{\text{Total-DTT}}$ $[(5.4 \pm 2.9) \times 10^2 \text{ nmol min}^{-1} \text{m}^{-3}]$ which was 60–140% greater than that of other tested stoves; however, it was only statistically different from ACE and TSF $[(2.2 \pm 1.4) \times 10^2 \text{ and } (2.5 \pm 0.5) \times 10^2 \text{ nmol min}^{-1} \text{m}^{-3}]$, respectively.

The most prominent result was that for each cookstove, the combustion of charcoal led to significant reductions of 61–86% for $OP_{\text{vol}}^{\text{Total-DTT}}$ compared to wood. The reduction of $OP_{\text{vol}}^{\text{Total-DTT}}$ for each cookstove due to the use of charcoal instead of wood is consistent with the study by Niu *et al.*, where for $PM_{2.5}$ emitted from a clean cookstove, combustion of charcoal fuel led to a reduction in cell injury by 10–20% and cellular inflammatory response by 62.7% compared to the use of (unprocessed) wood fuel.²⁵ Moreover, the results from the current study extend this reduction effect from the use of charcoal fuel instead of wood for other cookstoves (including the traditional three-stone stove), except for the use of ACE-wood to Dura-charcoal or Versa-charcoal, where the reductions were not significant. Since charcoal is a processed form of wood, results from the current study and those by Niu *et al.* suggest that the chemical components in wood that are responsible for the higher $OP_{\text{vol}}^{\text{Total-DTT}}$ are removed or converted to a less DTT active form during the production of charcoal.

3.5 WS and WI volume-normalized DTT activity

The contribution of WS fraction to total-DTT activity offers valuable insights into the nature of toxic components capable of reacting with DTT. In particular, the difference in $OP_{\text{vol}}^{\text{WS-DTT}}$ to $OP_{\text{vol}}^{\text{Total-DTT}}$ represents the contribution of WI components to $OP_{\text{vol}}^{\text{Total-DTT}}$. There is growing evidence that insoluble material in $PM_{2.5}$ contributes to oxidative stress and different $PM_{2.5}$ sources



have varying proportions of WS and WI components contributing to overall toxicity.^{40,66–68}

The fractional contribution of OP_{vol}^{WS-DTT} to $OP_{vol}^{Total-DTT}$ (Fig. 2c; hereafter referred to as $f_{ws-to-Total}$) varied tremendously for the tested stove–fuel combinations, with mean values ranging from 0.12 to 0.85. Traditional stove burning wood or charcoal fuels both resulted in significant contributions of WS fraction (0.85 ± 0.13), which is statistically greater compared to most improved cookstoves, suggesting the impacts of cookstove technology and fuel type on the variable contribution of WS and WI components to total-DTT activity for cookstove $PM_{2.5}$. Detailed comparison of $f_{ws-to-Total}$ across cookstove and fuel type is provided in Section S7.†

3.6 Association of OP^{DTT} with $PM_{2.5}$ chemical components

To investigate differences in the contribution of chemical components to OP^{DTT} , univariate linear regression (Spearman rank correlation coefficient, r_s) was initially used to examine correlations of $OP_{mass}^{Total-DTT}$ to the mass fraction of EC, OM, and inorganic ions for all cookstove/fuel combinations (see Table S3†). $OP_{mass}^{Total-DTT}$ was best correlated with the mass fraction of EC ($r_s: 0.62, p < 0.01$), whereas $OP_{mass}^{Total-DTT}$ had weak correlations with the mass fraction of OM ($r_s: -0.20, p > 0.05$), and all measured inorganic ions ($-0.30 \leq r_s \leq 0.19, p > 0.05$). The positive moderate correlation between the mass fraction of EC and $OP_{mass}^{Total-DTT}$ is in line with previous studies of household biomass combustion, in which EC is correlated with mutagenicity,²⁶ inflammatory response,²⁵ and environmental persistent free radicals.⁶⁹ The lack of a positive correlation between the mass fraction of OM to $OP_{mass}^{Total-DTT}$ may be attributed to different intrinsic OP^{DTT} between WSOM and WIOM,⁷⁰ where aggregate measures of OM and total-DTT activity (both metrics include WSOM and WIOM) may mask the variable contribution of WSOM and WIOM to $OP_{mass}^{Total-DTT}$.

PLSR models were developed to assess the importance of measured chemical components (EC, OM, and inorganic ions) on $OP_{vol}^{Total-DTT}$, OP_{vol}^{WS-DTT} , and OP_{vol}^{WI-DTT} . The three models demonstrated good explanatory power; each explaining 89%, 68%, and 87% of the variation in OP_{vol}^{DTT} , respectively (see Table S2† for a summary of model performance assessments and related discussion in Section S6†). Table 1 shows the variable importance in the projection (VIP) scores and the regression coefficients for key predictors whose VIP scores are greater than one (see Table S4† for all predictors). The VIP is a measure to

rank the relative contributions of each predictor in the model. A VIP score greater than one indicates that the predictor significantly contributes to the model predictive ability. The sign of the regression coefficient indicates the direction of influence of each predictor on OP_{vol}^{DTT} .⁷¹ For the $OP_{vol}^{Total-DTT}$ PLSR model, EC emerged as the most important predictor, consistent with the Spearman rank correlation analysis. In particular, EC is not only an important predictor in the OP_{vol}^{WI-DTT} model but also has larger regression coefficient in this model compared to the OP_{vol}^{WS-DTT} model. This aligns with the inherent insolubility of EC. Though EC is inherently insoluble, the enhanced importance of EC as a key predictor in OP_{vol}^{WI-DTT} model compared to the OP_{vol}^{WS-DTT} model could arise from DTT-active insoluble components associated with EC. For example, it has been previously suggested that soot, which can be detected as EC in OC/EC analysis,^{72,73} can have water-insoluble DTT active species on its surface.⁶⁶

OM is the most significant predictor of OP_{vol}^{WS-DTT} , with OM increasing with OP_{vol}^{WS-DTT} as indicated by the positive regression coefficient coupled with the observation that OM was not a significant predictor for OP_{vol}^{WI-DTT} , suggest that DTT active OM are predominately water-soluble. Strong positive correlations of water-soluble organic carbon to DTT have been reported previously for biomass burning.^{74–76} DTT active WS humic-like substances (HULIS) are abundant in biomass burning $PM_{2.5}$ and can account for $50 \pm 7\%$ of OP_{vol}^{WS-DTT} for $PM_{2.5}$ generated from the combustion of five different biomass fuels.⁷⁷ We note that certain organic compounds, which may be present in the water extract of cookstove $PM_{2.5}$ and have been identified in solid fuel combustion $PM_{2.5}$,^{63,78} could have contributed to the observed WS-DTT activity. Specifically, compounds such as quinones and oxygenated PAHs are known to be DTT active,^{79,80} and nitro-PAHs have been postulated to exhibit DTT activity.^{81,82} Future studies to investigate the contribution of these oxygenated and nitro-PAHs on the DTT activity of cookstove $PM_{2.5}$ are warranted. In fact, a deeper understanding of the molecular composition of WSOM emitted by different fuel types and cookstoves can shed light on the specific organic compounds that drive the observed OP^{DTT} .

Although OM was the most significant predictor for OP_{vol}^{WS-DTT} , its reduced importance in predicting $OP_{vol}^{Total-DTT}$ can be attributed to OM being a combined measure of WSOM and WIOM. This is further supported by the relatively low VIP score (0.47) with a positive regression coefficient (+0.14) for OM in the

Table 1 Key predictors (*i.e.*, VIP scores greater than one) and their corresponding VIP score and regression coefficient in PLSR models of OP_{vol}^{DTT}

$OP_{vol}^{Total-DTT}$			OP_{vol}^{WS-DTT}			OP_{vol}^{WI-DTT}		
Predictor	VIP	Coefficient	Predictor	VIP	Coefficient	Predictor	VIP	Coefficient
EC	2.25	+0.72	OM	2.51	+0.64	EC	2.69	+0.88
OM	1.33	+0.52	PO_4^{3-}	1.15	−0.17	PO_4^{3-}	1.01	+0.03
PO_4^{3-}	1.20	−0.07	NO_2^-	1.10	+0.02	Mg^{2+}	1.00	−0.06
NO_2^-	1.10	−0.05	NO_3^-	1.04	+0.03			
Ca^{2+}	1.02	−0.12	EC	1.03	+0.26			
NO_3^-	1.01	+0.14						



OP_{vol}^{WI-DTT} PLSR model, suggesting that the WIOM fraction may have considerably lower DTT activity compared to WSOM. As a result, the lower DTT activity of WIOM would reduce the influence that DTT-active WSOM has on OM in the $OP_{vol}^{Total-DTT}$ PLSR model. Therefore, the results from this study further emphasize that sample preparation approaches that separate $PM_{2.5}$ component based on solubility provide important insights into the relative contribution of various toxic WSOM components in $PM_{2.5}$ that might be otherwise overlooked.^{41,62}

Notably, certain inorganic ions, such as PO_4^{3-} , NO_2^- , NO_3^- , Ca^{2+} , and Mg^{2+} , which are not known to directly react with DTT, emerged as significant predictors of OP_{vol}^{DTT} . These inorganic ions may be co-emitted with species influencing DTT activity. The varying signs of their regression coefficients indicated associations with both increased and reduced DTT activities. However, compared to dominant predictors such as EC, the coefficients of these inorganic ions are smaller in magnitude, suggesting that they play a lesser role in DTT activity of cookstove $PM_{2.5}$.

While PLSR models can predict 68–89% of the variation in OP_{vol}^{DTT} using directly measured chemical components, the contributions of DTT-active transition metals are not considered in these models. For example, DTT-active metals copper and manganese,⁷⁹ may be present in the unknown component and have been identified in biomass burning $PM_{2.5}$ in minute fractions (typically <1% of $PM_{2.5}$ mass).^{83,84} As such, they may have contributed to the measured DTT activities in this study. To gain additional insights, more comprehensive chemical characterization of cookstove $PM_{2.5}$, coupled with OP_{mass}^{DTT} measurements, is warranted. This will enable the evaluation of the impact of cookstove and fuel type on the emission of DTT-active transition metals.

4 Implications

The results of this study, along with previous research, have shown that various improved cookstove designs can result in lower $PM_{2.5}$ mass concentrations and change the chemical composition of the $PM_{2.5}$ compared to traditional cookstoves. For an improved forced-draft cookstove, the decrease in $PM_{2.5}$ mass concentration effectively offsets the higher $OP_{mass}^{Total-DTT}$ DTT activity compared to a traditional stove, leading to a net reduction in the exposure to DTT active $PM_{2.5}$. However, improved natural-draft cookstoves resulted in slightly lower or similar $PM_{2.5}$ mass concentrations compared to a traditional stove, but the increase in $OP_{mass}^{Total-DTT}$ resulted in a similar or even greater exposure to DTT active $PM_{2.5}$. As such, reduced $PM_{2.5}$ emissions of improved cookstoves do not necessarily equate to reduced toxic $PM_{2.5}$ exposure. Although this study focused on the OP_{vol}^{DTT} of primary $PM_{2.5}$ emitted from biomass cookstoves, oxidation of gaseous emissions from wood fuel combustion in a traditional three-stone cookstove, improved natural- and forced-drafted cookstoves can give rise to secondary organic aerosol formation.^{85,86} Over time, as these secondary organic aerosol form and emissions disperse throughout the household, their contribution may become more significant, potentially leading to differences in exposure levels between the kitchen and the rest

of the household. The health impacts of this secondary organic aerosol remain unknown and should be examined.

This study also suggests that the switch to charcoal fuels from wood fuel (without the switch in cookstove technology) can potentially lead to a reduction of 61–86% in $OP_{vol}^{Total-DTT}$, which further supports recommendations by previous studies to consider the use of processed fuel as an alternative strategy to reduce cookstove emissions.^{18,25,87} However, several caveats associated with the use of charcoal should be considered. Compared to wood, charcoal combustion produces higher carbon monoxide concentrations;^{12,16,17} given the documented health impacts associated with carbon monoxide,² this represents a pollutant trade-off for the decreased exposure to DTT active $PM_{2.5}$. Additionally, the production of charcoal emits a significant amount of $PM_{2.5}$, resulting in greater emissions than wood over their lifecycle, thus the use of charcoal fuel instead of wood represents a trade-off between exposure and climate impacts associated with biomass cookstoves.^{5,88}

Furthermore, the results from this study suggest that insoluble EC and WSOM are the DTT active components of $PM_{2.5}$ emitted from the tested cookstove–fuel combinations. Therefore, variations in the emissions of these DTT active components, which are likely driven by differences in cookstove technology and fuel properties, can significantly influence the overall toxic impacts of biomass cookstove $PM_{2.5}$. From this perspective, reductions in $PM_{2.5}$ will not necessarily lead to reduced toxicity if the reduction in mass concentration is driven by lower emissions of non-toxic components. As such, this study emphasizes the need to consider not only reductions in $PM_{2.5}$ emissions, but also the changes in chemical composition and toxicity of $PM_{2.5}$ when evaluating the potential health impacts of biomass cookstoves.

Data availability

Experimental data and codes (in R language) used to develop the PLSR models are available on request.

Author contributions

Conceptualization, JPSW; methodology, JPSW, MMF, and BHI; investigation, BHI, JPD, SAW, and JPSW; writing – original draft, BHI and JPSW; writing – review and editing, BHI and JPSW; funding acquisition, JPSW; supervision, JPSW. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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