Thanks to the anonymous reviewer for their constructive comments on the manuscript and helpful suggestions for further improvement. Please find detailed responses below in blue-color font.

Response to Anonymous Referee #2

Summary:

In this scientific work, the authors have used excitation-emission matrix (EEM) fluorescence spectroscopy coupled with Fourier transform ion cyclotron resonance mass spectroscopy (FT-ICRMS) to study the chemical composition of water soluble organic carbon (WSOC) ambient aerosols in Karachi, Pakistan. In addition to that, they tried to postulate the different formation mechanisms of these organic compounds. They identified three broad components of moieties using parallel factor analysis (PARAFAC), of which two were humic-like (C1 and C2) and one was protein like (C3). They also tried to correlate these PARAFAC components to the extensive datasets of brown carbon (BrC) chromophores available in literature through the total number of carbon (C) atoms present in the molecular formula and double-bond equivalence (DBE). Except the discussion of formation mechanism, the rest of the methodology and findings of this work is quite trivial and another repetition of multiple EEM spectroscopy based ambient aerosol characterization studies.

Response: We appreciate your valuable comments and suggestions, which can help improve the manuscript.

EEM fluorescence spectroscopy was initially employed to study chromophoric dissolved organic matter (CDOM) in terrestrial and oceanic systems before being used for atmospheric aerosol research. It has been suggested that organic chromophores differ across various environments, including aquatic systems and aerosols (H. Jiang et al., 2022; Wu et al., 2022). Studying the molecular compositions of fluorophores, specifically the fluorescent components decomposed by PARAFAC analysis, is crucial for improving our understanding of their structures, sources, and chemical properties in the atmosphere. Although the molecular compositions of PARAFAC components in aquatic systems have been well described (Kellerman et al., 2015; Stubbins et al., 2014), only a few studies have been conducted on these components in the atmosphere. Chen et al. (2016) investigated the chemical compositions of water-soluble PARAFAC components. However, they were unable to provide the corresponding molecular formulas using high-resolution aerosol mass spectrometers (HR-AMS). F. Jiang et al. (2022) and H. Jiang et al. (2022) provided respective molecular formulas for PARAFAC

components. However, their primary focus was on the methanol-extracted fractions and not water-extracts, which can significantly differ. To the best of our knowledge, only a recent study has provided the molecular formulas associated with water-soluble PARAFAC components in $PM_{2.5}$ collected in Guangzhou, a city in Southern China (He et al., 2023). However, they did not investigate the potential formation pathways of PARAFAC components and the molecular-level correlation between them and BrC. It is important to constrain the optical properties of BrC aerosols.

Additionally, our investigation discovered molecular formulas detected in the ESI+ mode that exhibit a significant correlation with PARAFAC components, providing a more comprehensive molecular characterization of them. The main objective of our study is to identify the molecular compositions and formation mechanisms of PARAFAC components in water-soluble organic carbon (WSOC), with a particular on identifying the relevant pathways, which, to our knowledge, have not been reported previously. Additionally, identification of diverse molecular compositions and formation pathways of commonly detected fluorescent components in the atmosphere will provide valuable information, particularly when utilizing EEM in combination with PARAFAC analysis to study atmospheric BrC. Thus, our work assists in comprehending the composition and fate of PARAFAC components and enhances the utilization of the EEM-PARAFAC method in characterizing atmospheric BrC. This is the novelty and significance of our work.

References:

He, T., Wu, Y., Wang, D., Cai, J., Song, J., Yu, Z., Zeng, X., and Peng, P. a.: Molecular compositions and optical properties of water-soluble brown carbon during the autumn and winter in Guangzhou, China, Atmos. Environ., 296, https://doi.org/10.1016/j.atmosenv.2022.119573, 2023.

Jiang, F., Song, J., Bauer, J., Gao, L., Vallon, M., Gebhardt, R., Leisner, T., Norra, S., and Saathoff, H.: Chromophores and chemical composition of brown carbon characterized at an urban kerbside by excitation–emission spectroscopy and mass spectrometry, Atmos. Chem. Phys., 22, 14971-14986, https://doi.org/10.5194/acp-22-14971-2022, 2022.

Jiang, H., Tang, J., Li, J., Zhao, S., Mo, Y., Tian, C., Zhang, X., Jiang, B., Liao, Y., Chen, Y., and Zhang, G.: Molecular Signatures and Sources of Fluorescent Components in Atmospheric Organic Matter in South China, Environ. Sci. Technol. Lett., 9, 913-920, https://doi.org/10.1021/acs.estlett.2c00629, 2022.

Kellerman, A. M., Kothawala, D. N., Dittmar, T., and Tranvik, L. J.: Persistence of dissolved organic matter in lakes related to its molecular characteristics, Nat. Geosci., 8, 454-U452, https://doi.org/10.1038/ngeo2440, 2015.

Stubbins, A., Lapierre, J. F., Berggren, M., Prairie, Y. T., Dittmar, T., and del Giorgio, P. A.: What's in an EEM? Molecular signatures associated with dissolved organic fluorescence in boreal Canada, Environ. Sci. Technol., 48, 10598-10606, https://doi.org/10.1021/es502086e, 2014.

Wu, G., Fu, P., Ram, K., Song, J., Chen, Q., Kawamura, K., Wan, X., Kang, S., Wang, X., Laskin, A., and Cong, Z.: Fluorescence characteristics of water-soluble organic carbon in atmospheric aerosol, Environ. Pollut., 268, 115906, https://doi.org/10.1016/j.envpol.2020.115906, 2021.

Strength of this work:

The authors analysed the FT-ICR MS data and came up with the most probable formation mechanisms from different molecular signals in addition to EEM spectroscopic studies.

Response: We appreciate the reviewer for providing valuable suggestions.

Limitations of this work:

Lack of discussion on seasonal variation: As seen in Figure 1, the study site is located at a very interesting geographical location with great seasonal variation in wind direction. In pre-monsoon and monsoon, the wind flow is directed from middle-east Asia and Arabian Sea, whereas the wind trajectories arise from North Pakistan and North-West India during post-monsoon and winter. This will result in very different chemical compositions of ambient particulate matter (PM) reaching the study site. For example, in the months of May-June, the aerosol composition will be close to marine aerosol composition, but in Oct-Dec there will be molecular signals of biomass burning emissions as previous studies have pointed out extensive crop burning and biomass burning for heat generation during winter in that part of the world. Which suggests that the wintertime aerosols will probably have higher S content and less oxygenated organics because the OH radical photochemistry is limited during winter and the atmospheric transformation is driven by NOx chemistry. The authors have reported all the data in a combined way, which does not give the readers the broader picture of the regional specific atmospheric chemistry of the study site. Postulating molecular formation pathways without considering the meteorological conditions can also lead to erroneous assumptions.

Response: Thank you for your suggestions. We chose this station as a typical region to study this topic due to its special geographical location. The station experiences great seasonal variation in wind direction, resulting in varying chemical compositions. This is evident from the differences in light absorption and fluorescence intensities across different seasons. Additionally, the molecular composition also differs across different

seasons, a detail that was not shown in our original version. Our recent research has shown that continental-influenced WSOC has a higher composition of aromatic and highly oxidized compounds. Conversely, marine-influenced WSOC has a large availability of marine organic compounds that are saturated and have a lower degree of oxidation (Mo et al., 2022). This may highlight similarities in molecular characteristics between different air mass influences, compared to this study.

Because our primary objective is to obtain the molecular signatures and formation mechanisms of the fluorescent components decomposed by PARAFAC analysis, which is crucial for studying atmospheric BrC, we need subjective evaluations unless they are unambiguously marked as such. We appreciate your suggestions and as a result, we included the detailed molecular characteristics of each sample in different seasons in Tables S3 and S4 in the supplement for readers' reference.

Reference:

Mo, Y., Zhong, G., Li, J., Liu, X., Jiang, H., Tang, J., Jiang, B., Liao, Y., Cheng, Z., and Zhang, G.: The Sources, Molecular Compositions, and Light Absorption Properties of Water‐Soluble Organic Carbon in Marine Aerosols From South China Sea to the Eastern Indian Ocean, J. Geophys. Res.-Atmos., 127, https://doi.org/10.1029/2021jd036168, 2022.

Lack of relevant references: In continuation to the previous point, as the authors have not discussed the geographical context of this work, they have also failed to compare their findings with previous works carried out in similar locations. Although they have mentioned a few studies carried out in the Indo-Gangetic Plane (IGP) while reporting mass absorption efficiency (MAE) and Aerosol absorption exponent (AAE), this kind of comparisons have not been made for EEM spectroscopic studies. Previous EEM spectroscopic analyses of ambient aerosol in IGP and other parts of the world have found similar PARAFAC components (two HULIS and one protein like). These references from around the globe should be mentioned and compared with the findings of this work.

Response: Thank you for your suggestions. We regret our negligence in discussing the comparison with EEM spectroscopic studies. In order to improve the comprehension of the characteristics of fluorescent components in this geographic location, we have added a new discussion on the comparison in the revised manuscript. Please refer to the specified lines 290-307.

Structure of the Results and Discussion Section: The results and discussions section needs to be restructured. For the convenience of the readers, the discussion of

482 "Underlying implication of PARAFAC component to BrC absorption" should be done
483 under section 3.3 and the discussion on formation pathways should be under section
484 3.4. That way the flow of information will be more coherent.
485 Response: Thanks for the suggestions. In the revised manuscript, we have restructured
486 section 3.3 to discuss "Underlying implications of PARAFAC components on BrC
487 absorption" (lines 506-567) and section 3.4 to discuss "Potential formation mechanisms
488 of PARAFAC components" (lines 576-778).
489
490 The discussion on formation pathway of S-containing compounds should be more
491 condense and can also be moved to supplementary information (SI). The authors have
492 mentioned that S containing compounds have almost no effect on BrC chromophores.
493 They also reported that the two Humic-like PARAFAC components had very little S
494 containing compounds, mostly component C3 had the highest S containing compound.
495 Eventually it has also been shown that component C3 has the lowest overlap with the
496 BrC region in figure 5. Therefore, in terms of climate relevant BrC chromophores, this
497 pathway is not as important as CHO and CHON formation pathways.
498 Response: Thank you for your suggestion regarding the formation pathway of S-
499 containing compounds. According to previous studies, S-containing compounds have
500 little or no impact on BrC chromophores. Therefore, discussing S-containing
501 compounds may be confusing for readers. We have revised this section and moved it to
502 Text S7 in the revised supplement (lines 271-305).
503
504 The overall grammar and clarity of the current section 3.3 (proposed to be made section
505 3.4 after restructuring) is unsatisfactory. Too much statistics have been used in
506 sentences, which can instead be represented graphically. Sentences need to be written
507 with proper grammar, for example 407-408 is unclear and needs to be rewritten, so does
508 413. There are many occurrences like this, so the reviewer suggests a rewriting of this
509 whole section in a clear and concise manner. In the rewriting, the authors should also
510 elaborate and clarify how they reached conclusions made in 520-522.
511 **But most importantly in this section**, the authors have looked at some
512 molecular signals found through FT-ICR MS and compared those molecules with aged
513 byproducts of certain precursors reported in previous literature. And by doing that they
514 have tried to postulate these known reaction pathways to be present in their study
515 samples. These are good hypotheses, but there is no concrete way of establishing these
516 reaction pathways in the scope of this study. Therefore, it doesn't add up as novel new
517 information. If the authors can combine other analytical study with the collected filters
518 (if there is any remaining), for example ^{1}H NMR spectroscopy of WSOC, in which they

519 can quantitatively compare the spectra of the precursor and aged molecules with their
520 sample spectra and confirm its presence, that would be a much stronger argument for
521 the formation pathways.

522 Response: We apologize for the low quality of the language in our manuscript. We spent
523 a long time revising the manuscript, which involved repeatedly adding and removing
524 sentences and paragraphs. This led to a significant decrease in readability. We have
525 worked on improving both the language and the readability of the document.
526 Additionally, we have invited fellow experts to make professional language editing for
527 the manuscript. We hope that the flow and language level have been significantly
528 improved.

529 Furthermore, the sentence in original lines 407-408 was modified as follows: "For
530 instance, Mo et al. (2018) demonstrated that 98% of CHON compounds found in $PM_{2.5}$
531 collected from Beijing have O/N $\geq$ 3. This finding indicates that the compound has at
532 least one nitro ($-NO_2$) or nitrooxy ($-ONO_2$) group in addition to other oxygen-
533 containing groups (i.e., $-OH$ and $-COOH$)." Please refer to lines 618-622 in the revised
534 manuscript.

535 The sentence in original lines 413 was modified as follows: "However, oxidized CHON
536 formulas assigned to C1 and C2 may be underestimated as they contain two or more N
537 atoms and do not require three folds of O atoms to form $-NO_2$ or $-ONO_2$ groups."
538 Please refer to lines 626-629 in the revised manuscript.

539 We draw the conclusion (original version in lines 520-522) based on the elemental
540 compositions of the overlapping molecules assigned to C1 and C2, which consist
541 mainly of CHON and CHO compounds. Additionally, the formation pathways of these
542 overlapping molecules were found to be a significant part of oxidation-derived
543 pathways, with CHON compounds primarily attributed to N_2O_5 oxidation and the CHO
544 compounds were highly detected in the SOA formed from limonene ozonolysis. For the
545 revised sections, please refer to lines 763-778 in the revised manuscript.

546 Additionally, we hope to explore the formation pathways of PARAFAC components by
547 comparing the assigned molecules to those reported in previous studies, despite the
548 potential for uncertainty. This approach may offer additional clarity on the possible
549 formation pathways of PARAFAC components. For example, when comparing the
550 CHO compounds assigned to PARAFAC components to the SOA formed from
551 limonene ozonolysis, our focus is on the presence of oxidized CHO compounds rather
552 than on the precursors. Also, when comparing to the CHO compounds resulting from
553 the photooxidation of naphthalene, biomass burning emissions, and aqueous-phase
554 reactions of phenols, we have obtained new insight into the high aromatic structures
555 and the predominance of C2 formulas in an oxidative form. As we concluded, the

oxidation pathway appears to be the main pathway for the formation of C1 and C2. Indeed, the use of ^{1}H NMR spectroscopy is important to characterizing WSOC and obtaining insight into their structural characteristics. However, there are no remaining samples for subsequent analysis. This study presents preliminary findings on the formation pathways of PARAFAC components, given their complexity. In the future, other analytical techniques, such as ^{1}H NMR spectroscopy, should be combined to provide deeper insight into the formation pathways of fluorescent components.

Minor corrections:

Title: The title should be reconsidered. Instead of water soluble particulate matter, water soluble organic carbon is a preferable choice. The study location should be kept Karachi or mentioned a location in South East Asia.

Response: Thank you for the suggestions. The title has been revised as follows: "Molecular signatures and formation mechanisms of water-soluble chromophores in particulate matter from Karachi (Pakistan) in South Asia"

Corrections in SI:

Figure S2: The title of the figure mentions February 16, but the legend in the figure shows the trajectory starting from 17 February 2016.

Response: We apologize for the error. The figure has been revised by redrawing it due to the low resolution of the original version. We now present the updated figure below.
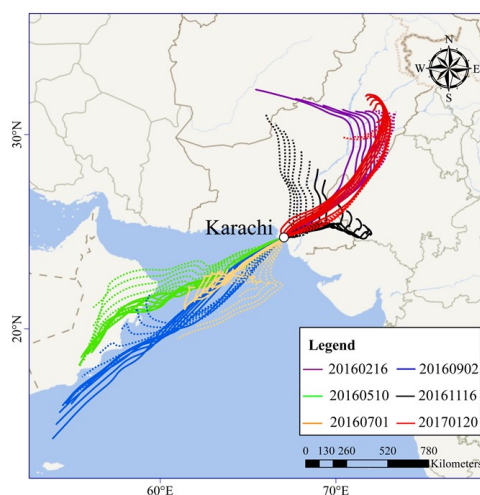
Figure S2. The 72 h back air-mass trajectories at Karachi from Pakistan for the selected samples via FT-ICR MS analysis on February 16, May 10, July 1, September 2, November 16, 2016, and January 20, 2017, correspond to different seasons. The trajectories with black dots represent the corresponding night, otherwise, it is the day. The air-mass trajectories were analyzed by the

582   HYSPLIT model. The map was created using Arcgis software, and the base map is from the National

583   Platform for Common Geospatial Information Services (www.webmap.cn).