The effects of diesel exhaust pollution on floral volatiles and the consequences for honey bee olfaction

Article

Supplemental Material

Supporting online information

THE EFFECTS OF DIESEL EXHAUST POLLUTION ON FLORAL VOLATILES AND THE CONSEQUENCES FOR HONEY BEE OLFACTION

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Comparison of peak areas of CFV and OSR volatiles measured by SPME

Due to the amount of volatile exposures samples in our study we needed to switch SPME fibers during data collection, however, the SPME fibers were from the same lot and the abundance data did not vary significantly (Fig. 6); especially when compared to the variation seen in the NOX exposure data that was collected using only one SPME fiber (Fig. 4).

For example the compound with the highest variation in the NOX exposure experiment from the ambient air treatment was limonene, its S.D. was 8.82% of the average abundance. Whereas, in the CFV blend exposures itself the S.D. of limonene was only 4.65% of the average abundance. This demonstrates that using SPME fibers from the same lot might allow comparative analyses of samples taken with more than one SPME fiber.

Comparative statistics were not conducted in our studies, but the peak area data of the CFV blend were plotted as bar graphs with 95% confidence intervals (C.I.) as error bars (Fig. 5). This allows a visual comparison between volatiles exposed to ambient air and diesel exhaust, since non overlapping error bars indicate a significant difference with a P-value less than 0.05 (Payton et al. 2003).

Payton ME, Greenstone MH, Schenker N (2003) Overlapping confidence intervals or standard error intervals: What do they mean in terms of statistical significance? J Insect Sci. 2003; 3: 34 doi:10.1093/jis/3.1.34

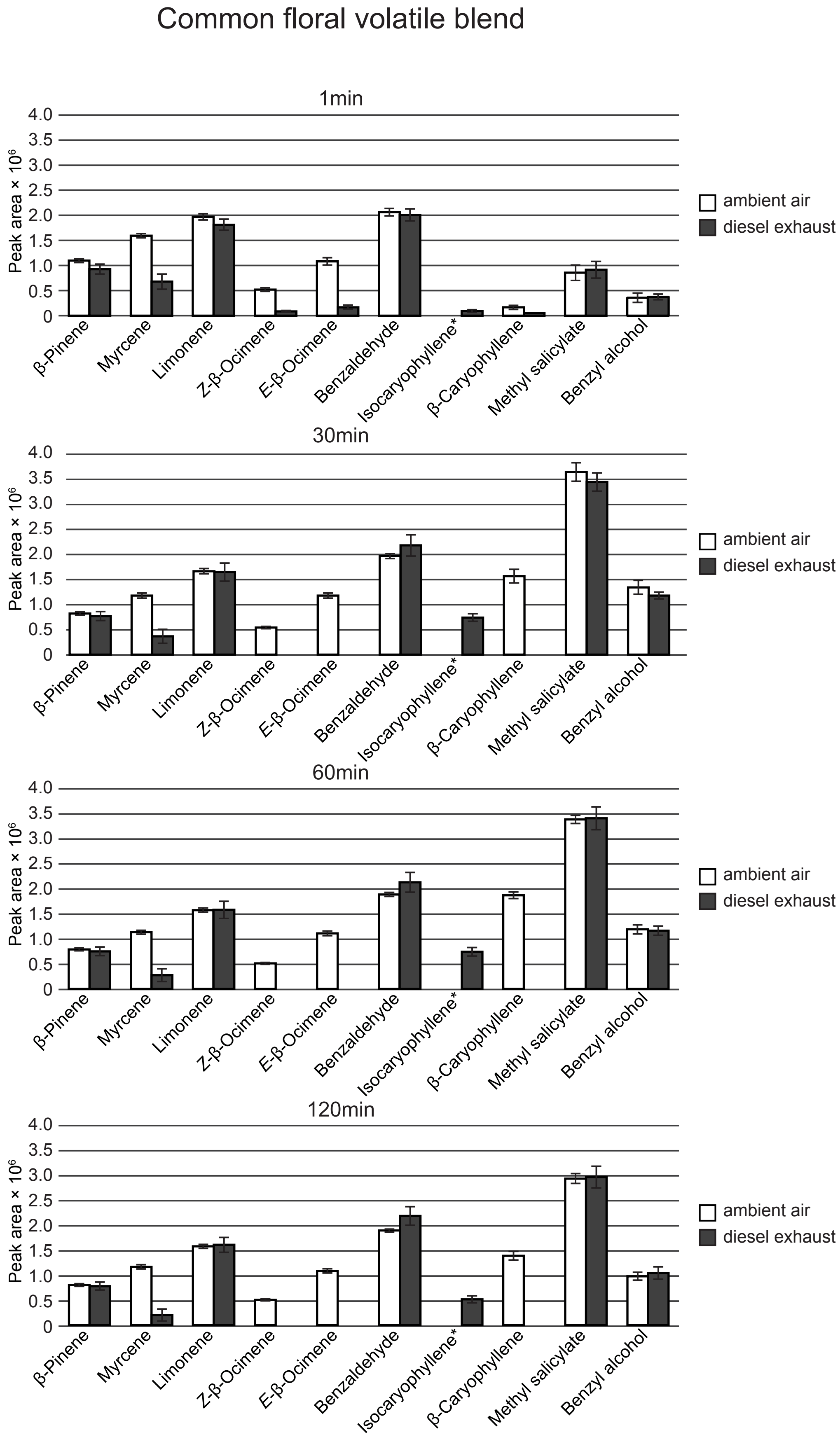
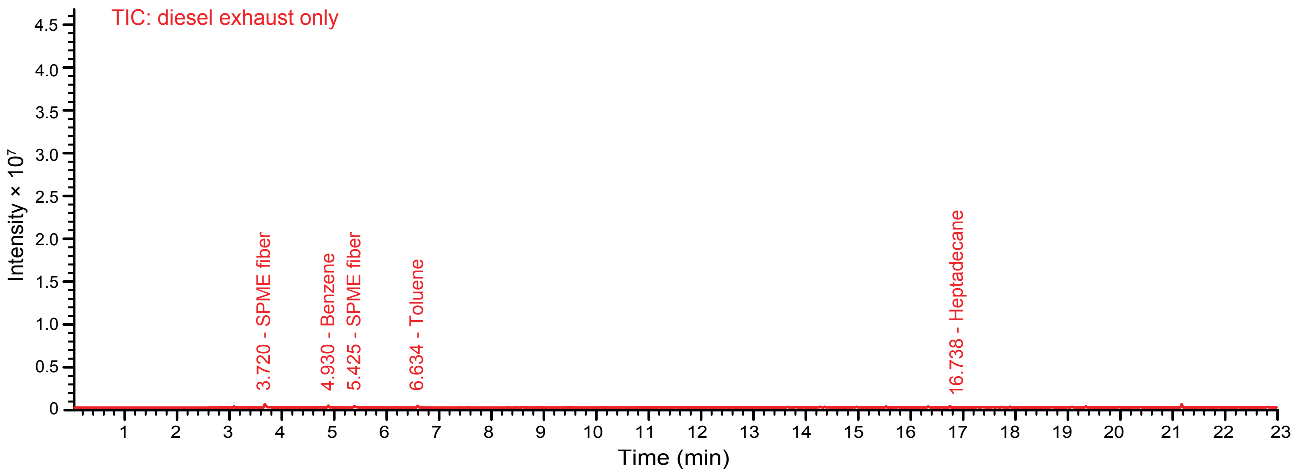


Fig. 5 Peak areas (± 95% C.I.) of the CFV blend under ambient air condition compared to peak areas after diesel exhaust exposure measured by SPME at different time points (1min, 30min, 60min, 120 min). Non overlapping error bars represent a significant difference. Isocaryophyllene* is a rearrangement product of β-caryophyllene and not part of the original blend.

Fate of individual floral volatiles

In order to determine the fate of all individual volatiles, we exposed every single compound to ambient air and diesel exhaust as mentioned in the main manuscript. For the analysis of the data we considered all peaks that were integrated by the chromatogram integration function in MSD ChemStation in either the ambient air or diesel exhaust TICs. Once a compound was found in either run, it was searched for manually in the other, if its abundance was below the detection limit of the integration function. As many compounds as possible were identified with standard injection or retention index and mass spectrum comparison.

The method used in this study did not allow to determine the possible destiny of all compounds in a diesel exhaust polluted environment (see all TICs on the following 2 pages; Fig. 6). See below for the TIC of diesel exhaust only, the measured abundance of all identified peaks was very low, therefore it appears almost like a flat line compared to all following TICs.



common flower volatile blend

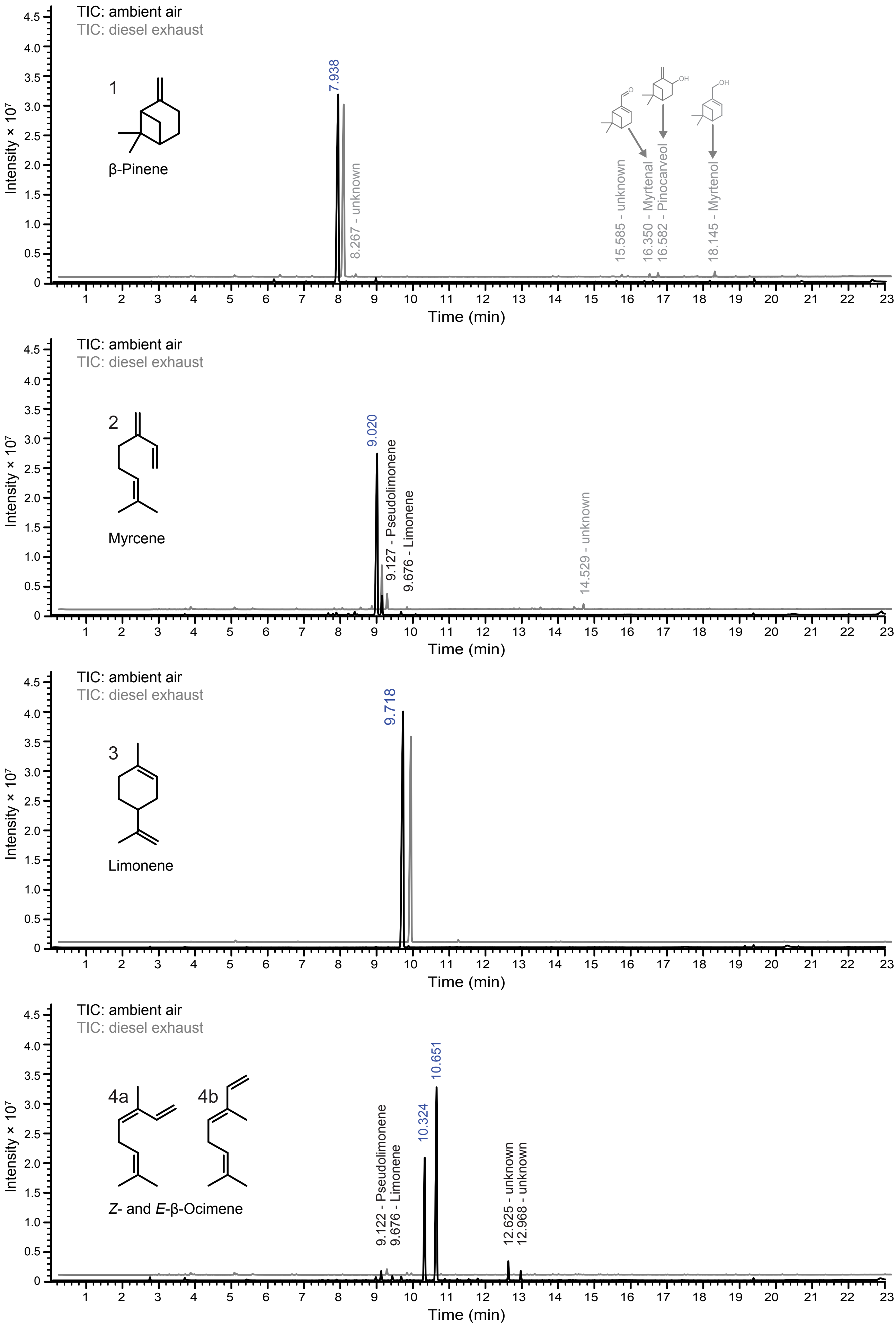
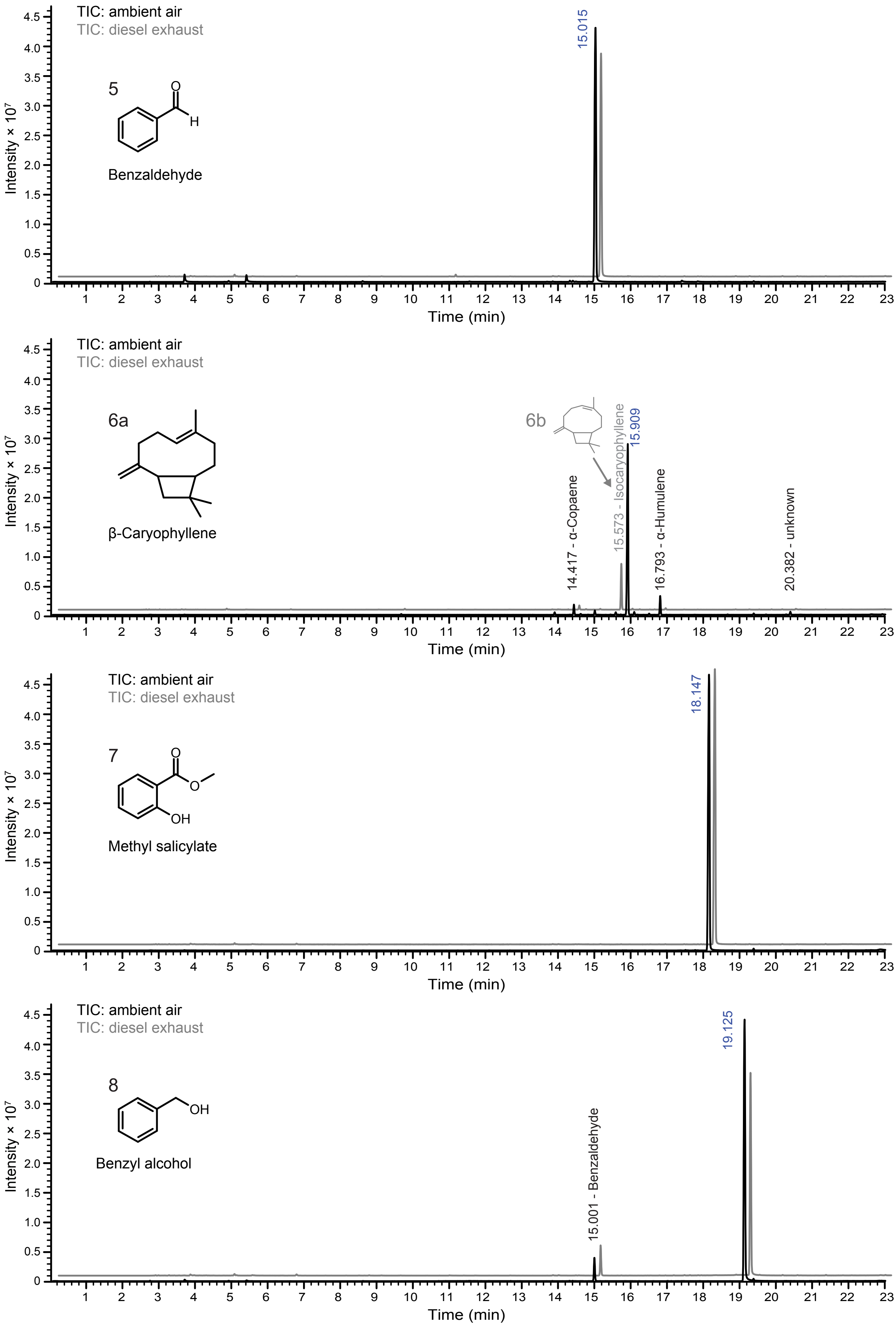


Fig. 6 TICs of all individual floral compounds in ambient air and after 30min exposure to diesel exhaust.

common flower volatile blend



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