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PAHS IN TREES FROM URBAN ENVIRONMENTS: RELATIONSHIP WITH LEAF TRAITS, INTRAINDIVIDUAL VARIABILITY AND INTERSPECIFIC DIFFERENCES

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Abstract in Galician (resumo), Spanish (resumen) and English

Resumo

Dende o inicio da revolución industrial, a calidade do aire deteriorouse considerablemente, o que conduciu a graves problemas de saúde e do medio ambiente. Este problema é moito máis grave en áreas urbanas.

Para contrarrestar isto, diferentes administracións públicas están tomando medidas para paliar este problema, pero a maioría delas baséanse, principalmente, na redución das emisións. Porén, existe outra opción moito menos explorada para a mellora da calidade do aire en ambientes urbanos: o emprego de vexetación arbórea como sumidoiro de contaminantes. Con esta idea, e a pesar de que na maioría das cidades do mundo as árbores restrínxense ás beirarrúas, os bulevares e aos parques, sendo plantados por motivos estéticos, nos últimos anos xurdiron multitude de iniciativas urbanísticas e arquitectónicas illadas que, co fin de reducir efectivamente a contaminación do aire en zonas urbanas, aumentan a superficie arborada que actúa como un filtro verde.

Non obstante, o impacto das árbores sobre a contaminación do aire non se estudiou en profundidade e non se tiveron en conta todos os contaminantes, prestando moi pouca atención aos hidrocarburos aromáticos policíclicos (HAPs). Así, na actualidade, existen grandes ocos no coñecemento sobre a capacidade de captación dos HAPs por parte das árbores. Hoxe en día non existe consenso sobre a relación entre as concentración destes contaminantes e as características das follas, a variabilidade intraindividual (dentro dunha árbore) das concentracións de HAPs e variación interespecífica das árbores para captar e retirar HAPs do aire. Nesta tese abordaremos esas cuestións en 3 estudos (cada unha delas nun estudo diferente).

Resumen

Desde el inicio de la revolución industrial, la calidad del aire se ha deteriorado considerablemente, lo que ha llevado a graves problemas de salud y medioambientales. Este problema es mucho más grave en áreas urbanas.

Para contrarrestar esto, diferentes administraciones públicas están tomando medidas para paliar este problema, pero la mayoría de ellas se basan, principalmente, en la reducción de

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las emisiones. Sin embargo, existe otra opción mucho menos explorada para mejorar la calidad del aire en ambientes urbanos: el uso de vegetación arbórea como sumidero de contaminantes. Con esta idea, y a pesar de que en la mayoría de las ciudades del mundo los árboles se limitan a las aceras, los bulevares y los parques, siendo plantados por motivos estéticos, en los últimos años han surgido numerosas iniciativas urbanísticas y arquitectónicas aisladas que, con el fin de reducir efectivamente la contaminación del aire en zonas urbanas, aumentan la superficie arbolada que actúa como un filtro verde.

No obstante, el impacto de los árboles sobre la contaminación del aire no se ha estudiado en profundidad y no se han tenido en cuenta todos los contaminantes, prestando muy poca atención a los hidrocarburos aromáticos policíclicos (HAPs). Así, en la actualidad, existen grandes lagunas en el conocimiento sobre la capacidad de captación de los HAPs por parte de los árboles. Hoy en día no existe consenso sobre la relación entre las concentraciones de estos contaminantes y las características de las hojas, la variabilidad intraindividual (dentro de un árbol) de las concentraciones de HAPs y la variación interespecífica de los árboles para captar y eliminar HAPs del aire. En esta tesis abordaremos esas cuestiones en 3 estudios (cada uno de ellos en un estudio diferente).

Abstract

Since the beginning of the industrial revolution, air quality has deteriorated considerably, leading to serious health and environmental issues. This problem is much more severe in urban areas.

To counter this, different public administrations are taking measures to alleviate this problem, but most of them are primarily focused on reducing emissions. However, there is another much less explored option for improving air quality in urban environments: the use of tree vegetation as a sink for pollutants. With this idea, and even though in most cities around the world, trees are limited to sidewalks, boulevards, and parks, being planted for aesthetic reasons, in recent years numerous urban planning and architectural initiatives have emerged that, in order to effectively reduce air pollution in urban areas, increase the tree-covered surface area acting as a green filter.

However, the impact of trees on air pollution has not been studied in depth, and not all pollutants have been taken into account, with very little attention paid to polycyclic aromatic

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hydrocarbons (PAHs). Thus, there are significant gaps in the knowledge regarding the ability of trees to capture PAHs. To date, there is no consensus on the relationship between the concentrations of these pollutants and leaf characteristics, intraindividual variability (within a tree) of PAH concentrations, and interspecific variation of trees in capturing and removing PAHs from the air. This thesis will address these issues in three studies (each in a separate study).

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Extended abstract in Galician

A contaminación atmosférica refírese á presenza no aire de substancias ou compostos en concentracións superiores ás naturais, procedentes de fontes industriais, transporte, agricultura, xestión de residuos, etc. Os efectos desta contaminación son diversos e inclúen dende impactos no clima, como o quecemento global, ata a xeración de choivas ácidas e danos aos ecosistemas. Con todo, un dos aspectos máis preocupantes é a súa repercusión na saúde humana. De feito, a Organización Mundial da Saúde (OMS) estima que, no ano 2019, a contaminación do aire foi responsable de 6,7 millóns de mortes prematuras a nivel mundial, asociadas a enfermidades como cardiopatías, accidentes cerebrovasculares, enfermidades pulmonares crónicas, infeccións respiratorias e distintos tipos de cancro. Todo isto ten, ademais da propia perda de vidas humanas, un elevado custo económico. Por exemplo o Banco Mundial, estima que a exposición a material particulado de 2.5 µm (PM2.5) en 2019 tivo un custo estimado de 8,1 mil millóns de dólares, equivalente ao 6,1% do PIB global.

Entre os contaminantes atmosféricos, destacan os hidrocarburos aromáticos policíclicos (HAPs), que serán estudados nesta tese e que se clasifican como compostos orgánicos semivolátiles (SVOCs). Estes compostos están formados por carbono e hidróxeno con estruturas que inclúen aneis de benceno, podendo conter aneis adicionais de 5 átomos de carbono (non bencénicos). A súa estrutura aromática, hidrofobicidade e estabilidade térmica fan que sexan persistentes e difíciles de degradar no medio ambiente. Os HAPs xéranse principalmente por procesos de descomposición térmica (pirólise) e recombinación (pirosíntese) de materia orgánica, aínda que tamén poden ter orixe bióxena, procedendo da actividade de plantas, fungos e microorganismos. Poden clasificarse segundo o número de aneis (2, 3, 4 ou máis) ou o peso molecular en HAPs de baixo (BPM), medio (MPM) e alto peso molecular (APM). Os HAPs BPM son máis volátiles, solubles e xéranse en procesos a temperaturas máis baixas, mentres que os HAPs APM son menos volátiles e solubles, e xéranse a temperaturas máis altas. Os HAPs MPM poden presentar comportamentos intermedios, pero existe certa controversia sobre a súa clasificación exacta. No aire, os HAPs poden atoparse en fase gasosa ou asociados a material particulado (PM), dependendo do seu peso molecular e da temperatura ambiental. Os HAPs BPM atópanse maioritariamente na fase gasosa, mentres que os HAPs APM adoitan estar ligados a partículas. Os HAPs MPM distribúense en ambas fases segundo as condicións atmosféricas.

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Os HAPs son contaminantes altamente tóxicos, mutaxénicos, carcinoxénicos, teratoxénicos e inmunotóxicos, con efectos nocivos tanto para os ecosistemas como para a saúde humana. Son emitidos principalmente a través da queima incompleta de materia orgánica en procesos industriais, transporte, combustión doméstica e incendios forestais. Nas zonas urbanas, as principais fontes son os vehículos e as actividades domésticas, seguidas, en menor medida, polas fontes industriais. A exposición aos HAPs pode ocorrer por inhalación, inxestión, e contacto directo e os seus efectos dependen da dose e da duración da exposición, podendo ir desde molestias leves ata problemas graves como o cancro. Un exemplo do elevado custo económico e social é a estimación feita en Canadá no 2011, segundo a cal os casos de cancro laboral asociados aos HAPs xeraron un custo de 183 millóns de dólares. En animais, os HAPs producen efectos similares aos observados en humanos, e en plantas poden alterar a xerminación, o crecemento e o metabolismo. Os HAPs están actualmente distribuídos por todo o ambiente de forma ubicua, xeralmente en mesturas complexas e non como compostos illados. A súa persistencia no medio ambiente fai que se acumulen nos ecosistemas, afectando tanto á flora como á fauna. É importante destacar que a toxicidade e os efectos dos HAPs varían segundo o seu peso molecular e número de aneis: os de baixo peso molecular son máis solubles e tóxicos para as plantas, mentres que os de alto peso molecular son máis carcinoxénicos pero teñen menos toxicidade aguda.

En base ao establecido anteriormente, podemos afirmar que a contaminación atmosférica, especialmente a causada por HAPs, é un problema ambiental serio que afecta á saúde humana e ao medio ambiente. Neste contexto, as estratexias de mitigación son esenciais, e unha delas é o uso de árbores como filtros naturais do aire. As árbores demostraron ser eficaces na mellora da calidade do aire mediante a captura de contaminantes como ozono, dióxido de nitróxeno, partículas e HAPs, especialmente en zonas urbanas onde as concentracións de contaminantes son máis altas. Non obstante, o impacto da súa función como filtros de HAPs aínda non está completamente cuantificado, con estimacións de eliminación que varían entre o 4% e o 44% dos HAPs emitidos nas zonas de estudos. Polo tanto, é necesario un maior coñecemento sobre os mecanismos de captación de HAPs e os factores que os regulan para optimizar o uso das árbores para esta función filtradora.

O proceso de captación de HAPs polas follas das árbores é complexo e está influído por catro factores principais: a concentración e forma dos HAPs no aire (gasosos ou particulados), factores ambientais (como temperatura, vento e precipitación), características das árbores e a

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presenza de microorganismos degradadores na superficie das follas. Ademais, existen dúas vías principais para a transferencia de HAPs desde o aire ás follas: mediante a súa deposición na superficie foliar ou pola difusión a través dos estomas. Porén, a importancia destas vías non é a mesma para todos os HAPs. Os que están en fase gasosa difunden principalmente a través dos estomas, aínda que tamén poden difundir na cutícula e, desde aí, ao mesófilo. Os HAPs máis pesados, ligados a partículas, depositanse principalmente na cutícula.

O proceso de transferencia dos HAPs está determinado polas vías de entrada e modulado polas características físicas e químicas das superficies foliares, así como pola fisioloxía e morfoloxía (macroscópica e ultrastructura) das follas. Os trazos ecolóxicos das follas estudados en relación á carga de HAPs poden clasificarse en cinco grupos: i) morfoloxía xeral da folla; ii) ultrastructura da superficie foliar; iii) intercambio gasoso; iv) características físicas da cutícula; e v) composición química da cutícula. Non obstante, ata o de agora existen discrepancias experimentais sobre o papel de case todos os trazos ecolóxicos no proceso de carga de HAPs e a importancia relativa destes trazos está lonxe de ser coñecida. Estas discrepancias poden deberse, en gran medida, a un problema de variabilidade intraindividual que se pasa por alto na maioría dos estudos existentes, poñendo en dúbida a representatividade das mostras. Ademais, a maior parte dos estudos que abordan esta cuestión só estudan algúns trazos ecolóxicos (1-3), o que ofrece unha visión moi parcial para explicar as diferenzas entre especies en termos de captación de HAPs. O estudo destes trazos é fundamental para obter unha idea clara de que especies de árbores son as máis adecuadas para actuar como filtros de HAPs nas rúas. Con todo, non sempre é necesario ter toda a información sobre os trazos ecolóxicos para estudar a capacidade das árbores para eliminar HAPs. Unha das formas máis eficientes é mediante variables como os coeficientes de partición planta-aire (K_{PA}), que indican a proporción, en equilibrio, entre a concentración dun composto químico nas follas das plantas e no aire. Esta variable, xunto con outros parámetros como a área foliar específica (SLA) e o índice de área foliar (LAI), permite aplicar modelos ambientais dinámicos de fluxo de contaminantes, incorporando as plantas ao balance de masas, para cuantificar o papel das plantas e especies concretas na eliminación de HAPs do aire. Non obstante, a información dispoñible sobre os K_{PA} e outros parámetros clave aínda é limitada, o que dificulta a avaliación precisa do potencial de distintas especies arbóreas para actuar como filtros de HAPs.

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En conclusión, aínda que as árbores representan unha estratexia custo-efectiva para mellorar a calidade do aire, é necesario continuar investigando para comprender mellor os mecanismos e

optimizar o seu uso na mitigación da contaminación por HAPs, especialmente en contornas urbanas. Concretamente, nesta tese centramos a nosa atención na relación entre a concentración de HAPs nas follas e os trazos foliares, así como na variabilidade intraespecífica (intra- e interindividual) e interespecífica. Todo isto concrétase no obxectivo xeral de aumentar o coñecemento sobre a captación de HAPs polas árbores, que se desenvolve en tres obxectivos específicos:

1. Examinar como os trazos ecolóxicos das follas asociados á morfoloxía foliar (área foliar, área foliar específica [SLA], grosor foliar e relación ancho/longo da folla) e ao intercambio gasoso (condutancia estomática por unidade de área foliar, sinatura isotópica de carbono nas follas [$\delta^{13}C$] e densidade estomática) afectan á captación de hidrocarburos aromáticos policíclicos (HAPs) por especies leñosas.

2. Determinar i) a variabilidade intraindividual nas concentracións de HAPs nas follas e ii) a relación entre esta variabilidade e os trazos foliares (SLA, densidade estomática, intercambio gasoso, ceras cuticulares e pilosidade) e a posición das follas (altura, distancia ao tronco e orientación).

3. Estimar os coeficientes de partición planta-aire (K_{PA}) para especies de árbores ornamentais habitualmente empregadas en parques e rúas de todo o mundo e comparar a súa capacidade para eliminar HAPs gasosos e asociados a partículas en áreas urbanas. Describir a variabilidade interespecífica dos trazos foliares (SLA, lonxitude, densidade estomática, condutancia estomática, $\delta^{13}C$ e contido relativo de ácidos graxos) e a súa relación coa captación de HAPs.

Para completar o obxectivo xeral de aumentar o coñecemento sobre a captación de HAPs polas árbores estruturamos esta tese en tres estudos, cada un deles deseñado para responder a un dos obxectivos específicos anteriores.

O primeiro estudo consistiu nunha revisión sistemática da literatura científica para identificar patróns entre as características morfolóxicas e de intercambio gasoso das follas e a súa capacidade de captar HAPs. Para a selección dos estudos empregamos a base de datos Scopus cunha busca específica e despois realizamos dous cribados. No primeiro, seleccionamos só os artigos en inglés que incluían datos numéricos sobre concentracións de HAPs en follas de árbores ou arbustos, excluíndo aqueles centrados en plantas herbáceas ou sen valores

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cuantitativos. No segundo, seleccionamos os artigos que comparaban concentracións de HAPs entre polo menos dúas especies de árbores ou arbustos en condicións similares, garantindo que as mostras se recollesen na mesma localización e período de tempo, baixo as mesmas fontes de contaminación. A continuación, para estudar a relación entre trazos ecolóxicos e concentracións de HAPs, recolléronse datos sobre trazos como área foliar, SLA, grosor foliar, relación ancho/largo da folla, densidade estomática, condutancia estomática e $\delta^{13}C$ da base de datos TRY Plant Trait Database. Por último, calculáronse as correlacións entre as diferenzas nos trazos foliares e as diferenzas de concentración de HAPs entre especies para identificar patróns significativos.

O segundo estudo centrouse en analizar as diferenzas nas concentracións de HAPs dentro dunha mesma especie de árbore. Para iso escollemos 15 árbores ornamentais de maceira (*Malus* × *moerlandsii* 'Profusion') situadas nunha rúa principal da cidade de A Coruña. De cada árbore, recolléronse mostras en 12 puntos da copa, variando en altura, distancia ao tronco e orientación. Isto permitiu avaliar a influencia da posición na acumulación de HAPs. En cada punto da copa, determinamos a concentración de HAPs e realizamos medicións de distintos trazos foliares como densidade estomática, SLA, $\delta^{13}C$, concentración de ácidos graxos na cutícula e pilosidade. Testamos a existencia de diferencias significativas nas concentracións de HAPs e nos trazos foliares entre as distintas posicións das follas na copa das árbores. Por último, estudamos a relación entre os trazos e as concentracións de HAPs.

Dividimos o terceiro estudo en dúas partes, na primeira estudamos a variabilidade interespecífica na concentración de HAPs nas follas de distintas coníferas perennifolias. Ademais, tamén estimamos as ecuacións dos K_{PA} destas especies e avaliamos a súa capacidade para eliminar HAPs asociados a gases e materia particulada en áreas urbanas. Mentres, na seguda parte determinamos a variabilidade interespecífica de distintos trazos foliares desas mesmas árbores e as súa relación coas concentracións de HAPs nas follas. Para abordar a primeira parte, determinamos as concentración de HAPs en 13 especies de coníferas perennifolias (i.e. *Cedrus atlantica, Cedrus deodara, Chamaecyparis pisifera, Cryptomeria japonica* e *Cryptomeria japonica* f. *elegans, Cupressus arizonica, Cupressus lusitanica, Cupressus sempervirens, Juniperus chinensis, Picea abies, Pinus pinaster, Sequoia sempervirens, Taxus baccata* e *Thujopsis dolabrata*) presentes no Campus Universitario da Universidade de Santiago de Compostela e que son frecuentemente usadas de maneira ornamental en xardíns e rúas. Para o calculo dos K_{PA} foi necesario estimar as concentracións

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de HAPs no aire, para o que se empregaron as concentracións de HAPs determinadas en *P. abies* e unhas ecuacións preexistentes para a estimación dos K_{PA} dos bifenilos policlorados (PCBs) nesta especie. A partir das concentracións estimadas de HAPs no aire e das concentracións determinadas de HAPs nas follas das árbores, desenvolvemos as ecuacións para estimar o K_{PA} en función do Log K_{OA} (coeficiente de partición octanol-aire). Estas ecuacións xunto cas concentración de HAPs determinadas nas follas permitíronnos comparar a capacidade das distintas especies para captar HAPs asociados a fase gasosa e en fase particulada. Para isto utilizamos as concentracións de HAPs determinadas orixinalmente (en función da masa da folla) e calculamos as concentracións en función da área da folla, é dicir, as concentracións corrixidas polo SLA (que tamén determinamos para cada especie). Para abordar a segunda parte do estudo, durante a mostraxe das árbores para a determinación dos HAPs medíronse outros trazos foliares a parte do SLA, concretamente a densidade estomática, a condutancia estomática, o $\delta^{13}C$ e contido relativo de ácidos graxos. Ademais destes trazos, tamén se incorporou ás análises a lonxitude das follas, que foi extraída da base de datos TRY Plant Trait Database. O contido relativo de ácidos graxos obtívose mediante a pirólise das follas e calculouse a porcentaxe para cada ácido graxo respecto ao total dos compostos extraídos mediante a pirólise. A relación entre os trazos foliares e as concentracións de HAPs nas follas estudouse a través do cálculo de correlacións entre ambos grupos de variables.

No primeiro estudo, como xa indicamos anteriormente, abordamos, a través dunha meta-análise, a relación entre a concentración de HAPs e os trazos relacionados coa morfoloxía foliar e o intercambio gasoso nas follas. Os resultados da meta-análise varían entre os diferentes trazos estudados e, en xeral, foron máis robustos e claros para os trazos morfolóxicos, e menos consistentes para os trazos de intercambio gasoso.

Os resultados mostraron correlacións positiva entre a captación de HAPs e a área foliar e o SLA, especialmente aqueles HAPs asociados ao material particulado. Isto parece confirmar a hipótese inicial de que un aumento na área foliar incrementa a concentración de HAPs nas follas ao aumentar a superficie de adsorción. Pola contra, o grosor foliar mostrou unha relación negativa coa captación de HAPs asociados ao material particulado e unha relación positiva cos HAPs lixeiros. A relación ancho/largo amosarou resultados mixtos, con correlacións negativas para HAPs lixeiros en fase gasosa e positivas para HAPs máis pesados.

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Os trazos relacionados co intercambio gasoso presentaron, en moitos casos, contrarios ás hipóteses principais na literatura. Por exemplo, a condutancia estomática mostrou correlacións positivas cos HAPs asociados a partículas, e o $\delta^{13}C$, que ten unha relación inversa co intercambio gasoso, mostrou correlacións positivas con HAPs lixeiros e negativas con HAPs pesados. A densidade estomática, mostrou correlacións negativas con todos os HAPs, pero estes resultados son pouco fiables, debido aos poucos datos dispoñibles. Ademais hai que ter en conta que esta variable non ten en conta cuestións como o tamaño do estoma ou a súa apertura.

Os resultados suxiren que os trazos morfolóxicos son máis determinantes para a captación de HAPs que os relacionados co intercambio gasoso, aínda que este último pode contribuír á captación de HAPs lixeiros en fase gasosa. A investigación destaca limitacións significativas, como a falta de datos específicos para as árbores nas que se determinaron as concentracións de HAPs, xa que os trazos foliares utilizados proveñen de valores medianos da base de datos TRY Plant Trait Database, o que obvia a variabilidade natural e introduce potenciais sesgos nos resultados. A pesar destas limitacións, este estudo ofrece unha visión completa do coñecemento actual sobre a relación entre os trazos foliares e as concentracións de HAPs nas follas. Ademais, reforza as hipóteses existentes sobre o papel dos trazos da morfoloxía foliar na captación de HAPs e ofrece novas preguntas sobre o papel do intercambio gasoso. Ademais, destaca a necesidade de enfoques máis detallados e multifactoriais en estudos futuros para comprender mellor a relación entre trazos foliares, fases dos HAPs e as condicións ambientais.

No segundo estudo abordamos a variabilidade intraespecífica (tanto inter- como intraindividual) nas concentracións de HAPs e nos trazos foliares, así como a relación entre ambos. Como resultado, observamos que esta variabilidade non é despreciable nin nas concentracións de HAPs nin para os trazos foliares. As concentracións de HAPs nas follas variaron entre 57 e 472 ng g^{-1} e a concentración total nas árbores máis contaminadas son ata o dobre que nas menos contaminadas. En canto aos trazos foliares, a variabilidade interindividual foi xeralmente menor ou similar á variabilidade intraindividual.

A análise estatística revelou diferenzas significativas nas concentracións de HAPs segundo a posición das follas, as follas situadas máis abaixo e máis próximas ao tronco presentaron concentracións significativamente máis altas en comparación coas follas situadas máis arriba e afastadas do tronco. Ao dividir as árboles en dous grupos en función da súa concentración de HAPs, observamos que no grupo 2, o máis pequeño e homoxeneo, as diferenzas estiveron

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relacionadas principalmente coa altura na copa, e só se observaron diferenzas segundo a distancia ao tronco para o fenantreno (Phe). Tamén se aplicaron modelos de regresión lineal para analizar estas diferenzas, cun rango de coeficientes de determinación (R^2) axustado entre 0,14 e 0,48 para todos as árbores e unha media de 0,26. No grupo 1, o rango foi similar, mentres que no grupo 2 foi maior (de 0,32 a 0,59), probablemente debido á menor variabilidade deste grupo, cuxos árbores estaban máis próximos entre si.

En canto aos trazos foliares, atopamos diferenzas significativas segundo a posición das follas na copa: as follas máis expostas (nas zonas superiores da copa) presentaron un menor SLA, maior densidade estomática e maior $\delta^{13}C$. Isto está en liña coa variación destas variables en función da altura e exposición á luz. Non se observaron diferenzas significativas na composición de ácidos graxos entre diferentes posicións da copa. En canto á pilosidade, observouse a simple vista que as follas superiores tiñan máis tricomas que as inferiores, con maior presenza na cara adaxial que na abaxial. Non obstante, debido a que a avaliación da pilosidade non se realizou de forma cuantitativa, non foi posible incluír esta variable nas análises estatísticas.

Do mesmo xeito que no primeiro estudo, analizamos a relación entre as concentracións de HAPs e os trazos foliares, e observamos que a maioría dos HAPs presentaron correlacións positivas co SLA e negativas coa densidade estomática e o $\delta^{13}C$ mentres que a inmensa maioría dos ácidos graxos da cutícula non amosaron correlacións significativas con ningunha variable. O SLA semella se o trazo con maior influencia na captación de HAPs, cas correlacións máis altas (0,16 a 0,58). O SLA, a densidade estomática e o $\delta^{13}C$ parecen estar moi influenciados pola posición das follas na copa, que os modifica segundo o gradiente vertical e a exposición á luz.

Neste estudo, do mesmo xeito que no primeiro, o SLA presentou correlacións negativas coa maioría dos HAPs e a densidade estomática amosou correlacións negativas. No caso do SLA, o rango de valores é semellante en ambos estudos. Porén, os valores para a densidade estomática no primeiro estudo son considerablemente maiores. En canto ao $\delta^{13}C$, as correlacións significativas no segundo estudo foron negativas en todos os casos, mentres que no primeiro estudo foron positivas para HAPs lixeiros.

Para analizar a importancia do SLA nas diferenzas observadas nas concentracións de HAPs, estas reexpresáronse en termos de área foliar, dividindo os datos polo SLA (e así eliminando o

seu efecto). Isto elimina case por completo as diferenzas atribuídas á distancia das follas ao tronco e diminúe o poder explicativo (R^2) dos modelos, o que suxire que boa parte da variación observada na primeira análise pode atribuírse á morfoloxía foliar. Porén, mesmo eliminando o efecto do SLA, observouse unha diminución significativa nas concentracións de HAPs foliares coa altura das follas, probablemente debido á maior distancia respecto á fonte de emisión de HAPs (vehículos) e a otros factores como o efecto barreira das follas e a iluminación/temperatura, que afectan tanto ao SLA como ao equilibrio entre volatilización, degradación e deposición de HAPs.

Como resultado da primeira parte do terceiro estudo, observamos unha elevada variabilidade interespecífica nas concentracións de HAPs nas follas das especies estudadas, que mostraron un diferencias importantes na acumulación de HAPs, tanto no total como de compostos individuais. As concentracións totais de HAPs variaron entre 845 ng g^{-1} dw en *Pinus pinaster* e 95 ng g^{-1} dw en *Picea abies*, cunha variación de ata unha orde de magnitude. Este rango é semellante ao observado nas follas das maceiras no segundo estudo.

A partir das concentración desenvolvéronse tres modelos para estimar os coeficientes de partición planta-ar (KPA): i) un modelo lineal para HAPs asociados á fase gasosa; ii) un modelo lineal para todos os HAPs (fase gasosa e particulada); e iii) un modelo polinómico de segundo grao para todos os HAPs. En todos os casos o Log K_{PA} incrementouse co Log K_{OA}, pero en valores superiores a 10, estabilizouse ou diminuíu, indicando un retraso cinético na partición dos compostos máis hidrofóbicos, ligados principalmente ás partículas (PM) que compiten coa biomasa foliar na absorción de HAPs. Ademais disto, é probable que as concentracións no aire de HAPs asociados a partículas foran sobreestimadas, resultando en estimacións máis baixas dos K_{PA} destes HAPs.

O modelo 1 mostrou valores de R^2 que oscilaron entre 0,56 (*P. pinaster*) e 0,92 (*J. chinensis*) considerando a temperatura de referencia. Pendentes suaves neste modelo sinalan diferenzas pequenas na afinidade dunha por HAPs con distinta hidrofobicidade (Log K_{OA}) e pendentes pronunciadas indican grandes diferencias nas afinidades. As pendentes máis baixas corresponderon a *J. chinensis* e *T. baccata*, especies que tamén estiveron entre as catro con menores concentracións totais de HAPs, o que indica unha baixa afinidade xeral por HAPs. As ecuacións de *P. pinaster*, *C. deodara*, *T. dolabrata* e *Cupressus spp.* mostraron as pendentes

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máis pronunciadas, aínda que a súa afinidade por HAPs totais varía. As pendentes do modelo 2 foron, en xeral, máis baixas debido ás incertezas nas concentracións de aire.

As especies cunha diferenza pequena na pendente entre os modelo 1 e o 3, mostran unha tendencia máis lineal na acumulación de HAPs, probablemente debido a unha maior capacidade de acumulación de HAPs asociados a PM en comparación con outras especies. Para realizar una avaliación preliminar da capacidade das especies para reter HAPs asociados a PM, calculamos para cada especie a porcentaxe de HAPs asociados a PM e a porcentaxe asociados a gas (moito maior). Como resultado, observamos que a porcentaxe de HAPs asociados a PM, e polo tanto a capacidade das follas para captar PM, varía considerablemente entre as especies estudadas, desde o 3,4 % en *P. pinaster* ata o 34,4 % en *C. lusitanica*. En base a todo o anterior, as agullas de *P. pinaster* demostraron a maior capacidade para capturar HAPs en fase gasosa, mentres que as follas de *C. sempervirens* foron as máis eficaces na captura de HAPs asociados a PM. Considerando os HAPs por unidade de superficie foliar, as follas de *C. sempervirens* destacaron pola mellor eficiencia de eliminación, sendo a segunda especie con maior captura de HAPs en fase gasosa e a primeira para HAPs asociados a PM. Con todo, hai que ter en conta as incertezas nos parámetros incluídos nas ecuacións dos K_{PA} e a existencia doutros factores non analizados neste estudo, como o índice de área foliar (LAI), a forma das árbores, a porcentaxe de biomasa foliar e o cambio desta porcentaxe ao longo do ciclo de vida da árbore, que serán investigados en futuros estudos.

Como resultado da segunda parte do terceiro estudo, observouse unha elevada variabilidade nos trazos analizados. Concretamente os rangos foron: 54 a 19 $cm^2\ g^{-1}$ (*T. baccata* e *P. abies*, respectivamente) para o SLA; 0,075 cm a 20 cm (*C. sempervirens* e *P. pinaster*) para a lonxitude; -23,7‰ a -30,2‰ (*T. dolabrata* e *P. pinaster*) para o $\delta^{13}C$; 3203 a 22813 estomas cm^{-2} (*C. arizonica* e *C. japonica*, respectivamente) para a densidade estomática; e 0,016 a 0,257 mol $H_2O\ m^{-2}\ s^{-1}$ (*J. chinensis* e *C. deodara*, respectivamente) para a conductancia estomática. Estes valores non son moi diferentes dos observados no segundo estudo para a variabilidade intraindividual. En canto ao contido relativo de ácidos graxos, tamén se detectou unha variación substancial entre especies. A porcentaxe total de ácidos graxos (Total F.A.) variou entre o 5,49% (*P. abies*) e o 22,20% (*J. chinensis*). Ademais, o ácido graxo maioritario tamén variou significativamente entre especies, por exemplo, o F.A. C12 representou o 4,24% dos compostos extraídos en *P. pinaster* e o 0,71% en *C. deodara*; en contraste, o F.A. C20:2 supuxo o 0,09% en *P. pinaster* e o 6,30% en *C. deodara*.

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A maioría destas correlacións non foron significativas, probablemente debido ao tamaño da mostra e á elevada variabilidade. Para o SLA, atopáronse correlacións positivas cun HAP BPM e cun HAP MPM en consonancia cos resultados obtidos nos Capítulos I e II. En canto á lonxitude, observáronse correlacións significativamente negativas para HAPs asociados principalmente a partículas (PM). Probablemente debido a que neste caso que a lonxitude das follas actuaría como un indicador para especies con follas escamosas (as de menor tamaño), que neste estudo amosan maior rugosidade superficial que as especies con follas máis longas, o que lles permitiría reter máis PM. Para a condutancia estomática atopouse unha única correlación positiva con ∑HAPs, o que parece apoiar a hipótese de que un aumento no intercambio gasoso incrementa a concentración de HAPs (principalmente en fase gasosa). Este resultado contrasta cos do Capítulo I. O $\delta^{13}C$ mostrou correlacións negativas con diferentes HAPs e con sumas de HAPs. Estes resultados parecen concordar coa hipótese anterior do intercambio gasoso e son totalmente opostos aos obtidos no Capítulo I e similares aos do Capítulo II. A densidade estomática, non parece estar relacionada cos HAPs en fase gasosa, xa que todas as correlacións significativas son negativas e refírense a HAPs asociados a PM. En todos os capítulos (I, II e IV) observáronse correlacións significativamente negativas con HAPs asociados a PM. En conxunto, todos estes resultados parecen indicar e reforzar a hipótese de que a densidade estomática non é un bo indicador do intercambio gasoso. En canto aos ácidos graxos, ao calcular as correlacións, obtivéronse resultados mixtos, con relacións tanto positivas (F.A. C12, F.A. C14 e F.A. C20:2) como negativas (F.A. C16, F.A. C18:2 e F.A. C18:1). É destacable que no Capítulo II só se obtiveron dúas correlacións significativas para ácidos graxos: unha para o ácido esteárico (F.A. C18) e outra para o ácido linolénico (F.A. C18:3), ambas con antraceno, mentres que no Capítulo IV non se atoparon correlacións significativas para ningún destes ácidos graxos.

En resumo, os resultados desta tese parecen indicar que a relación entre a concentración de HAPs nas follas e os trazos morfolóxicos destas é máis consistente e robusta que a relación cos trazos de intercambio gasoso. Ademais, os resultados dos trazos morfolóxicos parecen apoiar as hipóteses principais da literatura mentres que os resultados para os trazos de intercambio gasoso son contraditorios en todos os niveis e a necesitan una investigación máis profunda (tanto a nivel individual como multifactorial). Por outra banda, tamén sería necesario profundizar no rol dos ácidos graxos. De todos os trazos estudados nesta tese, o SLA foi o que mostrou resultados máis claros e coherentes e unha maior importancia con respecto ás

concentración de HAPs. En contraste, todos os resultados parecen indicar que a densidade estomática non sería un bo indicador para o intercambio gasoso. Nesta tese tamén demostramos a dimensión da variabilidade intraindividual das concentracións de HAPs nas follas e identificamos a altura das follas como a súa principal fonte de variabilidade, de tal forma que as follas inferiores absorben máis HAPs que as superiores. Isto depende da morfoloxía das follas (SLA), da distancia á fonte de emisión (coches), do efecto barreira e da iluminación/temperatura das follas. Por último, observamos a existencia dunha gran variabilidade interespecífica no potencial das coníferas para eliminar HAPs do aire e aportamos ecuacións para estimar os seus coeficientes de partición planta-aire. Estas ecuacións permítennos mellorar o coñecemento sobre o potencial das diferentes coníferas perennes para eliminar HAPs do aire, a pesar de algunhas incertezas restantes sobre os valores exactos dos parámetros incluídos neslas. Como resultado destes estudos, podemos dicir que a vexetación ideal para eliminar HAPs do aire en ambientes urbanos serían árbores perennes cunha alta biomasa foliar), non moi altas, cunha alta proporción de follas de sombra e cunha elevada capacidade para captar tanto HAPs en fase gasosa como asociados a partículas. Así e todo, para optimizar o uso das árbores como filtros de HAPs, é esencial investigar máis a fondo os factores que inflúen na acumulación de HAPs nas follas das árbores e realizar máis estudos comparativos entre diferentes especies, tanto experimentais como baseados en modelos, para comprender mellor o seu potencial como eliminadores de HAPs.

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Publications included in the thesis

1. Chapter I: Giráldez, P., Vázquez-Arias, A., de Nicola, F., Fernández, J. Á., & Aboal, J. R. (2025). Leaf ecological traits (morphology and gas exchange) and polycyclic aromatic hydrocarbons concentrations in shrubs and trees: A meta-analysis approach. *Environmental Pollution*, *364*, 125337. https://doi.org/10.1016/J.ENVPOL.2024.125337**.**

2. Chapter II: Giráldez, P., Varela, Z., Di Guardo, A., Terzaghi, E., Celeiro, M., García-Jares, C., Fernández, J. Á., & Aboal, J. R. (2024). Relationship between foliar polycyclic aromatic hydrocarbons (PAHs) concentrations and plant traits: Intracanopy variability for a broadleaf species in an urban environment. *Science of The Total Environment*, *940*, 173698. https://doi.org/10.1016/J.SCITOTENV.2024.173698.

3. Chapter III: Giráldez, P., Aboal, J. R., Fernández, J. Á., Di Guardo, A., & Terzaghi, E. (2022). Plant-air partition coefficients for thirteen urban conifer tree species: Estimating the best gas and particulate matter associated PAH removers. *Environmental Pollution*, *315*, 120409. https://doi.org/10.1016/J.ENVPOL.2022.120409.

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1. Introduction

1.1. Environmental pollution

Ecosystems and the life they harbor depend on maintaining a delicate balance in the flow of matter and energy, both within these ecosystems and between them and the surrounding world. However, this balance is often disturbed by the presence of contaminants, i.e. substances (or energy) that are above normal average values (Chapman, 2007). These disturbances do not intrinsically imply a negative effect on ecosystems, since they have buffer systems that make them resilient to external disturbances (e.g. Barling & Moore, 1994). Nevertheless, more often than anyone would like, contamination becomes pollution, i.e., it produces biologically adverse effects (Chapman, 2007). Thus, pollution could be defined as the addition of any substance (solid, liquid or gaseous) or any form of energy (such as heat, sound or radioactivity) to the environment at a faster rate than it can be dispersed, diluted, decomposed, recycled or stored in some harmless form (Hill, 2020; Yu & Tsunoda, 2004).

According to this definition, there are two possible sources of pollution, anthropogenic sources and natural sources, although in general the term pollution is reserved for pollutants of anthropic origin. The main types of pollution are usually classified by environment into atmospheric (air), terrestrial (soil) and aquatic (water) pollution, the first of which is the focus of this thesis.

1.2. Atmospheric pollution

1.2.1. Definition and main sources

Atmospheric pollution is, as we have indicated, pollution that occurs in the air and can therefore be defined as any substance emitted into the air from an anthropogenic, biogenic or geogenic source, which is either not part of the natural atmosphere or which is found in concentrations higher than those that are natural in the atmosphere, and which could cause adverse effects in the short or long term (Daly & Zannetti, 2007).

Atmospheric pollution has different sources, both natural (fires, volcanoes, biogenic, etc.) and anthropogenic. However, from now on, when we talk about ‘atmospheric pollution’, we will only refer to pollution generated by anthropogenic sources. Some of these main sources

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originate from industrial activities, transport, agricultural activities, waste management practices, residential energy use and construction and demolition of buildings (see Hill, 2020; Yu & Tsunoda, 2004). Each of these sources can produce a different set of pollutants that end up in the atmosphere, the main ones being particulate matter, carbon monoxide, sulphur dioxide, nitrogen oxides, volatile organic compounds (VOCs), etc. (WHO, 2024a). Apart from these directly emitted pollutants (primary pollutants), there are other pollutants that are not emitted, but are formed in the atmosphere itself from precursors, these compounds are called secondary pollutants and one example is tropospheric ozone.

1.2.2. Air pollution effects

Air pollution is associated with adverse effects on both environment and human health. Effects on the environment include, among others, acid rain, ozone layer depletion, damage to vegetation and long-term climate change (Newman, 2014). As if this were not serious enough, the effects of air pollution on human health are diverse and extensive. The World Health Organization (WHO, 2024b) has estimated that in 2019 there were 6.7 million premature deaths worldwide due to air pollution, 4.2 million of them due to ambient (outdoor) air pollution. Of these, WHO estimates that 68% of outdoor air pollution related premature deaths were due to ischemic heart disease and stroke, 14% were due to chronic obstructive pulmonary disease, 14% were due to acute lower respiratory infections, and 4% of deaths were due to lung cancers. According to the Global Burden of Disease 2019, PM2.5 (fine particulate matter) air pollution -in both outdoor environments and inside households that use solid fuels for cooking and heating- caused 6.4 million deaths and 93 billion days lived with illness in 2019, with an estimated welfare cost of $8.1 trillion, equivalent to 6.1% of global GDP (World Bank, 2022). In Europe, the European Environment Agency (EEA, 2020) estimates 400 000 deaths per year in EEA-39 (excluding Turkey) as a result of exposure to PM2.5.

1.3. Polycyclic Aromatic Hydrocarbons (PAHs)

1.3.1. Definition, origin and characteristics

This thesis focuses on a particular group of these air pollutants, polycyclic aromatic hydrocarbons (PAHs). These compounds can be classified as semi-volatile organic compounds (SVOCs, Chaturvedi et al., 2023), which are a subgroup of VOCs that tend to have a higher molecular weight and higher boiling point temperature than other VOCs (Weschler & Nazaroff,

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2008). The PAHs are a group of organic pollutants consisting of carbon and hydrogen with a structure containing at least two benzene rings joined in linear, angular or cluster arrangements (Mojiri et al., 2019). Other than the benzene rings, PAHs may also contain additional rings that do not have six sides (Ravindra et al., 2008). Depending on the presence of these additional rings they can be classified as alternants (only 6-carbon rings) or non-alternants (with some 5-carbon ring) (Gupte et al., 2016). The inherent properties of PAHs such as heterocyclic aromatic ring structures, hydrophobicity, and thermostability have made them recalcitrant and highly persistent in the environment (Patel et al., 2020).

PAHs are mainly generated from a process of thermal decomposition (pyrolysis) and subsequent recombination (pyrosynthesis) of different organic molecules (Kumar et al., 2016). Thus, PAHs are the most stable form of hydrocarbons having low hydrogen-to-carbon ratio and usually occur in complex mixtures rather than single compounds (Ravindra et al., 2008). Although little known, it is necessary to indicate that there are also PAHs that are not synthesized by this route but have a biogenic origin (Wakeham & Canuel, 2016) (formed mainly by the activity of plants, fungi and microorganisms).

PAHs are a group with common characteristics, however, they have heterogeneous behavior depending on their size. Thus, PAHs can be classified by the number of rings they are composed of (e.g. 2-ring, 3-ring, 4-ring PAHs, etc.) or by molecular weight. The molecular weight classification usually places PAHs into two groups, low molecular weight PAHs (LMW) and high molecular weight PAHs (HMW). LMW PAHs are those with 2-3 rings while HMW PAHs have 4 or more (e.g. Ali et al., 2021). Some authors include the 4-ring PAHs and some of the 3-ring PAHs in a third group of medium molecular weight (MMW) PAHs. However, there is controversy regarding the PAHs that integrate MMW PAHs, in some studies only 4-ring PAHs are considered MMW PAHs (e.g. WHO Regional Office for Europe, 2010). Furthermore, in some cases, only part of the 4-ring PAHs are considered MMW PAHs while the others are considered HMW PAHs (e.g. Pleijel et al., 2022). Something similar occurs to 3-ring PAHs, as the compounds of this group that are included in MMW PAHs may vary between studies (e.g. De Nicola et al., 2017; Jouraeva et al., 2002). In any case, lighter PAHs are more volatile, have higher solubility and are generated in processes that take place at lower temperatures. On the other hand, heavier PAHs are usually generated in higher temperature processes, have lower volatility and solubility (Mai et al., 2003; Şahin et al., 2022). In air, PAHs can be found in the gas phase or associated with particulate matter, with LMW PAHs

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predominantly associated to the gas phase and HMW PAHs mostly associated to the particulate phase (Slezakova et al., 2013). The MMW PAHs are partitioned in both phases depending on the temperature of the atmosphere (WHO Regional Office for Europe, 2010).

1.3.2. PAHs sources

The sources of PAHs can also be classified, like air pollution, into anthropogenic sources (industrial, mobile, domestic and agricultural sources) and natural sources (volcanic eruptions, natural forest fires...). It is noteworthy that the contribution of the latter is much lower and, depending on the study region, negligible compared to the contribution of the former (Shen et al., 2013). Another classification of emission sources is according to the form of emission, into pyrogenic (from incomplete combustion of organic matter), petrogenic (present in petroleum and its by-products that become widespread due to storage, transport, use and leakage of crude oil and its products) and biogenic (synthesized by biological entities such as microorganisms, phytoplankton, algae and plants and during the slow biological conversion of organic materials) (Patel et al., 2020).

The main sources of global PAHs emission are residential/commercial biomass burning (60.5%), open-field biomass burning (agricultural, waste burning, deforestation, and wildfire, 13.6%), and petroleum consumption by on-road motor vehicles (12.8%) (percentages estimated by Shen et al., 2013). However, the emission profiles of PAHs can vary quite a bit between countries and regions (Shen et al., 2013). In urban regions, the major sources are mobile (vehicles) and domestic sources and, to a lesser extent, industrial sources (Ali et al., 2021).

Once emitted PAHs have different routes of exposure through which they can reach humans: i) these being inhalation of contaminated air (most common); ii) ingestion of contaminated water and/or food; and iii) contact with contaminated air, soil and/or water (Venkatraman et al., 2024). Naturally, these same routes of exposure affect other animals (e.g. mammals) and may affect other living things (e.g. plants) with corresponding modifications to their life forms. Nowadays, PAHs are ubiquitous in the environment and, in general, they are not isolated but are found in mixtures of several of them. Moreover, their persistence may cause them to be a serious problem in the environments in which they are found.

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1.4. Impacts of PAHs on environment and health

PAHs are pollutants that have been determined to be highly toxic, mutagenic, carcinogenic, teratogenic, and immunotoxicogenic to various life forms (Patel et al., 2020), thus producing harmful effects on both ecosystems and human health. As already indicated in the previous section, PAHs have different routes of exposure to come into contact with humans, which can have different effects on people's health. However, it is also necessary to take into account the amount of PAHs people are exposed to and the time this exposure lasts. According to this, we can have acute effects on human health and chronic effects, with special mention to occupational exposures (to which workers are subjected in their jobs). Depending on this, PAHs can cause from slight discomfort, irritation or nausea to problems as serious as cancer (see Kim et al., 2013 and Venkatraman et al., 2024 for more details).

PAHs have different mechanisms or pathways by which they can affect human health, producing oxidative damage and inflammation, cardiovascular effects, endocrine disruption, immunotoxicity, neurotoxicity, and cancer (Jo et al., 2022; Mallah et al., 2021; Ramesh et al., 2011; Zhang et al., 2016). There are studies that have suggested that PAHs associated with ambient particulate matter generate reactive oxygen species (ROS), resulting in oxidative stress and inflammatory responses (Zhang et al., 2016). This ROS can be produced from the metabolization of PAH, where catalytic activities of cytochrome P450 are known to produce ROS directly and also generate biologic reactive intermediates, including quinones, which produce ROS by redox cycling (Bonvallot et al., 2012). Although the exact mechanisms that link exposure to PAHs to cardiovascular diseases (CVDs) are not known, it is suspected that one of the pathways could be the oxidative damage and inflammation that they produce. In any case, as pointed out by Mallah et al. (2021) in their systematic review, everything seems to indicate that there is a positive relationship between PAHs and CVDs, as well as with obesity and other cardio-metabolic risk factors. This is probably also related to the fact that PAHs are known endocrine disruptors that can alter the hormonal functioning of humans and animals through different action pathways (Zhang et al., 2016), having, for example, effects on sex hormones (estrogens and testosterone, Vondráček et al., 2018) and metabolic hormones such as thyroid hormones (S. Yang et al., 2023). In addition, as Patel et al. (2020) indicate, PAHs have some immune system-related adverse effects like inhibition of pre B, pre T, and myeloid cell development, B and T cell suppression, apoptosis of lymphoid tissues, disruption of

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myelopoiesis, and altered cytokine production by macrophages and monocytes. Moreover, both in vitro and animal in vivo studies have shown that PAHs can cause neurological problems and, although there are discrepancies, the results of most epidemiological studies seem to indicate that PAHs have a neurological effect and also cause neurodevelopmental toxicity (Xu et al., 2024). Finally, it is important to highlight the carcinogenic effect of PAHs, which is the effect on human health for which they are best known. In fact, PAHs were the first carcinogenic chemicals to be identified as such (Peters & Gonzalez, 2018). Nowadays it has been established that PAHs can cause different types of cancer such as lung, bladder, skin or gastrointestinals (see as example Ravanbakhsh et al., 2023). Among them, the most common is lung cancer, the second most reported cancer in the world and the first in terms of lethality (Stading et al., 2021). As indicated by Stading et al. (2021), 90% of these cancers are associated with smoking tobacco (although there are other contributing factors) and are directly related to the consequent inhalation of PAHs.

Besides being harmful to human health, PAHs also have a negative effect on the environment. In fact, there are numerous animal studies that show that PAHs have similar effects on animals as in humans: carcinogenic, teratogenic, genotoxic, immunotoxic, neurotoxic, produce oxidative stress and have endocrine and reproductive disruption effects (Sun et al., 2021). PAHs can also have effects on plants, negatively affecting their germination, growth and metabolism. However, hormesis has been detected in the response of some plants to some PAHs, so that although they are harmful in high doses, in low doses they could have a stimulating effect (Molina & Segura, 2021). Finally, it should be noted that PAHs also alter the composition of microorganism communities, more specifically of indigenous bacteria and, under certain conditions, could be related (along with other stressors) to the creation and proliferation of antibiotic resistance genes (Rodgers et al., 2018).

We have discussed the health effects of PAHs in general, but not all PAHs have the same effects and are not equally toxic. As indicated previously (section 1.3.1), PAHs have different characteristics and behavior depending on their number of rings and their molecular weight. In general, increasing molecular weight increases carcinogenicity and decreases acute toxicity (Ravindra et al., 2008). In fact, there are systems that evaluate the carcinogenic effect of the different PAHs where the differences between them can be appreciated (e.g. see Sun et al., 2021). Another example of the different effects of PAHs is that LMW PAHs have higher

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water solubility and bioavailability than HMW-PAHs, making them generally more toxic to plants than HMW-PAHs (Molina & Segura, 2021).

These harmful effects have high economic and social cost, but although there are many estimates on the economic impact of air pollution, there are hardly any estimates on the specific effect of PAHs. One of those available is the one made by CAREX Canada and OCRC (2019) on the burden of occupational cancer due to PAHs, where in Canada alone, the estimated cost of lung cancer, non-melanoma skin cancer, and suspected bladder cancer due to workplace PAH exposure in 2011 is estimated at $183 million. This could give an idea of the magnitude of this problem at a global level, considering other exposures.

The harmful effects of PAHs make necessary to monitor them in order to control their levels. For this purpose, the United States Environmental Protection Agency (US EPA) selected 16 “priority” PAHs (see Bojes & Pope, 2007), which in general are those studied in most of the research and included in most of the world's regulations. These PAHs range from 2 rings to 6 rings and therefore include LMW PAHs and HMW PAHs with different behaviors, some being mostly in gaseous form and others in particulate form, so they have different toxicities (see Sun et al., 2021). These PAHs were selected in 1976 and are the result of the circumstances of the moment in which they were selected, with the main mention of the conditions of knowledge and analytical techniques (Keith, 2015). However, the exclusive monitoring of these PAHs is currently being questioned despite their success worldwide for more than 40 years. Nowadays there are researchers who consider that this choice is obsolete and that there would be others that would be more representative or interesting when evaluating PAH contamination (Andersson & Achten, 2015). In any case, in this thesis we will stick to these 16 PAHs (with slight modifications) to keep in line with the literature, facilitating comparisons and maximizing their usefulness.

1.5. Trees as air and PAHs filters

Clearly, air pollution and more specifically PAH pollution is a serious problem that needs to be addressed in order to improve the quality of life of people and the condition of the environment. While it is true that initiatives are being taken in a large part of the world to reduce these emissions, it would be naïve to think that they can be completely eliminated in the near

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future. It is in this context that remediation and mitigation strategies come into play. Among these strategies, we will focus on the ability of trees to act as air filters.

Evidence has shown that trees are able to improve air quality by filtering the air, capturing pollutants (acting as sinks) and improving people's health to the tune of billions of dollars (Nowak et al., 2014). Thus, several studies (e.g. Jim & Chen, 2008; Selmi et al., 2016; and Nowak et al., 2018) report the capacity of trees to capture ozone, nitrogen dioxide, sulphur dioxide, ammonia, nitric acid, carbon monoxide and particulate matter. This is of particular importance in urban areas, where air pollutant concentrations are much higher, as is population density. Although trees function as a sink for pollutants, it must be considered that other factors influence local pollutant concentrations. For example, trees may even increase the local concentration of pollutants by disrupting/altering air flows (Air Quality Expert Group, 2018; Tong et al., 2015). Therefore, in order to make optimal use of this strategy, further research is needed to get a holistic view of the dynamics between the different pollutants and the trees.

PAHs in cities are mainly distributed through the air, as previously mentioned, and therefore they are also affected by the filtering function of trees. In fact, there are a multitude of studies on PAH concentrations in tree leaves (e.g. Kostić et al., 2024; Tremolada et al., 1996 and Sun et al., 2010). Despite this, there are hardly any estimates of the amounts or percentages of PAHs that trees remove from the atmosphere, making it difficult to assess their bioremediation impact. Estimates vary widely, ranging from 4% (Wagrowski & Hites, 1997) of PAHs emitted in the study area to 44% (Simonich & Hites, 1994) including the 10% estimated by Tian et al., (2008). Anyway, it is evident that trees are able to uptake PAHs from the atmosphere, as the leaves of trees exposed to higher concentrations of PAHs in the air have higher concentrations in their leaves. Therefore, planting trees in cities can be a cost-effective strategy to improve air quality with respect to PAHs concentration. However, for the optimal implementation of this strategy it is necessary to deepen the knowledge about the mechanisms of PAH uptake by tree leaves and the variables that regulate it.

1.6. State of the art

The uptake process of PAHs in trees is very complex and consists of four components and their interactions: i) the concentration and form of PAHs in the atmosphere (gases or particles); ii) environmental factors (especially temperature, wind -which affects dispersion and

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deposition-, precipitation, etc.); iii) the trees; and, iv) organisms living on the leaf surfaces (e.g. PAH-degrading bacteria, Franzetti et al., 2020; Terzaghi et al., 2021).

Two main pathways exist for the transfer of PAHs from the air to the leaves: through their deposition on leaf surfaces or through their diffusion through the stomata. Regarding the first pathway, this may be reversible as the particles can be released due to dew, heavy rainfall or wind action. PAHs may remain on leaf surfaces adsorbed to particles or may be desorbed from particles and transferred to epicuticular waxes. Once deadsorbed, PAHs can remain on the waxes or penetrate into the cuticle and/or mesophyll (e.g. Terzaghi et al., 2013). While in particles or cuticle, PAHs can be revolatilised or photodegraded (Fellet et al., 2016). In addition, cuticles can incorporate particles into their tissue through a process known as encapsulation (Terzaghi et al., 2013). Regarding the second entry pathway, the stomata do not offer any resistance to the uptake of PAHs in the gas phase and will not prevent the entry of small particles (Franzaring, 1997).

The importance of these pathways is not the same for all PAHs. Those in the gas phase will diffuse primarily through stomata, although they also diffuse into the cuticle and from there into the mesophyll. The heavier, particle-bound ones will be deposited on the cuticle. This has been confirmed experimentally, as Wang et al. (2008) observed similarities in PAH profiles between leaf cuticle and airborne particles and between PAH profiles of internal tissues and those of the gas phase.

The PAH transfer process, determined by the entry pathways, will be modulated by the physical and chemical characteristics of the leaf surfaces and the physiology and morphology (macroscopic and ultrastructure) of the leaves. Thus, Table 1 presents a review of the works studying the relationship between ecological traits of leaves and PAH concentrations, as well as the arguments for these concentrations. These works generally compare several species, in which both leaf PAHs and some of the ecological traits are quantified. In summary, the ecological traits of leaves that have been studied in relation to PAHs load (Table 1SM) can be classified into 5 groups: i) general leaf morphology; ii) ultrastructure of the leaf surface; iii) gas exchange; iv) physical characteristics of the cuticle; and, v) chemical composition of the cuticle. In addition, the uptake of particles will be modified by the shape, surface and roughness of the leaves (including trichomes, glands, wax crystalloids). The transfer of gas PAHs is also influenced by these characteristics, which determine the sorption area of the leaf. However,

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another important factor will be the characteristics of the leaf cuticle (especially the epicuticular waxes), mainly its chemical composition (proportion of constituents responsible for hydrophobicity) and its physical structure (e.g. thickness). To this day, there are experimental discrepancies about the role of almost all ecological traits in the PAH loading process and the relative importance of these traits is far from being known. These discrepancies may be largely due to a problem of intra-individual variability that is overlooked in almost all existing work (questioning the representativeness of the samples). In addition, most of the studies included in this table address only a few ecological traits (1-3), so that only a very partial view is available to explain the differences between species in terms of PAH uptake.

As we have already mentioned above, leaf uptake capacity is determined by the ecological traits of the leaves and, therefore, the study of these traits is fundamental to obtain a clear idea of which tree species are the most appropriate to act as PAHs filters in the streets. However, it is not always necessary to have all the information on ecological traits to study the capacity of trees to remove PAHs. One of the most efficient ways is through variables such as plant-air partition coefficients (K_{PA}) which indicates the ratio, at equilibrium, between the concentration of a chemical in plant leaves and in air (Kömp & Mclachlan, 1997). This variable, together with other parameters such as specific leaf area (SLA) and leaf area index (LAI), allows us to apply dynamic environmental fate models, incorporating plants in the mass balance, to quantify the role of plants and specific plant species in removing PAH from air (Terzaghi et al., 2017). However, until now a few works provided equations to estimate K_{PA} for a handful of species (Bacci et al., 1990; Bolinius et al., 2016; Horstmann & McLachlan, 1998; Nizzetto et al., 2008) and for most species and chemicals, this information is not readily available.

1.7. Reasoned justification of the unity of the study.

The lack of information on different aspects of PAH uptake by tree leaves limits the applicability of trees as a PAH filter. Therefore, it is necessary to further study the factors that affect or may influence the uptake of PAHs. Specifically, in this thesis we focus on the relationship between PAHs concentration in leaves and leaf traits, as well as intraspecific (intra- and interindividual) and interspecific variability. All this is embodied in the general objective of increasing the understanding of PAH uptake by trees, which is developed in the three specific objectives shown in section 2.

Table 1: Ecological traits and mechanisms determining PAHs (polycyclic aromatic hydrocarbons) loading in leaves. Arrows indicate causality between ecological traits, factors modifying PAH concentration and effect on PAH concentration (in particles, P; in gaseous phase, G; or in cuticle from particles P→C). ΣPAHs: sum of PAHs; LMW PAHs: low molecular weight PAHs; MMW PAHs: medium molecular weight PAHs; HMW PAHs: high molecular weight PAHs; XR ΣHAPs: sum of PAHs from benzene X-ring compounds. The number in brackets refers to the papers addressing each ecological trait, the arguments for or against and the experimental evidence for them: (1) De Nicola et al. (2017); (2) Domingos et al. (2015) and Días et al. (2016); (3) Fellet et al. (2016); (4) Guo et al. (2021); (5) Howsam et al. (2000); (6) Howsam et al. (2001); (7) Jouraeva et al. (2002); (8) Jouraeva et al. (2006); (9) Li et al. (2010); (10) Li et al. (2017); (11) Liang et al. (2017); (12) Mętrak et al. (2016); (13) Müller et al. (2001); (14) Murakami et al. (2012); (15) Peck & Hornbuckle (2003); (16) Pleijel et al. (2022); (17) Prigioniero et al. (2022); (18) Prigioniero et al. (2023); (19) Ray et al. (2021); (20) Tao & Hornbuckle (2001); (21) Terzaghi et al. (2013); (22) Tian et al. (2008); (23) Tian et al. (2019); (24) Wang et al. (2008); (25) Yang et al. (2017); (26) Zhao et al. (2018).

Ecological Traits	
General leaf morphology	**↑<u>COMPOUND LEAVES</u> (2)** ↓**Boundary layer:** the deposition of PAHs in compound leaves with small leaflets and parallel arrangement increases the deposition; in addition, there is a decrease of dew which will reduce losses of PAHs **(P↑↑) (G↑) ΣPAHs.** Counter-arguments: increased wind and heavy precipitation effect on the leaf surface and thus increased PAHs loss **(P↓↓) (G↓) ΣPAHs**. ↑**Gas exchange:** in compound leaves with small, parallel arranged leaflets, due to the thinning of the boundary layer **(G↑) ΣPAHs** • **Empirical evidence supporting ↑ΣPAHs: (2)**
	↑<u>WIDTH/LENGTH RATIO</u> (cm cm^{-1}) (11 and 23) ↑**Boundary layer**: large, flat leaves have thicker boundary layers than narrow ones, which decreases deposition; in addition, there is increased dew, which will cause losses of the compounds **(P↓↓) (G↓) ΣPAHs.** Counter-arguments: decrease of wind and heavy precipitation effect on the leaf surface and therefore decrease of PAHs loss **(P↑↑) (G↑) ΣPAHs**. ↓**Gas exchange:** in large, flat leaves, due to the increase of the boundary layer **(G↓) ΣPAHs.** • **Empirical evidence against ↑ΣPAHs: (11, 23)** • **Empirical evidence supporting ↑LMW y ↑MMW PAHs: (23); against ↑HMW PAHs: (23)**
	↑<u>LEAF AREA</u> (cm^2) (2, 5, 6, 7, 8, 25 and 26) ↑**Surface:** increase in adsorption surface area regardless of mass **(P↑↑) (G↑) ΣPAHs.** • **Empirical evidence against ↑ΣPAHs: (2, 6 and 26)**
	↑<u>SPECIFIC LEAF AREA</u> (cm^2 g^{-1}) (1, 2, 5, 6, 7, 8, 13, 16, 19, 22, 23 and 24) ↑**Surface/weight:** increased adsorption surface area for the same leaf weight **(P↑↑) (G↑) ΣPAHs**. Counter-arguments: smaller relative leaf mass in relation to leaf area represents a reduction in the sink for LMW (16) **(G↓).**

	↓**Molecular attraction PAHs:** decrease in the thickness of the leaf and decrease in the concentration of waxes responsible for the sorption of the PAHs (23) **(G↓) (P→C↓) ΣPAHs.** • **Empirical evidence supporting ↑ΣPAHs: (22); against ↑ΣPAHs: (2, 23 and 24)** • **Empirical evidence supporting ↑LMW and ↑MMW PAHs: (23); supporting ↑MMW and ↑HMW PAHs: (6, 7 and 13); supporting >3R ΣPAHs: (1); against ↑LMW (6, 16); against ↑LMW and ↑MMW PAHs: (7); against ↑HMW PAHs: (23)**
	↑**<u>LEAF THICKNESS</u> (μ) (2)** ↓**Surface/Weight:** decrease in adsorption surface area for the same leaf weight **(P↓↓) (G↓) ΣPAHs.** ↑ **Proportion of intercellular spaces in the spongy parenchyma:** thinner leaves with relatively more air space in the spongy parenchyma (2) **(G↑) (P→C↑) ΣPAHs.** ↑ **Molecular attraction PAHs:** increased leaf thickness and increased wax concentration (23) **(G↑) (P→C↑) ΣPAHs** • **Empirical evidence supporting ↑ΣPAHs: (2)**
Ultrastructure of the leaf surface	↑ **<u>ROUGHNESS DUE TO THE SHAPE OF THE EPIDERMAL CELLS</u> (2, 6, 14, 21, 22 and 23)** ↑ **Surface/weight:** in leaves with rough cell surface structure (wavy, papillose, etc.) there is an increased contact surface in relation to their mass (14) **(P↑↑) (G↑) ΣPAHs.** ↑**Boundary layer:** in leaves with rough surface cell structure (wavy, papillose, etc.) the boundary layer is thicker and there is a decrease in deposition. **(P↓↓) (G↓) ΣPAHs**. Counter-arguments: decrease of wind and heavy precipitation effect on the leaf surface and therefore decrease of PAHs loss. **(P↑↑) (G↑) ΣPAHs**. ↑ **Physical retention of particles**: in leaves with rough surface cell structure (wavy, papery, etc.) by increasing their possibility of retention decreasing their probability of rebound and of being blown away by precipitation or wind **(P↑) ΣPAHs.** Counter-arguments: in leaves with rough surface cell structure (wavy, papery, etc.) by decreasing the retention of particles impacting on the surface (6) or by increasing the self-cleaning properties and decreasing the self-cleaning properties of particles on the surface **(P↓ ΣPAHs).** ↓**Gas exchange:** on leaves with a rough surface cell structure (wavy, papillose, etc.) due to the increase of the boundary layer. **(G↓) ΣPAHs.** • **Empirical evidence supporting ↑ΣPAHs: (14 and 22); against ↑ΣPAHs: (23)** • **Empirical evidence supporting ↑HMW PAHs: (23)**
	↑ **<u>PUBESCENCE (presence, distribution, morphology, and size of the trichomes)</u> (1, 2, 5, 6, 17, 18, 21 and 24)** ↑ **Surface/weight:** increased contact surface on pubescent leaves (5) **(P↑) (G↑) ΣPAHs.** ↑ **Boundary layer**: in pubescent leaves with decreasing deposition the boundary layer is thicker and there is a decrease in deposition (5). **(P↓↓) (G↓) ΣPAHs**. Counter-arguments: (i) decrease of wind and heavy precipitation effect on the leaf surface and therefore decrease of PAHs loss; (ii) furthermore the existence of a boundary layer of relatively stagnant air on the leaf surface improves particle retention (5) **(P↑↑) (G↑) ΣPAHs**. ↑ **Physical retention of particles:** in pubescent leaves, in addition to increasing particle retention, they increase the likelihood of particle retention by decreasing the likelihood of rebounding and being blown away by precipitation or wind (5, 17, 18). **(P↑) ΣPAHs** ↓ **Gas exchange**: in pubescent leaves due to the increase of the boundary layer. **(G↓) ΣPAHs** ↓ **Cuticle irradiation**: on pubescent leaves, which reduces photolysis **(P↑) (G↑) ΣPAHs.** • **Empirical evidence supporting ↑ΣPAHs: (2, 5, 19); against ↑ΣPAHs: (24)** • **Empirical evidence supporting ↑>3R ΣPAHs: (1); against ↑MMW PAHs: (17)**

	↑ **<u>ROUGHNESS OF EPICUTICULAR WAX MORPHOLOGY</u> (amorphous and crystalloid structures) (1, 2, 19, 21)** ↑ **Surface/weight**: increased adsorption surface area for the same weight of leaf, leading to increased deposition (1, 19) **(P↑↑) (G↑) ΣPAHs.** ↑ **Physical particulate matter retention**: increased particle retention (1, 19) **(P↑) ΣPAHs.** ↑ **Boundary layer:** in leaves with structures such as wax crystalloids, they increase their roughness and the boundary layer is thicker, so there is a decrease in deposition **(P↓↓) (G↓) ΣPAHs**. Counter-arguments: (i) decrease of wind and heavy precipitation effect on the leaf surface and therefore decrease of PAHs loss; (ii) furthermore the existence of a boundary layer of relatively stagnant air on the leaf surface enhances particle retention. **(P↑↑) (G↑) ΣPAHs**. ↓ **Gas exchange:** with structures such as wax crystalloids due to the increase of the boundary layer **(G↓) ΣPAHs.** • **Empirical evidence supporting ↑ΣPAHs: (2, 19)** • **Empirical evidence supporting ↑>3R ΣPAHs: (1)**
Gas Exchange	↑ **<u>STOMATIC DENSITY</u> (stomata /mm^2) (1, 3, 17, 18, 23, 24 and 25)** ↑ **Gas exchange:** as the number of stomata increase (3) **(G↑) LMW y MMW PAHs.** Counter-arguments: (i) stomatal density does not consider the morpho-physiological characteristics of stomata (such as their size, protective structure or location) or opening rate and therefore does not determine stomatal conductance (1, 25); and (ii) gas exchange may favour PAH volatilisation from the stomatal chamber or mesophyll (23). **(G↓) LMW and MMW PAHs**. ↓ **PAHs molecular attraction:** increased stomatal density is related to a decrease in wax concentration**.** (23) **(G↓) (P→C↓) ΣPAHs.** • **Empirical evidence supporting ↑ΣPAHs: (3); against ↑ΣPAHs: (1, 23, 24 and 25)** • **Empirical evidence supporting ↑LMW and ↑MMW PAHs: (18 and 23); supporting ↑MMW PAHs: (17)**
	↑ **<u>STOMATAL SURFACE AREA</u> (mm^2 /mm^2) (17, 18 and 20)** ↑ **Gas exchange:** as the surface area of stomata per leaf area increases **(G↑) LMW y MMW PAHs**. Counter-arguments: (i) stomatal surface area does not consider the opening rate and therefore does not determine stomatal conductance; and (ii) gas exchange may favour PAH volatilisation from the stomatal chamber or mesophyll **(G↓) LMW and MMW PAHs**. ↓ **PAHs molecular attraction:** increased stomatal density is related to a decrease in wax concentration **(G↓).** • **Empirical evidence supporting ↑HMW: (17)**
	↑ **<u>STOMATIC CONDUCTANCE</u> (mol CO_2 m^{-2} s^{-1}) (1, 15)** ↑ **Gas exchange**: measured through stomatal conductance (1) **(G↑) LMW PAHs.** • **Scientific evidence supporting ↑2R and 3R ΣPAHs: (1) against ↑LMW: (15)**
Physical characteristics of the cuticle	↑ **<u>CUTICLE THICKNESS</u> (µ) (3, 17, 18, 21 and 24)** ↓ **Surface/weight:** increase in leaf thickness leads to a decrease in adsorption surface area for the same leaf weight. **(P↓↓) (G↓) ΣPAHs** ↑ **PAHs molecular attraction**: increased sorption capacity of PAHs **(G↑) (P→C↑) ΣPAHs.** • **Scientific evidence against ↑ΣPAHs: (3)** • **Scientific evidence supporting ↑HMW: (17); supporting ↑LMW, MMW and HMW: (18); against ↑2-4R and 5-6R ΣPAHs: (24); against ↑LMW: (17)**

	↑ **SURFACE ENERGY (23)** ↑ **Molecular attraction PAHs**: increase in particle retention capacity **(P↑) ΣPAHs and ↑HMW PAHs.** • **Evidence supporting ↑ΣPAHs: (23)**
Chemical composition of the cuticle	↑ **LIPID CONCENTRATION (mg g^{-1}) (6, 7, 12, 14, 22 and 24)** ↑ **PAHs molecular attraction:** increased PAHs sorption capacity, especially by cutin modifying the partition coefficient **(G↑) (P→C↑) ΣPAHs.** • **Inconclusive evidence supporting ↑ΣPAHs: (7, 14, 22 and 24); against ↑ΣPAHs: (6, in some species)** • **Empirical evidence supporting ↑3R ΣPAHs: (6, for 1 species); ↑>4R ΣPAHs: (6, in some species)**
	↑ **CONCENTRATION OF ALIPHATIC CUTICLE COMPOUNDS (cutin and waxes) (9)** ↑ **PAHs molecular attraction**: increased PAHs sorption capacity, especially by cutin. **(G↑) (P→C↑) ΣPAHs** • **Empirical evidence in favour for ↑ΣPAHs: (9)**
	↑ **CONCENTRATION OF THE CUTICULAR/EPICUTICULAR WAXES (mg dm^{-2}, mg g^{-1}) (7, 8, 10, 19, 23 and 25)** ↑ **PAHs molecular attraction**: increased sorption capacity of PAHs, especially due to waxes modifying the partition coefficient **(G↑) (P→C↑) ΣPAHs.** Counter-arguments: increased hydrophobic compounds, so that a large part of the PAHs cannot migrate from the cuticle to the mesophyll and therefore increased loss of PAHs by photodegradation or volatilisation (25) increased adsorption of PAHs on the cuticle that do not migrate to the mesophyll (23) **(G↓) (P→C↓) ΣPAHs**. • **Evidence supporting ↑ΣPAHs: (23 and 19); against ↑ΣPAHs: (7, 10 and 25)** • **Evidence supporting ↑LMW PAHs: (8) and ↑HMW PAHs: (23); against ↑MMW ΣPAHs: (23)**
	↑ **CONCENTRATION OF HYDROPHOBIC WAX COMPOUNDS (2, 4 and 21)** ↑ **PAHs molecular attraction:** increased sorption capacity of PAHs, especially due to the more hydrophobic compounds of waxes (n-alkanes) waxes modifying the partition coefficient **(G↑) (P→C↑) ΣPAHs.** Contrary arguments: increase of hydrophobic compounds, so that a large part of the PAHs cannot migrate into the cuticle and therefore increase the loss of PAHs by photodegradation or volatilisation, so the existence of less hydrophobic compounds (alcohols, carboxylic acids, steroids and triterpenes) would facilitate the increase in the concentration of PAHs. (21) **(G↓) (P→C↓) ΣPAHs**. • **Evidence supporting ↑ΣPAHs: (4); against ↑ΣPAHs: (2)**
	↑ **POLAR COMPONENTS (2 and 23)** ↑ **Molecular attraction PAHs:** increased sorption capacity of PAHs **(G↑) (P→C↑) ΣPAHs** Contraargumentos: disminuye la atracción molecular de los PAHs al disminuir la hidrofobicidad de la cutícula **↓ΣPAHs** **Evidence against for ↑ΣPAHs: (23)**
	↑ **DISPERSION COMPONENTS (23)** ↓ **PAHs molecular attraction:** increased PAHs sorption capacity of cuticle waxes allowing PAHs to enter the cuticle and mesophyll. **(G↑) (P→C↑) ΣPAHs** **Evidence in favour of ↑ΣPAHs: (23)**

2. Hypotheses and objectives

The general objective of this thesis is to improve the knowledge on the uptake of PAHs by trees by testing three hypotheses:

1. There is a certain relationship between the concentration of PAHs in the leaves and the ecological traits of the leaves. Specifically, traits related to leaf morphology and traits related to gas exchange.
2. There is intra-individual (intracanopy) variability in PAH concentrations in leaves and this is largely determined by the variation in leaf traits and the position of the leaves in the canopy.
3. There are species that capture PAHs better than others (interspecific variability) and this capacity depends on whether the PAHs are in gas or particulate phase and on the interspecific variability of leaf traits.

Each of these hypotheses relates to one of the three specific objectives pursued in this thesis:

1. To examine how polycyclic aromatic hydrocarbons (PAH) uptake by woody species is affected by leaf ecological traits associated with leaf morphology (i.e. leaf area, specific leaf area [SLA], leaf thickness and leaf width/length ratio) and gas exchange (i.e. stomatal conductance per leaf area, leaf carbon isotope signature [$\delta^{13}C$] and stomatal density).
2. To determine i) the intra-individual variability in foliar PAH concentrations and ii) the relationship between this variability and leaf traits (SLA, stomata density, $\delta^{13}C$, cuticular waxes and hairiness) and leaf position (height, distance to the trunk and orientation).
3. To estimate plant-air partition coefficients (K_{PA}) for ornamental tree species usually employed in parks and streets around the world and compare their ability in removing gas and particle associated PAHs in urban areas. To describe the interspecific variability of leaf traits (SLA, length, stomatal density, stomatal conductance, $\delta^{13}C$ and relative fatty acid content) and their relationship with PAHs uptake.

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Each objective will be addressed in a separate study. Specific objectives 1 and 2 will be explored in the first and second studies, corresponding to Chapters I and II, respectively. Specific objective 3 will be addressed in the third study, which is divided into two parts: the first part is covered in Chapter III, and the second in Chapter IV.

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3. General Methodology

The general objective of this thesis was achieved by dividing the thesis into three different studies, each one of which referred to one of the specific objectives. Thus, in the first study (Chapter I) we performed a literature meta-analysis in an attempt to find patterns relating the concentration of PAHs in leaves to different leaf traits. To this end, we selected studies that compared the concentration of PAHs in leaves of two or more species under the same environmental conditions. This means that both species were at the same sampling station, were collected at the same time, were subjected to the same sources of contamination, etc. To assess the relationship of PAH concentrations from these studies with leaf traits, we extracted trait data from the TRY Trait Database and correlated them with concentrations.

For the second study (Chapter II) we evaluated the intra-individual variability of PAHs concentration in ornamental apple trees in a main street of the city of A Coruña (a medium-sized city). We sampled 12 canopy points of 15 trees and determined the PAHs concentration at each of these points. In addition, we also measured several traits in each of the sampled points and evaluated the relationship between PAH concentrations and traits at the intra-individual level. Finally, in the third study, which we have divided into 2 parts (Chapter III and Chapter IV), we studied interspecific variability by comparing the PAHs uptake potential of different species frequently used for ornamental purposes. In the first part of the study, we sampled 13 species of evergreen conifers at the Campus of the University of Santiago de Compostela and, by estimating the equations of their plant-air partition coefficients (K_{PA}), we evaluated the capacity of each of them to remove PAHs from the air. In the second part of the study, we described the interspecific variability of leaf traits and evaluated their relationship with the variation of PAHs concentrations through the calculation of correlations between both sets of variables.

The order in which the studies are presented in this thesis does not coincide with the chronological order of their publications, but with the logical order for their exposition and general design. In other words, they have been ordered in such a way that they enhance the argumentative logic (coherence) of their objectives and contents.

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4. Results

4.1. Results: published or accepted works

Chapter I: Leaf ecological traits (morphology and gas exchange) and polycyclic aromatic hydrocarbons concentrations in shrubs and trees: A meta-analysis approach

Reference: Giráldez, P., Vázquez-Arias, A., de Nicola, F., Fernández, J. Á., & Aboal, J. R. (2025). Leaf ecological traits (morphology and gas exchange) and polycyclic aromatic hydrocarbons concentrations in shrubs and trees: A meta-analysis approach. *Environmental Pollution, 364*, 125337. https://doi.org/10.1016/J.ENVPOL.2024.125337

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Leaf ecological traits (morphology and gas exchange) and polycyclic aromatic hydrocarbons concentrations in shrubs and trees: A meta-analysis approach☆

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ABSTRACT

The leaves of trees and shrubs can capture atmospheric pollutants such as polycyclic aromatic hydrocarbons (PAHs), and the capacity of uptake depends on the leaf traits. Although numerous studies have measured PAH concentrations in leaves of woody plants and the variability in leaf traits, few have investigated the relationship between these factors. We conducted a literature review to summarize the available information on this topic and found that five types of leaf traits have been studied, with those associated with leaf morphology and gas exchange being the most common. However, the results of the studies are often contradictory. To address these discrepancies, we conducted a meta-analysis to examine how PAH uptake by woody species is affected by leaf ecological traits associated with morphology (leaf area, specific leaf area [SLA], leaf thickness and leaf width/length ratio [W/L]) and with gas exchange (stomatal conductance, leaf carbon isotopic signature [$\delta^{13}C$] and stomatal density). The meta-analysis included studies involving at least two different species with comparable PAH concentrations. Many of the studies did not examine the relationship between ecological traits and PAH concentration, and those that did often involve different traits. We therefore used the TRY Plant Trait Database data as the standard source of trait data. Relationships were analyzed by determining differences regarding PAHs and traits and calculating Spearman correlations and their significance. The leaf morphology traits were more closely correlated with PAH concentrations than the gas exchange traits. Thus, morphological traits such as SLA and leaf area can be considered significant predictors of PAH uptake, especially for particulate-associated PAHs. Gas exchange traits showed less consistent correlations, indicating the complexity of factors influencing PAH uptake in leaves. This study highlights the importance of considering multiple leaf traits in order to better understand and predict PAH uptake in woody plants.

1. Introduction

Trees can remove polycyclic aromatic hydrocarbons (PAHs) from the atmosphere, as there is general scientific consensus regarding the existence of atmosphere-plant (mainly leaves) partition coefficients and the capacity of plants to adsorb/absorb PAHs (see e.g. Kömp & Mclachlan, 1997; Giráldez et al., 2022). This is further demonstrated by hundreds of articles using chamaephyte and phanerophyte species in biomonitoring atmospheric PAHs. However, the PAH loading process is complex and is determined by the ecological characteristics of the leaf (i.e. leaf ecological traits). Leaf ecological traits are largely determined by the conditions and resources available in their environment and show adaptive responses (see e.g. Reich et al., 1999; Wright et al., 2004). Nevertheless, the way in which these traits interact with atmospheric PAHs is still the subject of scientific debate (less than two dozen studies, all conducted in the present century, most in the last ten years). Moreover, given the huge differences in the molecular size of the different PAHs and their dispersion forms (gaseous vs. particulate), the

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interactions are expected to be different for each.

The leaf ecological traits studied in relation to PAH concentrations can be divided into five categories: i) general leaf morphology; ii) leaf gas exchange; iii) ultrastructure of the leaf surface; iv) physical characteristics of cuticle; and v) chemical composition of the cuticle. Various studies have considered leaf ecological traits included in the general leaf morphology category, such as the type of leaf (simple or compound) and its orientation (Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016); width/length ratio (Tian et al., 2019); leaf area (Howsam et al., 2000; Jouraeva et al., 2002, 2006; Domingos et al., 2015; Dias et al., 2016; Yang et al., 2017; Zhao et al., 2018); specific leaf area (Howsam et al., 2001; Müller et al., 2001; Jouraeva et al., 2002, 2006; Tian et al., 2008, 2019; Wang et al., 2008; Domingos et al., 2015; Dias et al., 2016; De Nicola et al., 2017) and leaf thickness (Domingos et al., 2015; Dias et al., 2016). Studies involving traits related to gaseous exchange include stomatal density (Wang et al., 2008; Fellet et al., 2016; De Nicola et al., 2017; Yang et al., 2017; Tian et al., 2019; Prigioniero et al., 2022, 2023), stomatal surface area (Tao & Hornbuckle, 2001; Prigioniero et al., 2022, 2023) and stomatal conductance (De Nicola et al., 2017). Studies on the ultrastructure of the leaf surface have considered the epidermal cell shape and roughness (Howsam et al., 2001; Tian et al., 2008, 2019; Murakami et al., 2012; Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016); leaf hairiness (pubescence: presence, distribution, morphology and size) (Howsam et al., 2000, 2001; Wang et al., 2008; Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016; De Nicola et al., 2005, 2017; Prigioniero et al., 2022, 2023), and roughness and morphology of the epicuticular waxes (amorphic and crystalloid structures) (Terzaghi et al., 2013; Domingos et al., 2015; Dias et al., 2016; De Nicola et al., 2017). Studies concerning the physical characteristics of cuticles have considered the thickness (Wang et al., 2008; Terzaghi et al., 2013; Fellet et al., 2016; Prigioniero et al., 2022, 2023) and the surface free energy (Tian et al., 2019). Regarding the chemical composition, different studies have examined the lipid concentration (Howsam et al., 2001; Jouraeva et al., 2002; Tian et al., 2008; Wang et al., 2008; Murakami et al., 2012; Mętrak et al., 2016; De Nicola et al., 2011), the concentration of cuticle aliphatic compounds (cutin and waxes) (Li et al., 2010), the esterification index (Prigioniero et al., 2022, 2023), the cuticular/epicuticular wax concentration (Jouraeva et al., 2002, 2006; Yang et al., 2017; Tian et al., 2019), the concentration of hydrophobic compounds in the wax (Terzaghi et al., 2013; Domingos et al., 2015) and the polar and dispersion components (Tian et al., 2019).

Two conclusions can be drawn after examining all the previously cited studies: i) the findings are sometimes contradictory and vary according to the compounds; and ii) the leaf ecological traits most commonly studied are those associated with leaf morphology, although some attention has also been given to leaf traits associated with gas exchange. In addition, some of the studies analyze the concentrations of PAHs in leaves from trees or shrubs of more than one species occurring in the same sample site, which could enable comparison of the findings. However, the leaf ecological traits of the species involved have not been documented or studied. Nevertheless, combining the data from these articles with the existing information in the databases on leaf ecological traits (e.g. the TRY Plant Trait Database) enabled us to conduct a meta-analysis focusing on the current questions about the role of leaf ecological traits in the PAH load in leaves of woody species.

Therefore, in the present study, after compiling the PAH concentrations determined in species coexisting at the same sites, we performed a meta-analysis to examine how PAH uptake by woody species is affected by leaf ecological traits associated with leaf morphology (i.e. leaf area, specific leaf area, leaf thickness and leaf width/length ratio) and gas exchange (i.e. stomatal conductance per leaf area, leaf carbon isotope signature [$\delta^{13}C$] and stomatal density).

2. Material and Methods

The bibliographical review was conducted at the end of 2022 using the Scopus document search tool with the search terms "pah AND leaf". A total of 524 documents were initially selected. Two successive screenings were then carried out. In the first, articles written in English and containing useful information on the concentrations of one or more PAHs in phanerophyte leaves were selected, and those concerning herbaceous plants, those written in other languages and those not reporting numerical values of concentrations were disregarded. In the second screening, articles involving at least two different plant species and comparable PAH concentrations were selected as follows: i) tree/shrubs in the same sampling site or close to each other; ii) trees/shrubs affected by the same sources of contamination; iii) samples collected in the same period; and iv) leaves of the same age (to ensure the same length of exposure to the sources of contamination). From these articles, we compiled a dataset with the average concentrations provided for each of the 16 US EPA PAHs (see Table A.1). In cases where the exact concentration was not reported in the article but was represented by figures, the concentrations were estimated using the WebPlotDigitizer tool (https://apps.automeris.io/wpd/). In addition to the 16 individual PAHs (see Table A.1), we also calculated the total sum of PAHs, the sum of PAHs according to their number of rings (2–6) and the sum according to the molecular weight: low (LMW, PAHs with 2–3 rings), medium (MMW, PAHs with 4 rings) and high (HMW, PAHs with 5–6 rings). Different ways have been used to group PAHs according to their molecular weight (e.g. Amodu et al., 2013; De Nicola et al., 2017; Mukhopadhyay et al., 2020; Pleijel et al., 2022; Singh et al., 2023), but the approach used here is one of the most common (e.g. Zhang et al., 2020; Ray et al., 2021; Yang et al., 2022). The sums were only computed when values were available for each of the individual PAHs.

Many of the selected studies either did not study the relationship between leaf traits and PAH concentrations or did not consider the same traits. We therefore decided to use the traits included in the TRY Plant Trait Database (Kattge et al., 2020) as a standard source of data for the species in our database. Although this database includes an enormous number of traits, many of these have no known relationship with PAHs concentration and in other cases there was not enough information for the species studied. Most of the traits included in the databases were related to leaf morphology and gas exchange, which as highlighted above are those that have received most attention in the existing literature. Finally, we considered 7 traits (Table 1), 6 from the TRY Plant Trait Database and 1 (ratio leaf width/length) calculated from another two. In cases where there were different measurements for the same trait and species, we used the median as the statistical reference value.

For each article examined, we identified the comparable samples (samples collected at the same place and time) and calculated the differences between the PAH concentrations in each pair of species and the differences between their traits. By comparing trees sampled at the same place and time, we ensure that they are subjected to the same environmental variables and therefore diminish the differential effect they might have on PAH uptake. Still, if our aim was to predict or model the

Table 1
Traits selected for analysis.

Type of trait	Name of trait	Unit of measurement
General leaf morphology	Leaf area (in case of compound leaves: leaf, petiole excluded)	mm^2
	Leaf area per leaf dry mass (specific leaf area, SLA or 1/LMA): petiole excluded	$mm^2\ mg^{-1}$
	Leaf thickness	mm
	Ratio leaf width/length	–
Gas exchange	Stomatal conductance per leaf area	$mmol\ m^{-2}\ s^{-1}$
	Leaf carbon isotope signature (delta 13C)	‰
	Stomatal density	n^o stomata mm^2

uptake of PAHs by leaves, we could not ignore environmental conditions, but when studying the relationship between PAH concentrations and trait values considering differences between comparable data this is not necessary. Once we obtained the differences between PAHs and between traits, we used them to calculate Spearman correlations between them and determined the significance levels. We only considered relevant those correlations that were significant ($p < 0.05$) and had an absolute value greater than 0.20. In addition, as each correlation corresponded to a different number of observations, we also computed the number of data pairs for each. All statistical analyses were performed with RStudio version 4.1.1 (RStudio Team, 2021).

3. Results

We selected 24 articles (including 67 species and 162 sampling sites) in order to construct the dataset. We then calculated the differences by subtracting the corresponding value (PAH concentration or value of the trait) of species 2 from species 1, for a total of 480 comparisons (Table B.1).

Based on these comparisons, we calculated the correlations (Table 2) and the number of pairs available for each (Table 3). We found significant correlations for all traits, with absolute correlation values varying between 0.17 and 0.80. However, most of the absolute values are below 0.50 and the higher values generally correspond to a small number of available pairs of observations (see Table 3). The available comparisons vary widely, ranging from 17 to 168 pairs (Table 3).

Fig. 1 shows an example of the Spearman correlations. The graph shows the rankings of the differences obtained for the sum of 6 rings PAHs and leaf thickness, the line representing the perfect correlation (−1) and, in color, whether the differences of the observations for the PAH and for trait vary in the same direction (green) or not (red), i.e. whether the sign of the difference is the same or different. Ties in rankings were resolved by using the median value (midrank), as this is the same resolution as the Spearman correlation implemented in R.

4. Discussion

4.1. Leaf morphology

Leaf morphology determines some purely physical characteristics of leaves in relation to PAH uptake and accumulation. In general, the variables under study affect the area available for adsorption of PAHs, either exclusively (i.e. leaf area) by considering the mass (i.e. SLA and leaf thickness) or through the modification of the boundary layer thickness (i.e. leaf width/length ratio).

4.1.1. Leaf area

Some authors have studied the effect of leaf area (cm^2) on foliar concentrations of PAHs (Domingos et al., 2015; Dias et al., 2016; Howsam et al., 2000; Jouraeva et al., 2002; Jouraeva et al., 2006; Yang et al., 2017; Zhao et al., 2018). In this case, the increase in concentrations is unequivocally explained by the increase in adsorption surface area regardless of mass, so that it affects both PAHs emitted in the gas phase and particularly those in the particulate phase. Our results show that concentrations of 4-, 5- and 6-ring compounds (with the only exceptions of BbF, BaP and Ind) are positively and significantly correlated with leaf area, as well as with MMW, Σ6-rings PAHs and ΣPAHs. These results are based on a large number of observations (*n* ranging between 45 and 69, except for DbA – n = 29; Table 3), and they can therefore be considered robust. Thus, leaf area affected those PAHs that are mainly deposited together with particles (log $K_{OA} > 10$; Giráldez et al., 2022), confirming the initial hypothesis that the concentration of particle-bound PAHs deposited on leaf surfaces is related to leaf area. The results obtained contrast with some previous findings, as several authors did not find any relationship between this variable and ΣPAHs (which also includes gas-phase PAHs) for *Croton floribundus*, *Piptadenia gonoacantha* and *Astronium graveolens* (Domingos et al., 2015; Dias et al., 2016), *Tilia x euchlora* and *Pyrus calleryana* (Jouraeva et al., 2002) and *Salix matsudana* (Zhao et al., 2018).

4.1.2. Specific leaf area (SLA)

SLA ($cm^2\ g^{-1}$) was the leaf trait most frequently studied in relation to PAH concentrations (De Nicola et al., 2017; Domingos et al., 2015; Dias et al., 2016; Howsam et al., 2001; Jouraeva et al., 2002; Jouraeva et al., 2006; Müller et al., 2001; Tian et al., 2008; Tian et al., 2019; Wang et al., 2008). The initial hypothesis has a physical justification: as the surface area available for adsorption increases relative to the leaf weight (which implies a larger surface available for adsorption for the same leaf weight, Simonich & Hites, 1994), the concentration of PAHs will also increase, as the concentration includes the weight in the denominator. This will

Table 2
Spearman correlations, significant at p-value ≤0.05.

PAH	Leaf area	SLA	Leaf thickness	Ratio leaf width/length	Stomatal conductance	$\delta^{13}C$	Stomatal density
Naph				−0.29		0.26	
Acy		0.29					
Ace			−0.21				
Flu							
Phe			0.33	−0.32		0.30	−0.60
Ant			0.24				
Fla	0.40	0.17					−0.77
Pyr	0.37	0.25		0.22			−0.61
BaA	0.49	0.36	−0.29	0.48	0.25		
Chry	0.43	0.41	−0.31	0.31	0.24		−0.71
BbF		0.27	−0.24	0.43			
BkF	0.31	0.33	−0.33	0.30	0.36		
BaP		0.22			0.18		−0.53
Ind		0.22					
DbA	0.63	0.44		0.29			
BghiP	0.43	0.34	−0.20	0.47	0.21	−0.37	
∑LWM					0.29		
∑MMW	0.33	0.23	−0.16	0.28			−0.80
∑HWM		0.28	−0.23	0.42			
∑2rings				−0.29		0.26	
∑3rings							
∑4rings	0.33	0.23	−0.16	0.28			−0.80
∑5rings		0.21		0.30			
∑6rings	0.32	0.40	−0.26	0.28	0.21	−0.27	
∑PAHS	0.35	0.28		0.36		−0.27	

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Table 3
Number of pairs of available values for each significant correlation.

PAH	Leaf area	SLA	Leaf thickness	Ratio leaf width/length	Stomatal conductance	$\delta^{13}C$	Stomatal density
Naph				74		58	
Acy		63					
Ace			122				
Flu							
Phe			168	123		107	17
Ant			162				
Fla	69	139					18
Pyr	69	138		124			18
BaA	66	131	160	109	133		
Chry	67	135	165	121	137		17
BbF		114	138	100			
BkF	58	126	156	112	128		
BaP		135			137		17
Ind		90					
DbA	29	67		71			
BghiP	45	86	137	77	91	61	
∑LWM					81		
∑MMW	67	132	161	117			17
∑HWM		94	138	80			
∑2rings				74		58	
∑3rings							
∑4rings	67	132	161	117			17
∑5rings		94		80			
∑6rings	69	117	167	103	119	87	
∑PAHS	45	112		91		75	

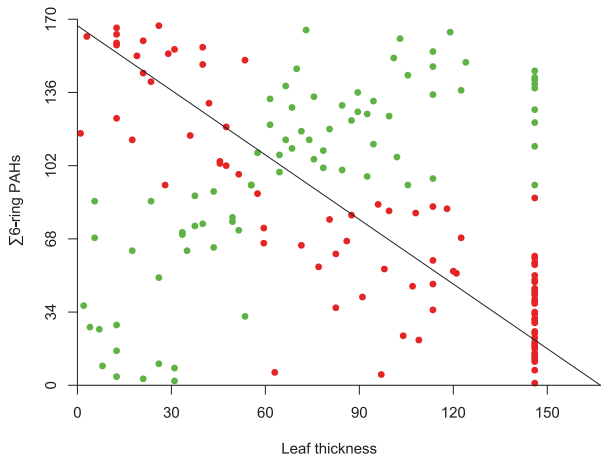


Fig. 1. Plot showing the rankings of the differences obtained for each variable. Each point represents a comparison of two comparable observations. The differences between the sums of 6-ring PAHs and between the leaf thickness of both observations were calculated and the rank of each of these differences was computed for each variable. The green dots represent cases in which the difference in both variables has the same sign (both are positive or negative), while the red dots represent cases in which the differences have opposite signs. The line represents a perfect negative correlation (−1). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

particularly affect the PAHs associated with particles, with a log K_{OA} > 10 (Jouraeva et al., 2002). However, a second hypothesis considers that the increase in SLA will imply a lower molecular attraction capacity of PAHs, as the decrease in leaf thickness may be associated with a reduction in the thickness of the leaf wax layer, which could lead to a decrease in the concentration of waxes responsible for the sorption of PAHs (Tian et al., 2019). In this case, higher SLA values will imply lower PAH concentrations.

The results obtained in the present study show positive and significant correlations for all MMW and HMW PAHs and also ΣMMW, ΣHMW, Σ4-rings, Σ5-rings and Σ6-rings PAHs, confirming the first hypothesis (Table 2). These results are based on a large number of observations (*n* ranging between 63 and 139, Table 3), and they can therefore be considered solid. Other authors have reached the same conclusion for these PAHs. For instance, Jouraeva et al. (2002) detected the same pattern for half of the MMW and all HMW PAHs studied in *Tilia x euchlora* and *Pyrus calleryana* leaves, all with log K_{OA} > 10, except for phenanthrene (log K_{OA} = 9.20 according to Jouraeva's calculations). De Nicola et al. (2017) observed the same effect in *Pinus pinaster vs. Quercus robur* leaves for PAHs with more than 3 rings, except for Ace, and Müller et al. (2001) reached the same conclusion comparing *Melaleuca* sp. with grasses leaves for B(a)P, Per, Ind and B(ghi)P.

Nevertheless, many other authors only detected positive relationships between SLA and some of the MMW or HMW PAHs, thus only partly confirming the first hypothesis. For example, Howsam et al. (2001) reported correlations between the SLA and the concentrations of 5- and 6-rings PAHs in *Quercus robur* and *Quercus petrea* leaves and 4- and 5-ring PAHs in *Fraxinus excelsior* leaves; and Tian et al. (2019) reported significant positive correlations in a group of 8 species for MMW. By contrast (and by way of exception), significant negative correlations between the SLA and HMW PAHs have also been reported, by Tian et al. (2019), who based the second hypothesis on their findings. Furthermore, an absence of correlation has been reported by Domingos et al. (2015) and Dias et al. (2016) for some of the species indicated above (lending more weight to other leaf traits such as epicuticular wax composition and leaf surface structure) or in Wang et al. (2008) for *Gingkgo biloba*, *Prunus persica*, *Sophora japonica*, *Prunus cerasifera* and *Syringa pekinensis*. The first hypothesis was also confirmed in the present meta-analysis by the absence of a relationship between the concentrations of LMW PAHs other than Acy and SLA, which is consistent with the results of Howsam et al. (2001) and Jouraeva et al. (2002) and is only contradicted (again exceptionally) by Tian et al. (2019), who found that LMW PAHs were affected by SLA.

Regarding the ΣPAHs, the relationship was also significant and positive, as previously reported by Tian et al. (2008) for *Pinus massoniana, Cunninghamia lanceolata, Eucalyptus citriodora, Acacia auriculaeformis, Acacia mangium* and *Schima superba*. By contrast, a negative correlation between ΣPAHs and SLA (Tian et al., 2019) and also no correlation (Domingos et al., 2015; Dias et al., 2016; Wang et al., 2008) have also been reported.

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4.1.3. Leaf thickness

Leaf thickness (mm) is closely related to SLA, which may explain why the relation to PAH concentrations has scarcely been studied (Domingos et al., 2015; Dias et al., 2016; in the species above indicated). Assuming a constant surface area, an increase in leaf thickness implies a decrease in SLA, leading to a decrease in the adsorption surface for the same leaf weight. This should lead to a reduction in particle-bound PAHs concentrations, as in the previous case. However, the increase in thickness could also be related to an increase in the concentration of waxes responsible for the sorption of PAHs (Tian et al., 2019) or to an increase in the proportion of intercellular spaces in the spongy parenchyma. Thus, the increase in leaf thickness could lead to an increase in PAH concentrations, both those deposited in the particulate phase and particularly those associated with the gas phase. The results obtained, although not as consistent as for SLA, suggest a similar pattern. For the individual 4-, 5- and 6-ring PAHs (with some exceptions that showed no significance, i.e. Fla, Pyr, BaP, Ind and DbA), and the ΣMMW, ΣHMW, Σ4-rings and Σ6-rings, significant, inverse correlations were obtained. Regarding the 3-ring PAHs, Phe and Ant were significantly, positively correlated with leaf thickness. These results are based on a large number of observations (n ranged between 122 and 168, Table 3), and they can therefore be considered solid. Thus, it appears that as leaf thickness increases (and therefore the SLA decreases), the concentrations of particle-bound PAHs with 4 or more rings will decrease. At the same time, the concentration of some of the 3-ring PAHs (gaseous phase) increases, either due to higher wax concentration, or by larger proportion of intercellular spaces in the parenchyma. The result for the sum of PAHs was not significant. In general, these results are consistent with previous findings, as Domingos et al. (2015) and Dias et al. (2016) reported empirical evidence supporting that the decrease in thickness produces an increase in the total concentration of PAHs.

4.1.4. Ratio leaf width/length

The leaf width/length ratio (cm cm ^{1}) has generally been neglected (Tian et al., 2019). These authors hypothesized that as the leaf width/length ratio (W/L) increases, the capacity to intercept dew drops also increases, which would wash the surface of the leaves and lead to low accumulation of PAHs. On the other hand, the thickness of the boundary layer can also vary; as the W/L ratio decreases, the thickness of the boundary layer increases, thus decreasing the gas exchange processes and decreasing the concentration of PAHs in the gas phase. The results obtained in the present meta-analysis show that light PAHs (2 and 3 rings) related to the gas phase, such as Naph and Phe, are significantly and negatively correlated with W/L. However, for the heavier PAHs of 4, 5 and 6 rings (Table 2), the respective correlation is positive. These results are based on a large number of observations (*n* ranged between 71 and 124, Table 3), and they can therefore be considered robust. Thus, the results obtained are consistent with a change in the boundary layer thickness. These findings are partly in agreement with those reported by Tian et al. (2019), who found that leaf W/L ratio was positively correlated with LMW and MMW, but not with ΣPAHs or HMW.

4.2. Leaf gas exchange

Gas exchange in plants is physiologically regulated by control of stomatal closure. Greater gas exchange can increase the intake of gas-phase PAHs into the stomatal cavity, potentially increasing the transfer of the compounds into the parenchyma. As access of particles through stomata is thought to be negligible, the selected variables should not be related to the concentrations of particle-bound PAHs. Conversely, gaseous exchange may favor volatilization of PAHs from the stomatal chamber or mesophyll (Tian et al., 2019). Therefore, all the variables studied are proxies which attempt to describe the magnitude of the leaf gas exchanges. Using leaf traits as proxies for gas exchange has the advantage that the traits are less variable over time and methodologically easier to determine than direct gas exchange; however, they can also introduce noise into the data.

4.2.1. Stomatal conductance (per leaf area)

The relationship between stomatal conductance and PAHs has only been addressed once in the published literature (De Nicola et al., 2017; species specified above). The lack of studies is probably due to the methodological difficulty of measurement, even though this relationship best describes the gas exchange defined as the transfer of water vapor or CO_2 through the stomata. According to the above authors, as the stomatal conductance increases (per leaf area, mol CO_2 m^{-2} s^{-1}), gas exchange and the concentration of PAHs linked to the gas-phase bioconcentration also increase. Significant correlations were obtained in a very small number of cases, in which they were always positive, e.g. for some MMW and HMW PAHs (i.e. BaA, Chry, BkF, BaP BghiP, and Σ6-rings PAHs). These correlations are based on a high *n* (80–137, Table 3). This result does not support the previously stated hypothesis, in which higher gas exchange would favor the uptake of gas phase PAHs, and there is no apparent causal relation between stomatal conductance and particle-bound PAHs concentration. This contrasts with previous findings, as De Nicola et al. (2017) demonstrated that stomatal conductance was related to an increase in Σ2-rings and Σ3-rings PAHs, in accordance with the proposed hypothesis.

4.2.2. Leaf carbon isotope signature ($\delta^{13}C$)

This variable has not previously been used in comparative studies about the relationship between gas exchange and PAH concentration. However, this isotopic ratio has been shown to be a suitable proxy for gas exchange. The greater affinity of the photosynthetic enzyme RuBisCo for $^{12}CO_2$ than for $^{13}CO_2$ and the greater diffusion of the former isotope through the stomata leads to differences in the $^{13}C/^{12}C$ ratio in plants and in the atmosphere. This variation is captured by the $\delta^{13}C$ (Farquhar et al., 1989). However, under lower stomatal conductance (greater stomatal closure) depletion of $^{12}CO_2$ leads to the use $^{13}CO_2$, yielding higher $\delta^{13}C$ values. Thus, as $\delta^{13}C$ is negatively correlated with gas exchange, we would expect it to be negatively correlated with the concentration of gas-associated PAHs. Nevertheless, only positive correlations were observed for Naph, Phe and Σ2-ring PAHs, and negative correlations were observed for BghiP, ΣPAHs and Σ6-ring PAHs. These correlations are based on a high *n* (58–107, Table 3). These results are not consistent with the main hypothesis claiming that higher gas exchange would favor the uptake of gas phase PAHs. Only two LMW PAHs were positively correlated with $\delta^{13}C$, partly supporting the hypothesis by Tian et al. (2019) that PAH volatilization from the stomatal chamber or mesophyll increases with gas exchange. There is no hypothesis to support the result for 6-ring PAHs and sums of PAHs. Although light PAHs have some weighting within the ΣPAHs, we do not believe that this is sufficient to explain the observed correlation between $\delta^{13}C$ and the ΣPAHs.

4.2.3. Stomatal density

Given the ease of measurement, the relationship between stomatal density (stomata/mm^2), as a proxy for gas exchange, and PAH concentrations is the most commonly studied (De Nicola et al., 2017; Fellet et al., 2016; Prigioniero et al., 2022; Prigioniero et al., 2023; Tian et al., 2019; Wang et al., 2008; Yang et al., 2017). According to these authors, as the stomata density increases, gas exchange and the PAHs associated with the gas-phase also increase. The absence of any relationship between the previous gas exchange proxies and the concentration of light PAHs established a low expectation for their correlation with stomatal density, but apart from gas exchange, the increase in this parameter is related to a decrease in wax concentration (Tian et al., 2019). Furthermore, its use as a proxy for gas exchange is not straightforward as stomatal density does not consider the morpho-physiological characteristics of stomata (such as their size, protective structure -as could be trichomes- or location) or the rate of opening, and therefore it

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does not determine stomatal conductance (De Nicola et al., 2017; Yang et al., 2017). The number of significant correlations was small and always negative. Significant correlations were found for half of the 4-ring PAHs (i.e. Fla, Pyr and Chry) and their sum, as well as the sum of the MMW and exceptionally for PAHs with other number of rings (Phe and BaP). These correlations are based on a low *n* (17–18, Table 3), which casts doubt on the reliability of the findings. In the previous literature, as in this meta-analysis, no relationship between stomatal density and ΣPAHs concentration was usually found (De Nicola et al., 2017, Tian et al., 2019; Wang et al., 2008; Yang et al., 2017; all species studied cited above, except in the last, *Cinnamomum camphora*), except by Fellet et al. (2016) (who studied leaves of *Elaeagnus x ebbingei, Ilex aquifolium, Laurus nobilis, Ligustrum japonimcum, Photinia x fraserii and Viburnum lucidum*). However, in contrast to our meta-analysis, Tian et al. (2019) observed a relationship between stomatal density and LMW and MMW PAH concentrations, supporting the starting hypothesis in this section. A study by Prigioniero et al. (2022) with *Chamaerops humilis*, *Citrus* × *aurantium*, *Magnolia grandiflora*, and *Quercus ilex* differentiated the upper and lower leaf surfaces, from which they also selectively removed waxes. In the study, the upper leaf surface (with waxes) was significantly and positively correlated with LMM. In addition, the lower leaf surface (with waxes and without them) was significantly and positively correlated with HMM and negatively with LMM (with waxes). Both this and the present meta-analysis coincide in finding a relationship between PAHs associated with particulate matter and stomatal density, although no hypothesis for the relationship was proposed. Prigioniero et al. (2023) obtained similar results in a study including 28 species, detecting positive correlations between stomatal density on the upper leaf surface and the concentration of MMM and LMM PAH in dewaxed leaves.

4.3. Study limitations and conclusions

This paper provides a comprehensive review of the current knowledge on the relationship between leaf traits and leaf PAH concentrations. It also provides further information obtained through a meta-analysis. However, without detracting from its value, we must be aware of the limitations of the study, many of which stem from the nature of the data available, particularly the trait data. Unlike the data on PAH concentrations, the trait data do not correspond to the trees studied but are median values of the data available for each species in the TRY Plant Trait Database. This obviates the natural variability in this type of data and leads to some pseudoreplication, which introduces many ties in the Spearman correlations, thus affecting the exact p-values. Another problem arises when assessing the importance of each species in the comparisons, as some species are included much more often, and some studies carry out many more comparisons than others.

It is interesting to note that PAH uptake by tree leaves is influenced by environmental conditions. Thus, although we minimize its effect in the analyses by making comparisons between "comparable" individuals (samples collected by the same authors in the same place, time period, with leaves of the same age and exposed to the same sources of contamination), in some cases interactions may occur. That is, it could happen that in one climate (or set of environmental conditions) species 1 captures more than species 2 and in another it is the other way around. However, such a scenario would only have the effect of increasing noise when calculating correlations. This would make more difficult to find significant correlations but would not invalidate the ones we have found. The same would be true for all factors that could affect the concentrations measured in each of the studies. Although the conditions are heterogeneous between studies, they are standardized in each of the comparisons. It is important to emphasize that our goal is not to accurately model the process by which PAHs are uptaken by tree leaves (this would require the inclusion of numerous environmental variables), but to examine the relationship between the concentration of PAHs in leaves with their ecological traits. Therefore, the non-assessment of environmental conditions cannot be considered a limitation of the study.

The results obtained for the traits related to leaf morphology are much more robust and are more consistent with the main hypotheses than those related to gas exchange. This may be due to several factors, including two main candidates. First, the morphological traits are more consistent possibly because they are less sensitive than gas exchange traits to intraindividual and intraspecific variability caused by the environmental conditions. Second, the morphological traits are measured directly, while gas exchange is estimated by proxy measures (gas exchange traits). Although there is a large variability in both intraindividual and interindividual traits (e.g. Giráldez et al., 2024; Herrera, 2009; Vinod et al., 2023), gas exchange is generally much more variable than morphological traits, and the effect of disregarding this variability in the analysis may therefore be much more pronounced. Although we can deduce from the results that gas exchange is not of great importance in relation to PAH uptake, it seems logical to think that it will probably contribute to the load of light, gas-phase PAHs. However, it is possible that these traits alone are not as decisive and are affected by other traits, as we focused on individual traits and many of these uptake processes may be multifactorial.

Despite these limitations, this study provides a complete view of the current knowledge on the relationship between leaf traits and leaf PAH concentrations. Moreover, it reinforces existing hypotheses on the relationship between leaf morphological traits and the different types of PAHs, while also challenging some of the hypotheses relating PAHs and gas exchange. Future studies on the effect of gas exchange should apply more careful and detailed approaches and consider the multifactorial aspects of PAH uptake.

CRediT authorship contribution statement

Pablo Giráldez: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Antón Vázquez-Arias:** Writing – review & editing, Investigation, Data curation. **Flavia De Nicola:** Writing – review & editing, Investigation. **J. Ángel Fernández:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Jesús R. Aboal:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125337.

Data availability

Data will be made available on request.

References

Amodu, O.S., Ojumu, T.V., Ntwampe, S.K.O., Amodu, O.S., Ojumu, T.V., Ntwampe, S.K. O., 2013. Bioavailability of high molecular weight polycyclic aromatic hydrocarbons

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using renewable resources. In: Petre, M. (Ed.), Environmental Biotechnology - New Approaches and Prospective Applications. IntechOpen. https://doi.org/10.5772/54727.

De Nicola, F., Concha Graña, E., López Mahía, P., Muniategui Lorenzo, S., Prada Rodríguez, D., Retuerto, R., Carballeira, A., Aboal, J.R., Fernández, J.Á., 2017. Evergreen or deciduous trees for capturing PAHs from ambient air? A case study. Environmental Pollution 221, 276–284. https://doi.org/10.1016/J.ENVPOL.2016.11.074.

De Nicola, F., Lancellotti, C., Prati, M.V., Maisto, G., Alfani, A., 2011. Biomonitoring of PAHs by using Quercus ilex leaves: source diagnostic and toxicity assessment. Atmos. Environ. 45 (7), 1428–1433. https://doi.org/10.1016/J.ATMOSENV.2010.12.022.

De Nicola, F., Maisto, G., Prati, M.V., Alfani, A., 2005. Temporal variations in PAH concentrations in Quercus ilex L. (holm oak) leaves in an urban area. Chemosphere 61 (3), 432–440. https://doi.org/10.1016/J.CHEMOSPHERE.2005.01.082.

Dias, A.P.L., Rinaldi, M.C.S., Domingos, M., 2016. Foliar accumulation of polycyclic aromatic hydrocarbons in native tree species from the Atlantic Forest (SE-Brazil). Sci. Total Environ. 544, 175–184. https://doi.org/10.1016/J.SCITOTENV.2015.11.092.

Domingos, M., Bulbovas, P., Camargo, C.Z.S., Aguiar-Silva, C., Brandão, S.E., Dafré-Martinelli, M., Dias, A.P.L., Engela, M.R.G.S., Gagliano, J., Moura, B.B., Alves, E.S., Rinaldi, M.C.S., Gomes, E.P.C., Furlan, C.M., Figueiredo, A.M.G., 2015. Searching for native tree species and respective potential biomarkers for future assessment of pollution effects on the highly diverse Atlantic Forest in SE-Brazil. Environmental Pollution 202, 85–95. https://doi.org/10.1016/J.ENVPOL.2015.03.018.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 503–537. https://doi.org/10.1146/ANNUREV.PP.40.060189.002443.

Fellet, G., Pošćić, F., Licen, S., Marchiol, L., Musetti, R., Tolloi, A., Barbieri, P., Zerbi, G., 2016. PAHs accumulation on leaves of six evergreen urban shrubs: a field experiment. Atmos. Pollut. Res. 7 (5), 915–924. https://doi.org/10.1016/J.APR.2016.05.007.

Giráldez, P., Aboal, J.R., Fernández, J.Á., Di Guardo, A., Terzaghi, E., 2022. Plant-air partition coefficients for thirteen urban conifer tree species: estimating the best gas and particulate matter associated PAH removers. Environmental Pollution 315, 120409. https://doi.org/10.1016/J.ENVPOL.2022.120409.

Giráldez, P., Varela, Z., Di Guardo, A., Terzaghi, E., Celeiro, M., García-Jares, C., Fernández, J.Á., Aboal, J.R., 2024. Relationship between foliar polycyclic aromatic hydrocarbons (PAHs) concentrations and plant traits: intracanopy variability for a broadleaf species in an urban environment. Sci. Total Environ. 940, 173698. https://doi.org/10.1016/J.SCITOTENV.2024.173698.

Herrera, C.M., 2009. In: Multiplicity in Unity: Plant Subindividual Variation and Interactions with Animals (University of Chicago Press. University of Chicago Press. https://digital.csic.es/handle/10261/38723.

Howsam, M., Jones, K.C., Ineson, P., 2000. PAHs associated with the leaves of three deciduous tree species. I — concentrations and profiles. Environmental Pollution 108 (3), 413–424. https://doi.org/10.1016/S0269-7491(99)00195-5.

Howsam, Mike, Jones, K.C., Ineson, P., 2001. PAHs associated with the leaves of three deciduous tree species. II: uptake during a growing season. Chemosphere 44 (2), 155–164. https://doi.org/10.1016/S0045-6535(00)00268-X.

Jouraeva, V.A., Johnson, D.L., Hassett, J.P., Nowak, D.J., 2002. Differences in accumulation of PAHs and metals on the leaves of Tilia×euchlora and Pyrus calleryana. Environmental Pollution 120 (2), 331–338. https://doi.org/10.1016/S0269-7491(02)00121-5.

Jouraeva, V.A., Johnson, D.L., Hassett, J.P., Nowak, D.J., Shipunova, N.A., Barbarossa, D., 2006. Role of sooty mold fungi in accumulation of fine-particle-associated PAHs and metals on deciduous leaves. Environ. Res. 102 (3), 272–282. https://doi.org/10.1016/J.ENVRES.2006.06.004.

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Tautenhahn, S., Werner, G.D.A., 2020. TRY plant trait database – enhanced coverage and open access. Global Change Biol. 26 (1), 119–188. https://doi.org/10.1111/GCB.14904.

Kömp, P., Mclachlan, M.S., 1997. Influence of temperature on the plant/air partitioning of semivolatile organic compounds. Environ. Sci. Technol. 31 (3), 886–890. https://doi.org/10.1021/ES960590U.

Li, Y., Chen, B., Zhu, L., 2010. Single-solute and bi-solute sorption of phenanthrene and pyrene onto pine needle cuticular fractions. Environmental Pollution 158 (7), 2478–2484. https://doi.org/10.1016/J.ENVPOL.2010.03.021.

Mętrak, M., Aneta, E., Wilkomirski, B., Staszewski, T., Suska-Malawska, M., 2016. Interspecific differences in foliar 1 PAHs load between Scots pine, birch, and wild rosemary from three polish peat bogs. Environ. Monit. Assess. 188 (8), 1–13. https://doi.org/10.1007/S10661-016-5465-2.

Mukhopadhyay, S., Dutta, R., Das, P., 2020. A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques. Chemosphere 251, 126441. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126441.

Müller, J.F., Hawker, D.W., McLachlan, M.S., Connell, D.W., 2001. PAHs, PCDD/Fs, PCBs and HCB in leaves from Brisbane, Australia. Chemosphere 43 (4–7), 507–515. https://doi.org/10.1016/S0045-6535(00)00402-1.

Murakami, M., Abe, M., Kakumoto, Y., Kawano, H., Fukasawa, H., Saha, M., Takada, H., 2012. Evaluation of ginkgo as a biomonitor of airborne polycyclic aromatic hydrocarbons. Atmos. Environ. 54, 9–17. https://doi.org/10.1016/J.ATMOSENV.2012.02.014.

Pleijel, H., Klingberg, J., Strandberg, B., Sjöman, H., Tarvainen, L., Wallin, G., 2022. Differences in accumulation of polycyclic aromatic compounds (PACs) among eleven broadleaved and conifer tree species. Ecol. Indicat. 145, 109681. https://doi.org/10.1016/J.ECOLIND.2022.109681.

Prigioniero, A., Postiglione, A., Zuzolo, D., Niinemets, Ü., Tartaglia, M., Scarano, P., Mercurio, M., Germinario, C., Izzo, F., Trifuoggi, M., Toscanesi, M., Sciarrillo, R., Guarino, C., 2023. Leaf surface functional traits influence particulate matter and polycyclic aromatic hydrocarbons air pollution mitigation: insights from Mediterranean urban forests. J. Clean. Prod. 418, 138158. https://doi.org/10.1016/J.JCLEPRO.2023.138158.

Prigioniero, A., Zuzolo, D., Niinemets, Ü., Postiglione, A., Mercurio, M., Izzo, F., Trifuoggi, M., Toscanesi, M., Scarano, P., Tartaglia, M., Sciarrillo, R., Guarino, C., 2022. Particulate matter and polycyclic aromatic hydrocarbon uptake in relation to leaf surface functional traits in Mediterranean evergreens: potentials for air phytoremediation. J. Hazard Mater. 435, 129029. https://doi.org/10.1016/J.JHAZMAT.2022.129029.

Ray, D., Ghosh, S.K., Raha, S., 2021. Seasonal foliar uptake of atmospheric polycyclic aromatic hydrocarbons by some local plants in a tropical metropolis in India. Atmos. Pollut. Res. 12 (2), 104–112. https://doi.org/10.1016/J.APR.2020.10.013.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C., Bowman, W.D., 1999. Generality of leaf trait relationships: a test across six biomes. Ecology 80 (6), 1955–1969. https://doi.org/10.1890/0012-9658(1999)080.

RStudio Team, 2021. *RStudio: Integrated Development Environment For R*. RStudio. PBC. http://www.rstudio.com/.

Simonich, S.L., Hites, R.A., 1994. Vegetation–atmosphere partitioning of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 28 (5), 939–943. https://doi.org/10.1021/ES00054A028.

Singh, B.P., Zughaibi, T.A., Alharthy, S.A., Al-Asmari, A.I., Rahman, S., 2023. Statistical analysis, source apportionment, and toxicity of particulate- and gaseous-phase PAHs in the urban atmosphere. Front. Public Health 10, 1070663. https://doi.org/10.3389/FPUBH.2022.1070663/BIBTEX.

Tao, Z., Hornbuckle, K.C., 2001. Uptake of polycyclic aromatic hydrocarbons (PAHS) by broad leaves: analysis of kinetic limitations. Water, Air and Soil Pollution: Focus 2001 1 (5), 275–283. https://doi.org/10.1023/A:1013136028586, 5, 1.

Terzaghi, E., Wild, E., Zacchello, G., Cerabolini, B.E.L., Jones, K.C., Di Guardo, A., 2013. Forest Filter Effect: role of leaves in capturing/releasing air particulate matter and its associated PAHs. Atmos. Environ. 74, 378–384. https://doi.org/10.1016/J.ATMOSENV.2013.04.013.

Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., Liu, C., 2019. Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: morphological characteristics have the greatest impact. Sci. Total Environ. 652, 1149–1155. https://doi.org/10.1016/J.SCITOTENV.2018.10.357.

Tian, X., Liu, J., Zhou, G., Peng, P., Wang, X., Wang, C., 2008. Estimation of the annual scavenged amount of polycyclic aromatic hydrocarbons by forests in the Pearl River Delta of Southern China. Environmental Pollution 156 (2), 306–315. https://doi.org/10.1016/J.ENVPOL.2008.02.012.

Vinod, N., Slot, M., McGregor, I.R., Ordway, E.M., Smith, M.N., Taylor, T.C., Sack, L., Buckley, T.N., Anderson-Teixeira, K.J., 2023. Thermal sensitivity across forest vertical profiles: patterns, mechanisms, and ecological implications. New Phytol. 237 (1), 22–47. https://doi.org/10.1111/NPH.18539.

Wang, Y.Q., Tao, S., Jiao, X.C., Coveney, R.M., Wu, S.P., Xing, B.S., 2008. Polycyclic aromatic hydrocarbons in leaf cuticles and inner tissues of six species of trees in urban Beijing. Environmental Pollution 151 (1), 158–164. https://doi.org/10.1016/J.ENVPOL.2007.02.005.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornellssen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., et al., 2004. The worldwide leaf economics spectrum. Nature 2004 428:6985 428 (6985), 821–827. https://doi.org/10.1038/nature02403.

Yang, B., Liu, S., Liu, Y., Li, X., Lin, X., Liu, M., Liu, X., 2017. PAHs uptake and translocation in Cinnamomum camphora leaves from Shanghai, China. Sci. Total Environ. 574, 358–368. https://doi.org/10.1016/J.SCITOTENV.2016.09.058.

Yang, M., Tian, S., Liu, Q., Yang, Z., Yang, Y., Shao, P., Liu, Y., 2022. Determination of 31 polycyclic aromatic hydrocarbons in plant leaves using internal standard method with ultrasonic extraction–gas chromatography–mass spectrometry. Toxics 10 (11), 634. https://doi.org/10.3390/TOXICS10110634/S1.

Zhang, M., Wang, J., Zhao, Q., Mishra, V., Fan, J., Sun, Y., 2020. Polycyclic aromatic hydrocarbons (PAHs) and esophageal carcinoma in Handan-Xingtai district, North China: a preliminary study based on cancer risk assessment. Environmental Monitoring and Assessment 2020 192 (9), 1–20. https://doi.org/10.1007/S10661-020-08499-5, 9, 192.

Zhao, X., He, M., Shang, H., Yu, H., Wang, H., Li, H., Piao, J., Quinto, M., Li, D., 2018. Biomonitoring polycyclic aromatic hydrocarbons by Salix matsudana leaves: a comparison with the relevant air content and evaluation of environmental parameter effects. Atmos. Environ. 181, 47–53. https://doi.org/10.1016/J.ATMOSENV.2018.03.004.

Chapter II: Relationship between foliar polycyclic aromatic hydrocarbons (PAHs) concentrations and plant traits: Intracanopy variability for a broadleaf species in an urban environment

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Relationship between foliar polycyclic aromatic hydrocarbons (PAHs) concentrations and plant traits: Intracanopy variability for a broadleaf species in an urban environment

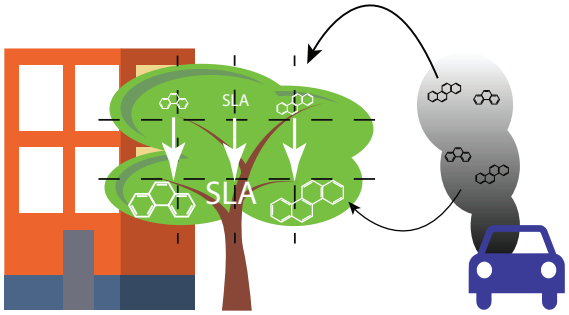
Pablo Giráldez [a,*], Zulema Varela [a], Antonio Di Guardo [b], Elisa Terzaghi [b], María Celeiro [c], Carmen García-Jares [c], J. Ángel Fernández [a], Jesús R. Aboal [a]

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HIGHLIGHTS

- Intra-canopy variation of PAH concentrations is not negligible.
- This variation is associated with the position of the leaves within the canopy.
- Leaf height is the main source of variation in PAH concentrations.
- Lower leaves take up more PAHs than upper leaves.
- PAHs levels depend on leaf morphology, exposure, and distance to the emission.

GRAPHICAL ABSTRACT



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ABSTRACT

The emission of potentially harmful compounds, including polycyclic aromatic hydrocarbons (PAHs), and the resulting air pollution is a serious problem in modern cities. It is therefore important to develop mitigation strategies, such as "smart" planting of trees that act as sinks for PAHs. However, the intra-individual (within-tree) variability in leaf PAH concentrations remains unknown. In this paper, we studied 15 ornamental apple trees (*Malus* × *moerlandsii* 'Profusion') growing on a main street in a medium-sized city in Galicia (NW Spain). We determined the PAH concentrations at 12 canopy positions in each tree (2 orientations and 2 distances from the trunk at 3 heights), measured various ecological traits (specific leaf area [SLA], $\delta^{13}C$, stomatal density, fatty acid contents and leaf hairiness) and analyzed the variability in traits within the canopy in relation to PAH concentrations. We observed high intra-individual variability in the PAH concentrations and the leaf traits. Statistical analyses revealed that leaf height was the main source of variability both in the PAH concentrations and in the traits, mainly due to the leaf morphology, particularly to the SLA. Therefore, the ideal vegetation to remove PAHs would be high leaf biomass trees, not too tall and with a high proportion of shade leaves.

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1. Introduction

Air pollution is a major environmental problem and has important effects on ecosystems and human health (Kampa and Castanas, 2008), in particular, in urban areas (EPA, 2023; Piracha and Chaudhary, 2022). The exposure to air pollution is responsible for approximately 300,000 premature deaths every year in Europe (EEA, 2022) and is estimated to cause over 4 million premature deaths annually, globally (World Health Organization, 2022). Air pollution is increasing along with the rapid rise in the urban population and 68 % of the world's population is expected to live in urban areas by 2050. This would represent an almost threefold increase in the world's urban population (United Nations Department of Economic and Social Affairs Population Division, 2019), which highlight the need to address urban development challenges in a sustainable manner. Therefore, it is essential to develop local strategies to reduce pollution in cities.

Plants are known to accumulate gaseous contaminants and particulate matter (PM) (Terzaghi et al., 2013). "Smart" tree planting, involving the use of trees as green filters to remove pollutants from the air, has thus been proposed as a measure to reduce pollution (Nowak et al., 2014). However, for this approach to be effective, more information about the mechanisms of the uptake of air pollutants in trees is required (Janhäll, 2015). This information could then be used to develop models and make informed decisions for selecting the types of trees that should be planted in urban environments, considering the capacity of the trees to remove pollutants from the air. Polycyclic aromatic hydrocarbons (PAHs) are included among the compounds that contribute to the deterioration of air quality in cities and that can be mitigated by trees (Wagrowski and Hites, 1997). PAHs are known carcinogens and primarily originate from the incomplete combustion of fossil fuels (IARC, 2010). The main sources of PAH emissions in urban areas include road traffic and building heating systems (Krugly et al., 2014).

Numerous studies have analyzed PAH concentrations in trees, mainly in the leaves (e.g. Ray et al., 2021; Pleijel et al., 2022); however, many aspects of the uptake of these pollutants by trees remain unknown. Some studies have compared foliar PAH concentrations (in different cohabiting species) in relation to leaf traits, with contradictory findings. For example, variable results have been obtained regarding the relationship between specific leaf area (SLA) and PAH concentrations, depending on the considered PAH compound. For heavy molecular weight (HMW) PAHs, Howsam et al. (2001) reported that an increase in SLA increases PAH uptake, while Tian et al. (2019) found the opposite, and Pleijel et al. (2022) did not observe any effect of SLA. The variable findings can probably be attributed to the complex interactions between traits -which determine the PAH concentration in leaves- and the difficulty in isolating the effects of individual traits. Thus, a novel approach to the study of the relationship between PAHs and traits is to focus on a single species; this will minimize the number of interactions between traits because the intra-specific (inter-individual) variability in some traits is lower than the inter-specific variability (Jiang et al., 2020).

Most researchers (with the exception Murakami et al., 2012) have ignored intra-individual variability in foliar PAH concentrations in relation to leaf position. Intra-individual variability is of paramount importance in modular organisms such as plants, where there may be greater variation within the same individual than between individuals of the same species (Herrera, 2009). This is a major restriction to accurately scaling up the concentration of PAHs from the leaves sampled to the total concentration in the tree, hindering estimation of the ability of plants to remove pollutants from the air.

Nowadays, modelling studies (e.g. Wania and McLachlan, 2001; Undeman et al., 2009; Trapp, 2015; and Terzaghi et al., 2017) generally use a bulk or generic plant/air partition coefficient (KPA) and/or particle deposition velocity to evaluate PAH and PM capture and ignore "within the canopy" variability of traits such as leaves position, SLA and Leaf Area Index (LAI), among others. Stochastic versions of such deterministic simulations are lacking due to the lack of measurements of the variability. Therefore, it is essential to characterize the magnitude and patterns of intra-individual variability and to establish standardized sampling designs to provide a representative estimate of PAH concentrations in the whole individual.

The main aims of the present study were to determine i) the intra-individual variability in foliar PAH concentrations and ii) the relationship between this variability and leaf traits (SLA, stomata density, gas exchange, cuticular waxes and hairiness) and leaf position (height, distance to the trunk and orientation). In addition to these aims, a further objective was to propose guidelines for selecting the most suitable trees and tree canopy treatments (such as pruning) that could be conducted to enhance PAH capture.

2. Material and methods

2.1. Sampling and sample processing

Sampling was conducted on 10–11 May 2021, on a main street in A Coruña (see Fig. A.1), a city in northern Galicia (NW Spain). The average elevation of the city is 21 m a.s.l., ranging from 0 to 291 m. The city occupies an area ca. 39 km^2 and has a population of ca. 245,000 inhabitants (2021). The climate in the area is oceanic, with an average annual temperature of 14.8 °C, mean precipitation of 1014 mm distributed across 130 rainy days (>1 mm) per year (climatological normal values in Coruña between 1981 and 2010; http://aemet.es/) and a wind speed at 10 m height of 5 m s^{-1} (mean value between 2007 and 2021; http://www.meteogalicia.gal/). The sampled street is one of the main one-way streets in the city, almost 1 km long, with three traffic lanes and cars parked on both sides. The slightly sloping street has an average traffic intensity of 12,262 vehicles/day.

The species selected for this study was an ornamental apple tree (*Malus* × *moerlandsii* 'Profusion'). This selection was made because the trees sampled met the characteristics that we considered relevant and necessary for the study, both in terms of location (the characteristics of the street where the trees were located and their arrangement in it) as well as the characteristics of the trees themselves (their age, foliage, canopy height…). Samples were obtained from 15 apple trees, of age at least 15 years, growing on the pavements on both sides of the road (see Fig. A.1). The average dimensions of the trees were as follows: canopy bottom height, 2.3 m; canopy top height, 7.3 m; canopy diameter, 5.4 m; and trunk diameter at breast height (DBH), 13.8 cm. Twelve samples were taken in each tree, i.e.: at three heights (from the bottom, middle and top of the canopy), two orientations (towards the road and towards the buildings) and at two distances from the trunk (near and far from the trunk) (see Fig. 1). Between 15 and 25 leaves were sampled at each point. The sampled leaves were wrapped in aluminum foil, placed in zip lock bags, which were then sealed and stored at 5 °C. The leaves were subsequently analyzed to measure different leaf traits and to determine the foliar PAH concentrations.

2.2. Trait measurements

The traits measured in this study are of interest in relation to understanding PAH loading mechanisms in tree leaves. The SLA is defined as the ratio of leaf surface area to leaf dry weight. Thus, for a given weight of leaf, higher SLA values will indicate larger areas available for deposition. Some authors (e.g. Desalme et al., 2013; Dias et al., 2016) have suggested that the opening of stomata during gas exchange may contribute to the diffusion of PAHs. To evaluate this possibility, the stomata density and $\delta^{13}C$ (a proxy for the intensity of gas exchange) were measured. The isotopic discrimination capacity of plants is determined by the length of time that stomata are open, which determines gas exchange and photosynthetic activity (carbon fixation). Thus, high $\delta^{13}C$ values correspond to low gas exchange and vice versa. The best known way in which PAHs bind to leaves is sorption to the hydrophobic cuticle (Barber et al., 2004). This phenomenon was evaluated by measuring the

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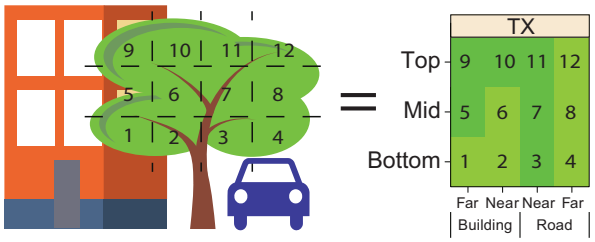


Fig. 1. Sketch showing the position of the 12 points studied in each tree (left) and the corresponding representation that will be used hereafter in this paper (right). The different shades of green indicate different hypothetical concentrations of PAHs at the sampled positions within the tree.

fatty acid content of leaves. Finally, the presence of trichomes on leaves, i.e., the pilosity, can increase the roughness of the leaf surface and modify the leaf boundary layer, thus altering the capacity of leaves to retain PAHs.

2.2.1. SLA

From each of the 180 samples, between 7 and 10 (depending on sample availability) fresh leaves were selected at random. The petioles were removed, and the projected area of each leaf was measured with a LI-3100 AREA METER scanner (LI-COR®). The leaves were then dried at 100 °C in an oven (Digitronic-TFT model; J.P. Selecta®) until constant weight and weighed with a precision balance (model C-3000CB; Cobos®).

2.2.2. Stomata density

One leaf was selected from each sample, and three impressions of the abaxial surface were made with nail varnish. A photograph of each impression was taken (CX41 Olympus® microscope), and stomata were counted over an area of 0.777 mm^2. The stomatal density was estimated as the average of the counts on the three impressions.

2.2.3. Gas exchange-δ^{13}CVPDV ‰

Leaves selected for carbon isotopic determination were dried at 100 °C until constant weight, before being homogenized in a ball mill (MM 400; Retsch®) at 12,000 rpm. The homogenized samples were stored in hermetically sealed glass vials at 20 °C in darkness until analysis. Carbon isotopic composition was determined by the Research Support Service (SAI, University of Coruña) with an EA1108 elemental analyzer (Carlo Erba Instruments) coupled to a MAT253 isotope ratio mass spectrometer (Thermo Finnigan) and a ConfloIII interface (Thermo Finnigan). The $\delta^{13}C$ ratios are expressed in parts per thousand (‰) relative to VPDV (Vienna Pee Dee Belemnite) according to standard notation ($\delta^{13}C = [(Rsample / Rstandard) - 1] \times 1000$, where R is the ratio $^{13}C/^{12}C$).

2.2.4. Fatty acid concentration in cuticular waxes

The fatty acid concentrations (μg g^{-1}_{fw}) in waxes were determined from a total of 60 samples, so that for each of the 12 locations on the tree, 5 samples were semi-randomly selected from the 15 samples (trees) available. Fresh leaves (0.2 g) were immersed in 2 mL of ethyl acetate and then sonicated for 2 min in an ultrasound bath (50 Hz, 25 °C). A 0.2 mL sample of the obtained extract was then diluted in ethyl acetate to a final volume of 1 mL. A 0.1 mL sample of this solution was evaporated to dryness under a gentle stream of nitrogen. The remaining solid was reconstituted in 0.5 mL of methyl tributyl ether (MTBE), and the fatty acids were derivatized by reaction with trimethyl sulfonium hydroxide (TMSH) according to a previously optimized procedure (Gómez-Brandón et al., 2008). After derivatization, the solution was filtered (0.22 μm) and analyzed by gas chromatography–mass spectrometry (GC–MS), in a chromatograph (Agilent 7890A) coupled to a mass spectrometer (Agilent 5975C) and an autosampler (Agilent 7693) (Palo Alto, CA, USA). Separation was carried out on a Zebron Semivolatiles column (30 m × 0.25 mm i.d. x 0.25 μm film thick) from Phenomenex (Torrance, CA, USA). The GC–MS instrumental parameters were previously optimized to achieve optimal separation and an unequivocal identification of the target compounds (Garcia-Jares et al., 2017).

The fatty acids determined in the samples were decanoic, myristic, palmitic, heptadecanoic, stearic, oleic, linoleic, and linolenic acid.

2.2.5. Characterization of leaf pilosity

In order to characterize the leaf pilosity, we took optical microscopy and electron microscopy pictures. Two leaves were selected from each tree -from the bottom and the top of the canopy- and were photographed in a stereoscopic microscope (Leica M205FA, equipped with a Leica DFC7000T CCD camera), in brightfield and fluorescence mode, with the ET GFP filter (ex: 470/40 nm, em: 525/50 nm) at 20× optical magnification. To get a better idea of the leaf structure, scanning electron microscopy (SEM) photomicrographs of two leaves were acquired, at different magnifications, with an electron microscope (SEM Zeiss EVO LS15). The morphology, size, and density of the trichomes were determined by visual inspection.

2.3. PAH determination in leaves

Each sample was dried in a paper envelope at 25 °C in a universal precision oven (model Digitronic-TFT; J.P. Selecta®), before being homogenized in an ultra-centrifugal mill (ZM 200; Retsch®) at 12,000 rpm and finally stored in hermetically closed glass vials at 20 °C in darkness until PAH analysis.

The following PAHs were analyzed: acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Ft), pyrene (Pyr), benzo(*a*)anthracene (BaA), chrysene (Chry), benzo(b+j)fluoranthene (B(b+j)F), benzo(*k*)fluoranthene (BkF), benzo(*e*)pyrene (BeP), benzo(*a*)pyrene (BaP), dibenzo(*a,h*)anthracene (DBahA), indeno(1,2,3-*cd*)pyrene (IP) and benzo(*ghi*)perylene (BghiP). The foliar contents of these PAHs were measured by the Applied Analytical Chemistry research group (QANAP, University of Coruña), with the MSPD-PTV-GC–MS/MS method (optimized for *Quercus robur*) developed by this group. The method involves matrix solid-phase dispersion extraction followed by programmed temperature vaporization-gas chromatography-tandem mass spectrometry (for a brief summary of the method and the equipment used see (De Nicola et al., 2017; for further details, see De Nicola et al., 2016). In cases where the concentration was below the limit of quantification, the middle-bound approach (0.5*LOQ) was applied.

2.3.1. Quality assurance/quality control (QA/QC)

Instrumental and procedural blanks, i.e. surrogates spiked on the C_{18} without matrices, were systematically evaluated (De Nicola et al., 2016). Quality control of the complete procedure and quantification was performed using labelled PAHs as surrogate standards. Chromatographic control was performed by injection of two standard solutions containing all target PAHs for each batch. Peak identification and integration were performed by Excalibur 2.0.7 software from Thermo Fisher Scientific Inc. The LOQs for each PAH correspond to those reported for *Q. robur* (De Nicola et al., 2016), and generally comprised between 0.13 and 1.46 ng/g dw.

2.4. PM measurement

It was not possible to measure particulate matter (PM) in the air at the same time as the leaves were sampled. The PM levels were sampled in 2023, on the same dates and under similar meteorological conditions. The concentrations of PM_{10} and $PM_{2.5}$ were determined with an M2000 2nd Generation Multi-functional Air Quality Detector (Temtop®), at each of the 12 points in trees 1, 2, 9, 10, 11, 12 and 14, on 23 and 24 May

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2023. The lack of leaves on the other trees meant that measurements could not be performed. However, we made measurements at all trees at a height of 1.70 m, close to the road and close to the buildings. The lack of leaves on some of the trees may have been due to pruning work carried out in the street. Tree number 15 was found to be dead.

2.5. Data analysis

In order to elucidate the differences between the different positions within the tree, different statistical analyses were performed according to the characteristics of the data (see Fig. A.2). In summary, the normality and homoscedasticity of the data were tested. When the data were not normally distributed, the optimal Box-Cox or Yeo-Johnson power transformation was applied. The interaction between canopy height, distance from the trunk and orientation (towards the building or the road) and their main effects were tested by ANOVA or permutation tests. Finally, linear regression models were fitted with the significant variables where the data allowed (normality and homoscedasticity). All tests were performed on PAHs with <10 % observations below the LOQ. The significance level for all tests was 0.05.

All statistical analyses were performed using different functions and packages in RStudio version 4.1.1 (RStudio Team, 2021). The shapiro.test function (stats package) was used to test for normality, and the leveneTest function (car package; Fox and Weisberg, 2019) was used to test for homoscedasticity. The Box-Cox transformation was performed with the boxcox function (MASS package; Venables and Ripley, 2002) and the interactions and main effects were determined using the aov (stats package) and perm.fact.test (asbio package; Aho, 2022) functions. The respective post hoc analyses were performed with the TukeyHSD function (stats package) and the pairwisePermutationTest function (rcompanion package; Mangiafico, 2022). Finally, the models were fitted using the lm function (stats package).

To determine the relationships between the PAHs and to observe how the trees clustered according to PAH concentrations, we performed a principal component analysis (PCA) and grouped the trees with a K-means algorithm. The mean values of the 12 concentrations of PAHS determined in each tree were used as input data in the analysis. The PCA was performed with the prcomp function (stats package). The functions of the factoextra package (Kassambara and Mundt, 2020) and the corrplot function (corrplot package; Wei and Simko, 2021) were used to visualize the results. Clustering was performed with the fviz_nbclust and eclust function (factoextra package).

Finally, the relationships between PAHs and traits were determined by Spearman correlations, implemented with the cor function (stats package). The significance of each correlation was determined with the cor.mtest function (corrplot package). The same package was used to visualize the results.

3. Results and discussion

3.1. PAH concentrations in leaves

The total PAH concentrations in leaves ranged from 57 to 472 ng/g dw (see Table A.1 for each sample). These results were in line with those reported in the literature for other plant species located in urban and semiurban areas in Europe (De Nicola et al., 2014; Fasani et al., 2016; Franzetti et al., 2020; Giráldez et al., 2022; Terzaghi et al., 2015a). Although our results are not directly comparable with them because they were not determined in the same spring/summer period, did not use deciduous trees, or were not located next to roads, they can give an idea of the range of concentrations. For example, Pleijel et al. (2022) in 2018 measured PAHs in leaves/needles of 11 species in the city of Gothenburg (Sweden), 800 m away from the main road, showing concentrations that ranged from 15 ng/g dw for *Fagus orientalis* to 160 ng/g dw for *Pinus nigra*. De Nicola et al. (2014) found 200 ng/g dw to 7800 ng/g dw in *Quercus ilex* leaves collected in Naples city (Italy) from 1998 to 2009, while the concentrations in Terzaghi et al. (2015b) in *Cornus mas* and *Acer pseudoplatanus* leaves, sampled in a semiurban site located in Como (Italy) in 2007, ranged from 30 to 170 ng/g dw. These numbers are more than one order of magnitude lower than those measured in megacities such as Shanghai (Liang et al., 2017; Yin et al., 2020).

In the present study, the most abundant PAH was Chry, which represented 17 % of the PAH fingerprint, followed by Ft (14 %) and Pyr (13 %). The sum of the isomers BbF and BjF (B(b+j)F) also accounted for 17 % of the total PAHs. The concentrations of Acy, Ace, Fl, Ant, BaP and DBahA were below the LOQ in several samples (>10 %) (Figs. A.3-A.8).

3.1.1. Inter- and intra-individual variation in PAH concentrations

The concentrations of PAHs differed between trees (inter-individual) and leaf positions (intra-individual) (Table A.1). Overall, both types of variation are of the same order of magnitude (Fig. 2). The differences in total PAH concentrations are illustrated in Fig. 2A; the foliar concentrations were about two times higher in trees 7, 8, 9, 11 and 12 than in the other trees. Differences between positions were also observed (Fig. 2B), mainly in relation to canopy height, with apparently higher concentrations in the lower areas of the canopy than in the upper areas.

3.1.2. Differences in PAH concentrations

The results of the statistical analyses (see Fig. A.2) used to test for differences in the PAH concentrations in the leaves according to their position in the trees are shown in Table 1. In most cases, there were significant differences (p-value <0.05) in the concentrations in relation to height in the canopy and distance from the trunk. Leaves lower in the canopy and closer to the trunk had significantly higher concentrations than those higher and further away from the trunk. Leaf orientation towards the road or buildings was only significant for BghiP, with leaves facing the road having higher concentrations of this compound than leaves facing buildings. The adjusted R^2 of the linear regression models performed with the significant variables are also shown in Table 1. The range is 0.14 to 0.48, with an average R^2 of 0.26 for individual compounds.

3.2. PAH concentration and tree clustering

PCA was used to examine patterns in PAH concentrations and relationships among the different compounds. The inclusion of PAHs with a high percentage (>10 %) of observations below the LOQ altered the natural variability of the data, so we decided to remove these compounds (Acy, Ace, Fl, Ant, BaP and DBahA) from the analysis (Fig. 3). All variables are well represented by 2 components, which explained ca. 80 % of the variation. The first component (Dim1 in Fig. 3) discriminated between trees with higher total PAH concentration and lower Phe concentration, and trees with lower total PAH concentrations and higher Phe concentrations. The presence of higher total concentrations is presumably associated with higher exposure to pollution sources (vehicles). The second component (Dim2 in Fig. 3) mainly explains part of the variability of Phe and BghiP and, to a lesser extent, seems to slightly discriminate the other PAHs according to whether they are associated with the gaseous phase or particulate matter. Nonetheless, the 3-ring PAHs produced different results than the other compounds, probably due to their higher volatility or the existence of an unknown secondary source contributing additional 3-ring PAHs to trees at the beginning of the street. The BghiP also produced slightly different results, as although it seems to follow the general trend of most PAHs (positive values in Dim1), it seems to be better represented by Dim2. On the basis of these results, we differentiated two clusters of trees (Fig. 3C): group 1 (Fig. 3D; trees 1 to 6, 10, and 13 to 15), with lower total PAH concentrations, but higher concentrations of light PAHs; and group 2 (Fig. 3D; trees 7 to 9, 11 and 12), with higher total PAH concentrations. These two groups of trees can be clearly observed in Fig. A.9, in which total PAHs, Chry (representative heavy molecular weight PAHs) and Phe (representative of the 3-ring fraction) concentrations are shown for each sample.

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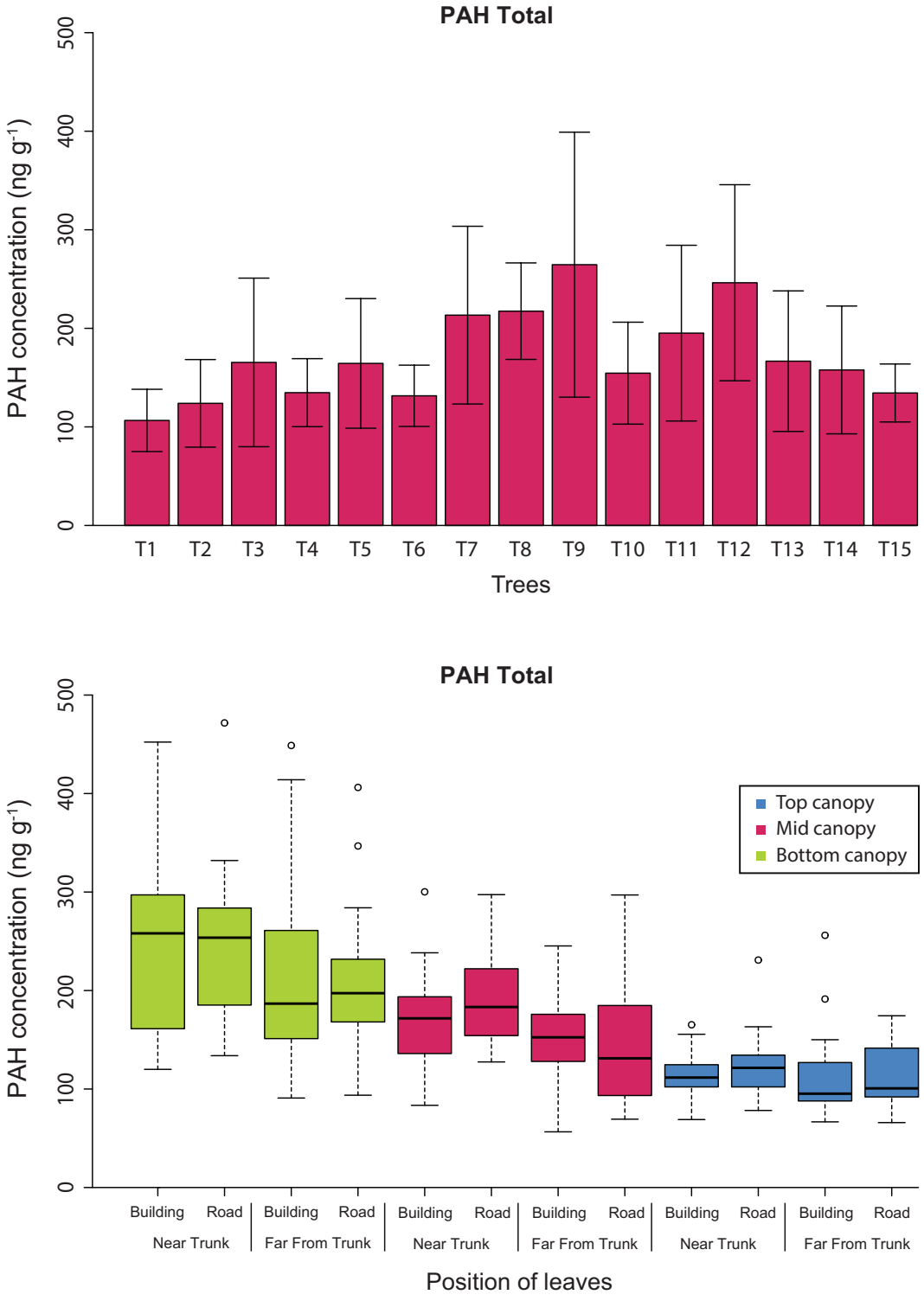


Fig. 2. A: Bar chart of the total PAH concentrations (ng g^{-1}) in each of the sampled trees. The error bars represent the standard deviation. B: Box plot of the total PAH concentration (ng g^{-1}) in each of the 12 sampled points within the tree canopy ($n = 15$).

Table 1
Statistical analysis results on the differences in PAH concentrations in leaves according to their position in the canopy ($n = 180$, i.e., all trees). The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^{2*}
Phe	A	–	C.H.	Bot>Mid, Bot>Top	0.18
			T.D.	N > F	
Ft	A	–	C.H.	Bot>Mid, Bot>Top	0.14
Pyr	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.34
			T.D.	N > F	
BaA	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.21
			T.D.	N > F	
Chry	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.26
			T.D.	N > F	
B(b+j)F	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.31
			T.D.	N > F	
BkF	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.19
BeP	P	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	–
			T.D.	N > F	
IP	P	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	–
			T.D.	N > F	
BghiP	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.48
			T.D.	N > F	
			O.	R > B	
3rings	A	–	C.H.	Bot>Mid, Bot>Top	0.15
			T.D.	N > F	
4rings	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.26
			T.D.	N > F	
5rings	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.29
			T.D.	N > F	
6rings	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.37
			T.D.	N > F	
Total	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.38
			T.D.	N > F	

A, ANOVA; P, permutation; C.H., Canopy Height; T.D., Trunk Distance; O, Orientation; –, indicates no significant interaction; Bot, Bottom canopy; Mid, Mid canopy; Top, Top canopy; N, Near to the trunk; F, Far from the trunk; R, Road orientation; B, Building orientation.
* Adjusted R^2 of the regression model constructed with the significant variables.

3.3. Intra-individual differences in PAHs by tree cluster and PM concentrations

3.3.1. Differences in PAH concentrations for each tree cluster

The results in Table 1 include all samples from all trees; however, as the concentrations of PAHs in the trees are not homogeneous and considering the clustering results in Fig. 3, the statistical analyses were repeated by separating the trees into two groups (Tables A.2 and A.3). In the first group (Table A.2), the results (significant differences, i.e. *p*-value <0.05) were similar to those reported in Table 1. The differences were mainly between canopy height and distance from the trunk. The R^2 values were also very similar (range 0.12 to 0.45; mean 0.27). In the second group (Table A.3), the differences were only related to canopy height, and differences according to distance from the trunk were only observed for Phe. The R^2 values of this group were much higher (range 0.32 to 0.59; mean 0.50). Orientation seems to have little effect in both cases, and significant differences were only detected for BghiP (Table A.2). An interaction between orientation and distance from the trunk was also detected for BaA (Table A.2). The observed differences between the two groups can probably be explained by the fact that group 2 is smaller and more homogeneous. In addition, the trees in group 2 are closer to each other, and they are therefore subject to less variability than the trees in group 1, which are distributed along the entire length of the street. These two factors could explain why the explanatory power of the linear models is higher in group 2. Note that BaP is included in group 2 analysis but not in group 1 due to the high percentage of samples < LOQ.

3.3.2. Differences in PM concentrations

The concentrations obtained for atmospheric $PM_{2.5}$ and PM_{10} in the different parts of the canopy and at a height of 1.70 m are shown in Fig. A.10. As can be seen from both figures and the test results (Table A.4), there does not seem to be any significant difference related to the different parts of the tree. This result was surprising, as previous findings and the results obtained for the PAH concentrations in the leaves suggested the opposite. In fact, several authors (see Hofman et al., 2013) have detected decreases in PM concentration with height. The following factors may explain this result: i) the time between sampling, the distribution of PAHs and PM is spatial and temporal, so by measuring at different times it is likely that environmental factors have been affected, e.g. the structure and cover of the trees, which has changed, affecting the barrier effect and their filtering capacity; ii) the wind turbulence that can occur in a street canyon, which may contribute to homogenizing the concentration of pollutants at different heights (De Nicola et al., 2013); and iii) single point measurements, which may not capture the differences that accumulate over time.

3.4. Trait variability

3.4.1. Inter- and intra-individual trait variability

The results for the different traits considered (SLA, $\delta^{13}C$, stomata density, concentration of different fatty acids in the leaf cuticle and leaf pilosity) are shown in Figs. 4 and A.11-A.15. These figures show that the inter-individual variability (Figs. A.11 and A.13) is lower or similar to the intra-individual variability (Figs. 4 and A.12). In fact, after calculating the inter-individual coefficient of variation (CV) of the traits and comparing it with the mean intra-individual CV, we found the former to be similar to (e.g. linolenic acid: inter-CV = 2.1 % and intra-CV = 2.0 %) or lower than (e.g. SLA: inter-CV = 6.8 % and intra-CV = 18.9 %) the latter.

The position of the leaves within the tree canopy seems to account for a large part of the intra-individual variability. Thus, the most exposed leaves (sun leaves, top canopy) seem to have a lower SLA, higher stomata density and higher $\delta^{13}C$ (Fig. 4) than the least exposed leaves (shade leaves, bottom canopy). These results are not surprising considering the variation in these variables in relation to height and

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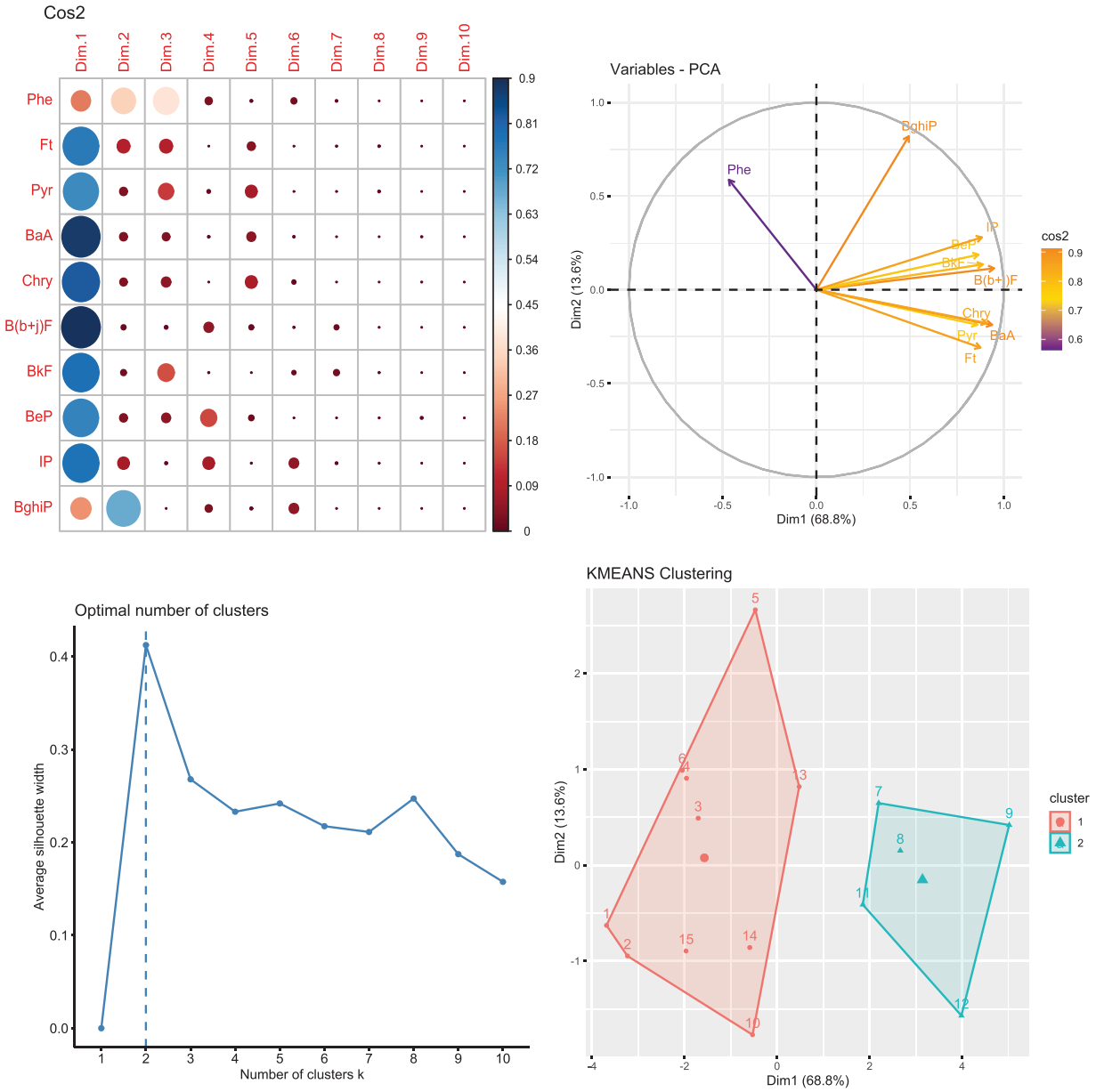


Fig. 3. Results obtained for PAHs for which <10 % of the observations were below the LOQ. From left to right and from top to bottom. A: Cosine-squared representation (Cos2), indicating the quality of representation of the variables by each of the PCA dimensions. B: Variable correlation plot. Positively correlated variables are grouped together, and negatively correlated variables are placed in opposite quadrants. The length of the arrows indicates the quality of representation of the variables (the longer the arrows, the better). C: Optimal number of clusters indicated by the dashed line. D: Result of clustering the trees with a KMEANS algorithm.

light exposure within the tree canopy (Vinod et al., 2023). The SLA indicates the leaf area per gram of leaf and is generally higher in low light conditions (Liu et al., 2016), i.e. leaves that are not as exposed to sunlight (leaves in the lower part of the canopy and close to the trunk). The $\delta^{13}C$ represents the $^{13}C/^{12}C$ isotope ratio, which is modified by the positive discrimination of ^{12}C against ^{13}C that occurs in C fixation processes in leaves. This discrimination is higher when stomata are open, with a continuous flow of CO_2, than when stomata are closed. Although the maximum stomatal conductance tends to increase with tree height, the stomata of sun leaves are more likely to close due to increased transpiration, which increases the δ13C value (Vinod et al., 2023). Stomatic density (stomata per unit area) is an indicator of possible gas exchange (although this can vary depending on the size of the stomata and duration of opening) and also tends to increase with height and light exposure (Camargo and Marenco, 2011). The fatty acid concentration (Fig. A.12) does not seem to depend on leaf position. In terms of pilosity, the leaves do not seem to be characterized by having many trichomes, which are filiform and mainly located on the leaf veins (Figs. A.14-A.15). A considerable difference between the number of trichomes on the bottom canopy leaves (less trichomes) and top canopy leaves (more trichomes) was observed by the naked eye. In addition, there also appeared to be more trichomes on the adaxial side than on the abaxial

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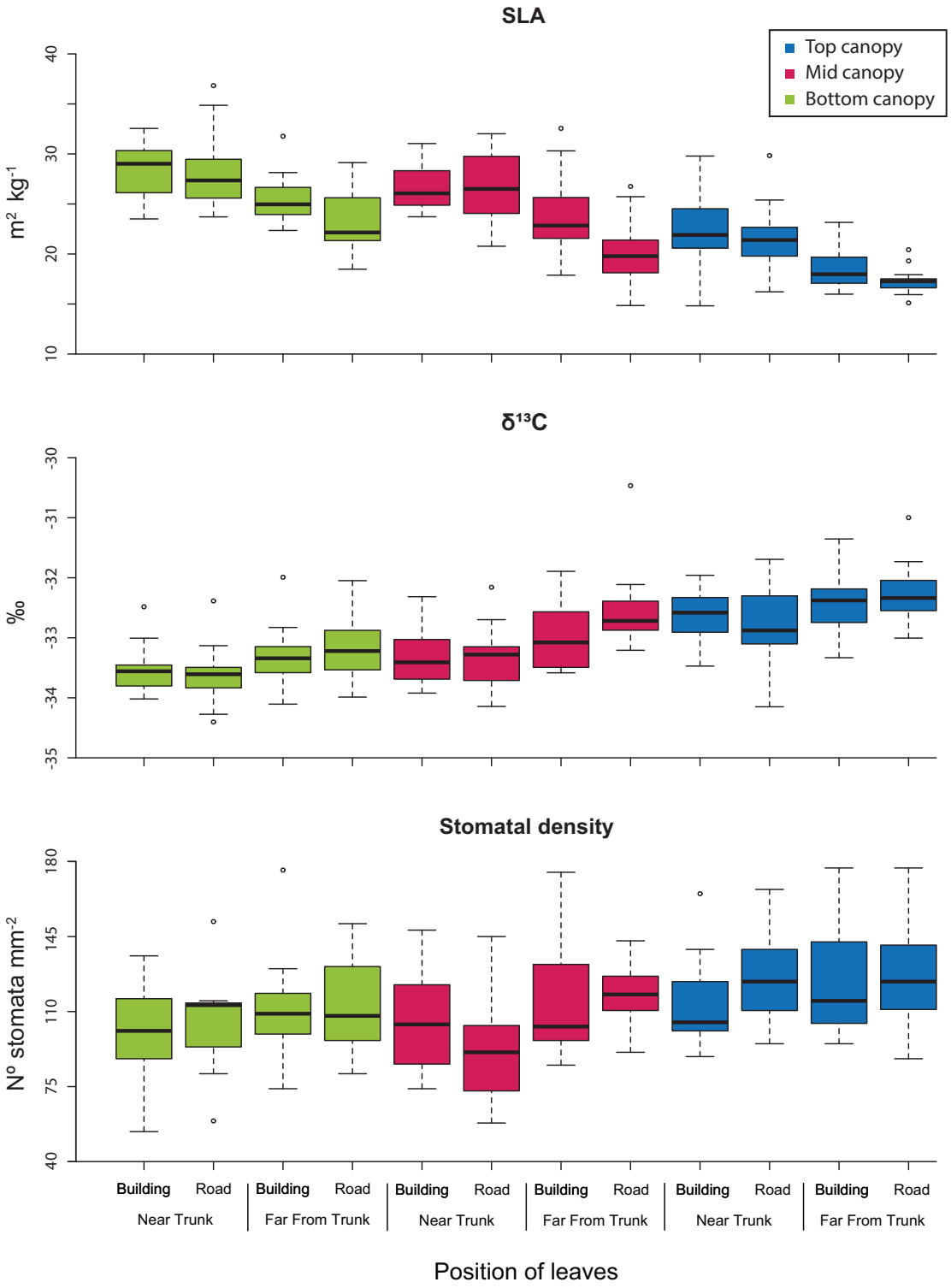


Fig. 4. Box plot of the SLA, $\delta^{13}C$ and stomatal density in each of the 12 sampled points within the tree canopy.

Table 2
Statistical analysis results on differences in leaf traits (SLA, δ13C, stomatal density and different fatty acids) according to their position in the canopy (all samples from all trees were used). The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^{2*}
SLA	A	T.D. O.	C.H.	Bot>Mid, Bot>Top, Mid < Top	0.58
$\delta^{13}C$	A	T.D. O.	C.H.	Bot<Mid, Bot<Top, Mid < Top	0.40
Stomata density	A	–	C.H. T.D.	Bot<Top, Mid < Top N < F	0.13
Heptadecanoic Ac.	A	–	–	–	–
Decanoic Ac.	A	–	–	–	–
Oleic Ac.	A	–	–	–	–
Linoleic Ac.	P	–	–	–	–
Linolenic Ac.	P	–	–	–	–
Myristic Ac.	A	–	–	–	–
Stearic Ac.	A	–	–	–	–
Palmitic Ac.	A	–	–	–	–

A, ANOVA; P, permutation; C.H., Canopy Height; T.D., Trunk Distance; O, Orientation; –, indicates no significant interaction; Bot, Bottom canopy; Mid, Mid canopy; Top, Top canopy; N, Near to the trunk; F, Far from the trunk; R, Road orientation; B, Building orientation.
* Adjusted R^2 of the regression model constructed with the significant variables.

side. This is not surprising as the position of trichomes varies greatly between species (Amada et al., 2023). However, it is not uncommon that, as in this case, there are more trichomes on leaves that are more exposed to sunlight (Vinod et al., 2023), where adaxial trichomes are associated, among others, with reflectance and protection functions against damaging ultraviolet-B (UV-B) radiation (Bickford, 2016). Trichomes can have many functions and can even influence gas exchange fluxes (Amada et al., 2023), in the trees under study, the trichomes were sparse and mainly located on the veins, and their effect on PAH uptake was not expected to be very important and was probably limited to retaining particulate matter, as seen in the lower left photo in Fig. A.15.

3.4.2. *Intra-individual trait differences*

The differences in traits among the different leaf positions in the trees were also tested. The results for the whole data set are shown in Table 2, while the results for each of the two groups of trees are shown in Tables A.5 and A.6. Significant differences were found in all cases for SLA, $\delta^{13}C$ and stomatal density. The R^2 (ca. 0.5) indicates that much of the variability in SLA and $\delta^{13}C$ depends on the position of the leaf on the tree. Regarding stomatal density, the position seems to have little influence (R^2 below 0.2). For fatty acids, no significant differences were detected between positions, only in group 1 for myristic acid, with a higher concentration in leaves close to the trunk.

3.5. *Correlations between PAH concentrations and traits*

It is well known that correlations generally reveal the strength and direction of the relationship between two variables, but do not indicate causality. Disentangling causal relationships among variables can be problematic in this field of study, in which a huge number of sources of variability must be considered. Numerous factors can affect the concentration of PAHs in tree leaves, and these factors can also interact and/or blend with each other. In addition to this complication, the variability among different tree species among trees of the same species (especially if they grow in different environments), and the variability within individual trees must be considered. By designing this study on trees of the same species located in the same street, we greatly reduced the variability in these traits, eliminating interspecific variability and limiting inter-individual variability while evaluating intra-individual variability. We were thus able to reduce the dimensions to be taken into account in this problem, allowing us to draw more robust conclusions from the results of the statistical tests applied.

The Spearman correlations that proved significant (p-value <0.05) for the PAHs determined and the traits measured, are shown in Fig. 5. The correlations clearly show a strong inverse relationship between SLA and $\delta^{13}C$ and, to a lesser extent, the nature of the relationship (inverse for SLA and direct for $\delta^{13}C$) of these variables with stomatal density. Likewise, most of the PAHs were positively correlated with SLA and negatively correlated with the other two traits. Most of the cuticle fatty acids were not significantly correlated with any variable except for other cuticle fatty acids. Considering the strong correlations between the traits

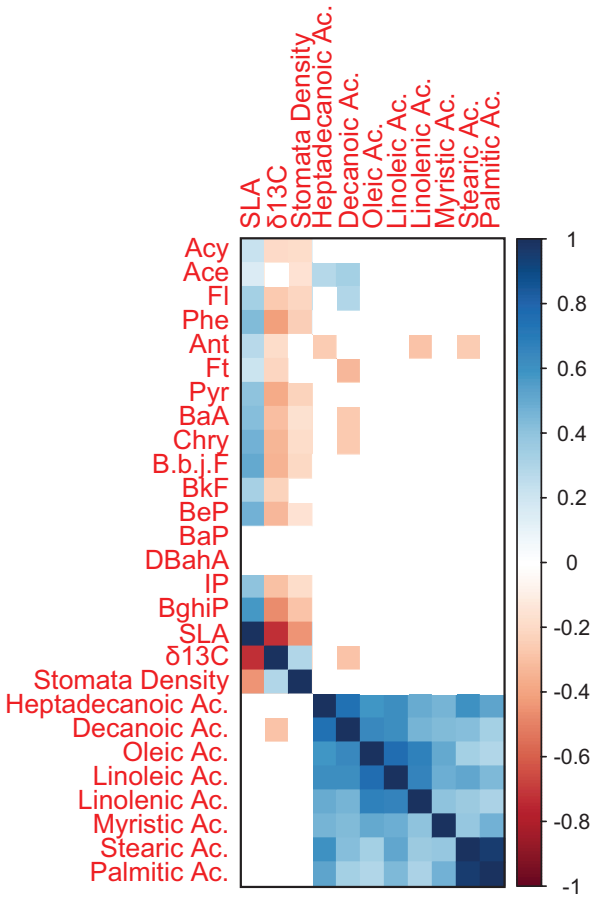


Fig. 5. Significant correlations (p-value <0.05) between PAH concentrations and traits.

Table 3
Statistical analysis results on differences in SLA-corrected PAH concentrations (PAH concentration/SLA) in leaves according to their position in the canopy (n = 180, i.e., all trees). The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^{2*}
Phe	A	–	C.H.	Bot>Mid, Bot>Top	0.06
Ft	A	–	C.H.	Bot>Mid	0.05
			O.	R > B	
Pyr	A	–	C.H.	Bot>Mid, Bot>Top	0.13
			O.	R > B	
BaA	A	–	C.H.	Bot>Top	0.07
Chry	P	–	C.H.	Bot>Top	–
B(b+j)F	A	–	C.H.	Bot>Mid, Bot>Top	0.12
BkF	A	–	C.H.	Bot>Mid, Bot>Top	0.12
BeP	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.12
IP	A	–	C.H.	Bot>Mid, Bot>Top	0.09
BghiP	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.31
			T.D.	N > F	
			O.	R > B	
3rings	A	–	C.H.	Bot>Mid, Bot>Top	0.05
4rings	A	–	C.H.	Bot>Mid, Bot>Top	0.07
5rings	A	–	C.H.	Bot>Mid, Bot>Top	0.12
6rings	A	–	C.H.	Bot>Mid, Bot>Top, Mid > Top	0.17
			O.	R > B	
Total	A	–	C.H.	Bot>Mid, Bot>Top	0.14

A, ANOVA; P, permutation; C.H., Canopy Height; T.D., Trunk Distance; O, Orientation; –, indicates no significant interaction; Bot, Bottom canopy; Mid, Mid canopy; Top, Top canopy; N, Near to the trunk; F, Far from the trunk; R, Road orientation; B, Building orientation.
* Adjusted R^2 of the regression model constructed with the significant variables.

related to PAHs concentration (SLA, $\delta^{13}C$ and stomatal density) it appears that these depend on one main source of variability, the leaf position, which modifies the traits according to the vertical gradient and light exposure. However, although the three traits vary according to these gradients, SLA seems to have the greatest influence on PAH uptake. This is the trait for which the highest correlations were obtained (0.16 to 0.58) and, furthermore, if gas exchange through the stomata would play a central role, differences should be clearly observed in the correlations with the lighter and heavier PAHs, which was not the case. The inverse correlation with stomatal density was probably due to (Prigioniero et al., 2022) the stomata being closed or, at least, not open sufficiently to significantly influence the concentration of PAHs.

3.6. Intra-individual differences in PAH concentrations expressed by leaf surface area

Considering the correlation between PAHs and SLA (Fig. 5) it is possible that some of the differences that we observed in Tables 1, A.2 and A.3 could be attributed to the concentrations being expressed in terms of mass (μg g^{-1}), without taking the leaf surface area into account. Therefore, we repeated the analyses with the data divided by the SLA, i.e., by expressing the concentration of PAHs expressed in terms of leaf surface area (μg m^{-2}). The results for all trees and for groups 1 and 2, are shown in Tables 3, A.7 and A.8. The most remarkable changes in the results are the disappearance of most of the differences due to the distance of the leaves from the trunk and the decrease in the explanatory power (R^2) of the models. In other words, a large part of the variation observed in the first analyses can be attributed to leaf morphology. This is consistent with the findings of other studies (Terzaghi et al., 2015b) showing the influence of SLA on the uptake of PAHs by trees.

3.7. Final remarks

In this study we have shown the importance of leaf morphology, mainly SLA, in explaining the variation in foliar PAH concentrations. (Terzaghi et al., 2015b) previously showed that the temporal variability of SLA could influence the PAH uptake rate of leaves of the same species during the growth season and therefore variations of SLA in time should be considered when evaluating the multimedia fate of chemicals using models accounting for a vegetation compartment (Terzaghi et al., 2017). However, the intraspecies (inter-individual) spatial variability was not considered yet.

Some researchers (Jouraeva et al., 2002) recommend expressing foliar PAH concentrations per unit of surface area rather than express them per unit of mass when comparing results for different plant species. Despite following this recommendation (Tables 3, A.7 and A.8), we still observed a significant decrease in leaf PAH concentrations due to leaf height. This observation is consistent with that of (Murakami et al., 2012) who reported a similar decrease, mainly associated with the distance from the source of emission of PAHs (cars). However, these researchers also detected differences between leaves over the road and those over the pavement, which we did not observe. This difference is probably due to the fact that the trees in the aforementioned study have "no obstacles to limit air circulation", while in the present study the buildings caused a street canyon effect that could enhance local air mixing. Finally, it is important to note that, although the distance from the PAH emission sources may be the main factor accounting for the observed variation between heights (once the effect of SLA is removed), other factors also play a role, such as the barrier effect of leaves and leaf illumination/temperature, because of both their influence on SLA (Gara et al., 2018) and their role in the volatilization/degradation-deposition balance of PAHs (Terzaghi et al., 2020). Interestingly, leaf pilosity is associated with higher uptake and retention of PM-associated PAHs (Prigioniero et al., 2023) and therefore we would expect higher concentration in exposed leaves; however, the slightly higher trichome density does not seem to be able to counteract the effect of factors that decrease PAH concentration with height.

Based on the findings of this study, although a mass balance of PAHs in the air- plant-soil environment was not performed, it is clear that not only SLA would be important as factor driving the uptake but also the leaf area index (LAI, m2/m2), which together compose plant biomass. In other terms to significantly reduce air concentrations in urban environments different measures would be needed: emission reduction, and mitigation measures such as the increase of plant foliar biomass in the streets. Rows of trees with high leaf biomass should be planted along roads in cities. Low trees are preferable for a given biomass, as lower leaves capture more pollutants (and remove them directly from the air that pedestrians breathe). These trees should have a dense canopy with a high proportion of shade leaves, characterized by a corresponding large SLA. This would increase the uptake of pollutants such as PAHs and

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possibly PM. Therefore, we recommend selecting species with a specific morphology and pruning practices that favour the maintenance of this morphology. Furthermore, as recommended by other authors (e.g. De Nicola et al., 2017), it is better to select perennial species that can remove pollutants throughout the year, especially in winter, when fossil fuel combustion is higher and the planet boundary layer decreases in height for large portion of the day, leading to an increase in the concentration of pollutants in the air of cities.

4. Conclusions

- This research study the intraindividual variability in foliar PAH concentrations in trees by limiting interspecific variation and reducing interindividual variation.
- The intraindividual variability is proved to be significant and should be considered in designing sampling schemes or contamination model development.
- The main source of intraindividual variability is leaf height (bottom, mid or top canopy), with lower leaves taking up more PAHs than higher leaves.
- This depends on leaf morphology (SLA), distance to the emission source (cars), the barrier effect and illumination/temperature of the leaves.
- Therefore, the ideal vegetation to remove PAHs from the air in urban environments similar to the one of this study would be leafy trees (with a high leaf biomass), not too tall and with a high proportion of shade leaves.

CRediT authorship contribution statement

Pablo Giráldez: Formal analysis, Investigation, Visualization, Writing – original draft. **Zulema Varela:** Conceptualization, Funding acquisition, Investigation, Resources, Writing – original draft. **Antonio Di Guardo:** Supervision, Writing – review & editing. **Elisa Terzaghi:** Supervision, Writing – review & editing. **Maria Celeiro:** Investigation, Writing – review & editing. **Carmen Garcia-Jares:** Funding acquisition, Investigation, Writing – review & editing. **J. Ángel Fernández:** Conceptualization, Resources, Validation, Visualization, Writing – review & editing. **Jesús R. Aboal:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Most of the data is available in the supplementary material, and the rest will be available on request.

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Appendix A. Supplementary data

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References

Aho, K., 2022. asbio: a Collection of Statistical Tools for Biologists (R package version 1.8-2). https://cran.r-project.org/package=asbio.
Amada, G., Yoshiko, K., Kitayama, K., Onoda, Y., 2023. Roles of lower-side leaf trichomes in diffusion resistance and gas-exchange characteristics across environmental gradients in Metrosideros polymorpha. Tree Physiol. 43 (8), 1400–1415. https://doi.org/10.1093/TREEPHYS/TPAD053.
Barber, J.L., Thomas, G.O., Kerstiens, G., Jones, K.C., 2004. Current issues and uncertainties in the measurement and modelling of air–vegetation exchange and within-plant processing of POPs. Environ. Pollut. 128 (1–2), 99–138. https://doi.org/10.1016/J.ENVPOL.2003.08.024.
Bickford, C.P., 2016. Ecophysiology of leaf trichomes. Funct. Plant Biol. 43 (9), 807–814. https://doi.org/10.1071/FP16095.
Camargo, M.A.B., Marenco, R.A., 2011. Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. Acta Amazon. 41 (2), 205–212. https://doi.org/10.1590/S0044-59672011000200004.
De Nicola, Flavia, Murena, F., Costagliola, M.A., Alfani, A., Baldantoni, D., Prati, M.V., Sessa, L., Spagnuolo, V., Giordano, S., 2013. A multi-approach monitoring of particulate matter, metals and PAHs in an urban street canyon. Environ. Sci. Pollut. Res. 20 (7), 4969–4979. https://doi.org/10.1007/S11356-012-1456-1.
De Nicola, F., Alfani, A., Maisto, G., 2014. Polycyclic aromatic hydrocarbon contamination in an urban area assessed by Quercus ilex leaves and soil. Environ. Sci. Pollut. Res. 21 (12), 7616–7623. https://doi.org/10.1007/S11356-014-2665-6 (2014 21:12).
De Nicola, F., Concha Graña, E., Aboal, J.R., Carballeira, A., Fernández, J., López Mahía, P., Prada Rodríguez, D., Muniategui Lorenzo, S., 2016. PAH detection in Quercus robur leaves and Pinus pinaster needles: a fast method for biomonitoring purpose. Talanta 153, 130–137. https://doi.org/10.1016/J.TALANTA.2016.01.067.
De Nicola, Flavia, Concha Graña, E., López Mahía, P., Muniategui Lorenzo, S., Prada Rodríguez, D., Retuerto, R., Carballeira, A., Aboal, J.R., Fernández, J.Á., 2017. Evergreen or deciduous trees for capturing PAHs from ambient air? A case study. Environ. Pollut. 221, 276–284. https://doi.org/10.1016/J.ENVPOL.2016.11.074.
Desalme, D., Binet, P., Chiapusio, G., 2013. Challenges in tracing the fate and effects of atmospheric polycyclic aromatic hydrocarbon deposition in vascular plants. Environ. Sci. Technol. 47 (9), 3967–3981. https://doi.org/10.1021/ES304964B.
Dias, A.P.L., Rinaldi, M.C.S., Domingos, M., 2016. Foliar accumulation of polycyclic aromatic hydrocarbons in native tree species from the Atlantic Forest (SE-Brazil). Sci. Total Environ. 544, 175–184. https://doi.org/10.1016/J.SCITOTENV.2015.11.092.
EEA, 2022. Health impacts of air pollution in Europe, 2022 — European Environment Agency. https://www.eea.europa.eu/publications/air-quality-in-europe-2022/health-impacts-of-air-pollution.
EPA, 2023. About Urban Air Toxics | US EPA. https://www.epa.gov/urban-air-toxics/about-urban-air-toxics.
Fasani, D., Fermo, P., Barroso, P.J., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2016. Analytical method for biomonitoring of PAH using leaves of bitter orange trees (Citrus aurantium): a case study in South Spain. Water Air Soil Pollut. 227 (10), 1–11. https://doi.org/10.1007/S11270-016-3056-Z.
Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression (Third). https://socialsciences.mcmaster.ca/jfox/Books/Companion/.
Franzetti, A., Gandolfi, I., Bestetti, G., Padoa Schioppa, E., Canedoli, C., Brambilla, D., Cappelletti, D., Sebastiani, B., Federici, E., Papacchini, M., Ambrosini, R., 2020. Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. J. Hazard. Mater. 384, 121021 https://doi.org/10.1016/J.JHAZMAT.2019.121021.
Gara, T.W., Darvishzadeh, R., Skidmore, A.K., Wang, T., 2018. Impact of vertical canopy position on leaf spectral properties and traits across multiple species. Remote Sens. 10 (2), 346. https://doi.org/10.3390/rs10020346.
Garcia-Jares, C., Sanchez-Nande, M., Lamas, J.P., Lores, M., 2017. Profiling the fatty acids content of ornamental Camellia seeds cultivated in Galicia by an optimized matrix solid-phase dispersion extraction. Bioengineering 4 (4), 87. https://doi.org/10.3390/BIOENGINEERING4040087, 2017, Vol. 4, Page 87.
Giráldez, P., Aboal, J.R., Fernández, J.Á., Di Guardo, A., Terzaghi, E., 2022. Plant-air partition coefficients for thirteen urban conifer tree species: estimating the best gas and particulate matter associated PAH removers. Environ. Pollut. 315, 120409 https://doi.org/10.1016/J.ENVPOL.2022.120409.
Gómez-Brandón, M., Lores, M., Domínguez, J., 2008. Comparison of extraction and derivatization methods for fatty acid analysis in solid environmental matrixes. Anal. Bioanal. Chem. 392 (3), 505–514. https://doi.org/10.1007/S00216-008-2274-7.
Herrera, C.M., 2009. Multiplicity in unity: plant subindividual variation and interactions with animals (University of Chicago Press (Ed.)). University of Chicago Press. https://digital.csic.es/handle/10261/38723.
Hofman, J., Stokkaer, I., Snauwaert, L., Samson, R., 2013. Spatial distribution assessment of particulate matter in an urban street canyon using biomagnetic leaf monitoring of

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tree crown deposited particles. Environ. Pollut. 183, 123–132. https://doi.org/10.1016/J.ENVPOL.2012.09.015.
Howsam, M., Jones, K.C., Ineson, P., 2001. PAHs associated with the leaves of three deciduous tree species. II: uptake during a growing season. Chemosphere 44 (2), 155–164. https://doi.org/10.1016/S0045-6535(00)00268-X.
IARC (Ed.), 2010. Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Occupational Exposures, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer.
Janhäll, S., 2015. Review on urban vegetation and particle air pollution – deposition and dispersion. Atmos. Environ. 105, 130–137. https://doi.org/10.1016/J.ATMOSENV.2015.01.052.
Jiang, Y., Chen, X., Ma, J., Liang, S., Huang, J., Liu, R., Pan, Y., 2020. Interspecific and Intraspecific Variation in Functional Traits of Subtropical Evergreen and Deciduous Broadleaved Mixed Forests in Karst Topography, Guilin, Southwest China. Doi: 10.1177/1940082916680211, 9(4). https://doi.org/10.1177/1940082916680211.
Jouraeva, V.A., Johnson, D.L., Hassett, J.P., Nowak, D.J., 2002. Differences in accumulation of PAHs and metals on the leaves of Tilia×euchlora and Pyrus calleryana. Environ. Pollut. 120 (2), 331–338. https://doi.org/10.1016/S0269-7491(02)00121-5.
Kampa, M., Castanas, E., 2008. Human health effects of air pollution. Environ. Pollut. 151 (2), 362–367. https://doi.org/10.1016/J.ENVPOL.2007.06.012.
Kassambara, A., Mundt, F., 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses (R package version 1.0.7). https://cran.r-project.org/package=factoextra.
Krugly, E., Martuzevicius, D., Sidaraviciute, R., Ciuzas, D., Prasauskas, T., Kauneliene, V., Stasiulaitiene, I., Kliucininkas, L., 2014. Characterization of particulate and vapor phase polycyclic aromatic hydrocarbons in indoor and outdoor air of primary schools. Atmos. Environ. 82, 298–306. https://doi.org/10.1016/J.ATMOSENV.2013.10.042.
Liang, J., Fang, H., Zhang, T., Wang, X., 2017. Polycyclic aromatic hydrocarbons in the leaves of twelve plant species along an urbanization gradient in Shanghai, China. Environ. Sci. Pollut. Res. 24 (10), 9361–9369. https://doi.org/10.1007/S11356-017-8552-1.
Liu, Y., Dawson, W., Prati, D., Haeuser, E., Feng, Y., Van Kleunen, M., 2016. Does greater specific leaf area plasticity help plants to maintain a high performance when shaded? Ann. Bot. 118 (7), 1329–1336. https://doi.org/10.1093/AOB/MCW180.
Mangiafico, S., 2022. rcompanion: Functions to Support Extension Education Program Evaluation (R package version 2.4.18). https://cran.r-project.org/package=rcompanion.
Murakami, M., Abe, M., Kakumoto, Y., Kawano, H., Fukasawa, H., Saha, M., Takada, H., 2012. Evaluation of ginkgo as a biomonitor of airborne polycyclic aromatic hydrocarbons. Atmos. Environ. 54, 9–17. https://doi.org/10.1016/J.ATMOSENV.2012.02.014.
Nowak, D.J., Hirabayashi, S., Bodine, A., Greenfield, E., 2014. Tree and forest effects on air quality and human health in the United States. Environ. Pollut. 193, 119–129. https://doi.org/10.1016/J.ENVPOL.2014.05.028.
Piracha, A., Chaudhary, M.T., 2022. Urban air pollution, urban heat island and human health: a review of the literature. Sustainability 14 (15), 9234. https://doi.org/10.3390/SU14159234, 2022, Vol. 14, Page 9234.
Pleijel, H., Klingberg, J., Strandberg, B., Sjöman, H., Tarvainen, L., Wallin, G., 2022. Differences in accumulation of polycyclic aromatic compounds (PACs) among eleven broadleaved and conifer tree species. Ecol. Indic. 145, 109681 https://doi.org/10.1016/J.ECOLIND.2022.109681.
Prigioniero, A., Zuzolo, D., Niinemets, Ü., Postiglione, A., Mercurio, M., Izzo, F., Trifuoggi, M., Toscanesi, M., Scarano, P., Tartaglia, M., Sciarrillo, R., Guarino, C., 2022. Particulate matter and polycyclic aromatic hydrocarbon uptake in relation to leaf surface functional traits in Mediterranean evergreens: potentials for air phytoremediation. J. Hazard. Mater. 435, 129029 https://doi.org/10.1016/J.JHAZMAT.2022.129029.
Prigioniero, A., Postiglione, A., Zuzolo, D., Niinemets, Ü., Tartaglia, M., Scarano, P., Mercurio, M., Germinario, C., Izzo, F., Trifuoggi, M., Toscanesi, M., Sciarrillo, R., Guarino, C., 2023. Leaf surface functional traits influence particulate matter and polycyclic aromatic hydrocarbons air pollution mitigation: insights from Mediterranean urban forests. J. Clean. Prod. 418, 138158 https://doi.org/10.1016/J.JCLEPRO.2023.138158.
Ray, D., Ghosh, S.K., Raha, S., 2021. Seasonal foliar uptake of atmospheric polycyclic aromatic hydrocarbons by some local plants in a tropical metropolis in India. Atmos. Pollut. Res. 12 (2), 104–112. https://doi.org/10.1016/J.APR.2020.10.013.
RStudio Team, 2021. RStudio: Integrated Development Environment for R. RStudio, PBC. http://www.rstudio.com/.
Terzaghi, E., Wild, E., Zacchello, G., Cerabolini, B.E.L., Jones, K.C., Di Guardo, A., 2013. Forest filter effect: role of leaves in capturing/releasing air particulate matter and its associated PAHs. Atmos. Environ. 74, 378–384. https://doi.org/10.1016/J.ATMOSENV.2013.04.013.
Terzaghi, E., Scacchi, M., Cerabolini, B., Jones, K.C., Di Guardo, A., 2015a. Estimation of polycyclic aromatic hydrocarbon variability in air using high volume, film, and vegetation as samplers. Environ. Sci. Technol. 49 (9), 5520–5528. https://doi.org/10.1021/ES5056929/SUPPL_FILE/ES5056929_SI_001.PDF.
Terzaghi, E., Zacchello, G., Scacchi, M., Raspa, G., Jones, K.C., Cerabolini, B., Di Guardo, A., 2015b. Towards more ecologically realistic scenarios of plant uptake modelling for chemicals: PAHs in a small forest. Sci. Total Environ. 505, 329–337. https://doi.org/10.1016/J.SCITOTENV.2014.09.108.
Terzaghi, E., Morselli, M., Semplice, M., Cerabolini, B.E.L., Jones, K.C., Freppaz, M., Di Guardo, A., 2017. SoilPlusVeg: an integrated air-plant-litter-soil model to predict organic chemical fate and recycling in forests. Sci. Total Environ. 595, 169–177. https://doi.org/10.1016/j.scitotenv.2017.03.252.
Terzaghi, E., De Nicola, F., Cerabolini, B.E.L., Posada-Baquero, R., Ortega-Calvo, J.J., Di Guardo, A., 2020. Role of photo- and biodegradation of two PAHs on leaves: modelling the impact on air quality ecosystem services provided by urban trees. Sci. Total Environ. 739, 139893 https://doi.org/10.1016/j.scitotenv.2020.139893.
Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., Liu, C., 2019. Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: morphological characteristics have the greatest impact. Sci. Total Environ. 652, 1149–1155. https://doi.org/10.1016/J.SCITOTENV.2018.10.357.
Trapp, S., 2015. Calibration of a plant uptake model with plant- and site-specific data for uptake of chlorinated organic compounds into radish. Environ. Sci. Technol. 49 (1), 395–402. https://doi.org/10.1021/ES503437P/SUPPL_FILE/ES503437P_SI_001.PDF.
Undeman, E., Czub, G., Mclachlan, M.S., 2009. Addressing temporal variability when modeling bioaccumulation in plants. Environ. Sci. Technol. 43 (10), 3751–3756. https://doi.org/10.1021/ES900265J/SUPPL_FILE/ES900265J_SI_001.PDF.
United Nations Department of Economic and Social Affairs Population Division, 2019. World Urbanization Prospects: The 2018 Revision. United Nations.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics With S (Fourth). Springer.
Vinod, N., Slot, M., McGregor, I.R., Ordway, E.M., Smith, M.N., Taylor, T.C., Sack, L., Buckley, T.N., Anderson-Teixeira, K.J., 2023. Thermal sensitivity across forest vertical profiles: patterns, mechanisms, and ecological implications. New Phytol. 237 (1), 22–47. https://doi.org/10.1111/NPH.18539.
Wagrowski, D.M., Hites, R.A., 1997. Polycyclic aromatic hydrocarbon accumulation in urban, suburban, and rural vegetation. Environ. Sci. Technol. 31 (1), 279–282. https://doi.org/10.1021/ES960419I.
Wania, F., McLachlan, M.S., 2001. Estimating the influence of forests on the overall fate of semivolatile organic compounds using a multimedia fate model. Environ. Sci. Technol. 35 (3), 582–590. https://doi.org/10.1021/ES0011919.
Wei, T., Simko, V., 2021. "corrplot": Visualization of a Correlation Matrix (Version 0.90). https://github.com/taiyun/corrplot.
World Health Organization, 2022. Ambient (outdoor) air pollution, World Health Organization. https://www.who.int/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health.
Yin, S., Tan, H., Hui, N., Ma, Y., Tian, L., Sun, N., Liu, C., 2020. Polycyclic aromatic hydrocarbons in leaves of Cinnamomum camphora along the urban–rural gradient of a megacity: distribution varies in concentration and potential toxicity. Sci. Total Environ. 732, 139328 https://doi.org/10.1016/J.SCITOTENV.2020.139328.

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Chapter III: Plant-air partition coefficients for thirteen urban conifer tree species: Estimating the best gas and particulate matter associated PAH removers

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Plant-air partition coefficients for thirteen urban conifer tree species: Estimating the best gas and particulate matter associated PAH removers

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ABSTRACT

Polycyclic Aromatic Hydrocarbons (PAHs) are an important class of pollutants which mostly come out from incomplete combustion of organic materials including fossil fuels. For this reason, they are often found at high concentrations in cities, contaminating air with their gas and particle phase. While European Union policies try to reduce their concentrations, huge efforts are still devoted to mitigate the pollution by PAHs. One such measure of mitigation is the use of plants in capturing PAHs and other chemicals due to the ability of leaves in filtering both gas and particle fractions. In this work thirteen conifer species among those frequently living in temperate and Mediterranean cities were selected and concentrations of 16 PAHs were measured in needles. The data for spruce were used to back-calculate air concentrations of PAHs which were later used to obtain specific equations of plant-air partition coefficient (K_{PA}). The results showed that the difference in total PAH uptake between the most (*Pinus pinaster*) and the least (*Picea abies*) efficient tree was close to an order of magnitude. Looking to the capability of the different species in enriching the particulate matter (PM) associated fraction of PAHs on their needles, *Pinus pinaster* was instead the least important (3.4% of total PAHs), while *Cupressus lusitanica* was the most important (34% of total PAHs). The new K_{PA} equations can be used to fine tune the PAH uptake of a specific amount of plant biomass on air concentration reduction.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced as a result of incomplete combustion of a variety of organic materials such as fossil fuels, wood, and biofuels (Kim et al., 2013). These pollutants seriously affect human health (IARC, 2010; WHO, 2000), reducing the quality of life and life span, which also entails a significant economic cost (WHO, 2021).

Policies against climate change such as the European Union Green Deal (EU, 2022) aim at abating the use of fossil fuels, which would reduce the anthropogenic concentration of PAHs in the atmosphere. Although the success of these guidelines could even cut off the anthropogenic emission budget of these compounds, PAHs still represent an environmental and human health issue. One of the strategies that could help to reduce and mitigate the concentrations of these pollutants in the atmosphere is their uptake by trees, called "filter effect" (McLachlan and Horstmann, 1998; Terzaghi et al., 2013). Thus, the choice of tree and shrub species used in urban environments should not be based solely on ornamental criteria but according to their air filtering potential.

The ability to filter the air of course depends not only on the chemical balance between the air and the leaf, but also on the characteristics and growth pattern of each species (De Nicola et al., 2017). One of the most important characteristics would be the presence of leaves throughout the year, which would allow pollutants to be continuously captured, unlike what happens with deciduous species (De Nicola et al., 2017). In addition, leaf fall occurs during the coldest months of the year, which coincides with the period of maximum concentration of PAHs in the air, due to the higher production derived from heating systems and traffic (Wang et al., 2017), as well as to low levels of photo- and thermo-degradation of compounds (Park et al., 2002), and to the decrease in the height of the planet boundary layer (PBL) (Chan and Wood, 2013; Morselli et al., 2011).

The lack of information and comparative studies, given the large number of ornamental species, complicate decision making in this regard. To quantify the role of plants and specific plant species in removing PAH from air, dynamic environmental fate models, incorporating plants in the mass balance could be employed (Terzaghi et al., 2017).

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These models would allow to identify which species are better, and to what extent, in capturing organic pollutants. These models require parameterization of the environmental scenario (including for example the atmospheric conditions, such PBL and other meteorological variables), emission strength (such as information on traffic, biomass burning, etc.), and the factors ruling the uptake of PAH in plant biomass. One of such parameters in these models is the plant-air partition coefficient (K_{PA}), which indicates the ratio, at equilibrium, between the concentration of a chemical in plant leaves and in air (Kömp and McLachlan, 1997). K_{PA}, together with parameters such as Leaf area Index (LAI) and Specific Leaf Area (SLA), allows to calculate the uptake capacity of plant biomass, in other terms the efficiency of the species in capturing PAHs and other chemicals from air (Terzaghi et al., 2015b). However, until now a few works provided equations to estimate K_{PA} for a handful of species (Bacci et al., 1990; Bolinius et al., 2016; Horstmann and McLachlan, 1998; Nizzetto et al., 2008) and for most species and chemicals, this information is not readily available.

The objective of this work was to estimate K_{PA} for 13 ornamental conifer species usually employed in parks and streets around the world and compare their ability in removing gas and particle associated PAHs in urban areas. This would allow to select the best conifers for PAH removal in cities.

2. Materials and methods

2.1. Sampling and samples processing

Sampling was conducted between November 6th and 8th 2017, at the University Campus of the University of Santiago de Compostela (see Fig. 1SM). Santiago de Compostela is a city in the NW of Galicia (NW Spain). The altitude of the city is 260 m a.s.l., it has an area of 220 km^2 and a population of 97 848 inhabitants (2020). It has an oceanic climate with an average annual temperature of 13.0 °C and a precipitation of 1787 mm distributed among 140 rainy days (>1 mm) per year (climatological normal values in Santiago de Compostela between 1981 and 2010; http://www.aemet.es/). Santiago de Compostela is a small-medium sized European city, with a residential character, far from heavy industrial pollution. The main sources of PAHs pollution are vehicle traffic and home heating during the coldest months of the year.

Twenty-three trees corresponding to thirteen species (plus 1 cultivar) and 3 families were sampled (see Table 1). In each tree, 5 branches located at different orientations and at a height of 5 m were cut and kept in a tray in cold storage at 5 °C (for up to 4 days). From these, the leaves of the current year were selected. In cases where the leaves were in scaly clusters (i.e., *Cupressus* sp.), the entire green (photosynthetically active) part was selected. Once selected, a composite sample (between 100 and 200 g f.w.) was made for each tree. These samples were dried in separated paper envelopes at 30 °C in a universal precision oven (model Digitronic-TFT; J.P. Selecta®), then homogenized in an ultra-centrifugal mill (ZM 200; Retsch®) at 12 000 rpm and finally stored at 20 °C in darkness in hermetically closed glass vials until PAH analysis.

Table 1
List of species and number of trees sampled. O: order (C: Cupresales; P: Pinales); F: family (Cu: Cupresaceae; Tx: Taxaceae; Pi: Pinaceae); n: number of sampled trees.

O	F	Scientific name	Abbr. in charts	Common name	n
C	Cu	*Chamaecyparis pisifera* (Siebold & Zucc.) Endl.	Cp	Sawara cypress	1
C	Cu	*Cryptomeria japonica* (Thunb. ex L.f.) D.Don	Cj	Japanese cedar	2
C	Cu	*Cryptomeria japonica* f. *elegans* (Jacob-Makoy) Beissn.	Cje	Elegant Japanese cedar	3
C	Cu	*Cupressus arizonica* Greene	Ca	Arizona cypress	2
C	Cu	*Cupressus lusitanica* Mill.	Cl	Mexican cypress	3
C	Cu	*Cupressus sempervirens* L.	Cs	Italian cypress	1
C	Cu	*Juniperus chinensis* L.	Jc	Chinese juniper	1
C	Cu	*Sequoia sempervirens* (D.Don) Endl.	Ss	Coastal Redwood	1
C	Cu	*Thujopsis dolabrata* (L.f.) Siebold & Zucc.	Td	Hiba	1
C	T	*Taxus baccata* L.	Tb	Common yew	3
P	Pi	*Cedrus atlantica* (Endl.) Manetti ex Carrière	Cat	Atlas cedar	1
P	Pi	*Cedrus deodara* (Roxb. ex D.Don) G.Don	Cd	Deodar cedar	1
P	Pi	*Picea abies* (L.) H.Karst.	Pa	Norway spruce	1
P	Pi	*Pinus pinaster* Aiton	Pp	Maritime pine	2

Samples were collected after 3 days without rainfall greater than 1 mm. The mean temperature during the sampling was 8.9 °C. Temperature data were available from a weather station located at a maximum distance of 650 m from the sampled trees (see Fig. 1SM). The weather station belongs to the regional government weather stations network (https://www.meteogalicia.gal/) which report temperature data each 10 min carried out with a HMP155 (Vaisala) sensor and a datalogger CR1000 (Campbell).

2.2. SLA measurement

SLA was measured to investigate its influence on PAH uptake in conifers. To determine the specific leaf area (SLA), 5 branches were pruned from each individual and 4 leaves per branch were selected. Photographs of the leaves were taken and processed with Image J (version 1.50i) digital image processing software (Rasband and ImageJ, 1997) to determine their area. The leaves were then dried at 100 °C in an oven for 3–5 days and weighed with precision balances (model C–3000CB; Cobos®). The final SLA used for each tree is the mean of the 20 leaves.

2.3. PAH determination in needles

The investigated PAHs were acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo(a)anthracene (B(a)A), chrysene (Chr), benzo(b+j)fluoranthene (B(b+j)F), benzo(k)fluoranthene (B(k)F), benzo(e)pyrene (B(e)P), benzo(a)pyrene (B(a)P), dibenzo(a,h)anthracene (D(ah)A), indeno(1,2,3-cd)pyrene (Ind) and benzo(ghi)perylene (B(ghi)P). The content of these PAHs in needles were measured by the Applied Analytical Chemistry research group (QANAP, University of Coruña) applying a MSPD-PTV-GC–MS/MS method, that is, a matrix solid-phase dispersion extraction method followed by programmed temperature vaporization-gas chromatography-tandem mass spectrometry developed by this group (De Nicola et al., 2017; De Nicola et al., 2016). Anthracene-d10 was used as internal standard to quantify all PAHs except dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene-d12 and benzo(g,h,i)perylene; whereas dibenz(a,h)anthracene-d14 was used as internal standard. Peak identification and integration were performed by Excalibur 2.0.7 software from Thermo Fisher Scientific Inc. This method, optimized for *P. pinaster*, was used for all species except *C. japonica* and *C. japonica* var. *elegans*, where some pigments interfered with the determinations, and it was necessary to modify the purification method and add 0.5 g of Primary Secondary Amine (PSA), because this sorbent has strong affinity and high capacity for removing fatty acids, organic acids, and some polar pigments and sugars (De Nicola et al., 2016). Despite this modification, in two of the *C. japonica* var. *elegans* trees and one of the *C. japonica* trees the mass at which phenanthrene and anthracene are quantified was interfered. Most of the rest of the species measured with the method optimized for *P. pinaster* did not present problems in the measurement. The only exceptions were the interference of naphtalene in *C. pisifera* and the low recoveries (20–30%) of B(a)P in *Taxus baccata* and B(ghi)P in *Taxus baccata* and *C. lusitanica*. These interfered or low recovery samples were eliminated from the rest of the analysis. In addition, in one of the

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C. lusitanica trees, B(b+j)F, B(k)F, B(e)P and B(a)P had abnormally high concentration, so they were also removed for data processing.

2.4. Quality assurance/quality control (QA/QC)

Instrumental and procedural blanks, i.e., surrogates spiked on the C_{18} without matrices, were systematically evaluated (De Nicola et al., 2016). Quality control of the complete procedure and quantification were performed using labeled PAHs as surrogate standards. Chromatographic control was performed by the injection of two standard solutions containing all target PAHs for each batch. The LOQs for each PAHs correspond to those shown in De Nicola et al. (2016).

2.5. PAH air concentration estimation

Since PAH air concentrations were not available for Santiago de Compostela during the sampling period, they were back-calculated from *P.abies* needle concentrations and K_{PA} equation available in (Nizzetto et al., 2008) for the same species (Eq. (1)):

$$\log K_{PA} = -1.75 + 0.33 \log K_{OA} \quad (1)$$

where Log K_{PA} is the plant-air partition coefficient on a mass/volume basis ($m^3\ g^{-1}$) at 25 °C and Log K_{OA} is the octanol-air partition coefficient calculated from each PAH physico-chemical properties (Mackay et al., 2006) (see Table 1SM).

K_{PA} was temperature updated according to the van't Hoff relationship reported in (Kömp and McLachlan, 1997) (Eq. (2)):

$$K_{PA}(T) = K_{PA}(T_0) exp\left[\left(\frac{1}{T} - \frac{1}{T_0}\right)\left(\frac{\Delta H_{PA}}{R}\right)\right] \quad (2)$$

where $K_{PA}(T)$ is the plant/air partition coefficient updated with temperature of the time of sampling (K), $K_{PA}(T_0)$ is plant/air partition coefficient at the reference temperature (T_0 = 298.15 K), ΔH_{PA} is the enthalpy of phase change between plant and air (kJ mol^{-1}) (estimated according to (Kömp and McLachlan, 1997) and R is the gas constant (kJ $mol^{-1}\ K^{-1}$).

Please note that the temperature of the time of sampling was chosen as the average temperature of the three days preceding sampling (i.e., 8.9 °C), according to the leaf-air PAH equilibration time (Terzaghi et al., 2015a).

PAH air concentrations were calculated considering that at equilibrium K_{PA} is the ratio between concentrations in plant leaves (C_P) (ng g^{-1} dw) and in air (C_A) (ng m^{-3}) (Eq. (3) **and** Eq. (4)):

$$K_{PA} = \frac{C_P}{C_A} \quad (3)$$

$$C_A = \frac{C_P}{K_{PA}} \quad (4)$$

Since the concentration of Acy, D(ah)A and B(ghi)P in *P. abies* were below the analytical limit of quantification (LOQ), the air concentrations of these congeners could not be calculated.

2.6. Development of new plant-air partition coefficient (K_{PA}) equations

K_{PA} for the investigated species was calculated considering the PAH leaf measured concentrations and calculated air concentrations (see Eq. (3)). New species-specific K_{PA} equations were then developed using Log K_{OA} as predictor of air-leaf partitioning of PAHs. More specifically, three different models were developed: i) a linear regression model with the PAHs mostly gas phase associated (Log $K_{OA} < 10$) (model 1); ii) a linear regression model with all PAHs (model 2); and iii) a 2nd degree polynomial regression model with all PAHs (model 3).

3. Results and discussion

3.1. PAH concentrations in leaves

The investigated plant species showed a different PAH accumulation behavior, considering total PAHs but also single compounds (Fig. 1). Total PAH concentrations ranged from 845 ng g^{-1} dw for *P.pinaster* to 95 ng g^{-1} dw for *P. abies*. Therefore, species belonging to the same family, showed concentrations that varied up to one order of magnitude. The most abundant gas phase PAHs were Phe, Fla and Pyr representing 49–89% of total PAH concentration in all species. Among particle phase PAHs, Chr was the most abundant, representing about 2–12% of total PAH concentration in all species.

Although PAH fingerprint were comparable among the investigated species, some few exceptions appeared: *C. japonica* f. *elegans* showed an higher percentage of Fle (25% of total PAHs with respect to an average of 9% for the rest of the species) and *C. lusitanica* was characterized by a higher contribution of B(b+j)F (17% of total PAHs with respect to an average of 3% for the rest of the species).

Most of the species used in this study are common ornamental species and their leaves have been previously used in studies on particulate matter (PM) content (Sæbø et al., 2012; Ozdemir, 2019; Tang et al., 2019), but for many of them PAH levels were here determined for the first time (i.e., *C. japonica* and *C. japonica elegans*, *C. sempervirens*, *S. sempervirens*, *C. pisifera*, *C. arizonica*, *C. lusitanica* and *T. dolabrata*). The concentrations measured were similar to those obtained in previous studies with *P. pinaster* in urban areas sampled in Portugal in 2007 (Ratola et al., 2010) and *P. abies* sampled in Norway (Borgulat et al., 2018), but higher than those measured for *P. pinaster* needles (average total PAH concentration of 320 and 137 ng g^{-1} dw) collected in rural sites located in the same region of the current study (De Nicola et al., 2016, 2017). Some measurements are also available for *C. atlantica* (average total PAH concentration of 9 ng g^{-1} dw) (Al-Alam et al., 2017), *C. deodara* (average total PAH concentration of 86 ng g^{-1} dw in autumn) (Franzetti et al., 2020), *J. chinensis* (total PAH concentration of 1975 ng g^{-1} dw) (Tian et al., 2019) and *T. baccata* (average total PAH concentration of 243 ng g^{-1} dw) (Walgraeve et al., 2017) collected in different countries (France, Italy, China and Belgium), urban sites and years (2015, 2016 and 2018) and therefore representative of different contamination levels.

3.2. Estimated PAH concentrations in air

Estimated PAH air concentrations ranged from 0.4 to 775 pg m^{-3} for B(e)P and Fle, respectively (Table 2), while total PAHs concentrations were 2672 pg m^{-3}. These numbers were generally lower than those measured in other European urban and semi-urban sites (Terzaghi et al.,

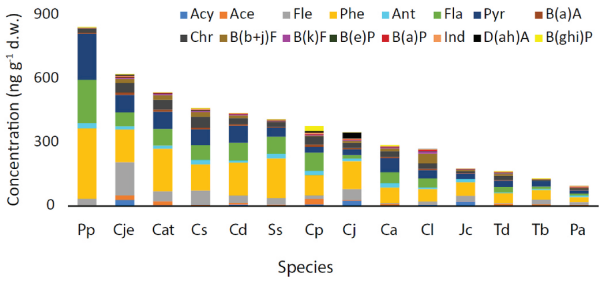


Fig. 1. PAH concentrations (ng g^{-1} dw) in leaves of the studied species. Pp: *Pinus pinaster*; Cje: *Cryptomeria japonica f. elegans*; Cat: *Cedrus atlantica*; Cs: *Cupressus sempervirens*; Cd: *Cedrus deodara*; Ss: *Sequoia sempervirens*; Cp: *Chamaecyparis pisifera*; Cj: *Cryptomeria japonica*; Ca: *Cupressus arizonica*; Cl: *Cupressus lusitanica*; Jc: *Juniperus chinensis*; Td: *Thujopsis dolabrata*; Tb: *Taxus baccata*; Pa: *Picea abies (L.)*.

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Table 2
Temperature updated plant-air partition coefficient (Log K_{PA}) obtained for Picea abies and PAH air concentrations.

PAH	Log K_{PA} T° updated (m^3 g^{-1})	Air concentration (pg m^{-3})
Ace	0.97	587.98
Fle	1.18	774.74
Ant	1.47	233.46
Phe	1.49	793.26
Fla	1.97	96.29
Pyr	1.99	156.90
B(a)A	2.46	5.24
Chr	2.67	15.94
B(e)P	2.99	0.41
B(a)P	2.97	1.92
B(b+j)F	3.12	3.11
B(k)F	3.12	1.21
Ind	3.44	1.20

2021). The University Campus resides in an open environment, characterized by many green spaces and few buildings (mainly faculties and some halls of residence). This reveals that the main PAH sources are vehicles circulating in the Campus and, to a lesser extent, the heating system of the Campus buildings. In addition, the contribution of polluted air masses from more populated areas of the city would be reduced since the Campus is located NW of these areas and the wind blows prevalently in the NE direction.

Very few works focusing on PAH air concentration measurement are available for Spain. Moreover, most of the investigations focused just on the PAH fraction associated with PM (e.g., Lara et al., 2022; Oleagoitia et al., 2019) or were carried out in sites characterized by different PAH sources (e.g., petrochemical area of Tarragona (Domínguez-Morueco et al., 2017),). The most comparable study was that performed in Ciudad Real by Villanueva et al. (2018) where the PAHs concentrations in outdoor air were obtained using passive samplers. The concentrations determined by Villanueva et al. (2018) were slightly higher than those estimated in this study (average total PAH concentration of about 12 ng m^{-3} vs 2.7 ng m^{-3}). This was expected since their sampling location is closer to an emission source (petrol station).

3.3. New K_{PA} equations

The results of the present study allowed to develop new K_{PA} equations useful for the prediction of PAH uptake by plant leaves from air, basing on the Log K_{OA} of the compounds. Fig. 2 shows the linear fit obtained considering gas phase PAHs (Log $K_{OA} < 10$) (model 1) and the 2nd degree polynomial model fitted on all data (i.e., gas + particle phase PAHs) (model 3). The parameters of the models 1, 2 (gas + particle phase PAHs, linear model) and 3 are shown in Table 3, Table 2SM and Table 3SM respectively. As expected, Log K_{PA} increased with the increase in Log K_{OA}. However, at Log K_{OA} larger than 10, Log K_{PA} reached a plateau and/or dropped indicating a kinetic delay in partitioning (Mackay et al., 2019) for the most hydrophobic compounds which are mainly bound to PM, which compete with leaf biomass for PAH uptake (McLachlan and Horstmann, 1998). Moreover, the equation used to predict K_{PA} for *Picea abies* and back-calculate air concentrations, was selected since it was developed for PCBs (Log K_{OA} ranging from 7.5 to 10.5) (Nizzetto et al., 2008) and allowed to better estimate gas phase PAH air concentration since it neglected the particle contribution. For the same reason, air concentrations of particle associated PAHs are probably overestimated, resulting in lower K_{PA} when the back-calculated air concentrations were used to derive species-specific K_{PA} equations (Fig. 2). The species are shown in decreasing order of the slope of the model 1. Fig. 2SM shows all linear models fitted on PAHs associated with the gas phase for all species. For the sake of comparison, species of the Cupressaceae family are represented by a solid line, those of the Pinaceae family by a dashed line and the Taxaceae family by a dotted line.

Equation developed considering gas phase PAHs (model 1, red shaded in Fig. 2), showed r^2 ranging from 0.66 (*Pinus pinaster*) to 0.98 (*Juniperus chinensis*) considering sampling time temperature and from 0.56 to 0.92 for the same species considering reference temperature. The low r^2 obtained for *Pinus pinaster* could be probably explained by the high concentrations of Phe, Fla and Pyr that resulted in higher K_{PA} compared to the other compounds with higher Log K_{OA}. This is probably due to the lipid content of pine epicuticular waxes. As commented by Ratola et al. (2011) and Fernández-Varela et al. (2015), when comparing *P. pinaster* and *P. pinea*, these waxes seem to be behind the higher affinity that *P. pinaster* has for lighter PAHs.

The investigated species showed a different accumulation efficiency for gas phase PAHs as shown by the different slopes of their K_{PA} equations. Gentle slopes indicate minor differences in the affinity of a species for PAHs characterized by different hydrophobicity (i.e., Log K_{OA}). Apart from the *P. abies* model, which was taken as the reference for estimating air concentrations, the models with the lowest slopes are those of *J. chinensis* and *T. baccata*. These species are among the 4 species with the lowest total PAH concentrations (Fig. 1), indicating their weak affinity for PAHs in general. Equations of the species *P. pinaster*, *C. deodara*, *T. dolabrata*, and *Cupressus* spp. showed the steepest slopes although their affinity for total PAHs varies. The case of *P. pinaster* is different, since the slope of its model is due to the high concentration of Phe, Fla and Pyr. As for PAH total concentrations in leaves, no relationships between slopes and any studied botanical families was found. The slope of the equations obtained considering also particle bound PAHs (model 2) are generally lower due to the uncertainties in air concentrations explained above (K_{PA} underestimation for the most hydrophobic PAHs).

3.4. Role of particulate matter in PAH leaf uptake

Particle associated PAH models (blue shaded areas) are also shown in Fig. 2. When comparing linear model 1 (gas-phase PAHs) and polynomial model (all PAHs) it can be noted that in some species the two models may diverge when Log K_{OA} is larger than 10. Species with a limited difference in slope in the two models show a more linear trend of PAH accumulation, probably due to the enhanced accumulation of particle associated PAHs compared to other species.

This means that the models developed in this study could provide new and important information about the loading of PAHs associated with the gas phase (Log $K_{OA} < 10$), as well as particle phase by trees, specifically ornamentally used perennial conifers. As seen in the previous section, basing on the information obtained from the K_{PA} of these PAHs, in conjunction with their concentrations in leaves, an initial evaluation of the suitability of the leaves and needles of these species as air filterers can be made. However, to make a complete evaluation it would be necessary to consider the PM and the PM associated PAHs (Log $K_{OA} > 10$). Since PM in air contains a great amount of the most hydrophobic PAHs (Omar et al., 2002; Moscoso-Pérez et al., 2010; Lara et al., 2022), a relevant part of the PAHs removed by trees are captured in the form of PM associated PAHs (e.g., Jouraeva et al., 2002). Although the information necessary to obtain reliable specific deposition velocity estimates for the PM associated PAHs present on leaves is lacking (e.g., Terzaghi et al., 2013), a rank of the capability of the species to retain particle associated PAHs is possible by analyzing the contribution of less or more hydrophobic compounds to the total PAH concentrations. Fig. 3 shows the percentage of PAHs with Log $K_{OA} > 10$ (blue bars) (particle phase) and with Log $K_{OA} < 10$ (red bars) (gas phase) in each species.

The percentage of PM associated PAHs is always smaller than that of gas phase PAHs. However, the percentage of PM associated PAHs, and therefore the leaf sequestration ability for PM varies greatly among the species in this study, ranging from 3.4% in *P. pinaster* to 34.4% in *C. lusitanica*.

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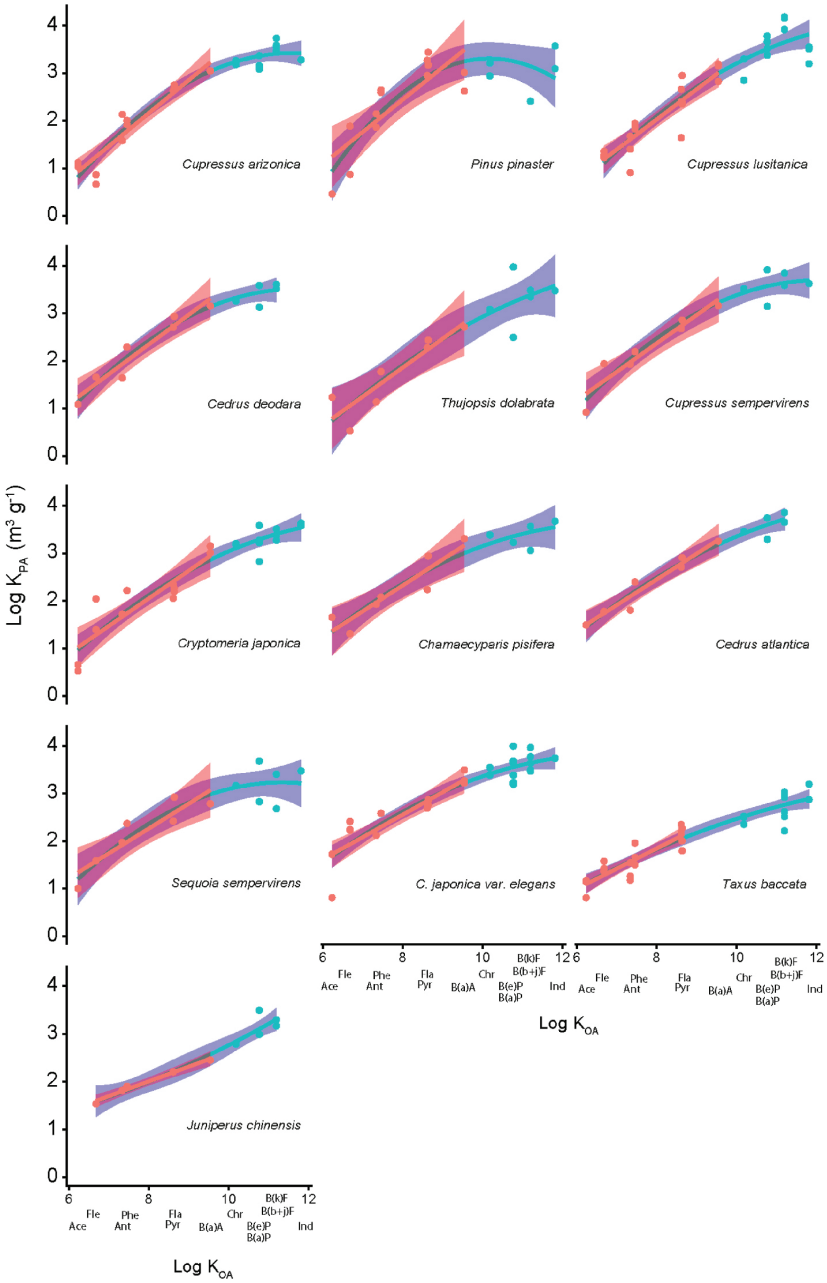


Fig. 2. Regression models relating Log K_{PA} (at sampling temperature 9 °C) with Log K_{OA} of the PAHs. PAHs are represented with dots, red for those associated with the gas phase and blue with particulate matter. Models are represented with lines and associated confidence intervals (shaded area). Linear models fitted to PAHs associated with the gas phase are plotted in red and 2nd polynomial models associated with all PAHs are plotted in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. Comparison of specific gas phase and PM PAH capture

Fig. 4 reports gas and particle phase concentrations normalized on leaf surface. This comparison allows to evaluate the role of each species in capturing PAHs basing on total leaf biomass or normalized on leaf surface (see Fig. 3SM for SLA boxplots). Results show that the ranking of species capturing gas-phase PAHs (Fig. 4A) varies from that of species capturing particle phase PAHs (Fig. 4B). The results obtained in this study show that *P. pinaster* needles have the largest capacity to capture gas phase PAHs, while *C. sempervirens* leaves have the largest capacity to capture PM associated PAHs. When considering the amount of PAHs per leaf surface, *C. sempervirens* leaves show the best removal performance, as it is the second species with the highest gas phase PAHs and the first one for PM associated PAHs. However, there are other factors that were not analyzed in this study, such as LAI, tree shape, percentage of leaves biomass, the change of this percentage during lifespan of the tree, which will be investigated in further studies.

3.6. K_{PA} comparison with literature

Few studies provide K_{PA} equations that can be used to estimate air concentrations. Some of these relationships are shown in Fig. 5 for comparison (Bolinius et al., 2016; McLachlan and Horstmann, 1998; Nizzetto et al., 2008), while a general review of plant uptake is available

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Table 3
Parameters of the equation Log K_{PA} = a + b log K_{OA} (model 1, gas phase PAHs linear) (a: intercept, b: slope, r^2: coefficient of determination).

Species	9 °C*			25 °C**		
	a	b	r^2	a	b	r^2
Cupressus arizonica	−3.39	0.69	0.90	−3.39	0.59	0.86
Pinus pinaster	−2.99	0.68	0.66	−2.99	0.57	0.56
Cupressus lusitanica	−3.05	0.63	0.80	−3.04	0.53	0.73
Cedrus deodara	−2.65	0.62	0.92	−2.65	0.52	0.88
Thujopsis dolabrata	−3.00	0.61	0.82	−3.00	0.50	0.74
Cupressus sempervirens	−2.41	0.60	0.90	−2.42	0.49	0.85
Cryptomeria japonica	−2.59	0.58	0.79	−2.59	0.47	0.73
Chamaecyparis pisifera	−2.03	0.54	0.85	−2.03	0.44	0.78
Cedrus atlantica	−1.86	0.54	0.94	−1.87	0.43	0.89
Sequoia sempervirens	−1.91	0.52	0.83	−1.92	0.42	0.73
C. japonica var. *elegans*	−1.22	0.47	0.79	−1.22	0.37	0.70
Picea abies	−1.75	0.44	–	−1.75	0.33	–
Taxus baccata	−1.54	0.42	0.77	−1.68	0.34	0.67
Juniperus chinensis	−0.45	0.31	0.98	−0.41	0.19	0.92

NOTES: * Sampling Temperature: ** Reference Temperature.
The species are shown in decreasing slope order; K_{PA} is expressed in ng g^{-1} of dry leaf/ ng m^{-3} of air; for *Picea abies* equation parameters reported in (Nizzetto et al., 2008) are shown.

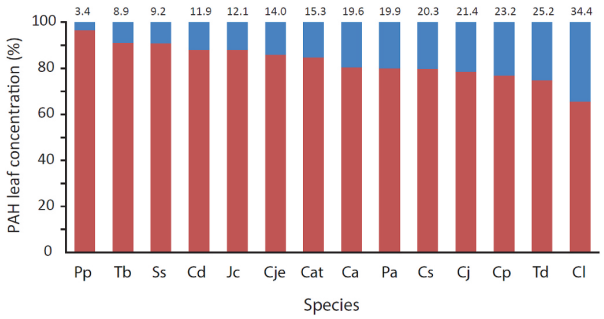


Fig. 3. Percentage of PM associated PAHs (blue) and gas phase associated PAHs (red). The numbers at the top of the bars correspond to the percentage of particle matter associated PAHs. Pp: *Pinus pinaster*; Tb: *Taxus baccata*; Ss: *Sequoia sempervirens*; Cd: *Cedrus deodara*; Jc: *Juniperus chinensis*; Cje: *Cryptomeria japonica f. elegans*; Cat: *Cedrus atlantica*; Ca: *Cupressus arizonica*; Pa: *Picea abies (L.)*; Cs: *Cupressus sempervirens*; Cj: *Cryptomeria japonica*; Cp: *Chamaecyparis pisifera*; Td: *Thujopsis dolabrata*; Cl: *Cupressus lusitanica*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in Doucette et al. (2018). For this exercise, we plotted the equations of the best and the worst gas phase PAH removers (i.e., *Cupressus arizonica* and *Juniperus chinensis)* obtained in this study (Table 3), which were compared to some examples of broadleaf and conifer species from the literature (Fig. 5).

The results for the conifers of this study occupy most of the space of the figure, where only *Rhododendron ponticum* is able to capture more chemical at high K_{OA} and less at K_{OA} lower than 7. The broadleaves are comprised within the range of K_{PA} of the studied conifers showing how broad is the span of plant-air partitioning for the conifer species. Although this comparison is referred to gas phase uptake, the results from previous findings (Fig. 3) also show the capability of conifers in retaining PM associated PAHs.

4. Conclusions

In this paper new species-specific K_{PA} equations for thirteen species are furnished. Although some uncertainty remains about the neat values of parameters included in K_{PA} equations, such as the difficulty of their estimation for particulate PAHs, these results allow improving the knowledge about PAH capturing ability of conifers. This information is relevant because these species carry leaves throughout the year and therefore can filter the air for PAHs and PM also in heavily impacted periods such as autumn and winter. The new equations produced in this study could be used in environmental fate models to further evaluate the mass balance of PAHs in urban and not urban scenarios and refine the strategy to reduce air contamination. Additional studies could include a more specific particle matter determination on needles to verify the uptake behavior of the different species for PM and PM associated PAHs.

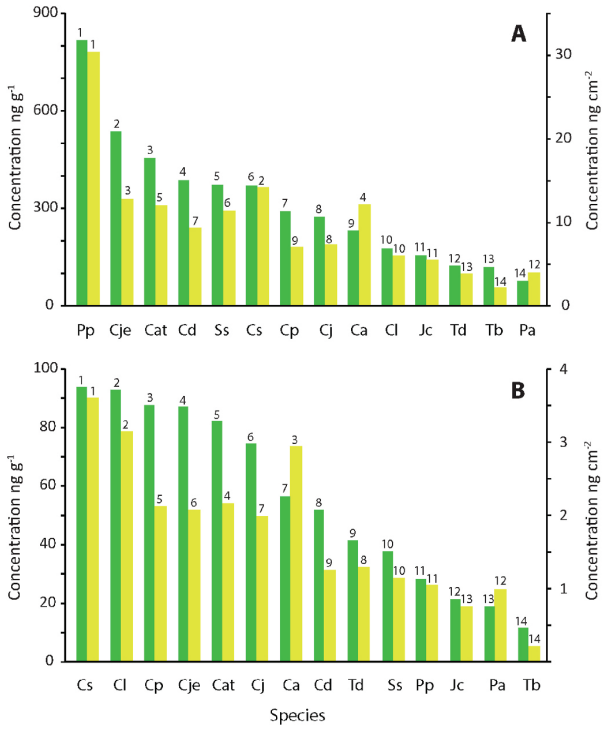


Fig. 4. A: Total concentration of gas phase PAHs (on a dry-weight basis (ng g^{-1}; green) and normalized on leaf surface (ng cm^{-2}; yellow); B: Total concentration of particle phase PAHs (on a dry-weight basis (ng g^{-1}; green) and normalized on leaf surface (ng cm^{-2}; yellow). Numbers represent the order of the species (more to less concentration). Cp: *Chamaecyparis pisifera*; Cj: *Cryptomeria japonica*; Cje: *Cryptomeria japonica f. elegans*; Ca: *Cupressus arizonica*; Cl: *Cupressus lusitanica*; Cs: *Cupressus sempervirens*; Jc: *Juniperus chinensis*; Ss: *Sequoia sempervirens*; Td: *Thujopsis dolabrata*; Tb: *Taxus baccata*; Cat: *Cedrus atlantica*; Cd: *Cedrus deodara*; Pa: *Picea abies (L.)*; Pp: *Pinus pinaster*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Credit author statement

Pablo Giráldez: Investigation, Writing – original draft preparation, Visualization; **Jesús R. Aboal**: Funding acquisition, Resources, Writing-Reviewing and Editing, Supervision; **J. Ángel Fernández**: Funding acquisition, Resources, Writing- Reviewing and Editing, Supervision; **Antonio Di Guardo**: Resources, Writing- Reviewing and Editing, Supervision; **Elisa Terzaghi**: Writing- Reviewing and Editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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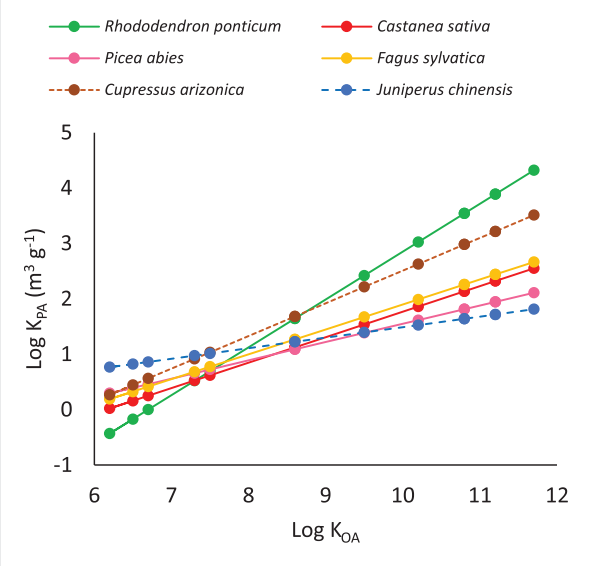


Fig. 5. Plant-air partition coefficient comparison: *Cupressus arizonica* and *Juniperus chinensis* (from this study); *Rhododendron ponticum* from (Bolinius et al., 2016); *Fagus sylvatica*, *Castanea sativa*, *Picea abies* from (Nizzetto et al., 2008). Density for *R. ponticum* was set to 187 700 g/m^3 (Castro-Díez et al., 2000) to convert the original K_{PA} expressed on a volume/volume basis.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120409.

References

Al-Alam, J., Fajloun, Z., Chbani, A., Millet, M., 2017. The use of conifer needles as biomonitor candidates for the study of temporal air pollution variation in the Strasbourg region. Chemosphere 168, 1411–1421. https://doi.org/10.1016/J.CHEMOSPHERE.2016.11.103.

Bacci, E., Cerejeira, M.J., Gaggi, C., Chemello, G., Calamari, D., Vighi, M., 1990. Bioconcentration of organic chemical vapours in plant leaves: the azalea model. Chemosphere 21, 525–535. https://doi.org/10.1016/0045-6535(90)90023-M.

Bolinius, D.J., Macleod, M., McLachlan, M.S., Mayer, P., Jahnke, A., 2016. A passive dosing method to determine fugacity capacities and partitioning properties of leaves. Environ. Sci. Process. Impacts 18, 1325–1332. https://doi.org/10.1039/C6EM00423G.

Borgulat, J., Staszewski, T., Łukasik, W., 2018. Monitoring of PAHs in the natural protected areas in non-heating season using Norway spruce (Picea abies (L.) Karst) needles. E3S Web Conf. 28, 01004 https://doi.org/10.1051/E3SCONF/20182801004.

Castro-Díez, P., Puyravaud, J.P., Cornelissen, J.H.C., 2000. Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types, 2000 Oecologia 476–486. https://doi.org/10.1007/PL00008873, 1244 124.

Chan, K.M., Wood, R., 2013. The seasonal cycle of planetary boundary layer depth determined using COSMIC radio occultation data. J. Geophys. Res. Atmospheres 118 (12). https://doi.org/10.1002/2013JD020147, 422-12,434.

De Nicola, F., Concha Graña, E., Aboal, J.R., Carballeira, A., Fernández, J., López Mahía, P., Prada Rodríguez, D., Muniategui Lorenzo, S., 2016. PAH detection in Quercus robur leaves and Pinus pinaster needles: a fast method for biomonitoring purpose. Talanta 153, 130–137. https://doi.org/10.1016/J.TALANTA.2016.01.067.

De Nicola, F., Concha Graña, E., López Mahía, P., Muniategui Lorenzo, S., Prada Rodríguez, D., Retuerto, R., Carballeira, A., Aboal, J.R., Fernández, J.Á., 2017. Evergreen or deciduous trees for capturing PAHs from ambient air? A case study. Environ. Pollut. 221, 276–284. https://doi.org/10.1016/J.ENVPOL.2016.11.074.

Domínguez-Morueco, N., Augusto, S., Trabalón, L., Pocurull, E., Borrull, F., Schuhmacher, M., Domingo, J.L., Nadal, M., 2017. Monitoring PAHs in the petrochemical area of Tarragona County, Spain: comparing passive air samplers with lichen transplants. Environ. Sci. Pollut. Res. 24, 11890–11900. https://doi.org/10.1007/S11356-015-5612-2/FIGURES/3.

Doucette, W.J., Shunthirasingham, C., Dettenmaier, E.M., Zaleski, R.T., Fantke, P., Arnot, J.A., 2018. A review of measured bioaccumulation data on terrestrial plants for organic chemicals: metrics, variability, and the need for standardized measurement protocols. Environ. Toxicol. Chem. 37, 21–33. https://doi.org/10.1002/ETC.3992.

EU, 2022. A European Green Deal | European Commission [WWW Document]. URL. https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en. accessed 8.4.22.

Fernández-Varela, R., Ratola, N., Alves, A., Amigo, J.M., 2015. Relationship between levels of polycyclic aromatic hydrocarbons in pine needles and socio-geographic parameters. J. Environ. Manag. 156, 52–61. https://doi.org/10.1016/J.JENVMAN.2015.03.019.

Franzetti, A., Gandolfi, I., Bestetti, G., Padoa Schioppa, E., Canedoli, C., Brambilla, D., Cappelletti, D., Sebastiani, B., Federici, E., Papacchini, M., Ambrosini, R., 2020. Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. J. Hazard Mater. 384, 121021 https://doi.org/10.1016/J.JHAZMAT.2019.121021.

Horstmann, M., McLachlan, M.S., 1998. Atmospheric deposition of semivolatile organic compounds to two forest canopies. Atmos. Environ. 32, 1799–1809. https://doi.org/10.1016/S1352-2310(97)00477-9.

IARC, 2010. In: Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Occupational Exposures, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer, Lyon, France.

Jouraeva, V.A., Johnson, D.L., Hassett, J.P., Nowak, D.J., 2002. Differences in accumulation of PAHs and metals on the leaves of Tilia×euchlora and Pyrus calleryana. Environ. Pollut. 120, 331–338. https://doi.org/10.1016/S0269-7491(02)00121-5.

Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80. https://doi.org/10.1016/J.ENVINT.2013.07.019.

Kömp, P., McLachlan, M.S., 1997. Influence of temperature on the plant/air partitioning of semivolatile organic compounds. Environ. Sci. Technol. 31, 886–890. https://doi.org/10.1021/ES960590U.

Lara, S., Villanueva, F., Martín, P., Salgado, S., Moreno, A., Sánchez-Verdú, P., 2022. Investigation of PAHs, nitrated PAHs and oxygenated PAHs in PM10 urban aerosols. A comprehensive data analysis. Chemosphere 294, 133745. https://doi.org/10.1016/J.CHEMOSPHERE.2022.133745.

Mackay, D., Celsie, A.K.D., Parnis, J.M., 2019. Kinetic delay in partitioning and parallel particle pathways: underappreciated aspects of environmental transport. Environ. Sci. Technol. 53, 234–241. https://doi.org/10.1021/ACS.EST.8B04514/SUPPL_FILE/ES8B04514_SI_001.PDF.

Mackay, D., Shiu, W.Y., Ma, Kuo-Ching, Lee, S.C., 2006. Handbook of physical-chemical properties and environmental fate for organic chemicals, 2nd ed.. CRC press, Taylor & Francis, Boca Raton, FL.

McLachlan, M.S., Horstmann, M., 1998. Forests as filters of airborne organic pollutants: a model. Environ. Sci. Technol. 32, 413–420. https://doi.org/10.1021/ES970592U.

Morselli, M., Ghirardello, D., Semplice, M., Di Guardo, A., 2011. Modeling short-term variability of semivolatile organic chemicals in air at a local scale: an integrated modeling approach. Environ. Pollut. 159, 1406–1412. https://doi.org/10.1016/J.ENVPOL.2010.12.034.

Moscoso-Pérez, C., Jiménez, A.C., Iglesias, M.P., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., De Bernabé, J.G., Picón, J.F.A., 2010. Evaluation of the degree of compliance of the eu target values for As, Pb, Cd, Ni and polycyclic aromatic hydrocarbons in PM10 at four urban sites of the Northwest of Spain. Chem. Eng. Trans. 22, 203–208. https://doi.org/10.3303/CET1022033.

Nizzetto, L., Pasture, C., Liu, X., Camporini, P., Stroppiana, D., Herbert, B., Boschetti, M., Zhang, G., Brivio, P.A., Jones, K.C., Di Guardo, A., 2008. Accumulation parameters and seasonal trends for PCBs in temperate and boreal forest plant species. Environ. Sci. Technol. 42, 5911–5916. https://doi.org/10.1021/ES800217M.

Oleagoitia, M.B.Z., Manterola, A.L., Maurolagoitia, J.I., de Dicastillo, M.D.M.L., Álvarez, J., Barandiaran, M.A., Loibide, A.I., Santa-Marina, L., 2019. Polycyclic aromatic hydrocarbons (PAHs) in air associated with particles PM 2.5 in the Basque Country (Spain). Air Qual. Atmos. Health 12, 107–114. https://doi.org/10.1007/s11869-018-0635-8.

Omar, N.Y.M.J., Abas, M.R. Bin, Ketuly, K.A., Tahir, N.M., 2002. Concentrations of PAHs in atmospheric particles (PM-10) and roadside soil particles collected in Kuala

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Lumpur, Malaysia. Atmos. Environ. 36, 247–254. https://doi.org/10.1016/S1352-2310(01)00425-3.
Ozdemir, H., 2019. Mitigation impact of roadside trees on fine particle pollution. Sci. Total Environ. 659, 1176–1185. https://doi.org/10.1016/J.SCITOTENV.2018.12.262.
Park, S.S., Kim, Y.J., Kang, C.H., 2002. Atmospheric polycyclic aromatic hydrocarbons in Seoul, Korea. Atmos. Environ. 36, 2917–2924. https://doi.org/10.1016/S1352-2310(02)00206-6.
Rasband, W.S., ImageJ, U. S. National institutes of health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2016.
Ratola, N., Amigo, J.M., Alves, A., 2010. Levels and sources of PAHs in selected sites from Portugal: biomonitoring with Pinus pinea and Pinus pinaster needles. Arch. Environ. Contam. Toxicol. 58, 631–647. https://doi.org/10.1007/S00244-009-9462-0.
Ratola, N., Amigo, J.M., Oliveira, M.S.N., Araújo, R., Silva, J.A., Alves, A., 2011. Differences between Pinus pinea and Pinus pinaster as bioindicators of polycyclic aromatic hydrocarbons. Environ. Exp. Bot. 72, 339–347. https://doi.org/10.1016/J.ENVEXPBOT.2011.04.012.
Sæbø, A., Popek, R., Nawrot, B., Hanslin, H.M., Gawronska, H., Gawronski, S.W., 2012. Plant species differences in particulate matter accumulation on leaf surfaces. Sci. Total Environ. 347–354. https://doi.org/10.1016/J.SCITOTENV.2012.03.084, 427–428.
Tang, J., Zha, Y., Cheng, Y., 2019. Heavy metals concentrations in deposited dust of typical Chinese tree species in different functional areas in Nanjing. Pol. J. Environ. Stud. 28, 2867–2876. https://doi.org/10.15244/PJOES/93296.
Terzaghi, E., Falakdin, P., Fattore, E., Di Guardo, A., 2021. Estimating temporal and spatial levels of PAHs in air using rain samples and SPME analysis: feasibility evaluation in an urban scenario. Sci. Total Environ. 762, 144184 https://doi.org/10.1016/J.SCITOTENV.2020.144184.
Terzaghi, E., Morselli, M., Semplice, M., Cerabolini, B.E.L., Jones, K.C., Freppaz, M., Di Guardo, A., 2017. SoilPlusVeg: an integrated air-plant-litter-soil model to predict organic chemical fate and recycling in forests. Sci. Total Environ. 595, 169–177. https://doi.org/10.1016/j.scitotenv.2017.03.252.
Terzaghi, E., Scacchi, M., Cerabolini, B., Jones, K.C., Di Guardo, A., 2015a. Estimation of polycyclic aromatic hydrocarbon variability in air using high volume, film, and vegetation as samplers. Environ. Sci. Technol. 49, 5520–5528. https://doi.org/10.1021/ES5056929.
Terzaghi, E., Wild, E., Zacchello, G., Cerabolini, B.E.L., Jones, K.C., Di Guardo, A., 2013. Forest Filter Effect: role of leaves in capturing/releasing air particulate matter and its associated PAHs. Atmos. Environ. 74, 378–384. https://doi.org/10.1016/J.ATMOSENV.2013.04.013.
Terzaghi, E., Zacchello, G., Scacchi, M., Raspa, G., Jones, K.C., Cerabolini, B., Di Guardo, A., 2015b. Towards more ecologically realistic scenarios of plant uptake modelling for chemicals: PAHs in a small forest. Sci. Total Environ. 505, 329–337. https://doi.org/10.1016/J.SCITOTENV.2014.09.108.
Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., Liu, C., 2019. Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: morphological characteristics have the greatest impact. Sci. Total Environ. 652, 1149–1155. https://doi.org/10.1016/J.SCITOTENV.2018.10.357.
Villanueva, F., Sevilla, G., Lara, S., Martín, P., Salgado, S., Albaladejo, J., Cabañas, B., 2018. Application of gas chromatography coupled with tandem mass spectrometry for the assessment of PAH levels in non industrial indoor air. Microchem. J. 142, 117–125. https://doi.org/10.1016/J.MICROC.2018.06.021.
Walgraeve, C., De Wispelaere, P., Van der Elst, F., Van Langenhove, H., 2017. Development of an analytical method to determine oxy-PAHs and PAHs in Taxus baccata leaves. Anal. Bioanal. Chem. 409, 335–347. https://doi.org/10.1007/s00216-016-0008-9.
Wang, J., Cao, J., Dong, Z., Guinot, B., Gao, M., Huang, R., Han, Y., Huang, Y., Ho, S.S.H., Shen, Z., 2017. Seasonal variation, spatial distribution and source apportionment for polycyclic aromatic hydrocarbons (PAHs) at nineteen communities in Xi'an, China: the effects of suburban scattered emissions in winter. Environ. Pollut. 231, 1330–1343. https://doi.org/10.1016/J.ENVPOL.2017.08.106.
WHO, 2021. Human health effects of polycyclic aromatic hydrocarbons as ambient air pollutants. In: Report of the Working Group on Polycyclic Aromatic Hydrocarbons of the Joint Task Force on the Health Aspects of Air Pollution. WHO Regional Office for Europe, Copenhagen.
WHO, 2000. Air quality guidelines for Europe, 2nd ed.. In: WHO Regional Publications. European Series ; No. 91). World Health Organization, Regional Office for Europe, Copenhagen.

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4.2. Results: unpublished works

Chapter IV: Interspecific variability of leaf traits and their relationship to leaf concentration of polycyclic aromatic hydrocarbons in conifer tree species

Introduction

In the previous chapters of this thesis we have established that understanding the effect of leaf traits on PAH uptake is fundamental to understanding PAH loading processes in tree leaves. Thus, during the study in Chapter III, while determining the concentration of PAHs in leaves, we also determined different leaf traits (i.e., SLA, mean stomatal density, stomatal conductance, carbon isotopic composition and pyrolytic composition). However, we did not include the results of the traits in the publication of Chapter III because we considered that this was an excessive amount of information for a single paper, which would make it difficult to understand an already complex article. Nevertheless, we consider that it would be regrettable not to make this data public.

Material and methods

Sampling was performed on the same days and on the same trees as the PAHs determination (see Chapter III). Apart from SLA, which was also included in Chapter III, we analyzed other foliar traits (i.e., stomatal density, stomatal conductance, carbon isotopic composition and pyrolytic composition) to investigate their influence on PAH uptake in conifers. Four leaves per tree were selected and surface impressions of each were obtained with nail varnish and adhesive tape for mean stomatal density determination. Mean stomatal density was obtained by counting the number of stomata in each leaf inside a square of 400x400 μm using an optical microscope (Olympus SZ2-ILST). In leaves with stomata on both sides, two prints were made on the adaxial side and two on the abaxial side. Stomatal conductance was measured by means of an infrared gas analyzer (LICOR-6400 XT), with a red and blue LED light source (LI6400-18 LCF) and a 6 cm^2 camera. Carbon isotopic composition, $\delta^{13}C$ (‰), was determined using a EA1108 elemental analyzer (Carlo Erba Instruments) coupled to an isotope ratio mass spectrometer MAT253 (ThermoFinnigan) using a ConfloIII interface (ThermoFinnigan). The leaves percentage of fatty acids was determined regarding the total compounds identified by means of a pyrolysis-gas chromatography/mass spectrometry using tetramethylammonium hydroxide for thermally-assisted hydrolysis and methylation

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(THM-Py-GC/MS; see Chaos et al., 2024 for more details). Scanning electron microscopy (SEM) photos of the leaf surface were also taken to check the roughness and pilosity of the leaves. In addition to the data sampled in this work, we also searched in the TRY Plant Trait Database (Kattge et al., 2020) the trait "Leaf length excluding petiole (leaf lamina length)" (code 940).

In order to detect possible relationships between PAH concentrations in leaves and leaf traits (including the SLA, see Chapter III), we calculated Spearman correlations between both groups of variables. The calculations were performed using RStudio software (RStudio Team, 2021). In addition to the PAHs determined in Chapter III, we also calculated the sum of the PAHs of each ring (3, 4, 5 and 6), the sum of the high molecular weight PAHs (HMW) and the sum of all the PAHs determined. We did not include the low and medium molecular weight PAHs (LMW and MMW, respectively) in the analysis because in this case the LMW PAHs would be the 3-ring PAHs and the MMW PAHs would be the 4-ring PAHs.

Results

Leaf traits

Even though all the species studied are evergreen conifers belonging to three botanical families, there is a great variability in the results obtained from the trait measurement (Table 1, Table 2, Fig. 1-2 and Fig. 3SM in Appendix III). The means range from -23.7‰ (*T. dolabrata*) to -30.2‰ (*P. pinaster*) for the $\delta^{13}C$. The length from TRY Plant Trait Database ranges from 0.075 cm (*C. sempervirens*) to 20 cm (*P. pinaster*). The mean stomatal density ranges from 3203 (*C. arizonica*) to 22813 stomata cm^{-2} (*C. japonica*). Mean stomatal conductance ranged from 0.016 (*J. chinensis*) to 0.257 mol $H2O\ m^{-2}\ s^{-1}$ (*C. deodara*). Mean SLA ranged from 19 (*P. abies*) to 54 $cm^2\ g^{-1}$ (*T. baccata*). On the other hand, in Table 2, we can see that the percentage of fatty acids in the total compounds extracted during pyrolysis of the leaves also varies greatly among the species under study. In fact, the total percentage of fatty acids ranges from 5.49% (*P. abies*) to 22.20% (*J. chinensis*). Moreover, the majority fatty acid also varies greatly between species, e.g., F.A. C12 accounts for 4.24% of the total extracted compounds in *P. pinaster* and 0.71% in *C. deodara*; in contrast, F.A. C20:2 accounts for 0.09% for *P. pinaster* and 6.30% for *C. deodara*). In addition to the interspecific variability in Fig. 1 and 2 we can

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also observe a high intraspecific variability in some variables and species (e.g. *S. sempervirens* for stomatal density or *C. japonica* for stomatal conductance).

Table 1. Mean values measured for specific leaf area (SLA), stomatal density (SDe), stomatal conductance (SC), $\delta^{13}C$, mean of available TRY Plant Trait Database and leaf length (TRY L) for each species.

Species	SLA ($cm^2\ g^{-1}$)	Sde (stomata cm^{-2})	SC (mol $H_2O\ m^{-2}\ s^{-1}$)	$\delta^{13}C$ (‰)	TRY L (cm)
Chamaecyparis pisifera	41.3	18438	0.04	-27.2	n.a.
Cryptomeria japonica	37.4	22813	0.15	-28.0	1.163
Cryptomeria japonica f. *elegans*	41.9	6458	0.19	-26.5	1.163
Cupressus arizonica	19.2	3203	0.21	-29.5	0.160
Cupressus lusitanica	29.5	3750	0.08	-28.7	0.175
Cupressus sempervirens	26.1	5000	0.03	-28.1	0.075
Juniperus chinensis	28.3	16719	0.02	-25.9	2.250
Sequoia sempervirens	32.9	6094	0.06	-28,0	1.550
Thujopsis dolabrata	32.0	21797	0.02	-23,7	0.567
Cedrus atlantica	37.9	3750	0.11	-28.1	2.000
Cedrus deodara	41.3	9219	0.26	-29.0	4.000
Picea abies	19.1	7656	0.09	-27.1	1.567
Pinus pinaster	26.9	9063	0.17	-30.2	20.00
Taxus baccata	54.3	7708	0.04	-27.0	2.500

NOTES: n.a.: not available

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Table 2. Determined values (when n=1) or mean values (when n>1) of the percentages (%) of each of the fatty acids (F.A.) listed in the table relative to the total of the compounds extracted in the pyrolysis of the leaves of the trees in the study.

Species	n	F.A. C10	F.A. C12	F.A. C14	F.A. C16	F.A. C18	F.A. C16:2	F.A. C18:2	F.A. C18:1	F.A. C20:2	F.A. C18:3	F.A. Total
Chamaecyparis pisifera	1	0.57	1.33	1.25	2.51	0.21	0.58	1.05	0.46	1.31	1.57	10.85
Cryptomeria japonica	2	0.52	1.08	1.72	5.13	0.27	1.03	1.31	0.48	3.40	0.50	15.44
Cryptomeria japonica elegans	3	0.47	0.83	2.43	3.39	0.19	0.60	0.77	0.27	2.22	5.11	16.28
Cupressus arizonic	2	0.44	0.64	1.30	3.33	0.20	0.86	1.06	0.43	0.59	3.19	12.05
Cupressus lusitanica	3	0.71	1.53	1.69	4.02	0.19	1.79	1.18	0.50	3.39	2.71	17.72
Cupressus sempervirens	1	0.78	2.12	2.14	4.75	0.39	1.75	1.75	0.59	4.18	0.83	19.30
Juniperus chinensis	1	0.91	3.60	2.25	5.35	0.31	1.53	2.54	1.31	2.66	1.73	22.20
Sequoia sempervirens	1	0.29	1.52	2.21	3.08	0.18	0.41	1.24	0.37	0.36	0.34	10.01
Thujopsis dolabrata	1	0.70	0.47	2.42	2.96	0.11	0.87	0.74	0.35	1.92	0.81	11.35
Cedrus atlántica	1	0.32	1.16	1.21	2.83	0.15	0.33	1.20	0.63	0.82	0.04	8.69
Cedrus deodara	1	0.44	0.71	2.57	3.62	0.17	0.90	1.46	0.82	6.30	1.03	18.02
Picea abies	1	0.13	0.36	1.01	2.02	0.11	0.12	0.72	0.49	0.26	0.27	5.49
Pinus pinaster	2	2.50	4.24	1.09	2.18	0.21	0.35	0.48	0.23	0.09	2.50	13.86
Taxus baccata	3	0.56	0.27	1.38	4.90	0.37	0.93	2.43	1.10	0.18	0.03	12.15

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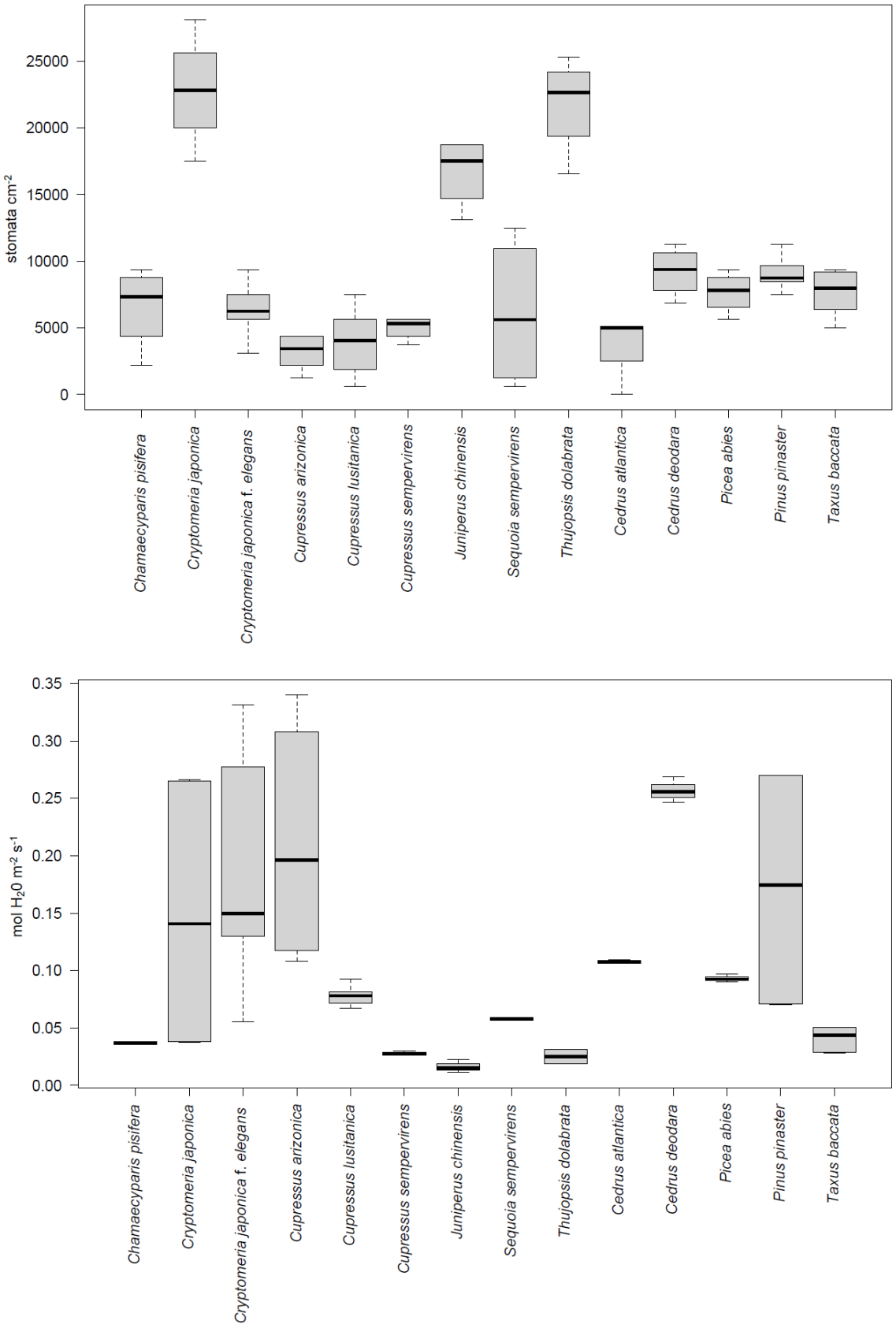


Fig. 1. Boxplots of stomatal density and stomatal conductance for each of the species in the study.

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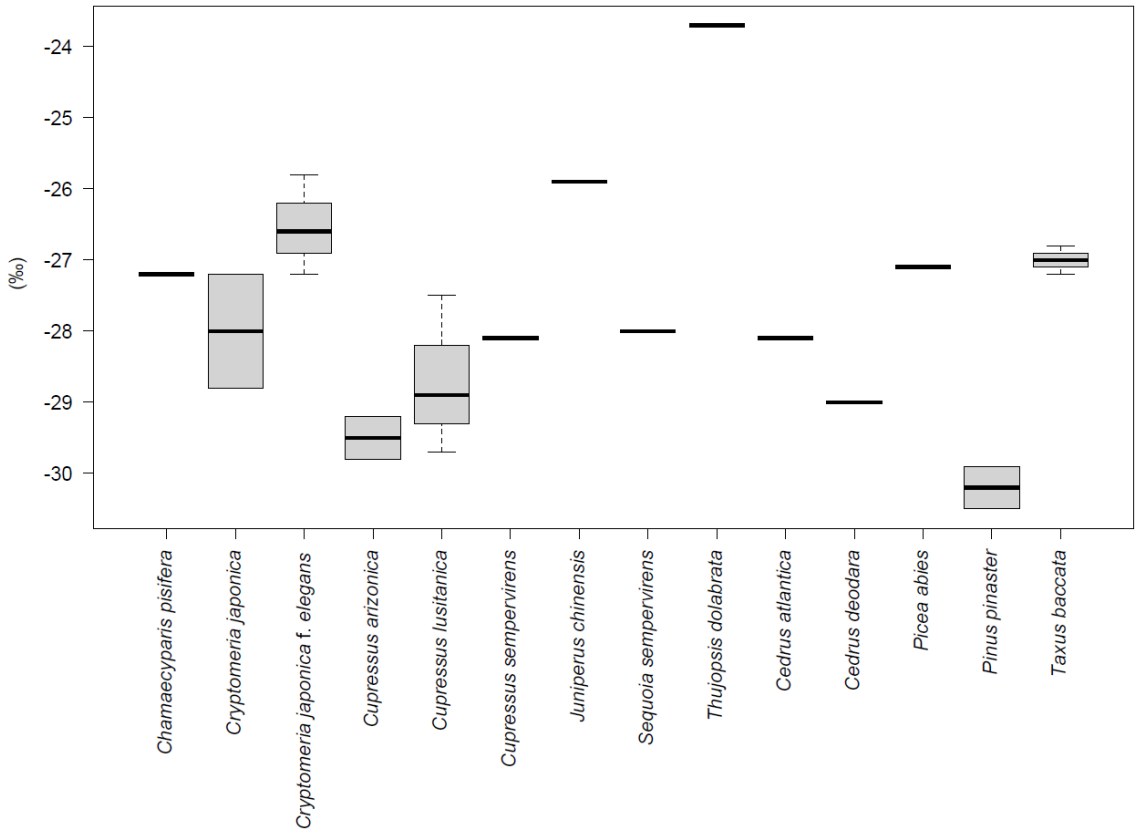


Fig. 2. Values of $\delta^{13}C$ determined for each of the species in the study. Boxplots of these values are shown for *C. japonica*, *C. japonica* f. *elegans*, *C. arizonica*, *P. pinaster* and *T. baccata*, while for the rest of the species (n=1) we show the single value determined for them.

Leaves surface characteristics

Figures 3-6 show the scanning electron microscopy photos of the leaf surface, where a multitude of leaf details can be clearly seen. Especially the apparent roughness of the leaves and the presence/absence of stomata, as well as their arrangement. Thus, for example, we can observe the amazing number of stomata on the surface of *T. dolabrata* or the roughness of *C. sempervirens*. However, these photos should be interpreted with care, as they can be misleading. An example of this would be *C. pisifera*, which in the photos does not seem to have too many stomata while in Table 1 we have estimated a very high stomatal density.

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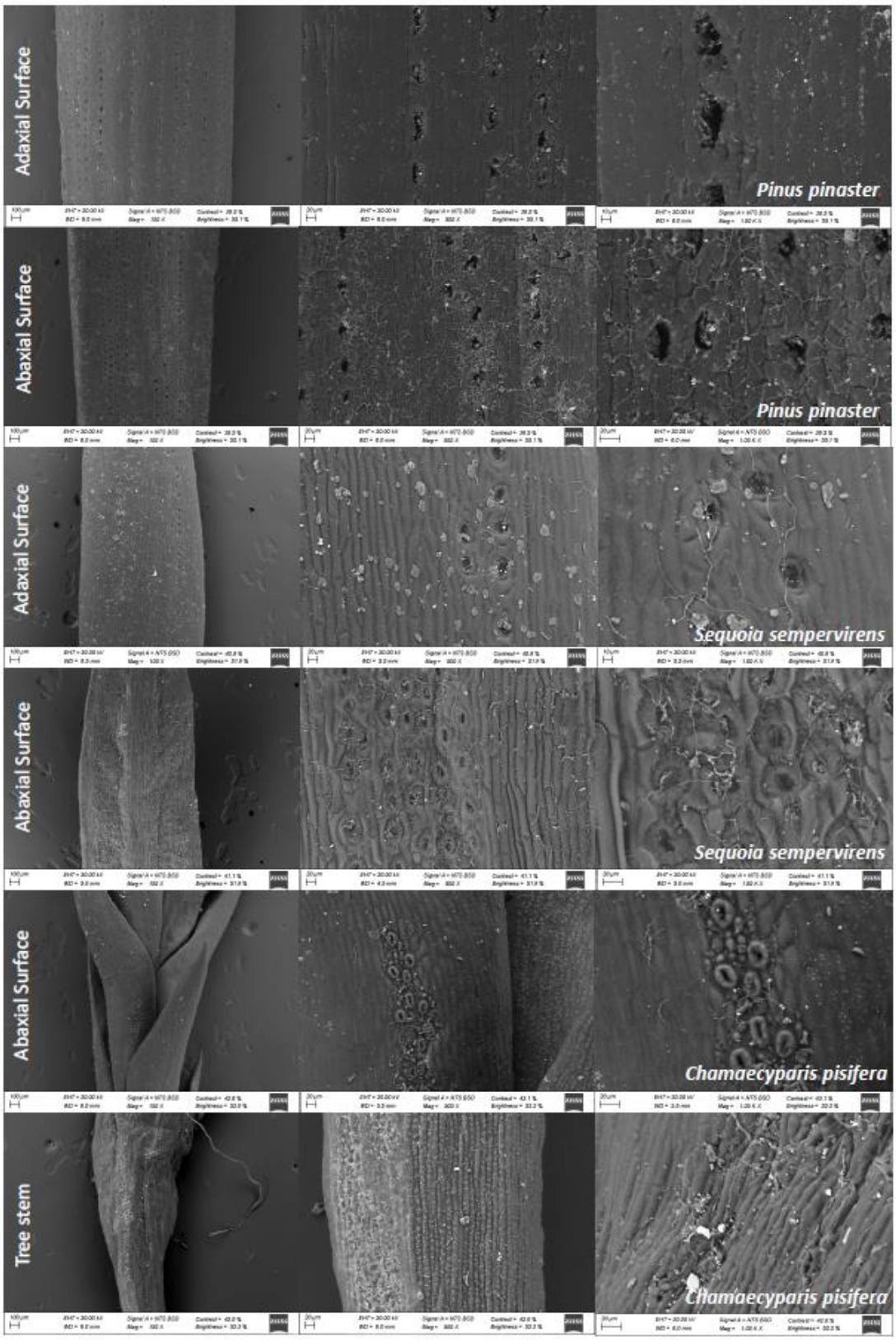


Fig. 3. Scanning electron microscopy photos of leaves of *Pinus pinaster*, *Sequoia sempervirens* and *Chamaecyparis pisifera*.

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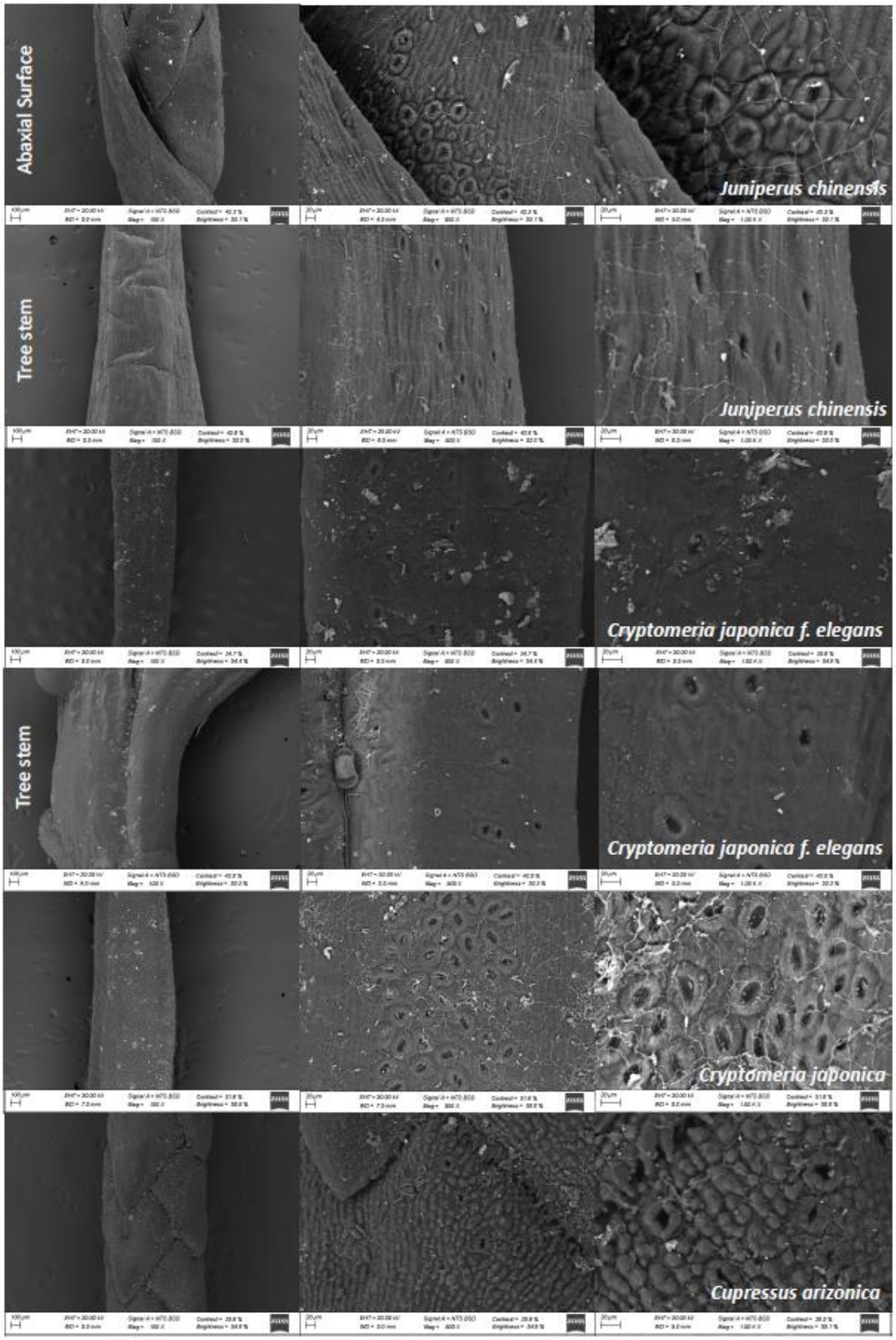


Fig. 4. Scanning electron microscopy photos of leaves of *Juniperus chinensis*, *Cryptomeria japonica and Cryptomeria japonica* f. *elegans*.

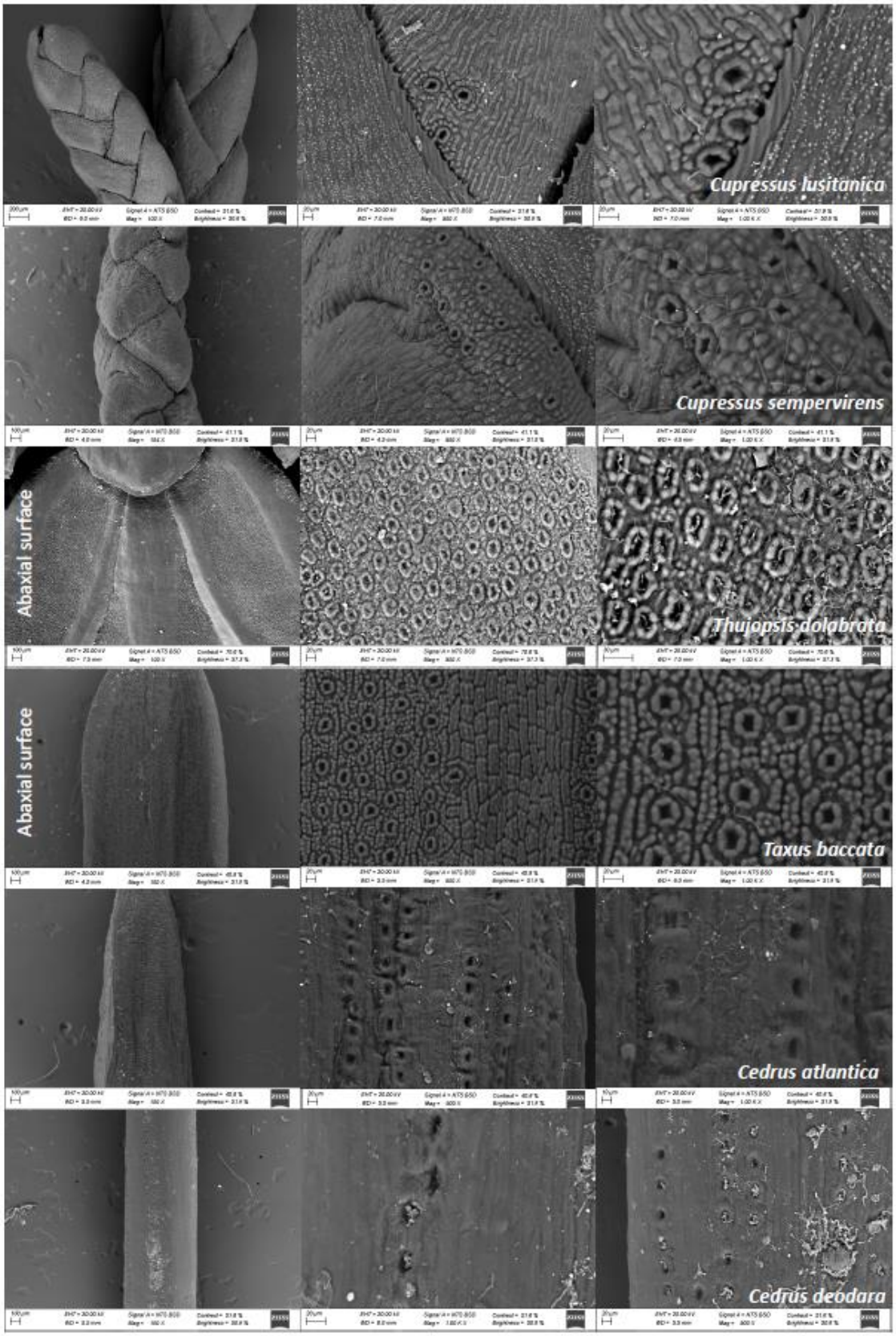


Fig. 5. Scanning electron microscopy photos of leaves of *Cupressus lusitanica, Cupressus sempervirens, Thujopsis dolabrata, Taxus baccata, Cedrus atlantica and Cedrus deodara*.

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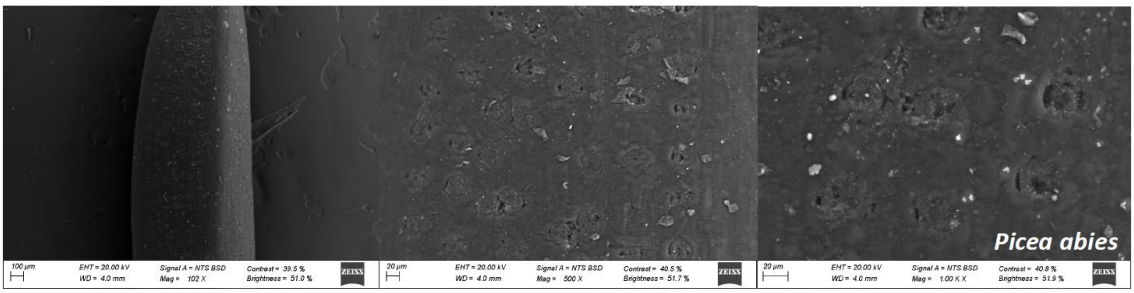


Fig. 6. Scanning electron microscopy photos of leaves of *Picea abies*.

Relationship between PAHs levels and leaf traits

The result of the correlations can be seen in Table 3. It is interesting to note that we found few significant correlations for the total number of correlations calculated. In fact, there are several fatty acids (i.e., F.A. C10, F.A. C18, F.A. C16:2, F.A. C18:3 and F.A. Total) for which we have not found any significant correlation with the PAHs studied. Morphological and gas exchange traits seem to have more significant relationships, although for stomatal conductance we found only one significant correlation with ∑PAHs and for SLA we found only two significant correlations, with Fle and with B(a)A. We also have to highlight the fact that there are some traits for which the direction of their relationship with PAHs (direct or inverse) seems quite clear (e.g. TRY L) or (F.A. C18:1), while for others there are opposite relationships (e.g. $\delta^{13}C$).

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Table 3. Significant correlations (p-value < 0.05) between leaf traits and leaf PAHs concentrations. Leaf traits included were specific leaf area (SLA), stomatal density (SDe), stomatal conductance (SC), $\delta^{13}C$, leaf length obtained from TRY Trait Database (TRY L) and different fatty acids (F.A.) with different number of carbons (Cx) and unsaturations (:x).

PAH	SLA	SDe	SC	$\delta^{13}C$	TRY L	F.A. C10	F.A. C12	F.A. C14	F.A. C16	F.A. C18	F.A. C16:2	F.A. C18:2	F.A. C18:1	F.A. C20:2	F.A. C18:3	F.A. Total
Acy														0.59		
Ace																
Fle	0.57							0.47								
Phe				-0.46												
Ant							0.49									
Fla				-0.44					-0.6			-0.43	-0.55			
Pyr									-0.41				-0.52			
B(a)A	0.54															
Chr					-0.45									0.48		
B(b+j)F		-0.58			-0.71									0.68		
B(k)F		-0.58			-0.69									0.65		
B(e)P								0.58						0.55		
B(a)P		-0.54			-0.53									0.46		
D(ah)A													-0.49			
Ind																
B(ghi)P																
∑3rings							0.41									
∑4rings				-0.41					-0.53			-0.51	-0.61			
∑5rings					-0.54									0.62		
∑6rings												-0.47	-0.59			
∑HMW					-0.57									0.58		
∑PAHs			0.43	-0.41					-0.44			-0.44	-0.58			

Discussion

In Chapter III, we have observed that there is a great variability in the foliar concentrations of PAHs among the study species. Specifically, the total concentration of PAHs ranges from 95 ng g^{-1} dry weight (dw) for *P. abies* to 845 ng g^{-1} dw for *P. pinaster*. In addition, variations can also be observed in the PAHs fingerprint, for example, *C. japonica* f. *elegans* and *C. lusitanica*, which in their fingerprint showed percentages of Fle and B(b+j)F considerably higher than the average of the remaining species. This variability may be due to a multitude of factors, but certainly one of them is the leaf traits. This makes even more sense if we look at the substantial differences we observed for traits among the species in this study (Tables 1-2, and Fig. 1-2). These traits can be divided into three groups: i) morphological traits (SLA, TRY length); ii) traits related to gas exchange (stomatal density, stomatal conductance and $\delta^{13}C$); and iii) traits related to leaf chemical composition (different fatty acids). We tried to evaluate the effect of these traits by obtaining the correlations of them with the concentration of PAHs in the leaves. Most of these correlations were not significant; but there were a few that yielded interesting results. The high number of non-significant correlations is probably due to the small sample size and the high intra- and interspecific variability at all levels (PAHs, traits, species position in the sampling area, etc.) which may increase the noise in the data. Thus, this would make it more difficult to obtain clear trends in the relationships between PAH concentrations and traits. It is important to keep in mind that the process of PAH uptake in trees is multifactorial and it is likely that there are interactions between traits that would make it difficult to identify the individual role of each one of them.

Leaf morphology traits are, in general, the most studied, especially SLA. In this case we only observed two significant correlations that are positive and with coefficients close to 0.6. One of the correlations was with a LMW PAH (Fle) and another with a MMW PAH (B(a)A), which is consistent with some of the literature (e.g. Tian et al., 2019) and the main hypotheses on the relationship of SLA with PAHs concentration. That is, as the leaf adsorption surface area increases for a given mass, the concentration of PAHs in the leaf expressed in mass (ng g^{-1}) will also increase (Simonich & Hites, 1994). Regarding length (retrieved from TRY Plant Trait Database), we observed significantly negative correlations for 4 PAHs mostly associated with PM, as well as with the ∑5-rings and ∑HMW PAHs. It is suggested in literature that the width/length ratio has an inverse relationship with the concentration of PAHs in leaves (e.g. Liang et al., 2017). Therefore,

it would be expected that the relationship between length and PAHs concentration would be direct and not inverse. However, in this case it is likely that leaf length behaves as a proxy for species with scaly leaf clusters (the species with the smallest leaves), which in this study have a higher surface roughness (see Fig. 3-6) than species with longer leaves, which would allow them to retain more PM (Shahrukh et al., 2024). As Tian et al. (2019) evaluated, the uptake of high molecular weight PAHs is highly dependent on leaf roughness and its wax content, while the uptakes of medium molecular weight PAHs depends on morphological characteristics.

The relationship between gas exchange traits and leaf PAH concentrations is not as clear as for morphological traits. The main hypothesis is that increasing gas exchange in leaves would increase the concentration of gas-phase PAHs inside the leaves as they would diffuse freely through the stomata. While a secondary hypothesis, contrary to the previous one, would be that increasing gas exchange could increase the volatilization of PAHs from inside the leaf. Regarding stomatal conductance, we only have one significant correlation (positive with ∑PAHs), but this seems to support the main hypothesis, as do the results of De Nicola et al. (2017). The $\delta^{13}C$ has an inverse relationship with gas exchange, thus the results of this study would also support the main hypothesis, as it has 4 significantly negative correlations with different PAHs and sums of PAHs. However, there is one unexpected result for which we have no explanation, which is the significantly positive correlation with B(e)P (a PAH mostly associated with PM). Concerning stomatal density, it does not seem to be related to the PAHs gas phase since the significant correlations are all negative and refer to PAHs associated with PM. This result is probably due to the fact that stomatal density does not really measure gas exchange, i.e., it measures the number of stomata, but not whether they are open. In fact, Prigioniero et al. (2022) found a “puzzling” relationship between stomatal traits and pollutant uptake and consider that this is probably due to the relationship of stomatal traits with other leaf traits and the absence of a relationship between stomatal traits and the opening of stomata.

Regarding traits related to leaf chemical composition, specifically fatty acids, we seem to have conflicting results, with positive (i.e. F.A. C12, F.A. C14 and F.A. C20:2) and negative (i.e. F.A. C16, F.A. C18:2 and F.A. C18:1) correlations. Out of the positive relationships F.A. C12 and F.A. C14 have only two significant correlations each, three of these 4 correlations are with gas phase PAHs and only one with a PAHs associated to PM. In contrast, F.A. C20:2 has 7 significant correlations, all with PAHs associated with

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PM. As for the negative correlations, all three fatty acids have at least 4 significant correlations and are mainly with four-ring PAHs, with ∑4-ring PAHs and with ∑PAHs. There are two main hypotheses on how waxes might affect PAH concentrations in leaves. One of them is that increasing the amount of waxes could facilitate the uptake of PAHs by increasing the hydrophobicity of the leaf (Ray et al., 2021). The other suggests that by increasing the amount of waxes, these could hinder the diffusion of PAHs inside the leaf and these, being retained in the cuticles, would be more exposed to photodegradation, revolatilization and/or removal from the surface (Yang et al., 2017; Terzaghi et al., 2013). However, the study conducted in this chapter does not allow us to determine the position of each of the different fatty acids within the leaf nor their specific interaction with PAHs. Therefore, it is necessary to carry out more studies in the future that would allow us to determine the role they play in the uptake of PAHs from the atmosphere.

Conclusions

This chapter completes Chapter III and further explores specific objective 3 of this thesis by examining the relationship between foliar traits and the concentration of PAHs in leaves. The results obtained seem to reinforce the positive relationship of PAHs concentrations (ng g^{-1}) with SLA, and its negative relationship with stomatal density, which seems to rule out stomatal density as a reliable proxy of gas exchange. On the other hand, stomatal conductance and $\delta^{13}C$ seem to be better indicators of gas exchange and in general it seems that increased gas exchange would have a positive relationship with PAHs concentration. The relationship between the uptake of PAHs associated with particulate matter and the arrangement of leaves in scaly clusters, which increase the roughness of the PAH uptake surface, also seems quite clear. Finally, we have observed relationships between PAHs concentration in leaves and relative fatty acid content in leaves, however further studies would be necessary to unravel the exact origin of these relationships and determine whether they are causal or spurious.

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References

Chaos, Z., Fernández, J. A., Balseiro-Romero, M., Celeiro, M., García-Jares, C., Méndez, A., Pérez-Alonso, P., Estébanez, B., Kaal, J., Nierop, K. G. J., Aboal, J. R., & Monterroso, C. (2024). What potential do mosses have as biomonitors of POPs? A comparative study of hexachlorocyclohexane sorption. *Science of The Total Environment*, *934*, 173021. https://doi.org/10.1016/J.SCITOTENV.2024.173021

De Nicola, F., Concha Graña, E., López Mahía, P., Muniategui Lorenzo, S., Prada Rodríguez, D., Retuerto, R., Carballeira, A., Aboal, J. R., & Fernández, J. Á. (2017). Evergreen or deciduous trees for capturing PAHs from ambient air? A case study. Environmental Pollution, 221, 276–284. https://doi.org/10.1016/J.ENVPOL.2016.11.074

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Tautenhahn, S., & Werner, G. D. A. (2020). TRY plant trait database – enhanced coverage and open access. Global Change Biology, 26(1), 119–188. https://doi.org/10.1111/GCB.14904

Liang, J., Fang, H., Zhang, T., & Wang, X. (2017). Polycyclic aromatic hydrocarbons in the leaves of twelve plant species along an urbanization gradient in Shanghai, China. Environmental Science and Pollution Research, 24(10), 9361–9369. https://doi.org/10.1007/S11356-017-8552-1

Prigioniero, A., Zuzolo, D., Niinemets, Ü., Postiglione, A., Mercurio, M., Izzo, F., Trifuoggi, M., Toscanesi, M., Scarano, P., Tartaglia, M., Sciarrillo, R., & Guarino, C. (2022). Particulate matter and polycyclic aromatic hydrocarbon uptake in relation to leaf surface functional traits in Mediterranean evergreens: Potentials for air phytoremediation. Journal of Hazardous Materials, 435, 129029. https://doi.org/10.1016/J.JHAZMAT.2022.129029

Ray, D., Ghosh, S. K., & Raha, S. (2021). Seasonal foliar uptake of atmospheric polycyclic aromatic hydrocarbons by some local plants in a tropical metropolis in India. Atmospheric Pollution Research, 12(2), 104–112. https://doi.org/10.1016/J.APR.2020.10.013

Shahrukh, S., Baldauf, R., Popek, R., Moniruzzaman, M., Huda, M. N., Islam, Md. M., Hossain, S. A., & Hossain, M. E. (2024). Removal of airborne particulate matter by evergreen tree species in Dhaka, Bangladesh. Environmental Pollution, 363, 125194. https://doi.org/10.1016/j.envpol.2024.125194

Simonich, S. L., & Hites, R. A. (1994). Importance of vegetation in removing polycyclic aromatic hydrocarbons from the atmosphere. Nature 1994 370:6484, 370(6484), 49–51. https://doi.org/10.1038/370049a0

Terzaghi, E., Wild, E., Zacchello, G., Cerabolini, B. E. L., Jones, K. C., & Di Guardo, A. (2013). Forest Filter Effect: Role of leaves in capturing/releasing air particulate matter and its associated PAHs. Atmospheric Environment, 74, 378–384. https://doi.org/10.1016/J.ATMOSENV.2013.04.013

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., & Liu, C. (2019). Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: Morphological characteristics have the greatest impact. Science of The Total Environment, 652, 1149–1155. https://doi.org/10.1016/J.SCITOTENV.2018.10.357

Yang, B., Liu, S., Liu, Y., Li, X., Lin, X., Liu, M., & Liu, X. (2017). PAHs uptake and translocation in Cinnamomum camphora leaves from Shanghai, China. Science of The Total Environment, 574, 358–368. https://doi.org/10.1016/J.SCITOTENV.2016.09.058

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5. General Discussion

The four chapters of this thesis address different aspects of PAH uptake by tree leaves that allow us to deepen our knowledge of PAH uptake. In Chapter I (specific objective 1) we evaluate, in broad terms (through a meta-analysis), the relationship between PAH concentrations in leaves and leaf traits, specifically morphological and gas exchange traits. In Chapter II (specific objective 2) we evaluate how PAHs concentration varies within an individual (tree) as well as the variability between individuals along a street. In addition, we also study the variability of various traits within an individual and relate them to PAH concentrations. In Chapter III (first part of specific objective 3) we study how PAHs concentration varies among different evergreen conifer species and assess their ability to remove PAHs from the air. In addition, we also provide estimates of the equations for calculating their K_{PA}. In Chapter IV (second part of specific objective 3) we evaluated the relationship between the concentration of PAHs in the trees of Chapter III and different foliar trait.

Chapter I shows that in previous literature there is a scientific consensus, empirically proven, that leaf traits affect the uptake of PAHs by leaves. However, although this seems to be clear, there is still a lot of uncertainty about which leaf traits affect the concentration of PAHs in the leaves. In addition, there are generally contradictory results on the effect of different traits on PAH concentration (whether they increase or decrease it). In this work we focus on reviewing some traits related to leaf morphology and others related to gas exchange. Leaf morphology determines some purely physical characteristics of leaves in relation to PAH adsorption and accumulation. In general, the variables under study affect the surface area available for PAH adsorption, either exclusively (i.e. leaf surface area) by considering the mass (i.e. SLA and leaf thickness) or by modifying the thickness of the boundary layer (i.e. leaf width/length ratio). Gas exchange in plants is physiologically regulated by the control of stomatal closure. Increased gas exchange can increase the gas-phase entry of PAH into the stomatal cavity, potentially increasing the transfer of compounds to the parenchyma. As particle access through stomata is thought to be negligible, the selected variables should not be related to particle-bound PAH concentrations. On the contrary, gas exchange may favour PAH volatilisation from the stomatal chamber or mesophyll (Tian et al., 2019). It should be noted that all the variables related to gas exchange studied in Chapter I are proxies that attempt to

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describe the magnitude of foliar gas exchanges. The use of foliar traits as proxies for gas exchange has the advantage that these traits are less time-varying and methodologically easier to determine than direct gas exchange. However, they can also introduce noise in the data. The results of the meta-analysis varied between the different traits studied and, in general, were more robust and clearer for morphological traits and less so for gas exchange traits.

The results of the meta-analysis show a positive correlation between leaf area and uptake of PAHs, mainly those associated with particulate matter. This seems to confirm the initial hypothesis that an increase in leaf area increases the concentration of PAHs in the leaf by increasing the adsorption surface independently of mass. Although the results of the meta-analysis seem to point to a relationship between this trait and the concentration of particle-associated PAHs, it would also be logical that it would affect PAHs associated with the gas phase. Although the results seem clear and logical, they contrast with some previous findings, as several authors did not find any relationship between this variable and ΣPAHs (which also includes gas-phase PAHs) for *Croton floribundus*, *Piptadenia gonoacantha* and *Astronium graveolens* (Domingos et al., 2015; Dias et al., 2016), *Tilia x euchlora* and *Pyrus calleryana* (Jouraeva et al., 2002) and *Salix matsudana* (Zhao et al., 2018).

The results for SLA were also robust and showed a positive correlation between this trait and the concentration of PAHs, mainly MMW and HMW PAHs. This would be in line with the hypothesis that as the leaf surface area increases for the same weight, the concentration in terms of weight also increases (the available adsorption surface area for the same weight increases). According to this hypothesis, the PAHs most affected would be those associated with particulate matter, as seems to be the case. Despite all this, the SLA ($cm^2\ g^{-1}$) was the leaf trait most frequently studied in relation to PAH concentrations (e.g. De Nicola et al., 2017; Domingos et al., 2015; Dias et al., 2016; Howsam et al., 2001) and therefore it is not surprising that there are contradictory results. The one that stands out the most is that of Tian et al. (2019), which finds that light PAHs are affected by SLA and a significantly negative correlation between SLA and HMW PAHs. The explanation Tian et al. (2019) give for this result is that increasing SLA could decrease the molecular attraction capacity towards PAHs, as a decrease in leaf thickness could reduce the thickness of the leaf wax layer, which is responsible for the sorption of PAHs.

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As indicated in the previous paragraph, leaf thickness is closely related to SLA, since for a constant leaf area, an increase in leaf thickness translates into a decrease in SLA. Therefore, following the first hypothesis proposed for SLA, leaf thickness should be negatively correlated with the concentration of PAHs associated with particulate matter, which is exactly the result obtained in our meta-analysis. Furthermore, as a result, we also obtained significantly positive correlations with LMW PAHs. This could be explained by the fact that an increase in leaf thickness could be related to an increase in the thickness of the wax layer or an increase in the proportion of intercellular spaces in the spongy parenchyma. In general, these results are consistent with previous findings, as Domingos et al. (2015) and Días et al. (2016) reported empirical evidence supporting that the decrease in thickness produces an increase in the total concentration of PAHs.

The last of the morphological leaf traits is leaf width/length ratio (W/L). This has been one of the least studied morphological traits and has generally been neglected (Tian et al., 2019). The results obtained en el Capítulo I showed a significantly negative relationship with some LMW PAHs, which could be explained by the thickness of the boundary layer. That is, as the W/L ratio decreases, the thickness of the boundary layer decreases, thus increases the gas exchange processes and increases the concentration of PAHs in the gas phase. However, for the heavier PAHs of 4, 5 and 6 rings (Table 2), the significative correlations are positive. These findings are partly in agreement with those reported by Tian et al. (2019), who found that leaf W/L ratio was positively correlated with MMW PAHs. However, in contrast to our results these authors found positive correlations also with LMW PAHs.

The results for the traits related to gas exchange were surprising, as they were mostly contrary to the main hypotheses on how the traits should affect the concentration of PAHs in the leaves. The starting hypothesis is that traits related to gas exchange would mainly affect gas PAHs and the higher the gas exchange, the higher the uptake of PAHs and hence the higher the concentrations would be. However, for stomatal conductance we obtained positive correlations with PAHs associated with PM, which contrasts with previous findings, as De Nicola et al. (2017) demonstrated that stomatal conductance was related to an increase in Σ2-rings and Σ3-rings PAHs, in accordance with the proposed hypothesis. For $\delta^{13}C$ we found positive correlations for LMW PAHs and negative correlations for HMW PAHs, which is contrary to expectations. The $\delta^{13}C$ has an inverse relationship with gas exchange and would therefore be

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expected to have a negative correlation with LMW and no significant correlations with PM-associated PAHs.

Finally, stomatal density is the most studied gas exchange trait because of its ease of measurement. However, it is the trait of the meta-analysis with the fewest available data pairs, so the results are questionable. The results show negative correlations between PAHs concentration and this variable. In the previous literature, as in this meta-analysis, no relationship between stomatal density and ΣPAHs concentration was usually found (e.g. De Nicola et al., 2017, Tian et al., 2019; Wang et al., 2008), except by Fellet et al. (2016). However, in contrast to our meta-analysis, Tian et al. (2019) observed a direct relationship between stomatal density and LMW and MMW PAH concentrations, supporting the starting hypothesis. A study by Prigioniero et al. (2022) found a positive correlation between the stomata density of the upper leaf surface (with waxes) and LMW PAHs. In addition, the lower leaf surface (with waxes and without them) was significantly and positively correlated with HMW PAHs and negatively with LMW PAHs (with waxes). Both this and the present meta-analysis coincide in finding a relationship between PAHs associated with particulate matter and stomatal density, although no hypothesis for the relationship was proposed. Prigioniero et al. (2023) obtained similar results in a study including 28 species, detecting positive correlations between stomatal density on the upper leaf surface and the concentration of MMW and LMW PAH in dewaxed leaves.

A possible hypothesis explaining the negative correlation between PAHs and gas exchange is the one put forward by Tian et al. (2019). According to this, the volatilization of PAHs from the stomatal chamber or mesophyll increases with gas exchange. Regarding stomatal density, it should also be noted that apart from gas exchange, the increase in this parameter is related to a decrease in wax concentration (Tian et al., 2019). Furthermore, stomatal density does not consider the morpho-physiological characteristics of stomata (such as their size, protective structure -as could be trichomes- or location) or the rate of opening, and therefore it does not determine stomatal conductance (De Nicola et al., 2017; Yang et al., 2017). In addition to this, it should be noted that these variables are proxy measures of gas exchange. Although there is a large variability in both intraindividual and interindividual traits (e.g. Herrera, 2009; Vinod et al., 2023), gas exchange is generally much more variable than morphological traits, and the effect of disregarding this variability in the analysis may therefore be much more pronounced. According to our results, we can deduce that gas exchange is not of

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great importance in relation to PAH uptake, however, it seems logical to think that it will probably contribute to the load of light, gas-phase PAHs. It should also be noted that even if these traits alone are not so decisive, they may affect or be affected by other traits that we do not consider. In this study we focus on individual traits and many of these absorption processes may be multifactorial.

In spite of the value of this work, we must be aware of the limitations of our study, many of which derive from the nature of the data available, in particular the trait data. Unlike the data on PAH concentrations, the trait data do not correspond to the trees studied but are median values of the data available for each species in the TRY Plant Trait Database. This obviates the natural variability in this type of data and leads to some pseudo replication, which introduces many ties in the Spearman correlations, thus affecting the exact p-values. Another problem arises when assessing the importance of each species in the comparisons, as some species are included much more often, and some studies carry out many more comparisons than others. Despite these limitations, this study provides a complete view of the current knowledge on the relationship between leaf traits and leaf PAH concentrations. Moreover, it reinforces existing hypotheses on the relationship between leaf morphological traits and the different types of PAHs, while also challenging some of the hypotheses relating PAHs and gas exchange. Future studies on the effect of gas exchange should apply more careful and detailed approaches and consider the multifactorial aspects of PAH uptake.

As explained above, in Chapter I we studied the general relationship between PAH concentrations and leaf traits based on the literature and a meta-analysis, while in Chapter II, we carried out an empirical study of the variation of these variables at the intra- and inter-individual level and the relationship between them. We sampled leaves from 12 different canopy positions (three heights, two distances from the trunk and two orientations - towards the road or towards buildings) of one type of ornamental apple tree. This was repeated in 15 apple trees along a main street in A Coruña, a medium-sized city in NW Spain. At each of these 12 positions we determined the concentration of PAHs in the leaves and several leaf traits (i.e. SLA, $\delta^{13}C$, stomata density, concentration of different fatty acids in the leaf cuticle and leaf pilosity).

Both leaf PAH concentrations and traits showed a marked intra- and inter-individual variation. PAHs concentrations in leaves ranged from 57 to 472 ng/g dw, while the total PAHs

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concentration in the most contaminated trees was up to twice the concentration determined in the least contaminated trees. In the case of traits, in general, inter-individual variability was lower or similar to intra-individual variability. An example are the inter- and intra-individual coefficients of variation (CV) for linolenic acid (inter-CV = 2.1 % and intra-CV = 2.0 %) and SLA (inter-CV = 6.8 % and intra-CV = 18.9 %). For both PAHs concentrations and traits, much of the intra-individual variability seems to come from the relative position of leaves within the tree canopy.

Statistical analysis of the PAHs concentration found, in general, significant differences between the different positions, mainly depending on the height of the leaves and their distance from the trunk. That is, leaves lower in the canopy and closer to the trunk had significantly higher concentrations than those higher and further away from the trunk. However, after analyzing the inter-individual variability (the differences between the sampled trees), we considered that the results of statistical analysis on the importance of leaf position on the concentration of PAHs might have been affected. Therefore, to reduce noise in the statistical analyses, we divided the trees into two groups. The first (group 1) contained the trees with the lowest total PAHs concentration and the highest concentration of light PAHs. The second (group 2), containing the trees with the highest total PAHs concentration. After repeating the statistical analyses, the results for group 1 were similar to those obtained in the analyses for all trees together. In other words, what most affected PAHs concentration was leaf height and distance from the trunk. Regarding group 2, the differences were only related to canopy height, and differences according to distance from the trunk were only observed for phenanthrene (Phe). In addition to these analyses, linear regression models were also applied when significant differences between leaf positions were observed. In the models with all trees the range of the adjusted R^2 was 0.14 to 0.48, with an average R^2 of 0.26 for individual compounds. For the group 1 models, the range was very similar, from 0.12 to 0.45, with an average of 0.27, while in group 2 it was much higher, from 0.32 to 0.59 with an average of 0.50. The observed differences between the two groups can probably be explained by the fact that group 2 is smaller and more homogeneous. In addition, the trees in group 2 are closer to each other, and they are therefore subject to less variability than the trees in group 1, which are distributed along the entire length of the street.

Regarding traits, it is expected and logical to find a significant variation between leaves in different canopy positions, especially for SLA, stomatal density and $\delta^{13}C$. In fact, after

statistical analysis, we found significant differences between the different positions. Thus, the most exposed leaves (sun leaves, top canopy) seem to have a lower SLA, higher stomata density and higher $\delta^{13}C$. These results are not surprising considering the variation in these variables in relation to height and light exposure within the tree canopy (Camargo & Marenco, 2011; Liu et al., 2016; Vinod et al., 2023). Despite the differences obtained for these variables, we found no significant differences in fatty acid composition between the different canopy positions, only in group 1 for myristic acid, with a higher concentration in leaves close to the trunk. Regarding pilosity, these leaves do not seem to be characterized by having many trichomes, which are filiform and mainly located on the leaf veins. A considerable difference between the number of trichomes on the bottom canopy leaves (less trichomes) and top canopy leaves (more trichomes) was observed by the naked eye. In addition, there also appeared to be more trichomes on the adaxial side than on the abaxial side. However, as the assessment of pilosity was not carried out on a quantitative basis, it was not possible to incorporate this variable into the statistical analyses.

After we confirmed the intra-individual variability (intracanopy) of PAH concentrations and traits studied, as well as their dependence on leaf position within the canopy, we thought it would be interesting to study the correlation between both groups of variables (PAHs and traits). This way, we would go further along the lines established in the first chapter of this thesis, analyzing the relationship between PAHs concentration in leaves and leaf traits. In this case in a more specific way and with less noise (concentrations and traits were determined on the same trees at the same time). Furthermore, while in Chapter I we only considered traits related to leaf morphology and gas exchange, in these correlations we included the fatty acids of leaf waxes.

The correlations clearly show a strong inverse relationship between SLA and $\delta^{13}C$ and, to a lesser extent, the nature of the relationship (inverse for SLA and direct for $\delta^{13}C$) of these variables with stomatal density. Likewise, most of the PAHs were positively correlated with SLA and negatively correlated with the other two traits. Most of the cuticle fatty acids were not significantly correlated with any variable except for other cuticle fatty acids. Considering the strong correlations between the traits related to PAHs concentration (SLA, $\delta^{13}C$ and stomatal density) it appears that these depend on one main source of variability, the leaf position, which modifies the traits according to the vertical gradient and light exposure. However, although the three traits vary according to these gradients, SLA seems to have the greatest influence on PAH

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uptake. This is the trait for which the highest correlations were obtained (0.16 to 0.58) and, furthermore, if gas exchange through the stomata would play a central role, differences should be clearly observed in the correlations with the lighter and heavier PAHs, which was not the case. The inverse correlation with stomatal density was probably due to (Prigioniero et al., 2022) the stomata being closed or, at least, not open sufficiently to significantly influence the concentration of PAHs. If we compare the results obtained for SLA, $\delta^{13}C$ and stomatal density with those obtained in the meta-analysis of Chapter I, we can observe similarities and discrepancies. The results for SLA and stomatal density are similar to those obtained in Chapter I. That is, the former variable shows positive correlations for most PAHs, while the latter shows negative correlations. In the case of SLA, moreover, the range of correlation values is similar in both cases. For stomatal density, the values obtained in Chapter I are considerably higher than those obtained in Chapter II. Regarding $\delta^{13}C$, in Chapter II the significant correlations were negative in all cases, while in Chapter I they were positive for light PAHs.

Considering the correlation between PAHs and SLA it is possible that some of the differences that we observed could be attributed to the concentrations of PAHs being expressed in terms of mass ($\mu g\ g^{-1}$), without taking the leaf surface area into account. Therefore, we repeated the analyses with the data divided by the SLA, i. e., by expressing the concentration of PAHs expressed in terms of leaf surface area ($\mu g\ m^{-2}$). The most remarkable changes in the results are the disappearance of most of the differences due to the distance of the leaves from the trunk and the decrease in the explanatory power (R^2) of the models. In other words, a large part of the variation observed in the first analysis can be attributed to leaf morphology. This is consistent with the findings of other studies (Terzaghi et al., 2015) showing the influence of SLA on the uptake of PAHs by trees.

In this chapter we have shown the importance of leaf morphology, mainly SLA, in explaining the variation in foliar PAH concentrations. Some researchers (Jouraeva et al., 2002) recommend expressing foliar PAH concentrations per unit of surface area rather than express them per unit of mass when comparing results for different plant species. Despite following this recommendation, we still observed a significant decrease in leaf PAH concentrations due to leaf height. This observation is consistent with that of Murakami et al. (2012) who reported a similar decrease, mainly associated with the distance from the source of emission of PAHs (cars). However, these researchers also detected differences between leaves over the road and those over the pavement, which we did not observe. This difference is probably due to the fact

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that the trees in the aforementioned study have “no obstacles to limit air circulation”, while in the present study the buildings caused a street canyon effect that could enhance local air mixing. Finally, it is important to note that, although the distance from the PAH emission sources may be the main factor accounting for the observed variation between heights (once the effect of SLA is removed), other factors also play a role, such as the barrier effect of leaves and leaf illumination/temperature, because of both their influence on SLA (Gara et al., 2018) and their role in the volatilization/degradation-deposition balance of PAHs (Terzaghi et al., 2020). Interestingly, leaf pilosity is associated with higher uptake and retention of PM-associated PAHs (Prigioniero et al., 2023) and therefore we would expect higher concentration in exposed leaves; however, the slightly higher trichome density does not seem to be able to counteract the effect of factors that decrease PAH concentration with height. Based on the findings of our study, although a mass balance of PAHs in the air-plant-soil environment was not performed, it is clear that not only SLA would be important as factor driving the uptake but also the leaf area index (LAI, m^2/m^2), which together compose plant biomass. In other terms to significantly reduce PAH air concentrations in urban environments different measures would be needed: emission reduction, and mitigation measures such as the increase of plant foliar biomass in the streets. Rows of trees with high leaf biomass should be planted along roads in cities. Low trees are preferable for a given biomass, as lower leaves capture more pollutants (and remove them directly from the air that pedestrians breathe). These trees should have a dense canopy with a high proportion of shade leaves, characterized by a corresponding large SLA. This would increase the uptake of pollutants such as PAHs and possibly PM. Therefore, we recommend selecting species with specific morphology and pruning practices that favour the maintenance of this morphology. Furthermore, as recommended by other authors (e.g. De Nicola et al., 2017), it is better to select perennial species that can remove pollutants throughout the year, especially in winter, when fossil fuel combustion is higher and the planet boundary layer decreases in height for large portion of the day, leading to an increase in the concentration of pollutants in the air of cities.

In Chapters I and II, we have studied the relationship between leaf traits and PAHs concentration in leaves, both on a general level from the literature reviewed (Chapter I) and on an intra-individual level through our own measurements (Chapter II). However, this information is not enough to select optimal species for the removal of PAHs from the atmosphere. The lack of information and comparative studies, given the large number of

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ornamental species, complicates decision making in this regard. To quantify the role of plants and specific plant species in removing PAH from air, dynamic environmental fate models, incorporating plants in the mass balance could be employed (Terzaghi et al., 2017). However, these models need numerous parameters to make accurate predictions. One of these parameters is the plant-air partition coefficient (K_{PA}), which indicates the ratio, at equilibrium, between the concentration of a chemical in plant leaves and in air. In Chapter III, we estimate the K_{PA} equations of 13 conifer species and study their ability to remove PAHs associated with gas and particulate matter in urban areas. All the selected species are commonly (but not exclusively) found as ornamental trees in temperate and Mediterranean cities and, moreover, all of them are perennial species since, as indicated at the end of Chapter II, "it is better to select perennial species that can remove pollutants throughout the year, especially in winter".

Although most of the species used in this study are common ornamental species and their leaves have been previously used in studies on particulate matter (PM) content (Sæbø et al., 2012; Ozdemir, 2019; Tang et al., 2019), for many of them PAH levels were here determined for the first time (i.e., *Cryptomeria japonica* and *Cryptomeria japonica* f. *elegans*, *Cupressus sempervirens*, *Sequoia sempervirens*, *Chamaecyparis pisifera*, *Cupressus arizonica*, *Cupressus lusitanica* and *Thujopsis dolabrata*). The species investigated in this study showed a different PAH accumulation behavior, considering total PAHs but also single compounds. Total PAH concentrations ranged from 845 ng g^{-1} dw for *Pinus pinaster* to 95 ng g^{-1} dw for *Picea abies* (both belonging to the same family). Therefore, species belonging to the same family showed concentrations that varied up to one order of magnitude. It is also interesting that the range is similar to the concentrations observed in the leaves of the apple trees studied in Chapter II, where variation in total PAHs concentrations is in the same order of magnitude. It is remarkable that, in general, the PAH fingerprints were comparable among the species investigated. However, there were some exceptions, i.e., *C. japonica* f. *elegans* and *C. lusitanica*. The concentrations measured were similar to those obtained in previous studies with *P. pinaster* in urban areas sampled in Portugal in 2007 (Ratola et al., 2010) and *P. abies* sampled in Norway (Borgulat et al., 2018), but higher than those measured for *P. pinaster* needles (average total PAH concentration of 320 and 137 ng g^{-1} dw) collected in rural sites located in the same region of the current study (De Nicola et al., 2016, 2017). Some measurements are also available for *Cedrus atlantica*, *Cedrus deodara*, *Juniperus chinensis* and *Taxus baccata*

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collected in different countries (France, Italy, China and Belgium), urban sites and years (2015, 2016 and 2018) and therefore representative of different contamination levels.

In order to calculate the $K_{PA,}$ it was necessary to estimate the concentrations of PAHs in the air. The estimated concentrations were generally lower than those determined at other urban and semi-urban sites in Europe. However, the conditions at the sampling site make this result not surprising. From these estimated concentrations of PAHs in air and the determined concentrations of PAHs in tree leaves, we developed equations to estimate K_{PA} as a function of Log K_{OA} (octanol-air partition coefficient). The equations developed were 3: i) a linear model fitted only for PAHs associated with the gas phase; ii) a linear model fitted for all PAHs (gas + particle phase PAHs); and iii) a 2nd degree polynomial model fitted to all PAHs. As expected, Log K_{PA} increased with the increase in Log K_{OA}. However, at Log K_{OA} larger than 10, Log K_{PA} reached a plateau and/or dropped indicating a kinetic delay in partitioning (Mackay et al., 2019) for the most hydrophobic compounds which are mainly bound to PM, which compete with leaf biomass for PAH uptake (Mclachlan & Horstmann, 1998). Moreover, the equation used to predict K_{PA} for *P. abies* and back-calculate air concentrations, was selected since it was developed for PCBs (Log K_{OA} ranging from 7.5 to 10.5) (Nizzetto et al., 2008) and allowed to better estimate gas phase PAH air concentration since it neglected the particle contribution. For the same reason, air concentrations of particle associated PAHs are probably overestimated, resulting in lower K_{PA} when the back-calculated air concentrations were used to derive species-specific K_{PA} equations.

Equation developed considering gas phase PAHs (model 1), showed R^2 ranging from 0.66 (*P. pinaster*) to 0.98 (*J. chinensis*) considering sampling time temperature and from 0.56 to 0.92 for the same species considering reference temperature. The low R^2 obtained for *P. pinaster* could be probably explained by the high concentrations of Phe, Fla and Pyr that resulted in higher K_{PA} compared to the other compounds with higher Log K_{OA}. This is probably due to the lipid content of pine epicuticular waxes. As commented by Ratola et al. (2011) and Fernández-Varela et al. (2015), when comparing *P. pinaster* and *Pinus pinea*, these waxes seem to be behind the higher affinity that *P. pinaster* has for lighter PAHs.

The investigated species showed a different accumulation efficiency for gas phase PAHs as shown by the different slopes of their K_{PA} equations. Gentle slopes indicate minor differences in the affinity of a species for PAHs characterized by different hydrophobicity (i.e., Log K_{OA}).

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Apart from the *P. abies* model, which was taken as the reference for estimating air concentrations, the models with the smallest slopes are those of *J. chinensis* and *T. baccata*. These species are among the 4 species with the lowest total PAH concentrations, indicating their weak affinity for PAHs in general. Equations of the species *P. pinaster*, *C. deodara*, *T. dolabrata*, and *Cupressus spp*. showed the steepest slopes although their affinity for total PAHs varies. The case of *P. pinaster* is different, since the slope of its model is due to the high concentration of Phe, Fla and Pyr. As for PAH total concentrations in leaves, no relationships between slopes and any studied botanical families were found. The slope of the equations obtained considering also particle bound PAHs (model 2) are generally smaller due to the uncertainties in air concentrations explained above (K_{PA} underestimation for the most hydrophobic PAHs).

When comparing linear model 1 (gas-phase PAHs) and polynomial model (all PAHs) it can be noted that in some species the two models may diverge when Log K_{OA} is larger than 10. Species with a limited difference in slope in the two models show a more linear trend of PAH accumulation, probably due to the enhanced accumulation of particle associated PAHs compared to other species.

This means that the models developed in this study could provide new and important information about the loading of PAHs associated with the gas phase (Log K_{OA} < 10), as well as particle phase by trees, specifically ornamentally used perennial conifers. As seen above, based on the information obtained from the K_{PA} of these PAHs, in conjunction with their concentrations in leaves, an initial evaluation of the suitability of the leaves and needles of these species as air filterers can be made. However, to make a complete evaluation it would be necessary to consider the PM and the PM associated PAHs (Log K_{OA} > 10). Since PM in air contains a great amount of the most hydrophobic PAHs (Omar et al., 2002; Moscoso-Pérez et al., 2010; Lara et al., 2022), a relevant part of the PAHs removed by trees are captured as PAHs bound to PM (e.g., Jouraeva et al., 2002). Although the information necessary to obtain reliable specific deposition velocity estimates for the PM associated PAHs present on leaves is lacking (e.g., Terzaghi et al., 2013), a rank of the capability of the species to retain particle associated PAHs is possible by analyzing the contribution of less or more hydrophobic compounds to the total PAH concentrations. By calculating the percentage of PAHs associated with PM and the percentage of PAHs associated with gas, we can observe that the former is always lower than the latter. However, the percentage of PM associated PAHs, and therefore the leaf sequestration

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ability for PM varies greatly among the species in this study, ranging from 3.4% in *P. pinaster* to 34.4% in *C. lusitanica*.

Similar to Chapter II, we determined the SLA for each of the species and calculated the SLA-corrected concentrations, i.e., the concentrations in terms of leaf surface area. Comparison of the original and the SLA-corrected concentrations allows evaluation of the role of each species in capturing PAHs based on total leaf biomass or normalized on leaf surface. Results show that the ranking of species capturing gas-phase PAHs varies from that of species capturing particle phase PAHs. The results obtained in this study show that *P. pinaster* needles have the largest capacity to capture gas phase PAHs, while *C. sempervirens* leaves have the largest capacity to capture PM associated PAHs. When considering the amount of PAHs per leaf surface, *C. sempervirens* leaves show the best removal performance, as it is the second species with the highest gas phase PAHs and the first one for PM associated PAHs. However, there are other factors that were not analyzed in this study, such as LAI, tree shape, percentage of leaves biomass, the change of this percentage during lifespan of the tree, which will be investigated in further studies.

Few studies provide K_{PA} equations that can be used to estimate air concentrations. Some of these relationships were reported by Bolinius et al. (2016), McLachlan and Horstmann (1998), and Nizzetto et al. (2008); while a general review of plant uptake is available in Doucette et al. (2018). We compared the model 1 equations (gas phase PAHs) with the steepest slope and the smallest slope (i.e., *C. arizonica* and *J. chinensis*) obtained in our study, with some examples of broadleaf and conifer species from literature. As result, only *Rhododendron ponticum* is able to capture more chemical at high K_{OA} and less at K_{OA} lower than 7. The broadleaves are comprised within the range of K_{PA} of our studied conifers showing how broad is the span of plant-air partitioning for the conifer species. Although this comparison refers to gas phase uptake, the results from previous findings also show the capability of conifers in retaining PM associated PAHs.

Although some uncertainty remains about the neat values of parameters included in K_{PA} equations, such as the difficulty of their estimation for particulate PAHs, our results allow improving the knowledge about PAH capturing ability of conifers. This information is relevant because these species carry leaves throughout the year and therefore can filter the air for PAHs and PM also in heavily impacted periods such as autumn and winter. Additional studies could

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include a more specific particle matter determination on needles to verify the uptake behavior of the different species for PM and PM associated PAHs.

In specific objective 3, as in the studies corresponding to specific objectives 1 and 2, we also address foliar traits and their relationship with PAHs concentration in leaves. The initial plan was to produce a single publication addressing specific objective 3 and including the trait data. However, in the end we did not include them in the publication of Chapter III, but we considered these data interesting and relevant to provide greater cohesion to the thesis. Thus, in Chapter IV we present the trait data corresponding to the trees of Chapter III and study their relationship with the PAH concentrations presented in Chapter III.

Even though all the species studied are evergreen conifers belonging to three botanical families, in Chapter III, we observed that the concentrations of PAHs vary greatly among the different species. This variability may be due to a multitude of factors, but certainly one of them is the leaf traits. This makes even more sense if we look at the substantial differences we observed for traits among the species in Chapter IV. However, not all traits have the same ranges of variability among species. Thus, in the morphological leaf traits, the highest SLA is almost three times higher than the lowest SLA (54 vs 19 cm^2 g^{-1}, *T. baccata* and *P. abies*, respectively) while length (extracted from the TRY Plant Trait Database) varies by two orders of magnitude (0.075 cm for *C. sempervirens* to 20 cm for *P. pinaster*). Similar is also true for the gas exchange traits, for the $\delta^{13}C$ the range is small (-23.7‰ for *T. dolabrata* to -30.2‰ for *P. pinaster*), whereas for the stomatal density it is of an order of magnitude (3203 vs 22813 stomata cm^{-2}, *C. arizonica* and *C. japonica*, respectively) and for the stomatal conductance it is two orders of magnitude (from 0.016 to 0.257 mol H2O m-2 s-1, *J. chinensis* and *C. deodara*, respectively). However, it is important to understand that even if one trait has a greater range of variation than another, it does not mean that it has greater importance or influence on the concentration of PAHs than another with less variation. In addition, it should be noted that although in some cases it may seem that the variation is considerable, the values are not very different from those observed in Chapter II for intraindividual variability (ca. 150 to 370 cm^2 g^{-1} for SLA, ca. -30‰ to -34‰ for $\delta^{13}C$ and ca. 4000 to 18000 stomata cm^{-2} for stomatal density). In fact, although it is not the objective of the study, in Chapter IV we can also appreciate a high intraspecific variability for some traits and species (e.g. *S. sempervirens* for stomatal density or *C. japonica* for stomatal conductance). Regarding traits related to leaf chemical composition (relative fatty acid content), we also see substantial variation among

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species. The percentage of the sum of fatty acids (Total F.A) ranges from 5.49% (*P. abies*) to 22.20% (*J. chinensis*). Moreover, the majority fatty acid also varies greatly between species, e.g., F.A. C12 accounts for 4.24% of the total extracted compounds in *P. pinaster* and 0.71% in *C. deodara*; in contrast, F.A. C20:2 accounts for 0.09% for *P. pinaster* and 6.30% for *C. deodara*). These values, unlike the concentrations obtained in Chapter II, are percentages of the total compounds extracted by pyrolysis and, therefore, cannot be compared.

We tried to evaluate the effect of these traits by obtaining their correlations with the concentration of PAHs in the leaves. Most of these correlations were not significant; but there were a few that yielded interesting results. The high number of non-significant correlations is probably due to the small sample size and the high intra- and interspecific variability at all levels (PAHs, traits, species position in the sampling area, etc.) which may increase the noise in the data. Thus, this would make it more difficult to obtain clear trends in the relationships between PAH concentrations and traits. Actually, all significant correlations had coefficients greater than 0.40.

For SLA we found two significantly positive correlations with coefficients close to 0.6: one with an LMW PAH (fluorene) and one with an MMW PAH (benz[a]anthracene). This is in agreement with some of the literature (e.g., Tian et al., 2019; see the Discussion of Chapter I for details) and with the results obtained for Chapter I and II. Regarding length, we observed significantly negative correlations for 4 PAHs mostly associated with PM, as well as with the ∑5-rings and ∑HMW PAHs. It is suggested in literature that the width/length (W/L) ratio has an inverse relationship with the concentration of PAHs in leaves (e.g. Liang et al., 2017). Therefore, it would be expected that the relationship between length and PAHs concentration would be direct and not inverse. However, in this case it is likely that leaf length behaves as a proxy for species with scaly leaf clusters (the species with the smallest leaves), which in this study have a higher surface roughness than species with longer leaves, which would allow them to retain more PM (Shahrukh et al., 2024). As Tian et al. (2019) evaluated, the uptake of HMW PAHs is highly dependent on leaf roughness and its wax content, while the uptakes of MMW PAHs depends on morphological characteristics. The result in Chapter IV is similar to the one obtained in Chapter I, where we observed significantly positive correlations for the W/L ratio (and therefore negative for the length) with PAHs associated mainly with PM. Furthermore, in Chapter I the PAHs associated with gas phase had significantly negative correlations with the W/L ratio, evidencing a different relationship with this trait to that of the PAHs associated with

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PM. This distinctive relationship between both groups of PAHs is also in line with the results obtained for Chapter IV, where gas phase PAHs do not present any significant correlation with length, unlike PM-associated PAHs.

Regarding gas exchange, as mentioned above, we have two hypotheses for its relationship with the concentration of PAHs in the leaves. The first and main hypothesis is that as gas exchange in the leaves increases, the concentration of the gas phase PAHs inside the leaf would also increase, since they would diffuse freely through the stomata. While a secondary hypothesis, contrary to the previous one, would be that increasing gas exchange could increase the volatilization of PAHs from inside the leaf. Regarding stomatal conductance, we only have one significant correlation (positive with ∑PAHs), but this seems to support the main hypothesis, as do the results of De Nicola et al. (2017). This is not the case for the results in Chapter I, where significantly positive correlations only occur with PAHs associated with PM. However, as we explained previously in this section, this may be due to the nature of the data used in Chapter I. The $\delta^{13}C$ has an inverse relationship with gas exchange, thus the results of this study would also support the main hypothesis, as it has 4 significantly negative correlations with different PAHs (phenanthrene and fluoranthene) and sums of PAHs (∑4-rings PAHs and ∑PAHs). These results are totally contrary to those obtained in Chapter I, where correlations for light PAHs were positive and correlations with heavy PAHs were negative. In addition, the results of Chapter IV were similar to those of Chapter II, although in the latter there were also significantly negative correlations with the PAHs associated with PM. Concerning stomatal density, it does not seem to be related to the PAHs gas phase since the significant correlations are all negative and refer to PAHs associated with PM. This result is probably due to the fact that stomatal density does not really measure gas exchange, i.e., it measures the number of stomata, but not whether they are open. In fact, Prigioniero et al. (2022) found a "puzzling" relationship between stomatal traits and pollutant uptake and consider that this is probably due to the relationship of stomatal traits with other leaf traits and the absence of a relationship between stomatal traits and the opening of stomata. In all three chapters (I, II and IV) we observed significant negative correlations for PAHs associated with PM. Unlike the other chapters, in Chapter IV we found no significant correlations for gas phase PAHs. Overall, all these results seem to indicate and reinforce the hypothesis that stomatal density is not really a good proxy for gas exchange.

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Regarding traits related to leaf chemical composition, there are two main hypotheses on how waxes might affect PAH concentrations in leaves. One of them is that increasing the amount of waxes could facilitate the uptake of PAHs by increasing the hydrophobicity of the leaf (Ray et al., 2021). The other suggests that by increasing the amount of waxes, these could hinder the diffusion of PAHs inside the leaf and these, being retained in the cuticles, would be more exposed to photodegradation, revolatilization and/or removal from the surface (Yang et al., 2017; Terzaghi et al., 2013). In Chapter IV the variable we evaluated in this regard was the relative composition of the fatty acids with respect to the total compounds extracted from the leaf by pyrolysis. When calculating the correlations we obtained mixed results, with positive (i.e. F.A. C12, F.A. C14 and F.A. C20:2) and negative (i.e. F.A. C16, F.A. C18:2 and F.A. C18:1) correlations. Out of the positive relationships F.A. C12 and F.A. C14 have only two significant correlations each, three of these 4 correlations are with gas phase PAHs and only one with a PAHs associated to PM. In contrast, F.A. C20:2 has 7 significant correlations, all with PAHs associated with PM. As for the negative correlations, all three fatty acids have at least 4 significant correlations and are mainly with four-ring PAHs, with ∑4-ring PAHs and with ∑PAHs. It is noteworthy that in Chapter II we only obtained two significant correlations for fatty acids, one for stearic acid (F. A. C18) and one for linolenic acid (F. A. C18:3), both with anthracene, and in Chapter IV we found no significant correlation for these fatty acids.

In short, Chapter IV completes Chapter III and further explores specific objective 3 of this thesis by examining the relationship between foliar traits and the concentration of PAHs in leaves. The results obtained seem to reinforce the positive relationship of PAHs concentrations (ng g^{-1}) with SLA, and its negative relationship with stomatal density, which seems to rule out stomatal density as a reliable proxy of gas exchange. On the other hand, stomatal conductance and $\delta^{13}C$ seem to be better indicators of gas exchange and in general it seems that increased gas exchange would have a positive relationship with PAHs concentration. The relationship between the uptake of PAHs associated with particulate matter and the arrangement of leaves in scaly clusters, which increase the roughness of the PAH uptake surface, also seems considerably clear. Finally, we have observed relationships between PAHs concentration in leaves and relative fatty acid content in leaves, however further studies would be necessary to unravel the exact origin of these relationships and determine whether they are causal or spurious.

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6. General Conclusions

- The relationship between PAHs concentration in the leaves and leaf morphology traits is more consistent and more robust than the relationship with gas exchange traits.
- The results obtained for the morphological traits seem to support the main hypotheses on literature about their relationship with PAHs concentration, while the results for the gas exchange traits are contradictory at all levels: within the available literature, within our meta-analysis and between the studies carried out in this thesis (including the meta-analysis).
- The importance of gas exchange traits and fatty acid composition need to be further investigated (both at the individual and multifactorial level) in order to better understand their contribution to leaf PAH concentration.
- Out of all the traits studied in this thesis, SLA was the one with the clearest and most coherent results. It has also been the most important due to its relationship with PAH concentrations and with the variation of these concentrations. In contrast, all results seem to indicate that stomatal density would not be a suitable proxy for gas exchange.
- The intraindividual variability leaf PAH concentration is proved to be significant and should be considered in designing sampling schemes or contamination model development.
- The main source of intraindividual variability is leaf height (bottom, mid or top canopy), with lower leaves taking up more PAHs than higher leaves. This depends on leaf morphology (SLA), distance to the emission source (cars), the barrier effect and illumination/temperature of the leaves.
- There is a large interspecific variability in the potential of conifers to remove PAHs from the air. Despite some remaining uncertainties about the neat values of the parameters including in K_{PA} equations, these equations allow us to improve the knowledge on the potential of different perennial conifers to remove PAHs from the air.

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- The ideal vegetation for removing PAHs from the air in urban environments would be perennial, leafy trees (with a high leaf biomass), not too tall, with a high proportion of shade leaves and with a high capacity to remove both gas and particulate matter PAHs.
- To optimize the use of trees as filters for PAHs, it is essential to further investigate the factors that influence PAH accumulation in tree leaves. Additionally, more comparative studies across different species are needed, both experimental and modeled, to better understand their potential as PAH removers.

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7. References

Air Quality Expert Group. (2018). *Effects of Vegetation on Urban Air Pollution*.

Ali, M. U., Siyi, L., Yousaf, B., Abbas, Q., Hameed, R., Zheng, C., Kuang, X., & Wong, M. H. (2021). Emission sources and full spectrum of health impacts of black carbon associated polycyclic aromatic hydrocarbons (PAHs) in urban environment: A review. *Critical Reviews in Environmental Science and Technology*, *51*(9), 857–896. https://doi.org/10.1080/10643389.2020.1738854

Andersson, J. T., & Achten, C. (2015). Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes. *Polycyclic Aromatic Compounds*, *35*(2–4), 330. https://doi.org/10.1080/10406638.2014.991042

Bacci, E., Cerejeira, M. J., Gaggi, C., Chemello, G., Calamari, D., & Vighi, M. (1990). Bioconcentration of organic chemical vapours in plant leaves: The azalea model. *Chemosphere*, *21*(4–5), 525–535. https://doi.org/10.1016/0045-6535(90)90023-M

Barling, R. D., & Moore, I. D. (1994). Role of buffer strips in management of waterway pollution: A review. *Environmental Management*, *18*(4), 543–558. https://doi.org/10.1007/BF02400858/METRICS

Bojes, H. K., & Pope, P. G. (2007). Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas. *Regulatory Toxicology and Pharmacology*, *47*(3), 288–295. https://doi.org/10.1016/J.YRTPH.2006.11.007

Bolinius, D. J., Macleod, M., McLachlan, M. S., Mayer, P., & Jahnke, A. (2016). A passive dosing method to determine fugacity capacities and partitioning properties of leaves. *Environmental Science: Processes & Impacts*, *18*(10), 1325–1332. https://doi.org/10.1039/C6EM00423G

Bonvallot, V., Baeza-Squiban, A., Baulig, A., Brulant, S., Boland, S., Muzeau, F., Barouki, R., & Marano, F. (2012). Organic Compounds from Diesel Exhaust Particles Elicit a Proinflammatory Response in Human Airway Epithelial Cells and Induce Cytochrome p450 1A1 Expression. *Https://Doi.Org/10.1165/Ajrcmb.25.4.4515*, *25*(4), 515–521. https://doi.org/10.1165/AJRCMB.25.4.4515

Borgulat, J., Staszewski, T., & Łukasik, W. (2018). Monitoring of PAHs in the natural protected areas in non-heating season using Norway spruce (Picea abies (L.) Karst) needles. *E3S Web of Conferences*, *28*, 01004. https://doi.org/10.1051/E3SCONF/20182801004

Camargo, M. A. B., & Marenco, R. A. (2011). Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. *Acta Amazonica*, *41*(2), 205–212. https://doi.org/10.1590/S0044-59672011000200004

CAREX Canada and OCRC. (2019). *Polycyclic Aromatic Hydrocarbons Burden of Occupational Cancer Fact Sheet*.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Chapman, P. M. (2007). Determining when contamination is pollution — Weight of evidence determinations for sediments and effluents. *Environment International*, *33*(4), 492–501. https://doi.org/10.1016/J.ENVINT.2006.09.001

Chaturvedi, S., Kumar, A., Singh, V., Chakraborty, B., Kumar, R., & Min, L. (2023). Recent Advancement in Organic Aerosol Understanding: a Review of Their Sources, Formation, and Health Impacts. *Water, Air, and Soil Pollution*, *234*(12), 1–21. https://doi.org/10.1007/S11270-023-06772-0/METRICS

Daly, A., & Zannetti, P. (2007). An Introduction to Air Pollution - Definitions, Classifications, and History. In P. Zannetti, D. Al-Ajmi, & S. Al-Rashied (Eds.), *Ambient Air Pollution* (pp. 1–14). The Arab School for Science and Technology and The EnviroComp Institute.

De Nicola, F., Concha Graña, E., Aboal, J. R., Carballeira, A., Fernández, J., López Mahía, P., Prada Rodríguez, D., & Muniategui Lorenzo, S. (2016). PAH detection in Quercus robur leaves and Pinus pinaster needles: A fast method for biomonitoring purpose. *Talanta*, *153*, 130–137. https://doi.org/10.1016/J.TALANTA.2016.01.067

De Nicola, F., Concha Graña, E., López Mahía, P., Muniategui Lorenzo, S., Prada Rodríguez, D., Retuerto, R., Carballeira, A., Aboal, J. R., & Fernández, J. Á. (2017). Evergreen or deciduous trees for capturing PAHs from ambient air? A case study. *Environmental Pollution*, *221*, 276–284. https://doi.org/10.1016/J.ENVPOL.2016.11.074

Dias, A. P. L., Rinaldi, M. C. S., & Domingos, M. (2016). Foliar accumulation of polycyclic aromatic hydrocarbons in native tree species from the Atlantic Forest (SE-Brazil). *Science of The Total Environment*, *544*, 175–184. https://doi.org/10.1016/J.SCITOTENV.2015.11.092

Domingos, M., Bulbovas, P., Camargo, C. Z. S., Aguiar-Silva, C., Brandão, S. E., Dafré-Martinelli, M., Dias, A. P. L., Engela, M. R. G. S., Gagliano, J., Moura, B. B., Alves, E. S., Rinaldi, M. C. S., Gomes, E. P. C., Furlan, C. M., & Figueiredo, A. M. G. (2015). Searching for native tree species and respective potential biomarkers for future assessment of pollution effects on the highly diverse Atlantic Forest in SE-Brazil. *Environmental Pollution*, *202*, 85–95. https://doi.org/10.1016/J.ENVPOL.2015.03.018

Doucette, W. J., Shunthirasingham, C., Dettenmaier, E. M., Zaleski, R. T., Fantke, P., & Arnot, J. A. (2018). A review of measured bioaccumulation data on terrestrial plants for organic chemicals: Metrics, variability, and the need for standardized measurement protocols. *Environmental Toxicology and Chemistry*, *37*(1), 21–33. https://doi.org/10.1002/ETC.3992

EEA. (2020). *Air quality in Europe — 2020 report*.

Fellet, G., Pošćić, F., Licen, S., Marchiol, L., Musetti, R., Tolloi, A., Barbieri, P., & Zerbi, G. (2016). PAHs accumulation on leaves of six evergreen urban shrubs: A field experiment. *Atmospheric Pollution Research*, *7*(5), 915–924. https://doi.org/10.1016/J.APR.2016.05.007

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Fernández-Varela, R., Ratola, N., Alves, A., & Amigo, J. M. (2015). Relationship between levels of polycyclic aromatic hydrocarbons in pine needles and socio-geographic parameters. *Journal of Environmental Management*, *156*, 52–61. https://doi.org/10.1016/J.JENVMAN.2015.03.019

Franzaring, J. (1997). Temperature and concentration effects in biomonitoring of organic air pollutants. *Environmental Monitoring and Assessment*, *46*(3), 209–220. https://doi.org/10.1023/A:1005719901961/METRICS

Franzetti, A., Gandolfi, I., Bestetti, G., Padoa Schioppa, E., Canedoli, C., Brambilla, D., Cappelletti, D., Sebastiani, B., Federici, E., Papacchini, M., & Ambrosini, R. (2020). Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. *Journal of Hazardous Materials*, *384*, 121021. https://doi.org/10.1016/J.JHAZMAT.2019.121021

Gara, T. W., Darvishzadeh, R., Skidmore, A. K., & Wang, T. (2018). Impact of vertical canopy position on leaf spectral properties and traits across multiple species. *Remote Sensing*, *10*(2), 346. https://doi.org/10.3390/rs10020346

Guo, S., Wei, C., Zhu, Y., & Zhang, Y. (2021). The distribution and retained amount of benzo[a]pyrene at the micro-zones of mangrove leaf cuticles: Results from a novel analytical method. *Environmental Pollution*, *287*, 117589. https://doi.org/10.1016/J.ENVPOL.2021.117589

Gupte, A., Tripathi, A., Patel, H., Rudakiya, D., & Gupte, S. (2016). Bioremediation of Polycyclic Aromatic Hydrocarbon (PAHs): A Perspective. *The Open Biotechnology Journal*, *10*(1), 363–378. https://doi.org/10.2174/1874070701610010363

Herrera, C. M. (2009). *Multiplicity in unity: plant subindividual variation and interactions with animals* (University of Chicago Press, Ed.). University of Chicago Press.

Hill, M. K. (2020). *Understanding Environmental Pollution* (4th edition). Cambridge University Press. https://doi.org/10.1017/9781108395021

Horstmann, M., & McLachlan, M. S. (1998). Atmospheric deposition of semivolatile organic compounds to two forest canopies. *Atmospheric Environment*, *32*(10), 1799–1809. https://doi.org/10.1016/S1352-2310(97)00477-9

Howsam, M., Jones, K. C., & Ineson, P. (2000). PAHs associated with the leaves of three deciduous tree species. I — Concentrations and profiles. *Environmental Pollution*, *108*(3), 413–424. https://doi.org/10.1016/S0269-7491(99)00195-5

Howsam, M., Jones, K. C., & Ineson, P. (2001). PAHs associated with the leaves of three deciduous tree species. II: uptake during a growing season. *Chemosphere*, *44*(2), 155–164. https://doi.org/10.1016/S0045-6535(00)00268-X

Jim, C. Y., & Chen, W. Y. (2008). Assessing the ecosystem service of air pollutant removal by urban trees in Guangzhou (China). *Journal of Environmental Management*, *88*(4), 665–676. https://doi.org/10.1016/J.JENVMAN.2007.03.035

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Jo, Y. S., Ko, H. S., Kim, A. Y., Jo, H. G., Kim, W. J., & Choi, S. K. (2022). Effects of polycyclic aromatic hydrocarbons on the proliferation and differentiation of placental cells. *Reproductive Biology and Endocrinology*, *20*(1), 1–7. https://doi.org/10.1186/S12958-022-00920-7/FIGURES/2

Jouraeva, V. A., Johnson, D. L., Hassett, J. P., & Nowak, D. J. (2002). Differences in accumulation of PAHs and metals on the leaves of Tilia×euchlora and Pyrus calleryana. *Environmental Pollution*, *120*(2), 331–338. https://doi.org/10.1016/S0269-7491(02)00121-5

Jouraeva, V. A., Johnson, D. L., Hassett, J. P., Nowak, D. J., Shipunova, N. A., & Barbarossa, D. (2006). Role of sooty mold fungi in accumulation of fine-particle-associated PAHs and metals on deciduous leaves. *Environmental Research*, *102*(3), 272–282. https://doi.org/10.1016/J.ENVRES.2006.06.004

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Tautenhahn, S., & Werner, G. D. A. (2020). TRY plant trait database – enhanced coverage and open access. *Global Change Biology*, *26*(1), 119–188. https://doi.org/10.1111/GCB.14904

Keith, L. H. (2015). The Source of U.S. EPA's Sixteen PAH Priority Pollutants. *Polycyclic Aromatic Compounds*, *35*(2–4), 147–160. https://doi.org/10.1080/10406638.2014.892886

Kim, K. H., Jahan, S. A., Kabir, E., & Brown, R. J. C. (2013). A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environment International*, *60*, 71–80. https://doi.org/10.1016/J.ENVINT.2013.07.019

Kömp, P., & Mclachlan, M. S. (1997). Influence of Temperature on the Plant/Air Partitioning of Semivolatile Organic Compounds. *Environmental Science and Technology*, *31*(3), 886–890. https://doi.org/10.1021/ES960590U

Kostić, S., Kebert, M., Teslić, N., Stojanović, D. B., Zorić, M., Kovačević, B., & Orlović, S. (2024). Polycyclic aromatic hydrocarbon (PAH) phytoaccumulation in urban areas by Platanus × acerifolia, Celtis australis, and Tilia grandifolia leaves and branches. *Environmental Science and Pollution Research*, *31*(21), 31273–31286. https://doi.org/10.1007/s11356-024-33280-3

Kumar, V., Kothiyal, N. C., Saruchi, Vikas, P., & Sharma, R. (2016). Sources, distribution, and health effect of carcinogenic polycyclic aromatic hydrocarbons (PAHs) – current knowledge and future directions. *Journal of the Chinese Advanced Materials Society*, *4*(4), 302–321. https://doi.org/10.1080/22243682.2016.1230475

Lara, S., Villanueva, F., Martín, P., Salgado, S., Moreno, A., & Sánchez-Verdú, P. (2022). Investigation of PAHs, nitrated PAHs and oxygenated PAHs in PM10 urban aerosols. A comprehensive data analysis. *Chemosphere*, *294*, 133745. https://doi.org/10.1016/J.CHEMOSPHERE.2022.133745

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Li, Q., Li, Y., Zhu, L., Xing, B., & Chen, B. (2017). Dependence of Plant Uptake and Diffusion of Polycyclic Aromatic Hydrocarbons on the Leaf Surface Morphology and Micro-structures of Cuticular Waxes. *Scientific Reports 2017 7:1*, *7*(1), 1–11. https://doi.org/10.1038/srep46235

Li, Y., Chen, B., & Zhu, L. (2010). Single-solute and bi-solute sorption of phenanthrene and pyrene onto pine needle cuticular fractions. *Environmental Pollution*, *158*(7), 2478–2484. https://doi.org/10.1016/J.ENVPOL.2010.03.021

Liang, J., Fang, H., Zhang, T., & Wang, X. (2017). Polycyclic aromatic hydrocarbons in the leaves of twelve plant species along an urbanization gradient in Shanghai, China. *Environmental Science and Pollution Research*, *24*(10), 9361–9369. https://doi.org/10.1007/S11356-017-8552-1

Liu, Y., Dawson, W., Prati, D., Haeuser, E., Feng, Y., & Van Kleunen, M. (2016). Does greater specific leaf area plasticity help plants to maintain a high performance when shaded? *Annals of Botany*, *118*(7), 1329–1336. https://doi.org/10.1093/AOB/MCW180

Mackay, D., Celsie, A. K. D., & Parnis, J. M. (2019). Kinetic Delay in Partitioning and Parallel Particle Pathways: Underappreciated Aspects of Environmental Transport. *Environmental Science and Technology*, *53*(1), 234–241. https://doi.org/10.1021/ACS.EST.8B04514/SUPPL_FILE/ES8B04514_SI_001.PDF

Mai, B., Qi, S., Zeng, E. Y., Yang, Q., Zhang, G., Fu, J., Sheng, G., Peng, P., & Wang, Z. (2003). Distribution of Polycyclic Aromatic Hydrocarbons in the Coastal Region off Macao, China: Assessment of Input Sources and Transport Pathways Using Compositional Analysis. *Environmental Science and Technology*, *37*(21), 4855–4863. https://doi.org/10.1021/ES034514K/SUPPL_FILE/ES034514KSI20030815_120628.PDF

Mclachlan, M. S., & Horstmann, M. (1998). Forests as Filters of Airborne Organic Pollutants: A Model. *Environmental Science and Technology*, *32*(3), 413–420. https://doi.org/10.1021/ES970592U

Mętrak, M., Aneta, E., Wiłkomirski, B., Staszewski, T., & Suska-Malawska, M. (2016). Interspecific differences in foliar 1 PAHs load between Scots pine, birch, and wild rosemary from three polish peat bogs. *Environmental Monitoring and Assessment*, *188*(8), 1–13. https://doi.org/10.1007/S10661-016-5465-2

Mojiri, A., Zhou, J. L., Ohashi, A., Ozaki, N., & Kindaichi, T. (2019). Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. *Science of The Total Environment*, *696*, 133971. https://doi.org/10.1016/J.SCITOTENV.2019.133971

Molina, L., & Segura, A. (2021). Biochemical and Metabolic Plant Responses toward Polycyclic Aromatic Hydrocarbons and Heavy Metals Present in Atmospheric Pollution. *Plants 2021, Vol. 10, Page 2305*, *10*(11), 2305. https://doi.org/10.3390/PLANTS10112305

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Moscoso-Pérez, C., Jiménez, A. C., Iglesias, M. P., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., De Bernabé, J. G., & Picón, J. F. A. (2010). Evaluation of the degree of compliance of the eu target values for As, Pb, Cd, Ni and polycyclic aromatic hydrocarbons in PM10 at four urban sites of the Northwest of Spain. *Chemical Engineering Transactions*, *22*, 203–208. https://doi.org/10.3303/CET1022033

Müller, J. F., Hawker, D. W., McLachlan, M. S., & Connell, D. W. (2001). PAHs, PCDD/Fs, PCBs and HCB in leaves from Brisbane, Australia. *Chemosphere*, *43*(4–7), 507–515. https://doi.org/10.1016/S0045-6535(00)00402-1

Murakami, M., Abe, M., Kakumoto, Y., Kawano, H., Fukasawa, H., Saha, M., & Takada, H. (2012). Evaluation of ginkgo as a biomonitor of airborne polycyclic aromatic hydrocarbons. *Atmospheric Environment*, *54*, 9–17. https://doi.org/10.1016/J.ATMOSENV.2012.02.014

Newman, M. C. (2014). *Fundamentals of Ecotoxicology: the science of pollution* (4th Edition). CRC Press. https://doi.org/10.1201/b17658

Nizzetto, L., Pasture, C., Liu, X., Camporini, P., Stroppiana, D., Herbert, B., Boschetti, M., Zhang, G., Brivio, P. A., Jones, K. C., & Di Guardo, A. (2008). Accumulation parameters and seasonal trends for PCBs in temperate and boreal forest plant species. *Environmental Science and Technology*, *42*(16), 5911–5916. https://doi.org/10.1021/ES800217M/SUPPL_FILE/ES800217M-FILE002.PDF

Nowak, D. J., Hirabayashi, S., Bodine, A., & Greenfield, E. (2014). Tree and forest effects on air quality and human health in the United States. *Environmental Pollution*, *193*, 119–129. https://doi.org/10.1016/J.ENVPOL.2014.05.028

Nowak, D. J., Hirabayashi, S., Doyle, M., McGovern, M., & Pasher, J. (2018). Air pollution removal by urban forests in Canada and its effect on air quality and human health. *Urban Forestry & Urban Greening*, *29*, 40–48. https://doi.org/10.1016/J.UFUG.2017.10.019

Omar, N. Y. M. J., Abas, M. R. Bin, Ketuly, K. A., & Tahir, N. M. (2002). Concentrations of PAHs in atmospheric particles (PM-10) and roadside soil particles collected in Kuala Lumpur, Malaysia. *Atmospheric Environment*, *36*(2), 247–254. https://doi.org/10.1016/S1352-2310(01)00425-3

Ozdemir, H. (2019). Mitigation impact of roadside trees on fine particle pollution. *Science of The Total Environment*, *659*, 1176–1185. https://doi.org/10.1016/J.SCITOTENV.2018.12.262

Patel, A. B., Shaikh, S., Jain, K. R., Desai, C., & Madamwar, D. (2020). Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. *Frontiers in Microbiology*, *11*, 562813. https://doi.org/10.3389/FMICB.2020.562813/BIBTEX

Peck, A. M., & Hornbuckle, K. C. (2003). Use of a climate-controlled chamber to investigate the fate of gas-phase anthracene. *Water, Air, and Soil Pollution*, *145*(1–4), 17–34. https://doi.org/10.1023/A:1023656023898/METRICS

USC DE COMPOSTELA

Peters, J. M., & Gonzalez, F. J. (2018). The Evolution of Carcinogenesis. *Toxicological Sciences*, *165*(2), 272. https://doi.org/10.1093/TOXSCI/KFY184

Pleijel, H., Klingberg, J., Strandberg, B., Sjöman, H., Tarvainen, L., & Wallin, G. (2022). Differences in accumulation of polycyclic aromatic compounds (PACs) among eleven broadleaved and conifer tree species. *Ecological Indicators*, *145*, 109681. https://doi.org/10.1016/J.ECOLIND.2022.109681

Prigioniero, A., Postiglione, A., Zuzolo, D., Niinemets, Ü., Tartaglia, M., Scarano, P., Mercurio, M., Germinario, C., Izzo, F., Trifuoggi, M., Toscanesi, M., Sciarrillo, R., & Guarino, C. (2023). Leaf surface functional traits influence particulate matter and polycyclic aromatic hydrocarbons air pollution mitigation: Insights from Mediterranean urban forests. *Journal of Cleaner Production*, *418*, 138158. https://doi.org/10.1016/J.JCLEPRO.2023.138158

Prigioniero, A., Zuzolo, D., Niinemets, Ü., Postiglione, A., Mercurio, M., Izzo, F., Trifuoggi, M., Toscanesi, M., Scarano, P., Tartaglia, M., Sciarrillo, R., & Guarino, C. (2022). Particulate matter and polycyclic aromatic hydrocarbon uptake in relation to leaf surface functional traits in Mediterranean evergreens: Potentials for air phytoremediation. *Journal of Hazardous Materials*, *435*, 129029. https://doi.org/10.1016/J.JHAZMAT.2022.129029

Ramesh, A., Archibong, A. E., Hood, D. B., Guo, Z., & Loganathan, B. G. (2011). Global Environmental Distribution and Human Health Effects of Polycyclic Aromatic Hydrocarbons. In B. G. Loganathan & P. K.-S. Lam (Eds.), *Global Contamination Trends of Persistent Organic Chemicals* (First, pp. 95–125). CRC Press. https://doi.org/10.1201/B11098-7

Ratola, N., Amigo, J. M., & Alves, A. (2010). Levels and sources of PAHs in selected sites from portugal: Biomonitoring with pinus pinea and pinus pinaster needles. *Archives of Environmental Contamination and Toxicology*, *58*(3), 631–647. https://doi.org/10.1007/S00244-009-9462-0/FIGURES/6

Ratola, N., Amigo, J. M., Oliveira, M. S. N., Araújo, R., Silva, J. A., & Alves, A. (2011). Differences between Pinus pinea and Pinus pinaster as bioindicators of polycyclic aromatic hydrocarbons. *Environmental and Experimental Botany*, *72*(2), 339–347. https://doi.org/10.1016/J.ENVEXPBOT.2011.04.012

Ravanbakhsh, M., Yousefi, H., Lak, E., Ansari, M. J., Suksatan, W., Qasim, Q. A., Asban, P., Kianizadeh, M., & Mohammadi, M. J. (2023). Effect of Polycyclic Aromatic Hydrocarbons (PAHs) on Respiratory Diseases and the Risk Factors Related to Cancer. *Polycyclic Aromatic Compounds*, *43*(9), 8371–8387. https://doi.org/10.1080/10406638.2022.2149569

Ravindra, K., Sokhi, R., & Van Grieken, R. (2008). Atmospheric polycyclic aromatic hydrocarbons: Source attribution, emission factors and regulation. *Atmospheric Environment*, *42*(13), 2895–2921. https://doi.org/10.1016/J.ATMOSENV.2007.12.010

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Ray, D., Ghosh, S. K., & Raha, S. (2021). Seasonal foliar uptake of atmospheric polycyclic aromatic hydrocarbons by some local plants in a tropical metropolis in India. *Atmospheric Pollution Research*, *12*(2), 104–112. https://doi.org/10.1016/J.APR.2020.10.013

Rodgers, K., McLellan, I., Peshkur, T., Williams, R., Tonner, R., Hursthouse, A. S., Knapp, C. W., & Henriquez, F. L. (2018). Can the legacy of industrial pollution influence antimicrobial resistance in estuarine sediments? *Environmental Chemistry Letters 2018 17:2*, *17*(2), 595–607. https://doi.org/10.1007/S10311-018-0791-Y

Sæbø, A., Popek, R., Nawrot, B., Hanslin, H. M., Gawronska, H., & Gawronski, S. W. (2012). Plant species differences in particulate matter accumulation on leaf surfaces. *Science of The Total Environment*, *427–428*, 347–354. https://doi.org/10.1016/J.SCITOTENV.2012.03.084

Şahin, T., Dalğa, S., & Ölmez, M. (2022). Polycyclic Aromatic Hydrocarbons (PAHs) and Their Importance in Animal Nutrition. In S. Kukovic (Ed.), *Animal Husbandry*. IntechOpen. https://doi.org/10.5772/intechopen.101816

Selmi, W., Weber, C., Rivière, E., Blond, N., Mehdi, L., & Nowak, D. (2016). Air pollution removal by trees in public green spaces in Strasbourg city, France. *Urban Forestry & Urban Greening*, *17*, 192–201. https://doi.org/10.1016/J.UFUG.2016.04.010

Shahrukh, S., Baldauf, R., Popek, R., Moniruzzaman, M., Huda, M. N., Islam, Md. M., Hossain, S. A., & Hossain, M. E. (2024). Removal of airborne particulate matter by evergreen tree species in Dhaka, Bangladesh. *Environmental Pollution*, *363*, 125194. https://doi.org/10.1016/j.envpol.2024.125194

Shen, H., Huang, Y., Wang, R., Zhu, D., Li, W., Shen, G., Wang, B., Zhang, Y., Chen, Y., Lu, Y., Chen, H., Li, T., Sun, K., Li, B., Liu, W., Liu, J., & Tao, S. (2013). Global atmospheric emissions of polycyclic aromatic hydrocarbons from 1960 to 2008 and future predictions. *Environmental Science and Technology*, *47*(12), 6415–6424. https://doi.org/10.1021/ES400857Z

Simonich, S. L., & Hites, R. A. (1994). Importance of vegetation in removing polycyclic aromatic hydrocarbons from the atmosphere. *Nature 1994 370:6484*, *370*(6484), 49–51. https://doi.org/10.1038/370049a0

Slezakova, K., Pires, J. C. M., Castro, D., Alvim-Ferraz, M. C. M., Delerue-Matos, C., Morais, S., & Pereira, M. C. (2013). PAH air pollution at a Portuguese urban area: Carcinogenic risks and sources identification. *Environmental Science and Pollution Research*, *20*(6), 3932–3945. https://doi.org/10.1007/S11356-012-1300-7/TABLES/4

Stading, R., Gastelum, G., Chu, C., Jiang, W., & Moorthy, B. (2021). Molecular mechanisms of pulmonary carcinogenesis by polycyclic aromatic hydrocarbons (PAHs): Implications for human lung cancer. *Seminars in Cancer Biology*, *76*, 3–16. https://doi.org/10.1016/J.SEMCANCER.2021.07.001

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Sun, F., Wen, D., Kuang, Y., Li, J., Li, J., & Zuo, W. (2010). Concentrations of heavy metals and polycyclic aromatic hydrocarbons in needles of Masson pine (Pinus massoniana L.) growing nearby different industrial sources. *Journal of Environmental Sciences*, *22*(7), 1006–1013. https://doi.org/10.1016/S1001-0742(09)60211-4

Sun, K., Song, Y., He, F., Jing, M., Tang, J., & Liu, R. (2021). A review of human and animals exposure to polycyclic aromatic hydrocarbons: Health risk and adverse effects, photo-induced toxicity and regulating effect of microplastics. *Science of The Total Environment*, *773*, 145403. https://doi.org/10.1016/J.SCITOTENV.2021.145403

Tang, J., Zha, Y., & Cheng, Y. (2019). Heavy metals concentrations in deposited dust of typical chinese tree species in different functional areas in Nanjing. *Polish Journal of Environmental Studies*, *28*(4), 2867–2876. https://doi.org/10.15244/PJOES/93296

Tao, Z., & Hornbuckle, K. C. (2001). Uptake of Polycyclic Aromatic Hydrocarbons (PAHS) by Broad Leaves: Analysis of Kinetic Limitations. *Water, Air and Soil Pollution: Focus 2001 1:5*, *1*(5), 275–283. https://doi.org/10.1023/A:1013136028586

Terzaghi, E., De Nicola, F., Cerabolini, B. E. L., Posada-Baquero, R., Ortega-Calvo, J. J., & Di Guardo, A. (2020). Role of photo- and biodegradation of two PAHs on leaves: Modelling the impact on air quality ecosystem services provided by urban trees. *Science of The Total Environment*, *739*, 139893. https://doi.org/10.1016/J.SCITOTENV.2020.139893

Terzaghi, E., Morselli, M., Semplice, M., Cerabolini, B. E. L., Jones, K. C., Freppaz, M., & Di Guardo, A. (2017). SoilPlusVeg: An integrated air-plant-litter-soil model to predict organic chemical fate and recycling in forests. *Science of the Total Environment*, *595*, 169–177. https://doi.org/10.1016/j.scitotenv.2017.03.252

Terzaghi, E., Posada-Baquero, R., Di Guardo, A., & Ortega-Calvo, J. J. (2021). Microbial degradation of pyrene in holm oak (Quercus ilex) phyllosphere: Role of particulate matter in regulating bioaccessibility. *Science of The Total Environment*, *786*, 147431. https://doi.org/10.1016/J.SCITOTENV.2021.147431

Terzaghi, E., Wild, E., Zacchello, G., Cerabolini, B. E. L., Jones, K. C., & Di Guardo, A. (2013). Forest Filter Effect: Role of leaves in capturing/releasing air particulate matter and its associated PAHs. *Atmospheric Environment*, *74*, 378–384. https://doi.org/10.1016/J.ATMOSENV.2013.04.013

Terzaghi, E., Zacchello, G., Scacchi, M., Raspa, G., Jones, K. C., Cerabolini, B., & Di Guardo, A. (2015). Towards more ecologically realistic scenarios of plant uptake modelling for chemicals: PAHs in a small forest. *Science of The Total Environment*, *505*, 329–337. https://doi.org/10.1016/J.SCITOTENV.2014.09.108

Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., & Liu, C. (2019). Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: Morphological characteristics have the greatest impact. *Science of The Total Environment*, *652*, 1149–1155. https://doi.org/10.1016/J.SCITOTENV.2018.10.357

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Tian, X., Liu, J., Zhou, G., Peng, P., Wang, X., & Wang, C. (2008). Estimation of the annual scavenged amount of polycyclic aromatic hydrocarbons by forests in the Pearl River Delta of Southern China. *Environmental Pollution*, *156*(2), 306–315. https://doi.org/10.1016/J.ENVPOL.2008.02.012

Tong, Z., Whitlow, T. H., Macrae, P. F., Landers, A. J., & Harada, Y. (2015). Quantifying the effect of vegetation on near-road air quality using brief campaigns. *Environmental Pollution*, *201*, 141–149. https://doi.org/10.1016/J.ENVPOL.2015.02.026

Tremolada, P., Burnett, V., Calamari, D., & Jones, K. C. (1996). Spatial distribution of PAHs in the U.K. atmosphere using pine needles. *Environmental Science and Technology*, *30*(12), 3570–3577. https://doi.org/10.1021/ES960269B/ASSET/IMAGES/LARGE/ES960269BF00005.JPEG

Venkatraman, G., Giribabu, N., Mohan, P. S., Muttiah, B., Govindarajan, V. K., Alagiri, M., Abdul Rahman, P. S., & Karsani, S. A. (2024). Environmental impact and human health effects of polycyclic aromatic hydrocarbons and remedial strategies: A detailed review. *Chemosphere*, *351*, 141227. https://doi.org/10.1016/J.CHEMOSPHERE.2024.141227

Vinod, N., Slot, M., McGregor, I. R., Ordway, E. M., Smith, M. N., Taylor, T. C., Sack, L., Buckley, T. N., & Anderson-Teixeira, K. J. (2023). Thermal sensitivity across forest vertical profiles: patterns, mechanisms, and ecological implications. *New Phytologist*, *237*(1), 22–47. https://doi.org/10.1111/NPH.18539

Vondráček, J., Pivnička, J., & Machala, M. (2018). Polycyclic aromatic hydrocarbons and disruption of steroid signaling. *Current Opinion in Toxicology*, *11–12*, 27–34. https://doi.org/10.1016/J.COTOX.2018.12.003

Wagrowski, D. M., & Hites, R. A. (1997). Polycyclic aromatic hydrocarbon accumulation in urban, suburban, and rural vegetation. *Environmental Science and Technology*, *31*(1), 279–282. https://doi.org/10.1021/ES960419I/SUPPL_FILE/ES279.PDF

Wakeham, S. G., & Canuel, E. A. (2016). Biogenic polycyclic aromatic hydrocarbons in sediments of the San Joaquin River in California (USA), and current paradigms on their formation. *Environmental Science and Pollution Research*, *23*(11), 10426–10442. https://doi.org/10.1007/S11356-015-5402-X/TABLES/3

Wang, Y. Q., Tao, S., Jiao, X. C., Coveney, R. M., Wu, S. P., & Xing, B. S. (2008). Polycyclic aromatic hydrocarbons in leaf cuticles and inner tissues of six species of trees in urban Beijing. *Environmental Pollution*, *151*(1), 158–164. https://doi.org/10.1016/J.ENVPOL.2007.02.005

Weschler, C. J., & Nazaroff, W. W. (2008). Semivolatile organic compounds in indoor environments. *Atmospheric Environment*, *42*(40), 9018–9040. https://doi.org/10.1016/J.ATMOSENV.2008.09.052

WHO. (2024a). *Air quality, energy and health. Health Impacts. [Fact sheet]*.

USC DE COMPOSTELA

WHO. (2024b). *Ambient (outdoor) air pollution [Fact sheet]*.

WHO Regional Office for Europe. (2010). *WHO guidelines for indoor air quality: selected pollutants* (World Health Organization, Ed.).

World Bank. (2022). *The Global Health Cost of PM2.5 Air Pollution: A Case for Action Beyond 2021*. . The World Bank. https://doi.org/10.1596/978-1-4648-1816-5

Xu, P., Liu, B., Chen, H., Wang, H., Guo, X., & Yuan, J. (2024). PAHs as environmental pollutants and their neurotoxic effects. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *283*, 109975. https://doi.org/10.1016/J.CBPC.2024.109975

Yang, B., Liu, S., Liu, Y., Li, X., Lin, X., Liu, M., & Liu, X. (2017). PAHs uptake and translocation in Cinnamomum camphora leaves from Shanghai, China. *Science of The Total Environment*, *574*, 358–368. https://doi.org/10.1016/J.SCITOTENV.2016.09.058

Yang, S., Sun, J., Wang, S., Limei, E., Zhang, S., & Jiang, X. (2023). Association of exposure to polycyclic aromatic hydrocarbons with thyroid hormones in adolescents and adults, and the influence of the iodine status. *Environmental Science: Processes & Impacts*, *25*(9), 1449–1463. https://doi.org/10.1039/D3EM00135K

Yu, M.-H., & Tsunoda, H. (2004). *Environmental Toxicology : Biological and Health Effects of Pollutants* (2nd edition). CRC Press. https://doi.org/10.1201/9780203495469

Zhang, Y., Dong, S., Wang, H., Tao, S., & Kiyama, R. (2016). Biological impact of environmental polycyclic aromatic hydrocarbons (ePAHs) as endocrine disruptors. *Environmental Pollution*, *213*, 809–824. https://doi.org/10.1016/J.ENVPOL.2016.03.050

Zhao, X., He, M., Shang, H., Yu, H., Wang, H., Li, H., Piao, J., Quinto, M., & Li, D. (2018). Biomonitoring polycyclic aromatic hydrocarbons by Salix matsudana leaves: A comparison with the relevant air content and evaluation of environmental parameter effects. *Atmospheric Environment*, *181*, 47–53. https://doi.org/10.1016/J.ATMOSENV.2018.03.004

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Supplementary material

Appendix I: Chapter I Supplementary Material

Appendix A

Table 1SM. Physico-chemical properties of the PAHs analyzed. n rings: number of rings. Abbreviations for properties: MW (molecular weight), VP (vapor pressure), WS (water solubility), Log K_{OW} (octanol-water partition coefficient), VapE (vaporization enthalpy). Abbreviations of PAHs: Nap (Naphthalene), Acy (acenaphthylene), Ace (acenaphthene), Fle (fluorene), Phe (phenanthrene), Ant (anthracene), Fla (fluoranthene), Pyr (pyrene), B(a)A (benz(a)anthracene), Chr (chrysene), B(b)F (benzo(b)fluoranthene), B(k)F (benzo(k)fluoranthene), B(a)P (benzo(a)pyrene), D(ah)A (dibenz(a,h)anthracene), Ind (indeno(1,2,3-cd)pyrene), B(ghi)P (benzo(ghi)perylene).

PAH	Formula	n rings	MW* (g mol^{-1})	VP* (Pa)	WS* (mol m^{-3})	LogK_{OW}*	VapE** (kJ mol^{-1})	Log K_{OA}***
Nap	$C_{10}H_8$	2	128.18	1.04E+01	3,10E+01	3.37	55.4	5.1
Acy	$C_{12}H_8$	3	150.19	9.00E-01	1.07E-01	4.00	66.9	6.5
Ace	$C_{12}H10$	3	154.21	3.00E-01	2.46E-02	3.92	66.5	6.2
Fle	$C_{13}H_{10}$	3	166.22	9.00E-02	1.14E-02	4.18	72.1	6.7
Ant	$C_{14}H_{10}$	3	178.23	1.00E-03	2.52E-04	4.54	79.9	7.3
Phe	$C_{14}H_{10}$	3	178.23	2.00E-02	6.17E-03	4.57	78.3	7.5
Fla	$C_{16}H_{10}$	4	202.25	1.23E-03	1.29E-03	5.22	87.1	8.6
Pyr	$C_{16}H_{10}$	4	202.25	6.00E-04	6.53E-04	5.18	89.4	8.6
B(a)A	$C_{18}H_{12}$	4	228.29	2.80E-05	4.82E-05	5.91	105.8	9.5
Chr	$C_{18}H_{12}$	4	228.29	5.70E-07	8.76E-06	5.60	106.2	10.2
B(a)P	$C_{20}H_{12}$	5	252.31	7.00E-07	1.51E-05	6.04	116.7	10.8
B(b)F•	$C_{20}H_{12}$	5	252.31	5.20E-08	5.95E-06	5.80	117.4	11.3
B(k)F	$C_{20}H_{12}$	5	252.31	5.20E-08	3.17E-06	6.00	117.4	11.2
D(ah)A	$C_{22}H_{14}$	5	278.36	3.70E-10	2.16E-06	6.75	131.1	13.9
Ind••	$C_{22}H_{12}$	6	276.33	1.00E-08	6.88E-07	6.50	128.9	11.8
B(ghi)P	$C_{22}H_{12}$	6	276.33	1,39E-08	2,60E-04	6.50	128.9	11.7

NOTES: * from (Mackay et al., 2006); ** from (Roux et al., 2008); *** $K_{OA}=K_{OW}/K_{AW}$, $K_{AW}=H/RT$, H=VP/WS; • the same VP and VapE of B(k)F was assumed; •• the values of Log K_{OW} and VapE of B(ghi)P were assumed to be the same

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References

Mackay, D., Shiu, W.-Y., Shiu, W.-Y., Lee, S.C., 2006. Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Handb. Phys.-Chem. Prop. Environ. Fate Org. Chem. **https://doi.org/10.1201/9781420044393**

Roux, M. V., Temprado, M., Chickos, J. S., & Nagano, Y. (2008). Critically Evaluated Thermochemical Properties of Polycyclic Aromatic Hydrocarbons. Journal of Physical and Chemical Reference Data, 37(4), 1855–1996. https://doi.org/10.1063/1.2955570

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Appendix B

The supplementary material in Appendix B can be downloaded and accessed from the following link: https://ars.els-cdn.com/content/image/1-s2.0-S0269749124020542-mmc2.xlsx

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Appendix II: Chapter II Supplementary Material

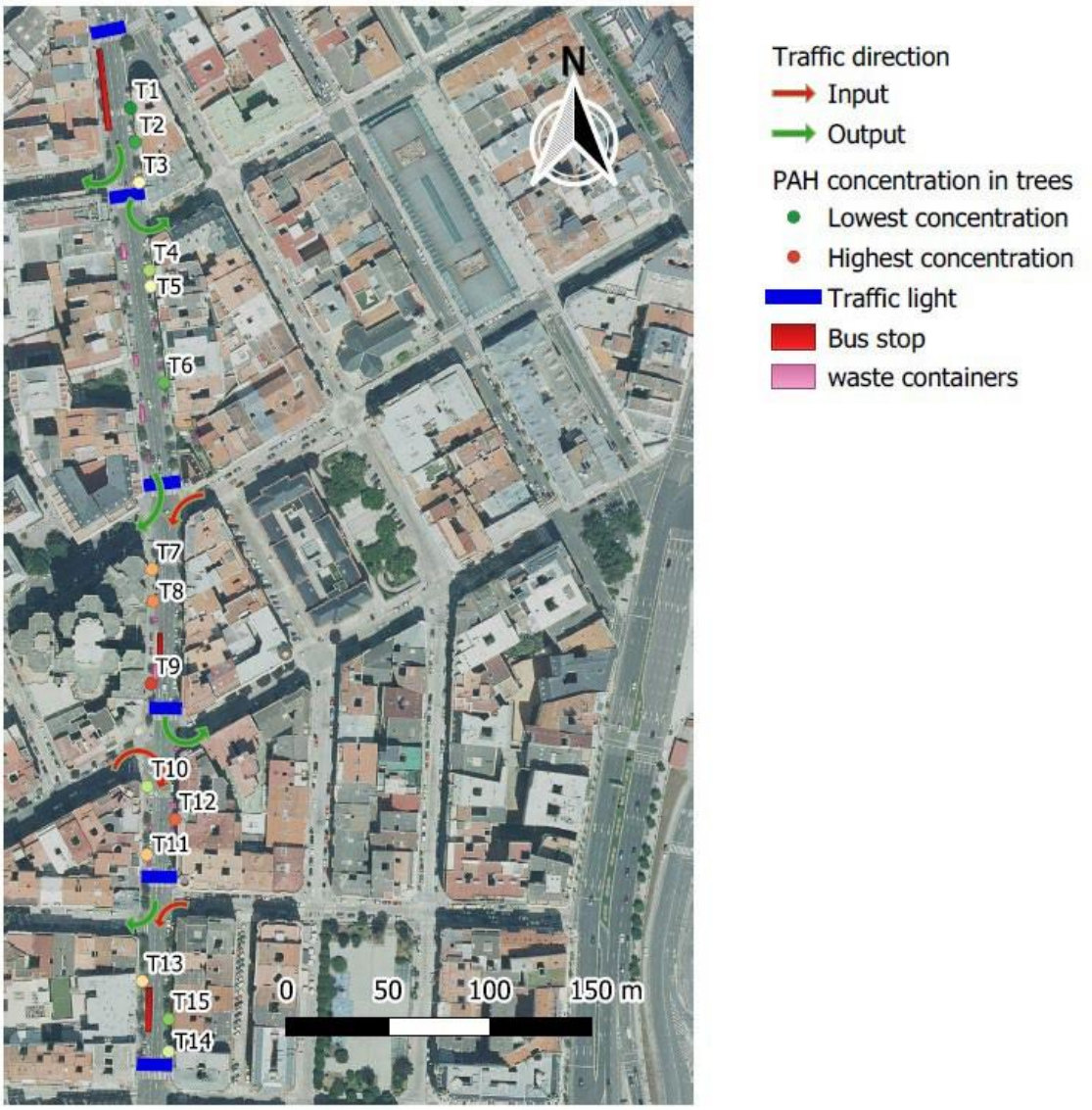
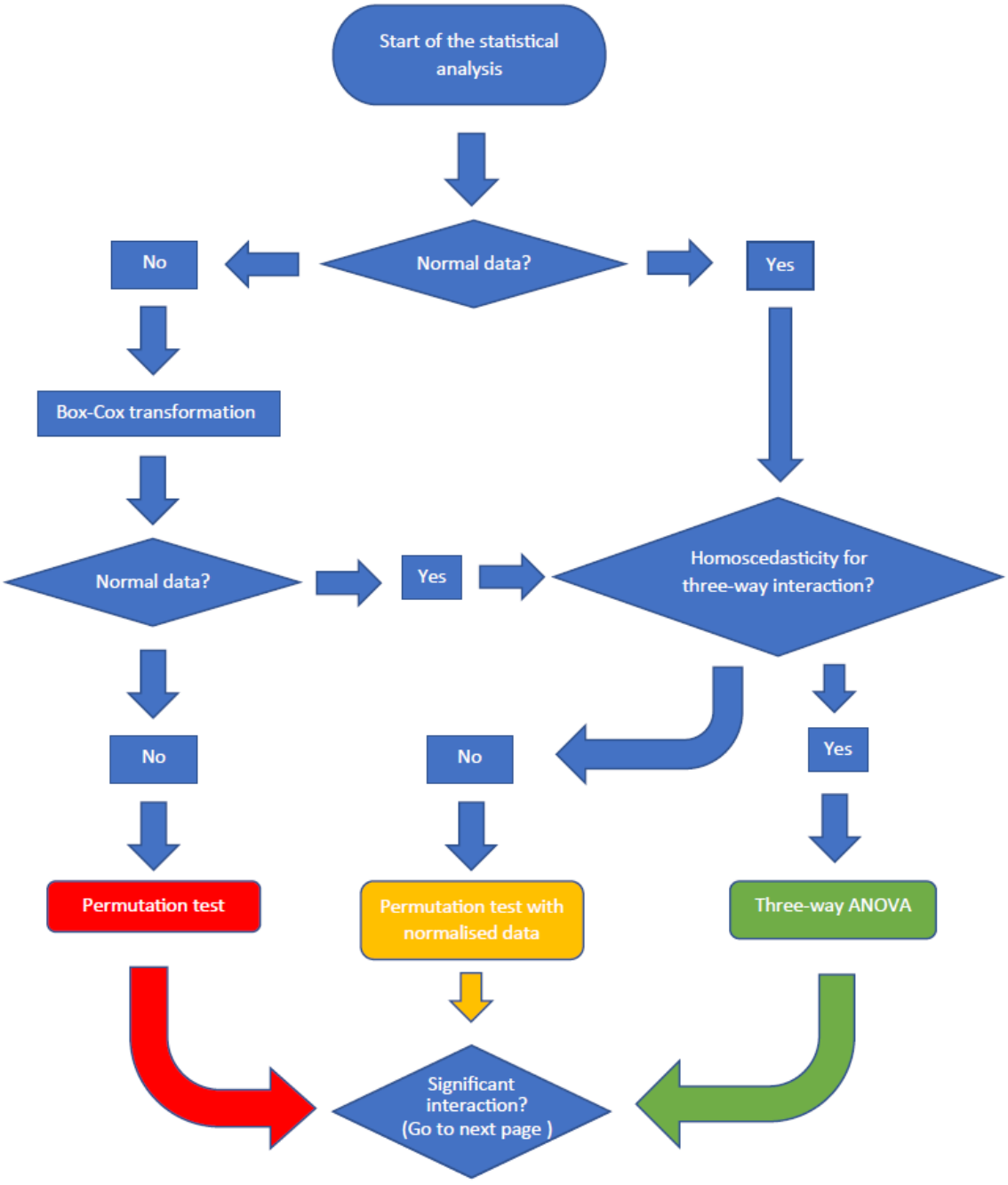


Figure A.1. Aerial image of the study street with the position of the 15 trees represented as circles and categorized according to their total PAHs concentration from lowest (dark green) to highest (red) concentration. The arrows indicate the traffic flow at each junction, with green representing the outputs and red the inputs. The rectangles represent bus stops (red) and containers (pink), and the blue lines represent the traffic lights.

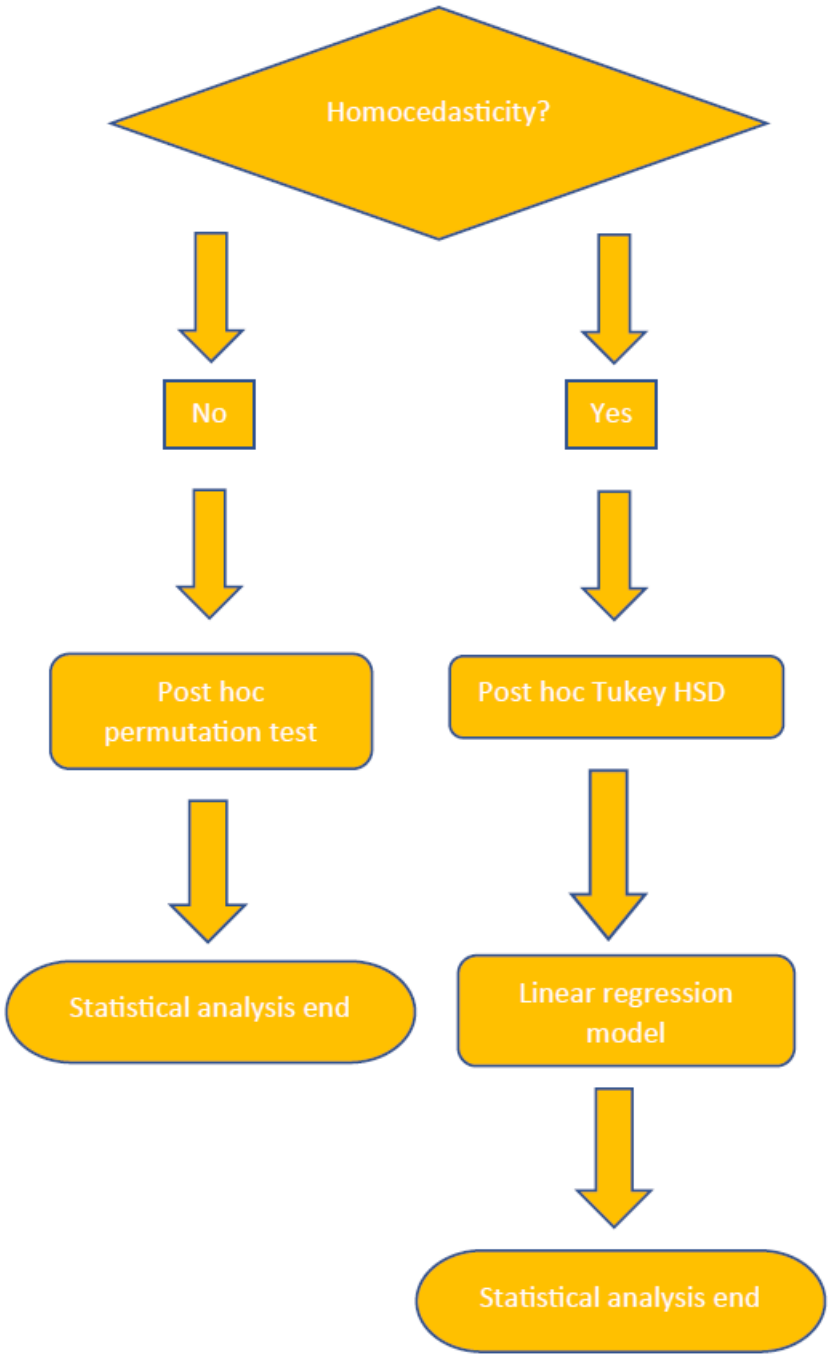
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Figure A.2.Flowchart of the statistical analysis.



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Significant interaction?
Yes
No
Main effects permutation test
Main effects permutation test
Three-way ANOVA with no interaction
Significant main effects not included in the interaction?
Significant main effects?
No
No
Statistical analysis end
Yes
Post hoc permutation test
Post hoc Tukey HSD
Linear regression model
Homocedasticity? (Go to next page)
Linear regression model
Statistical analysis end
Statistical analysis end
Statistical analysis end



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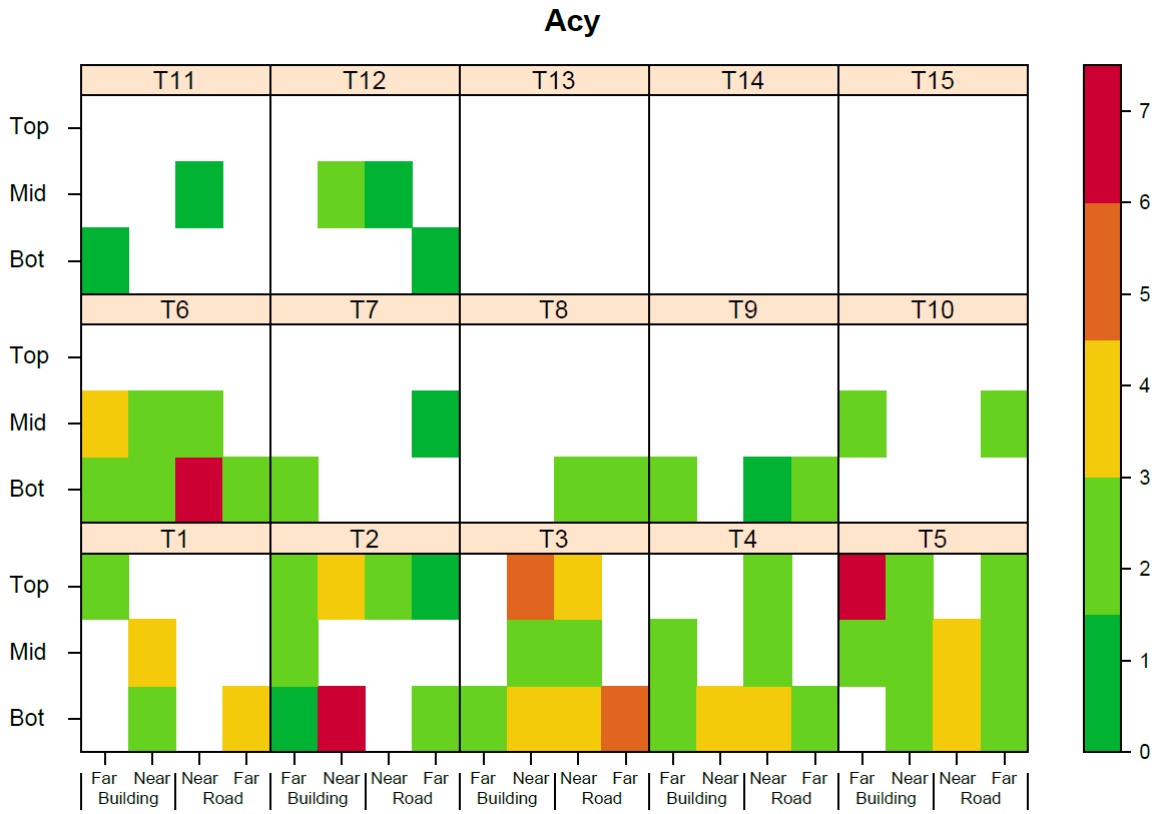


Figure A.3. Diagram of the sampled positions in each tree where the concentration of acenaphthylene (Acy; ng g^{-1}) in each of the analyzed samples is indicated by color coding. Observations below LOQ are represented in white.

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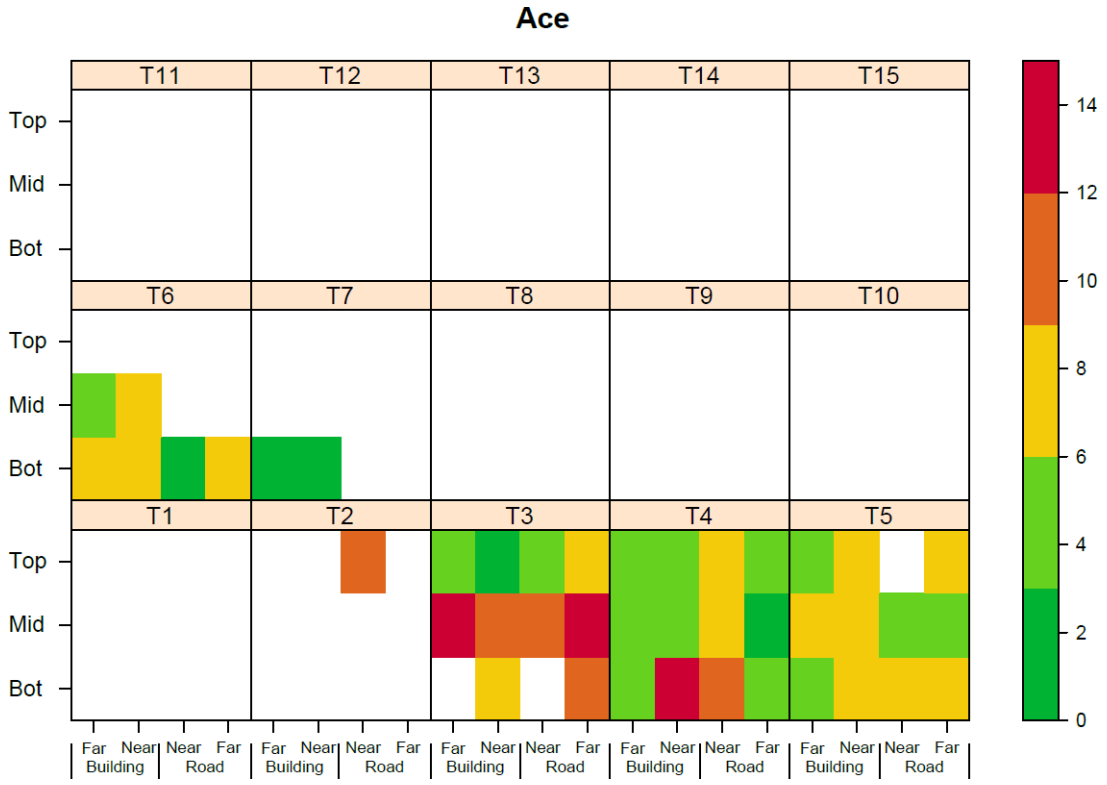


Figure A.4. Diagram of the sampled positions in each tree where the concentration of acenaphthene (Ace; ng g^{-1}) in each of the analyzed samples is indicated by color coding. Observations below LOQ are represented in white.

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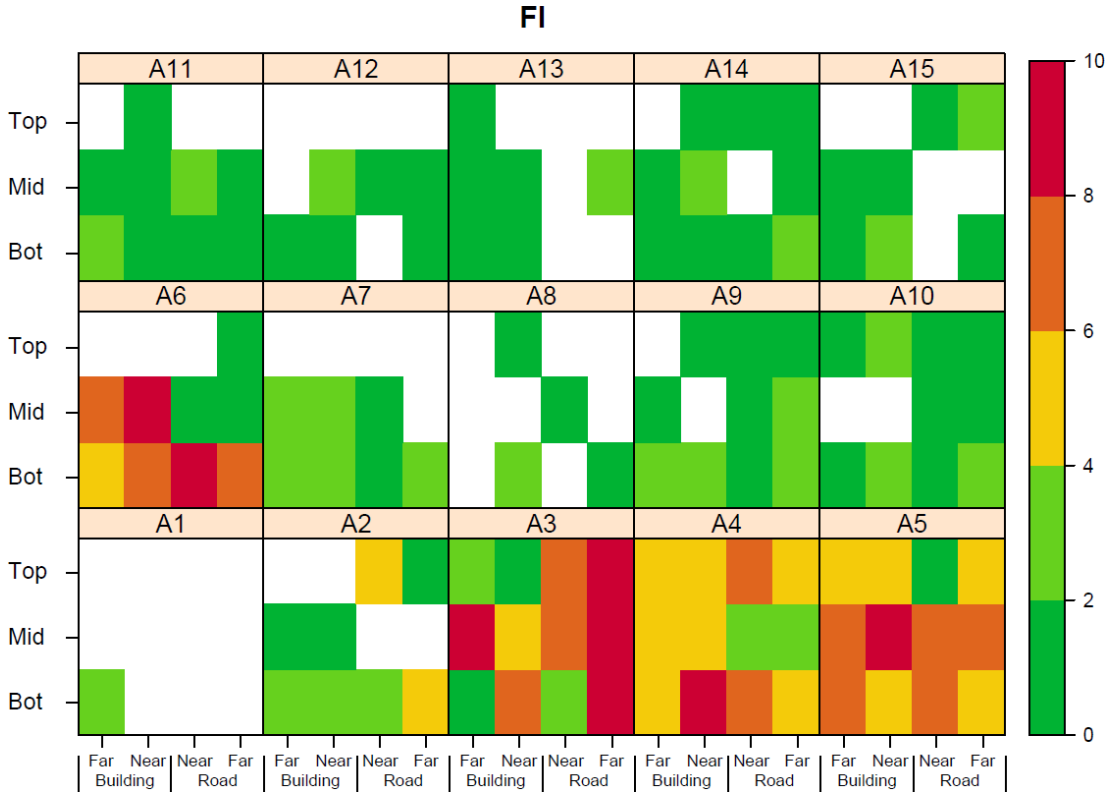


Figure A.5. Diagram of the sampled positions in each tree where the concentration of fluorene (Fl; ng g^{-1}) in each of the analyzed samples is indicated by color coding. Observations below LOQ are represented in white.

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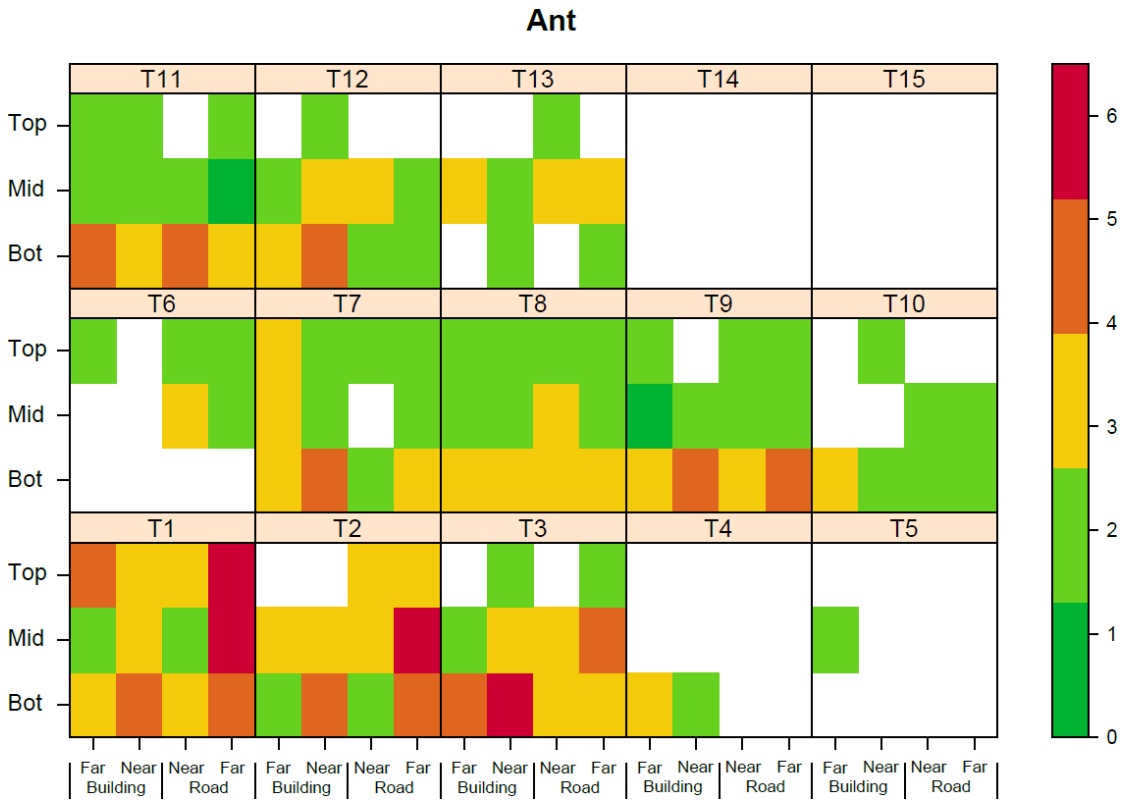


Figure A.6. Diagram of the sampled positions in each tree where the concentration of anthracene (Ant; ng g^{-1}) in each of the analyzed samples is indicated by color coding. Observations below LOQ are represented in white.

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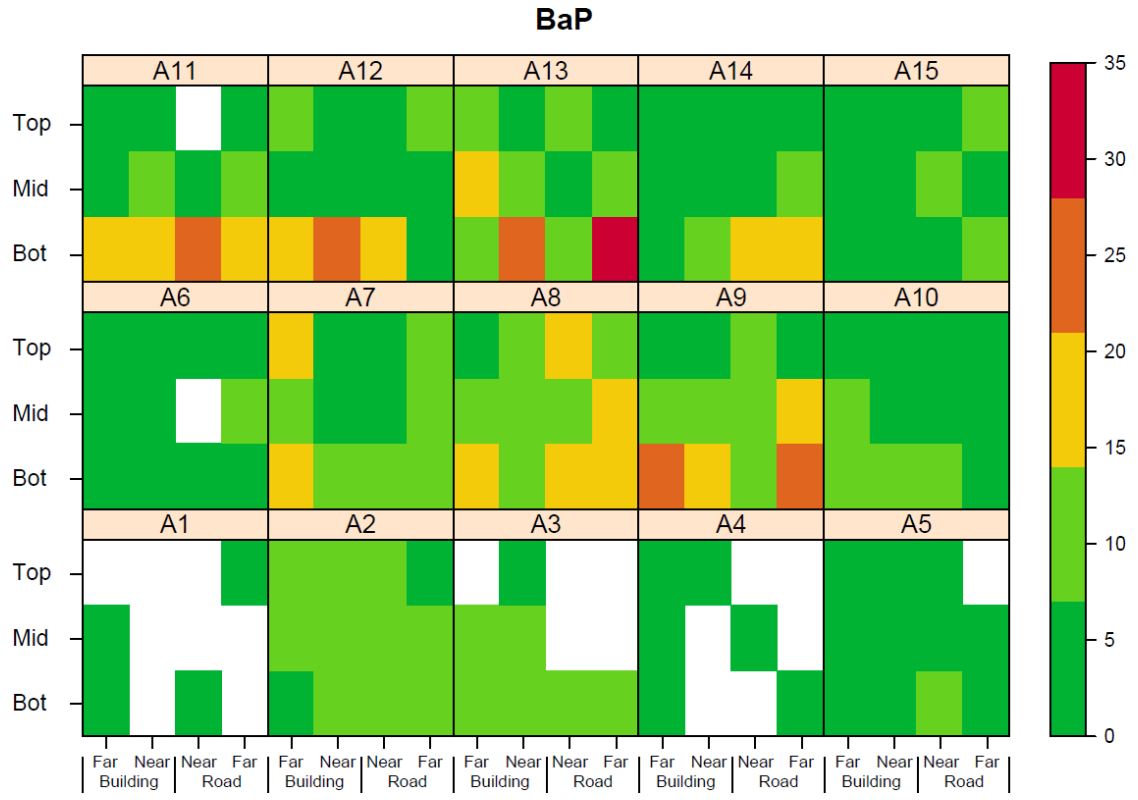


Figure A.7. Diagram of the sampled positions in each tree where the concentration of benzo(a)pyrene (BaP; ng g^{-1}) in each of the analyzed samples is indicated by color coding. Observations below LOQ are represented in white

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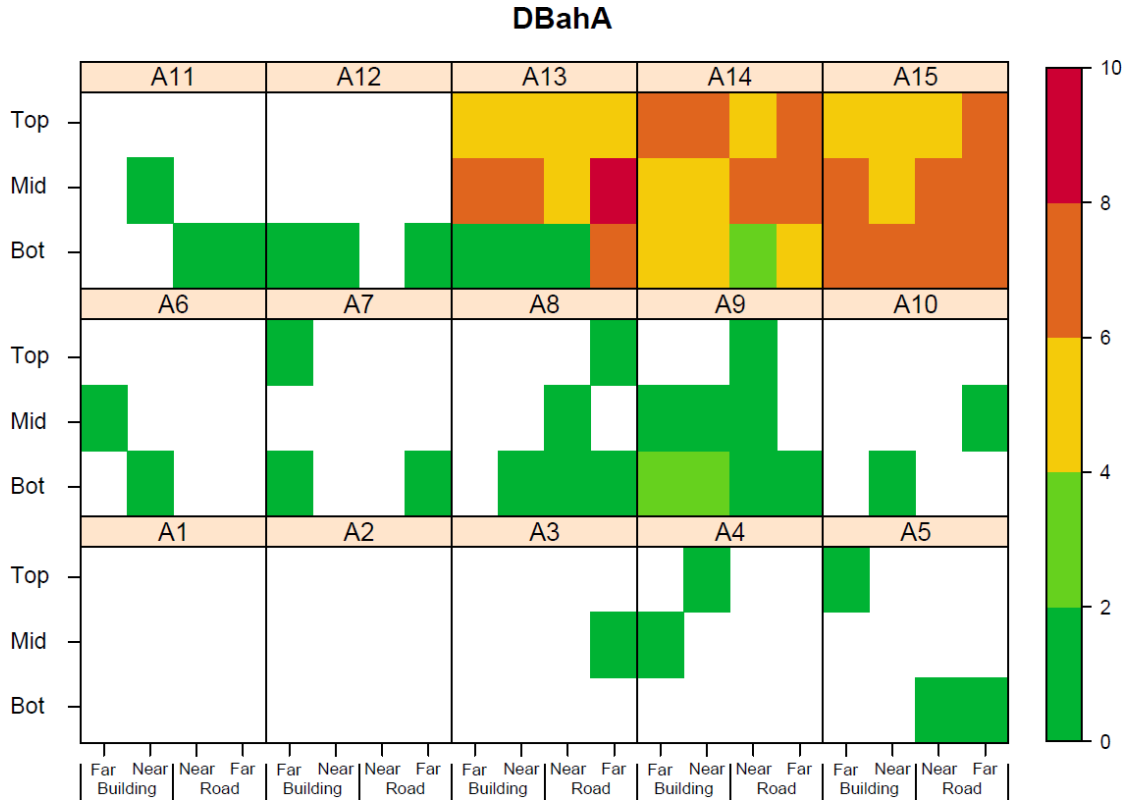


Figure A.8. Diagram of the sampled positions in each tree where the concentration of dibenzo(a,h)anthracene (DBahA; ng g^{-1}) in each of the analyzed samples is indicated by color coding. Observations below LOQ are represented in white.

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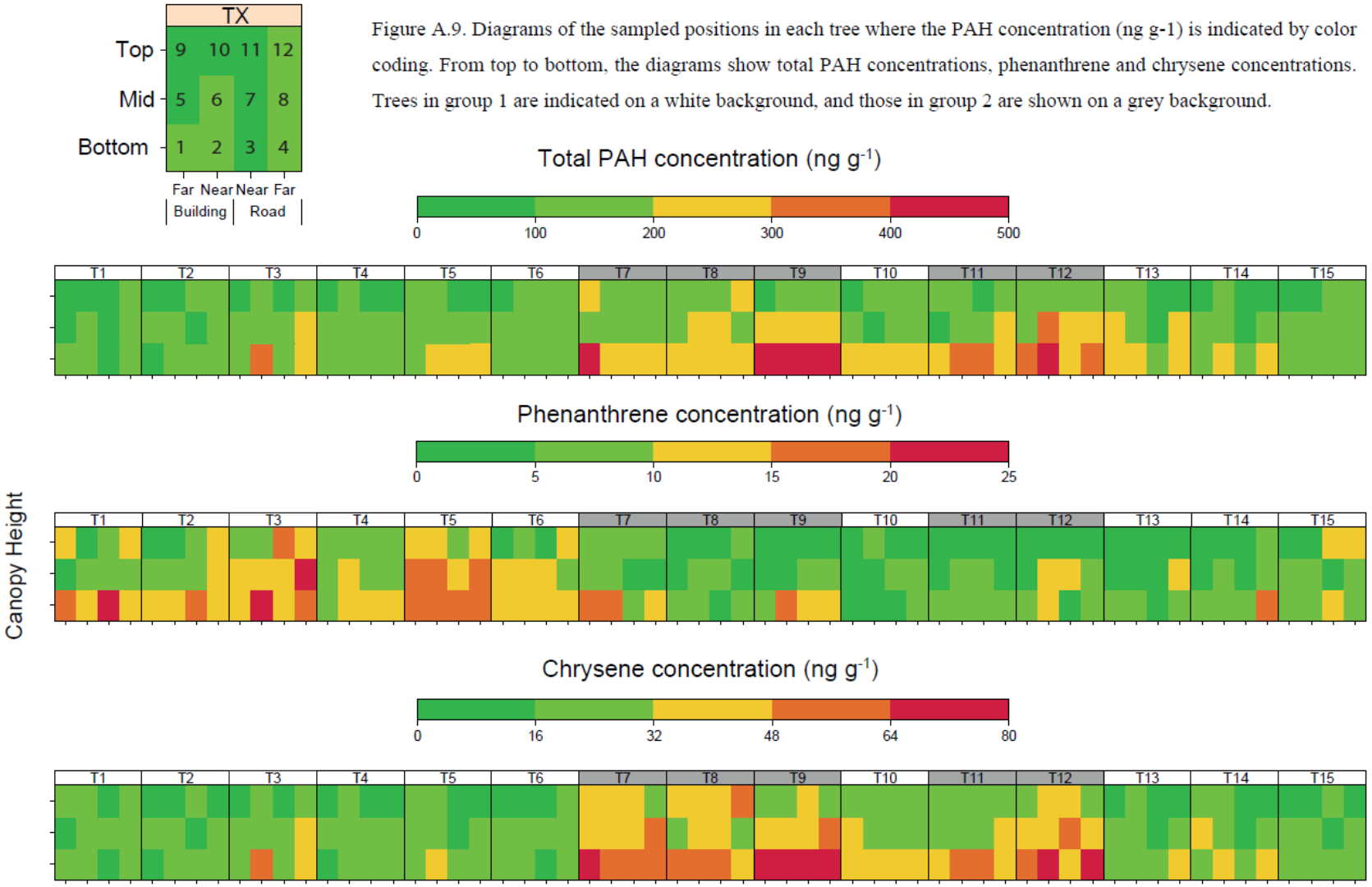


Figure A.9. Diagrams of the sampled positions in each tree where the PAH concentration (ng g-1) is indicated by color coding. From top to bottom, the diagrams show total PAH concentrations, phenanthrene and chrysene concentrations. Trees in group 1 are indicated on a white background, and those in group 2 are shown on a grey background.

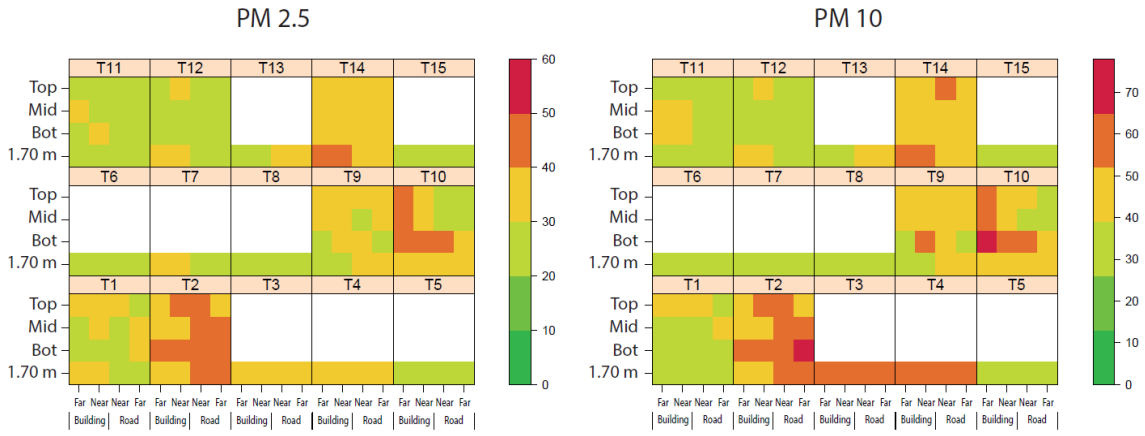


Figure A.10. Diagrams of PM2.5 and PM10 concentration (µg m^{-3}) in the 12 sampled positions in each canopy and below canopy (1.70 m). Non available data (NA) are represented in white.

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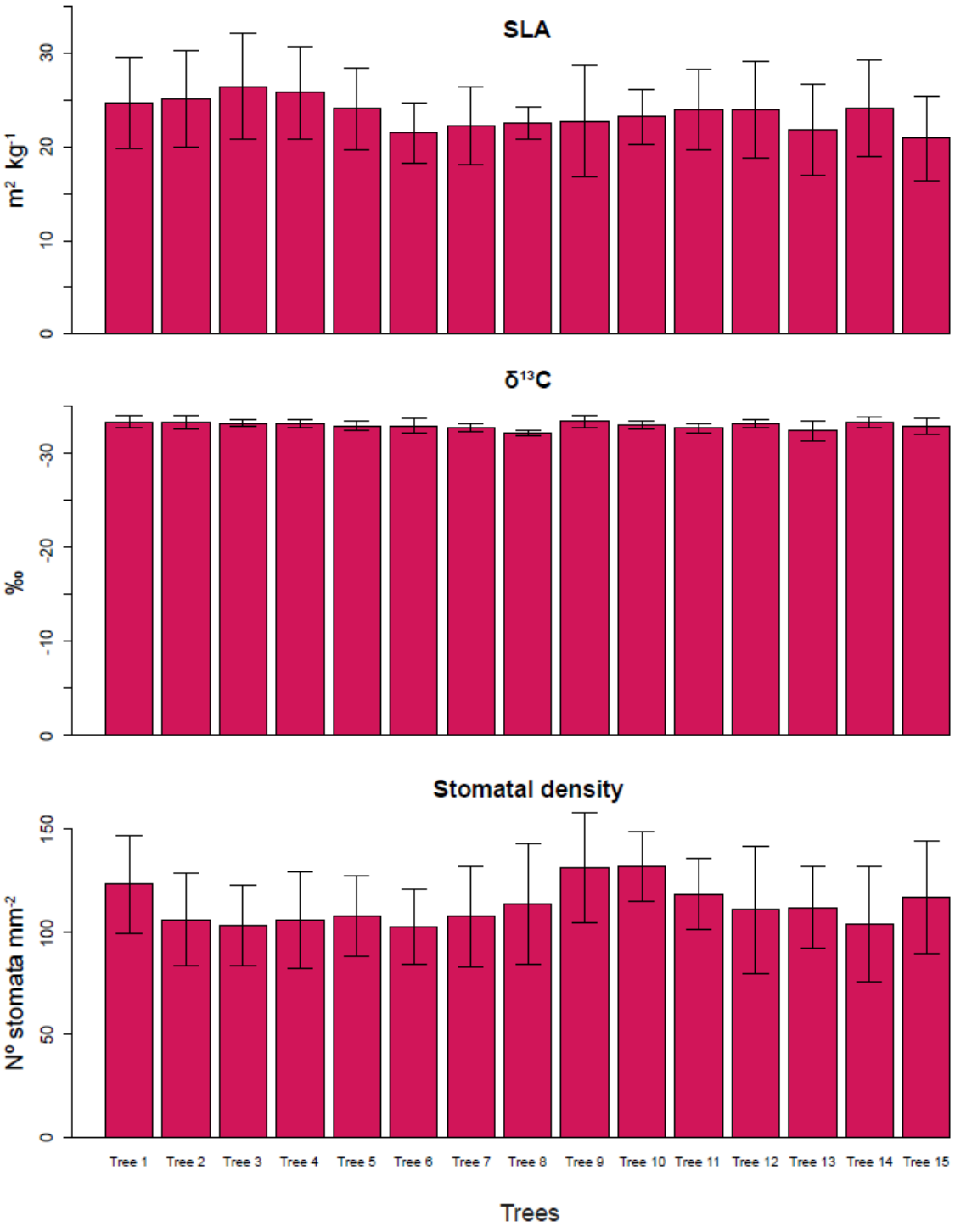


Figure A.11. Barplot with the mean (n=12, i.e. the 12 sampled positions) of SLA, $\delta^{13}C$ and stomatal density for each of the 15 trees. Error bars represent the standard deviation.

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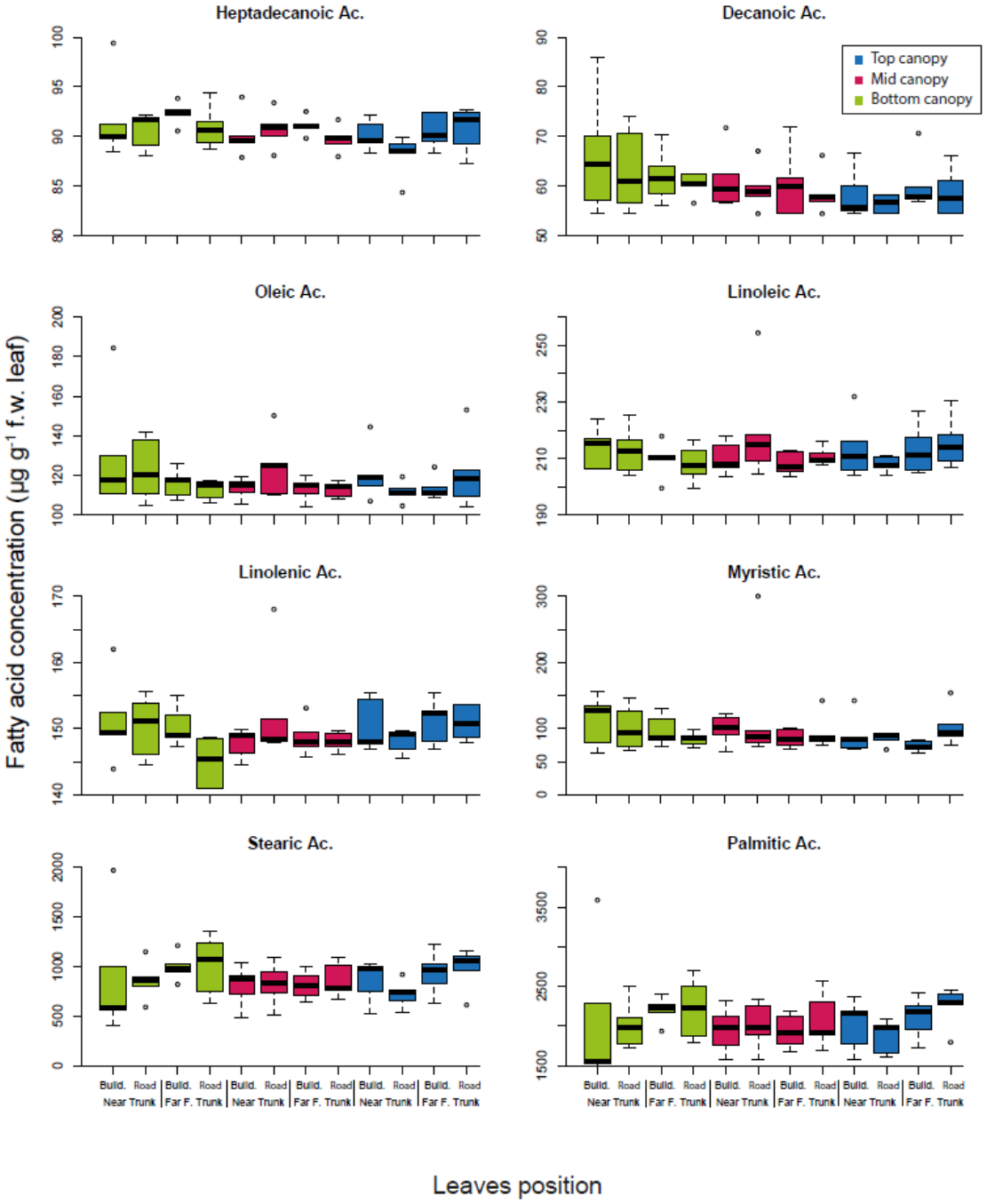


Figure A.12. Boxplots (n=5) of fatty acid concentration determined in cuticle waxes in leaves at each of the 12 studied canopy positions.

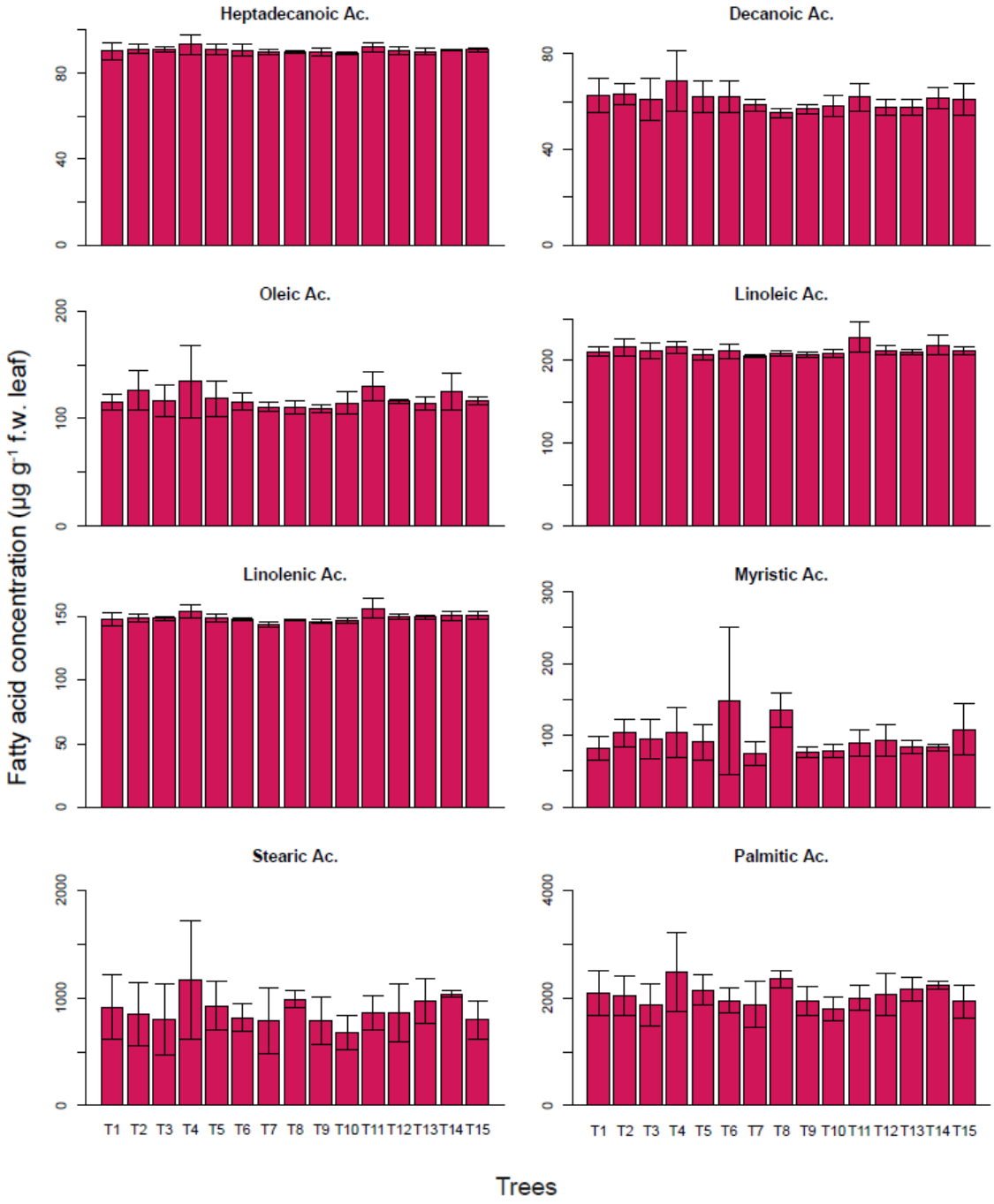


Figure A.13. Barplot with the mean (n ≈ 4) of fatty acid concentration for each of the 15 trees. Error bars represent the standard deviation.

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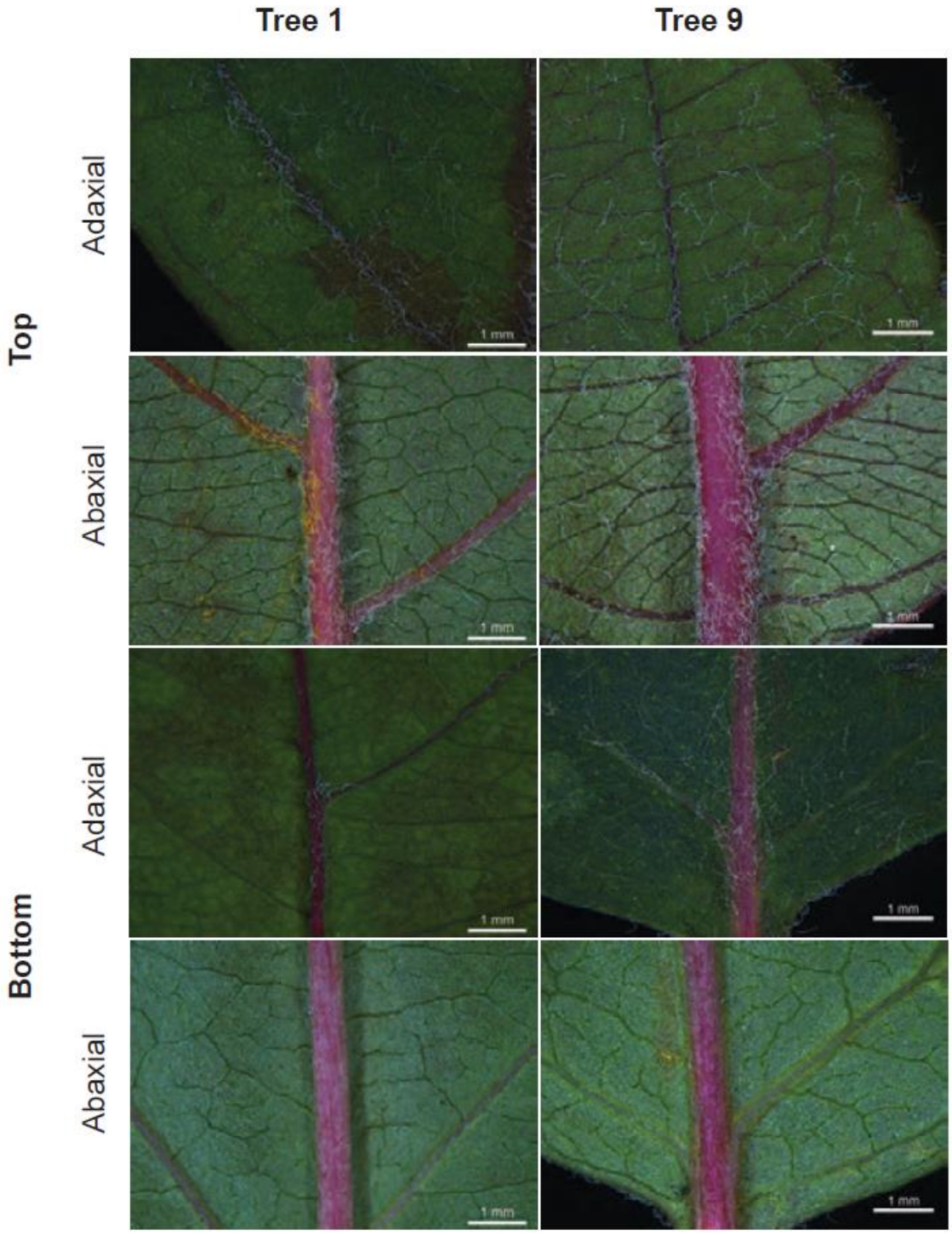


Figure A.14. Optical microscopy photographs of apple tree leaves. They show the trichomes in the adaxial and abaxial part, in the lower and upper part of the crown and in two different trees, tree 1, belonging to group 1, and tree 9, belonging to group 2.

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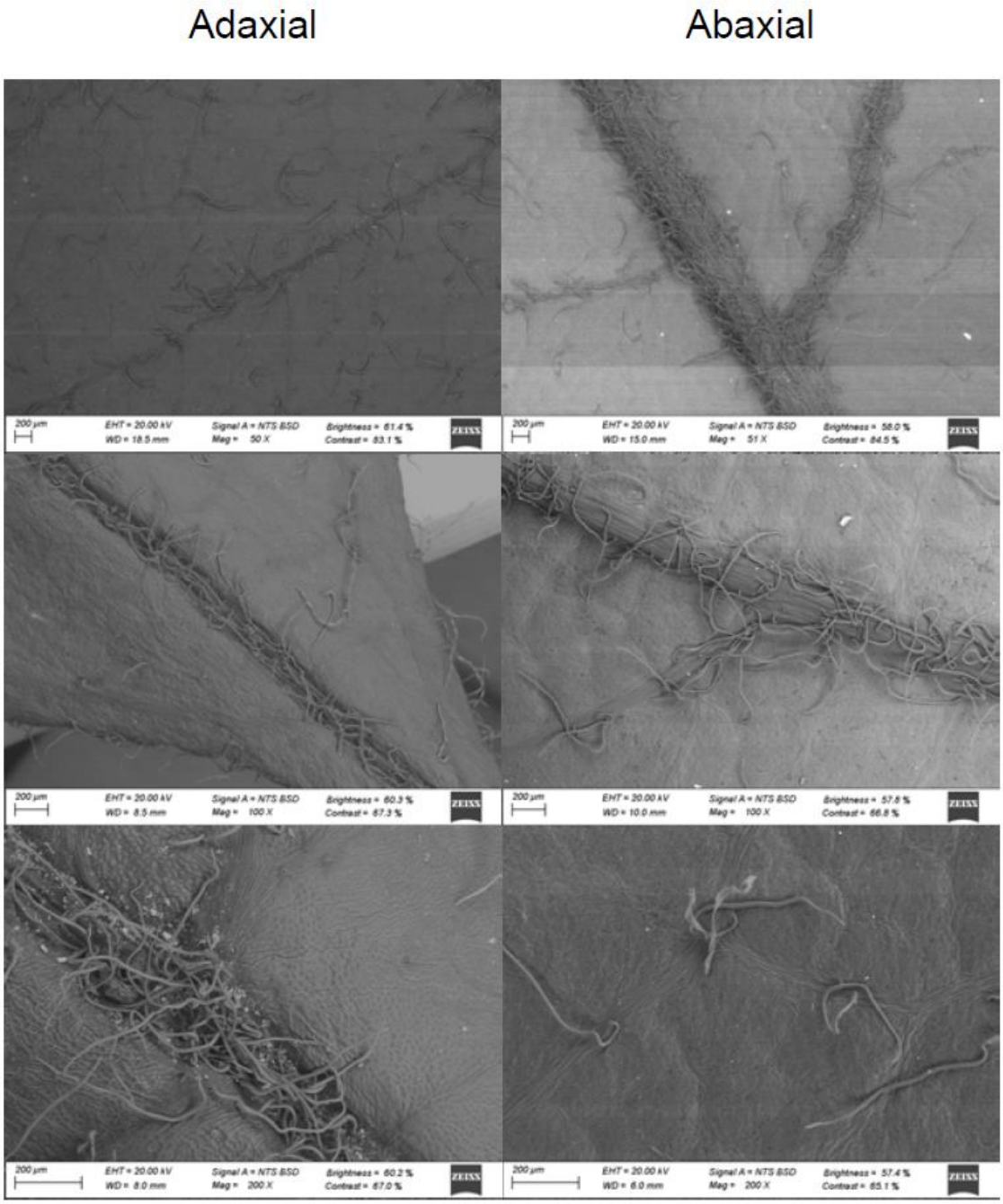


Figure A.15. Scanning electron microscopy pictures, with different magnifications, of the adaxial and abaxial part of an apple leaf.

Tabla A.1. Concentrations ng g-1 of PAHs in the analysed samples. T.: number of the analysed tree (T1-T15). O.: orientation of the leaves on the tree, towards the road (R) or buildings (B). D.T.: leaf distance to the trunk, near (N) or far (F). C.H.: height of the leaf within the canopy, bottom (Bot), middle (Mid) and top (Top).

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T1	B	F	Bot	0.65	0.3	2.6	17.9	3.5	24.4	19.6	7.1	23.1	13.3	2	3	1.9	0.2	0.45	2.2	24.95	74.2	20.2	2.85
T1	B	N	Bot	1.6	0.3	0.35	12.1	4.3	15.8	16.2	11.9	31.1	15.8	2	1.8	0.1	0.2	1.5	4.9	18.65	75	19.7	6.6
T1	R	F	Bot	0.65	0.3	0.35	22.1	3.6	8.6	14.1	5.1	13.1	10.1	0.9	3.7	3.7	0.2	2	5.2	27	40.9	18.4	7.4
T1	R	N	Bot	3.1	0.3	0.35	10.2	5.2	21.5	19.4	11.1	30.4	16.2	1.8	4.6	0.1	0.2	3.6	7.6	19.15	82.4	22.7	11.4
T1	B	F	Mid	0.65	0.3	0.35	4.4	2	7.4	11.1	3.3	11.4	8.9	1.1	2.5	0.4	0.2	0.9	1.6	7.7	33.2	12.9	2.7
T1	B	N	Mid	4.5	0.3	0.35	9.1	3.9	14	17.1	11.8	29.7	17.9	2	5.9	0.1	0.2	4.5	8.6	18.15	72.6	25.9	13.3
T1	R	F	Mid	0.65	0.3	0.35	6.3	2.1	12.6	14	6	16.2	13.8	0.6	3.3	0.1	0.2	1.5	3.5	9.7	48.8	17.8	5.2
T1	R	N	Mid	0.65	0.3	0.35	8.8	5.7	19.9	23.2	11	29.5	22.8	2.2	6.5	0.1	0.2	5.7	11.2	15.8	83.6	31.6	17.1
T1	B	F	Top	2.5	0.3	0.35	12.4	5.2	13.3	16	5.6	17.9	12	0.9	3.4	0.1	0.2	0.9	4.2	20.75	52.8	16.4	5.3
T1	B	N	Top	0.65	0.3	0.35	4.1	2.7	7.9	11	7.9	18.2	15.4	1	3.7	0.1	0.2	3.2	5	8.1	45	20.2	8.4
T1	R	F	Top	0.65	0.3	0.35	6.5	2.7	16.1	12.6	2.9	9.7	8.4	0.1	1.8	0.1	0.2	1.6	1.9	10.5	41.3	10.4	3.7
T1	R	N	Top	0.65	0.3	0.35	12.8	6.5	17.8	18.8	11	30	25	5.2	7	0.4	0.2	3.3	8.9	20.6	77.6	37.6	12.4
T2	B	F	Bot	1.4	0.3	3.5	10.3	2.2	15.4	13.8	5.3	15.1	10.8	0.1	2.5	6.9	0.2	0.45	2.6	17.7	49.6	20.3	3.25
T2	B	N	Bot	7.3	0.3	3	11.9	4.1	30	27.8	8.6	22.8	25.7	1.1	6.2	7.7	0.2	2.4	6	26.6	89.2	40.7	8.6
T2	R	F	Bot	0.65	0.3	3.3	17.5	1.9	26.5	32.3	8.2	21.8	26.8	3.3	8.2	9.8	0.2	3.9	10.5	23.65	88.8	48.1	14.6
T2	R	N	Bot	1.9	0.3	5	11.2	4.8	26.8	28.1	10.7	29.4	33.3	2.8	8.5	7.5	0.2	4.7	10.5	23.2	95	52.1	15.4
T2	B	F	Mid	3	0.3	1.6	8.6	3.4	21.5	23.8	8.5	23.3	18.8	2.3	5.1	8.6	0.2	2.1	5	16.9	77.1	34.8	7.3
T2	B	N	Mid	0.65	0.3	1.2	8	3.3	15.4	16	9.5	30.6	19.6	1.8	4.8	9.3	0.2	2	4.7	13.45	71.5	35.5	6.9
T2	R	F	Mid	0.65	0.3	0.35	7.5	2.8	9.8	10.5	3	9.6	13.6	0.5	1.3	7.3	0.2	0.45	1.5	11.6	32.9	22.7	2.15
T2	R	N	Mid	0.65	0.3	0.35	12.9	6.3	19.1	25	10.2	25.3	30.6	5.6	11.8	10.4	0.2	5	11.8	20.5	79.6	58.4	17
T2	B	F	Top	2	0.3	0.35	3.7	0.65	9.1	10.1	4	13.8	9	0.3	2.4	9.9	0.2	0.45	0.4	7	37	21.6	1.05
T2	B	N	Top	4.2	0.3	0.35	2	0.65	8.2	8.4	4.3	14.3	10.2	1.2	2.6	9.1	0.2	1.1	1.9	7.5	35.2	23.1	3.2
T2	R	F	Top	2.4	9.9	5.7	8.4	3.1	17	12.7	4.4	16.2	10.8	0.1	2.9	7.6	0.2	1.2	1.7	29.5	50.3	21.4	3.1

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T2	R	N	Top	1.3	0.3	0.7	14.9	2.8	19.4	19.1	5.8	15.8	21.6	1.5	7.1	5.2	0.2	1.3	4.5	20	60.1	35.4	6
T3	B	F	Bot	2	0.3	2	11.1	4.7	23.2	22.7	9.4	24.7	33.2	6.4	9.2	9.3	0.2	1.9	6.9	20.1	80	58.1	9
T3	B	N	Bot	4.4	8.9	7.5	24.3	5.9	61.8	58.3	18.2	50.7	67.1	6.3	21.3	9.3	0.2	9.4	17.2	51	189	104	26.8
T3	R	F	Bot	3.1	0.3	2.4	13	3	25.1	31.5	9.5	27.9	40.8	5.4	12.5	10.2	0.2	3.6	8.8	21.8	94	68.9	12.6
T3	R	N	Bot	4.7	9.9	8.8	17.6	2.7	29.6	31.6	11.6	38.2	36	7.1	11.1	11.6	0.2	4.2	8.5	43.7	111	65.8	12.9
T3	B	F	Mid	0.65	13.7	10	14	1.8	9.1	10.4	6.7	20.5	18.1	1.9	4.4	9.5	0.2	1.5	2.9	40.15	46.7	33.9	4.6
T3	B	N	Mid	2.1	9.8	5.8	10.6	2.9	14.9	18.8	7.5	23.8	20.1	1.8	6.2	7.4	0.2	2.5	5.4	31.2	65	35.5	8.1
T3	R	F	Mid	2.7	11.2	7.5	11.3	2.8	13.1	15.7	6.4	19.5	17	2.6	3.9	0.1	0.2	1.1	4.3	35.5	54.7	23.6	5.6
T3	R	N	Mid	0.65	12.4	9.8	23.3	4.8	41.8	44.7	13.7	33.2	36.1	4.2	10.7	0.1	0.7	4.8	12.1	50.95	133.4	51.1	17.6
T3	B	F	Top	0.65	4.7	3.5	8.1	0.65	12.4	11.3	4.8	15.7	12.9	0.1	3	0.1	0.2	0.45	1.1	17.6	44.2	16.1	1.75
T3	B	N	Top	4.9	2.9	1.9	7	1.5	15	12.4	4.5	20.1	17.7	2.4	4.6	1.8	0.2	1.8	3.2	18.2	52	26.5	5.2
T3	R	F	Top	4.3	3.7	7.8	17.1	0.65	14.7	10.3	2.8	12.3	8.8	0.1	2.6	0.1	0.2	0.45	1.4	33.55	40.1	11.6	2.05
T3	R	N	Top	0.65	6.2	9.6	14.9	1.7	20.1	12.1	4.5	16.5	16.4	0.4	3.8	0.1	0.2	0.45	2.2	33.05	53.2	20.7	2.85
T4	B	F	Bot	1.6	3.6	4.9	9.7	2.9	8.3	10.7	5.1	14.6	22.5	2.1	6.6	0.6	0.2	1.8	5.6	22.7	38.7	31.8	7.6
T4	B	N	Bot	3.3	12.9	9.5	14.9	2.4	12.3	14	6.3	20.5	25.4	1.3	5.6	0.1	0.2	2.6	7.4	43	53.1	32.4	10.2
T4	R	F	Bot	3.1	9.3	8	13.1	0.65	15.4	19.8	5.6	16.8	29.6	0.1	9.7	0.1	0.2	5.6	11.9	34.15	57.6	39.5	17.7
T4	R	N	Bot	2.9	4.3	5.6	10.5	0.65	17.2	21.8	8.7	29.5	41.9	12.5	11.5	2.6	0.2	5.8	9.1	23.95	77.2	68.5	15.1
T4	B	F	Mid	2.8	3.3	4.5	9.7	0.65	10.6	15.3	9.3	25.4	41	11.2	10.7	0.3	0.7	5.6	7	20.95	60.6	63.2	13.3
T4	B	N	Mid	0.65	5.6	5.1	11.5	0.65	10	14.9	6.3	21.4	34.8	10.3	10.3	0.1	0.2	4.4	7.8	23.5	52.6	55.5	12.4
T4	R	F	Mid	2.5	8.8	3.5	9.5	0.65	17.2	20.8	11	30.4	44.3	16.2	14.4	3.1	0.2	5.4	7.5	24.95	79.4	78	13.1
T4	R	N	Mid	0.65	2.3	3.7	8.4	0.65	10.3	13.4	7	24.6	34.3	5	8.1	0.1	0.2	4.5	4.3	15.7	55.3	47.5	9
T4	B	F	Top	0.65	3.2	5.9	9.7	0.65	10.4	9.7	4.8	15.3	19.2	4.7	0.3	0.8	0.2	2.3	3.2	20.1	40.2	25	5.7
T4	B	N	Top	0.65	5.8	4.7	10	0.65	10.3	13.1	6.4	17.6	34.2	11.4	9.7	1.1	0.5	2.5	6.1	21.8	47.4	56.4	9.1
T4	R	F	Top	2.3	6.1	6.1	9.4	0.65	11	11.8	4.4	13.1	19.7	2	4.7	0.1	0.2	2.7	1.7	24.55	40.3	26.5	4.6
T4	R	N	Top	0.65	5.5	5.2	8.6	0.65	7.7	9.4	4.3	14	24.1	3.2	5.5	0.1	0.2	4.1	3.7	20.6	35.4	32.9	8
T5	B	F	Bot	0.65	5.9	7.3	16.9	0.65	14.6	18.1	7.2	21.7	31.2	13	10	2	0.2	5.9	5.9	31.4	61.6	56.2	12
T5	B	N	Bot	1.8	8.2	5.7	16.6	0.65	18	26.1	11.2	34	61.1	27.3	20.9	1.6	0.2	10.4	14.3	32.95	89.3	110.9	24.9
T5	R	F	Bot	4.4	8.2	7.2	16	0.65	15	20.9	7	20.3	46.2	19.9	16.3	11.7	0.7	3.3	9.4	36.45	63.2	94.1	13.4

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T5	R	N	Bot	2.2	6.3	5.9	18.7	0.65	27.9	37.6	14.4	30	59.5	26.9	20.5	6.3	1.6	18.2	22.5	33.75	109.9	113.2	42.3
T5	B	F	Mid	2.6	6.3	6.8	16.1	1.5	14.4	16.6	6.4	18.5	21.9	2.9	6.8	0.5	0.2	3.5	2.8	33.3	55.9	32.1	6.5
T5	B	N	Mid	2.4	8.8	9.8	19.2	0.65	12.8	17.9	7.3	20.8	35	18.7	10.4	2.8	0.2	7.1	7.9	40.85	58.8	66.9	15.2
T5	R	F	Mid	3.3	5.6	7.7	10.8	0.65	8.8	9.5	4.2	11.6	15.5	7.3	0.1	4.2	0.2	6.1	5.3	28.05	34.1	27.1	11.6
T5	R	N	Mid	1.8	5.3	7.4	15.6	0.65	11.3	15.5	6.5	20.5	24.2	9.7	3.2	0.6	0.2	5.7	5.4	30.75	53.8	37.7	11.3
T5	B	F	Top	7.1	4.6	5	11.2	0.65	43.2	35.7	14.2	21.7	19.1	8.2	4.5	5.1	0.4	6.6	4.1	28.55	114.8	36.9	11.1
T5	B	N	Top	3	7	5.6	12.4	0.65	12.1	13.6	8.3	18.9	18.3	8	1	2.1	0.2	6.8	4.3	28.65	52.9	29.4	11.3
T5	R	F	Top	0.65	0.3	0.8	6.7	0.65	13.7	15.8	5.3	13.2	14.8	7.8	2.1	0.6	0.2	2.8	6.1	9.1	48	25.3	9.1
T5	R	N	Top	1.8	7.3	5.9	10.6	0.65	11.3	10.8	3.1	13.4	14.9	3	4.2	0.1	0.2	6.2	5.1	26.25	38.6	22.2	11.5
T6	B	F	Bot	1.6	8	5.8	11.8	0.65	11.7	17.4	9.4	22.3	19	9.9	3.5	3	0.2	7.9	9	27.85	60.8	35.4	17.1
T6	B	N	Bot	3	6.3	7	10.7	0.65	11.4	20.9	3.7	17.3	16.7	7.2	1.8	0.5	0.9	5.6	7.4	27.65	53.3	26.2	13.9
T6	R	F	Bot	6.7	1	8.4	11	0.65	14.9	21.3	3.6	14.1	14.4	5.8	1.9	2.2	0.2	3.9	6.7	27.75	53.9	24.3	10.8
T6	R	N	Bot	2.7	7.5	6.5	14.1	0.65	17.8	24.6	7.8	17.4	18.1	6.3	0.4	0.3	0.2	4	5.5	31.45	67.6	25.1	9.7
T6	B	F	Mid	3.5	4.1	6.6	12.1	0.65	11.5	15.4	7.1	19.9	17.5	8.9	3.3	2.7	0.4	6.9	8.5	26.95	53.9	32.4	15.8
T6	B	N	Mid	2.8	8.3	8.6	15	0.65	24.3	28.7	8.3	23.1	27.5	14.6	5	3.4	0.2	9.3	10.8	35.35	84.4	50.5	20.3
T6	R	F	Mid	1.9	0.6	1.7	11.1	3.1	25	28.1	8	23.3	20.1	0.7	12.3	0.1	0.2	5.8	6.6	18.4	84.4	33.2	12.6
T6	R	N	Mid	0.65	0.8	1	7.7	2.2	21.4	29.2	9.2	26.1	33.8	12.4	9.5	12.4	0.2	6.1	10.6	12.35	85.9	68.1	16.9
T6	B	F	Top	0.65	0.3	0.6	3.8	1.7	11.5	13.6	4.1	12.2	15.8	4.1	4.7	6.1	0.2	3.4	4.8	7.05	41.4	30.7	8.4
T6	B	N	Top	0.65	0.3	0.6	5.6	0.65	13.6	16.3	5.4	14.7	18.6	6.5	5.3	6	0.2	3.6	5.7	7.8	50	36.4	9.5
T6	R	F	Top	0.65	0.3	0.6	5	2.1	16	18.7	6.7	15	16	2.7	4.6	3.9	0.2	4	4.2	8.65	56.4	27.2	8.4
T6	R	N	Top	0.65	0.8	1.5	14.6	2.4	18	20.4	5.8	16.1	19.4	3.8	5.7	2.8	0.2	4.5	5.8	19.95	60.3	31.7	10.5
T7	B	F	Bot	2.4	1	3.7	17.3	3.7	43	36.6	31.7	72.3	111.8	57.9	25.9	15.2	1.6	9.8	14.9	28.1	183.6	210.8	26.3
T7	B	N	Bot	0.65	1	3.9	18.6	4.5	34.7	32	25.6	59.9	28.6	17.4	8.5	10.8	0.2	12.5	16.7	28.65	152.2	65.3	29.4
T7	R	F	Bot	0.65	0.3	1.4	8.9	1.9	29.3	26.6	19.1	49.3	43	11.1	9.6	11	0.2	7.6	9.7	13.15	124.3	74.7	17.5
T7	R	N	Bot	0.65	0.3	2.7	12.5	2.9	33.5	27.5	23.8	52.7	42.2	15.6	9.6	11.8	0.5	8.2	9.2	19.05	137.5	79.2	17.9
T7	B	F	Mid	0.65	0.3	2.3	7.6	2.7	30.7	22.8	13.5	37.5	25.3	8.8	6.6	7.3	0.2	4.3	4.3	13.55	104.5	48	8.8
T7	B	N	Mid	0.65	0.3	2.4	7.4	1.4	20.4	16.2	15.1	41	34.7	9.7	7.7	3.1	0.2	4.4	7.1	12.15	92.7	55.2	11.7
T7	R	F	Mid	0.65	0.3	0.8	2.7	0.65	24.3	17.1	13.1	38.3	23.4	6.4	5.3	5.2	0.2	3.9	2.6	5.1	92.8	40.3	6.7

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T7	R	N	Mid	1.3	0.3	0.6	3.2	1.7	27.5	21.1	18.6	52.8	36.1	9.1	7.1	7.4	0.2	6.6	5.4	7.1	120	59.7	12.2
T7	B	F	Top	0.65	0.3	0.6	7.3	2.7	40.5	30.4	19.6	45.4	43.5	18.1	12.3	17.9	0.6	7.6	8.6	11.55	135.9	91.8	16.8
T7	B	N	Top	0.65	0.3	0.6	7.4	2	17.5	16.2	11.7	32.2	21.6	2.3	4.6	3.9	0.2	2.9	3.1	10.95	77.6	32.4	6.2
T7	R	F	Top	0.65	0.3	0.6	7.6	2	17.7	14.8	13.6	34.3	24.4	6.9	5.6	6.7	0.2	4.5	3.5	11.15	80.4	43.6	8.2
T7	R	N	Top	0.65	0.3	0.6	7.6	1.9	21.4	16.2	10.4	26.6	21.4	10.7	4.8	7.2	0.2	3	2.9	11.05	74.6	44.1	6.1
T8	B	F	Bot	0.65	0.3	NA	5.8	2.8	36.8	30.3	23.4	51.1	52.6	20.1	10.8	17.9	0.2	9.5	8.6	9.55	141.6	101.4	18.3
T8	B	N	Bot	0.65	0.3	3.5	9.2	3.5	32.8	32.5	20.5	48.2	48.2	19.8	10.9	12.8	0.7	10.3	10	17.15	134	91.7	21
T8	R	F	Bot	2.5	0.3	0.6	4.8	2.8	30.7	27.2	24.2	55.7	55	30.9	12.2	17.3	1.7	9.2	8.9	11	137.8	115.4	19.8
T8	R	N	Bot	2.2	0.3	1.2	7.8	3.3	33.2	29.6	18.8	47	46.5	28.2	11.8	18.2	0.7	8.9	10.9	14.8	128.6	104.7	20.5
T8	B	F	Mid	0.65	0.3	0.6	1.8	1.7	22.9	21.5	11.8	30	28.7	10.6	5.5	8.7	0.2	3.9	3.6	5.05	86.2	53.5	7.7
T8	B	N	Mid	0.65	0.3	0.6	7.1	1.7	30.2	30.8	14.2	35	37.5	12.4	9.6	13.1	0.2	9	8.5	10.35	110.2	72.6	17.7
T8	R	F	Mid	0.65	0.3	2	9.3	3.1	36.2	34.9	20.4	42.2	39.7	8	9.7	13.3	0.6	8.6	9.1	15.35	133.7	70.7	18.3
T8	R	N	Mid	0.65	0.3	0.6	4.6	1.9	27.1	25.8	14.4	31.6	40.5	12	9.1	14.2	0.2	8.1	8.6	8.05	98.9	75.8	16.9
T8	B	F	Top	0.65	0.3	0.6	3.8	2.3	28.3	22	13.5	32.2	23	6.2	5	6.4	0.2	2.7	2.9	7.65	96	40.6	5.8
T8	B	N	Top	0.65	0.3	1.6	3.9	1.7	29.6	22.7	13.2	32.9	29.8	5.6	6.4	7.9	0.2	4.1	4.7	8.15	98.4	49.7	9
T8	R	F	Top	0.65	0.3	0.6	2.6	2.4	26.6	24.5	13.7	33.2	29.7	5.4	6.5	19.3	0.2	4.6	4.1	6.55	98	60.9	8.9
T8	R	N	Top	0.65	0.3	0.6	8.5	2.6	41.6	36.1	21.8	48.6	33.5	4.3	7.5	8.5	0.5	7	8.9	12.65	148.1	53.8	16.4
T9	B	F	Bot	2.3	0.3	3.5	9.8	2.8	54.8	43.7	35.9	77.3	78.6	35.2	18.6	23.3	3.1	12	12.8	18.7	211.7	155.7	27.9
T9	B	N	Bot	0.65	0.3	3.3	15.7	4.2	64.5	52.5	32.8	69.9	67.1	35.7	16.6	20.9	2.4	11	10.3	24.15	219.7	140.3	23.7
T9	R	F	Bot	1.5	0.3	1.8	12.9	3.6	107.4	69.9	31.2	69	46.6	15.3	13.4	8.4	0.8	12.2	11.9	20.1	277.5	83.7	24.9
T9	R	N	Bot	1.8	0.3	2.9	10.7	4.7	88.8	61.8	37	78.8	75.5	35.1	19.8	24.4	1	14.9	14.2	20.4	266.4	154.8	30.1
T9	B	F	Mid	0.65	0.3	1.1	4.8	1.3	32.5	30.6	23.1	47.6	44.2	13.9	11.5	10.4	1	12.3	10	8.15	133.8	80	23.3
T9	B	N	Mid	0.65	0.3	0.35	4.4	2	25.9	24.6	18.6	44.5	44.9	25.2	13	13.2	0.5	11.1	9.1	7.7	113.6	96.3	20.7
T9	R	F	Mid	0.65	0.3	1.2	7.1	1.9	40.6	30.3	15.7	37.4	46.6	8	10.9	13.1	0.4	7.3	7.6	11.15	124	78.6	15.3
T9	R	N	Mid	0.65	0.3	2.2	9.1	2.6	52	38.3	20.7	50.8	52.4	20.1	13.7	17.9	0.2	9	7.4	14.85	161.8	104.1	16.6
T9	B	F	Top	0.65	0.3	0.35	2.6	2	14.4	12.7	8.3	20.6	18	4.3	4.3	3.8	0.2	3.2	3.2	5.9	56	30.4	6.6
T9	B	N	Top	0.65	0.3	0.7	3.8	0.65	26.3	17.9	6.5	17.7	17.6	4.7	4.3	4.4	0.2	2.7	2.2	6.1	68.4	31	5.1
T9	R	F	Top	0.65	0.3	1.2	3.6	1.4	25.9	17	12.7	32.1	25.1	8.2	5.7	7.5	0.4	4.6	3.6	7.15	87.7	46.5	8.6

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T9	R	N	Top	0.65	0.3	1.1	3.6	1.4	26.3	17.7	6.2	18.1	14	4.3	4	4.7	0.2	1.9	1.3	7.05	68.3	27	3.4
T10	B	F	Bot	0.65	0.3	1.2	4	2.8	28.6	24.2	15.4	39.6	38.9	18.2	8.8	10.7	0.2	5.8	5	8.95	107.8	76.6	11
T10	B	N	Bot	0.65	0.3	2.7	4.7	2.4	37.6	30.5	19.4	47.5	36.9	14.1	9.4	9.8	0.4	6.6	6.6	10.75	135	70.2	13.6
T10	R	F	Bot	0.65	0.3	0.8	4	2.2	33.1	31.9	16.3	38.9	38.4	18	10.5	11.3	0.2	7.6	7.6	7.95	120.2	78.2	15.4
T10	R	N	Bot	0.65	0.3	2.3	8.2	2.5	37.1	30.3	15.3	37.9	35.3	10.2	9	4.2	0.2	5.2	6.7	13.95	120.6	58.7	12.1
T10	B	F	Mid	1.6	0.3	0.35	1	0.65	16.6	15	12.4	34.2	40.3	23	9.7	10.8	0.2	5.5	5	3.9	78.2	83.8	10.7
T10	B	N	Mid	0.65	0.3	0.35	0.5	0.65	10.2	11.3	6.4	18.2	14.7	6.5	3.4	4.3	0.2	2.8	3	2.45	46.1	28.9	6
T10	R	F	Mid	0.65	0.3	0.7	6.2	2.3	19.7	17.5	9.3	23.4	24.5	4.7	5.7	3.7	0.2	6.4	5.9	10.15	69.9	38.6	12.5
T10	R	N	Mid	1.8	0.3	1.3	7.4	1.5	23	21	12.1	29.8	28.5	7.8	7.8	3.9	0.5	6.5	5.2	12.3	85.9	48	12.2
T10	B	F	Top	0.65	0.3	1.3	4.1	0.65	13.3	10	7.7	20.7	15.6	5.5	4.3	2.8	0.2	3.3	2.4	7	51.7	28.2	5.9
T10	B	N	Top	0.65	0.3	2.1	5.1	2.1	21.9	16.4	6.9	20.2	17.7	4	4.6	4.5	0.2	3.1	1.9	10.25	65.4	30.8	5.2
T10	R	F	Top	0.65	0.3	1.7	4.6	0.65	18.4	16.1	8.9	22.4	22.9	6.8	4.8	5.4	0.2	4.1	2.9	7.9	65.8	39.9	7.2
T10	R	N	Top	0.65	0.3	1.3	4.5	0.65	17.8	15.8	8.5	23.8	23.5	8.1	5	2.5	0.2	3.2	2.4	7.4	65.9	39.1	5.8
T11	B	F	Bot	1.3	0.3	3.5	8	4.2	24	24.6	18.8	42.9	50.8	26.3	11.8	18.3	0.2	8.3	7.8	17.3	110.3	107.2	16.3
T11	B	N	Bot	0.65	0.3	1.7	8.3	3.3	45.4	38.6	24.7	49.7	63.1	31	16.1	20.3	0.2	8	7.3	14.25	158.4	130.5	15.5
T11	R	F	Bot	0.65	0.3	1.7	6.8	4.5	58.7	42.9	24	52.2	59.6	33.3	15.3	25	0.8	10.8	10.2	13.95	177.8	133.2	21.8
T11	R	N	Bot	0.65	0.3	0.8	5.3	3.3	32.9	28.5	19.9	45.4	51.8	24.1	12.3	18.7	0.6	8.4	9.1	10.35	126.7	106.9	18.1
T11	B	F	Mid	0.65	0.3	1	0.5	1.9	16.7	14.9	7.6	20	14.7	3.6	3.3	3.6	0.2	2	0.9	4.35	59.2	25.2	3.1
T11	B	N	Mid	0.65	0.3	1.7	6.1	2.6	21.6	22.9	13.5	29.4	38	15	12	13.2	1	9.3	9.5	11.35	87.4	78.2	19.8
T11	R	F	Mid	1.3	0.3	2.1	5.6	2.3	16.2	18.4	12.4	30.4	42.2	18.1	9.1	4.2	0.2	5.1	6.2	11.6	77.4	73.6	11.5
T11	R	N	Mid	0.65	0.3	1.5	4.3	1.3	25.5	24.5	19.5	41.2	47.5	25.8	11.7	11.6	0.2	9.5	9.3	8.05	110.7	96.6	19
T11	B	F	Top	0.65	0.3	0.35	0.5	2.2	22.8	16.9	8	22.2	16.8	5.5	4.6	4.6	0.2	3.1	1.9	4	69.9	31.5	5.2
T11	B	N	Top	0.65	0.3	0.9	2.4	1.5	15.9	14.1	9.3	22.3	26	8.6	6.3	1.8	0.2	5	4.2	5.75	61.6	42.7	9.4
T11	R	F	Top	0.65	0.3	0.35	0.5	0.65	12.2	11.6	5.9	16.8	13.4	2.8	3.2	0.1	0.2	2.1	1.8	2.45	46.5	19.5	4.1
T11	R	N	Top	0.65	0.3	0.35	3.7	2	22.1	19.8	13.3	31.6	30.4	11.3	8.1	7	0.2	6.8	5.6	7	86.8	56.8	12.6
T12	B	F	Bot	0.65	0.3	0.9	7.9	3.3	64.1	42.5	28.3	62.6	58.2	27.4	13.8	16.6	0.5	8.9	6.2	13.05	197.5	116	15.6
T12	B	N	Bot	0.65	0.3	1	13	4	91.6	69.3	35.9	74.1	66.2	32	17.6	21.8	1.1	13.3	10.4	18.95	270.9	137.6	24.8
T12	R	F	Bot	0.65	0.3	0.35	2	2.2	43	30.3	17.1	39	41.2	18.4	10.5	15.3	0.2	7.1	6.3	5.5	129.4	85.4	13.6

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T12	R	N	Bot	1.5	0.3	1.1	7.2	2.5	54.9	46.1	33.9	69.9	53.7	17.8	14.4	1.1	0.9	13.6	13.1	12.6	204.8	87	27.6
T12	B	F	Mid	0.65	0.3	0.35	3.8	1.7	30.1	20.8	14.7	37	35.3	10.5	8.4	1.9	0.2	5.7	3.7	6.8	102.6	56.1	9.6
T12	B	N	Mid	1.7	0.3	2.4	10.5	3	85.4	60.8	20	48	37.3	11	8.7	1.4	0.2	5.1	4.4	17.9	214.2	58.4	9.7
T12	R	F	Mid	1.3	0.3	1.2	11.4	3.3	58.2	46.1	23.6	49.9	46.3	19.1	13.2	5.8	0.2	8.8	8.3	17.5	177.8	84.4	17.3
T12	R	N	Mid	0.65	0.3	1.5	6.9	2.3	57.4	38.6	18.6	41.4	31.9	8.1	7.8	2.8	0.2	6	4.9	11.65	156	50.6	11.1
T12	B	F	Top	0.65	0.3	0.35	0.5	0.65	22	16	10	24.3	32	12.4	7.8	8.3	0.2	4.3	3.4	2.45	72.3	60.5	7.9
T12	B	N	Top	0.65	0.3	0.35	2.4	1.6	28.7	21	13.8	33	26.5	9.1	5.5	6	0.2	3.9	2.6	5.3	96.5	47.1	6.7
T12	R	F	Top	0.65	0.3	0.35	3.2	0.65	36.9	22.6	15.3	33.2	27.1	5.2	5.9	3	0.2	4	2.8	5.15	108	41.2	7
T12	R	N	Top	0.65	0.3	0.35	1.9	0.65	17.4	14.7	11.2	25.2	27.5	11.1	6.4	9	0.2	3	3.4	3.85	68.5	54	6.6
T13	B	F	Bot	0.65	0.3	2	7.3	0.65	20.7	22.7	12.7	29.5	48	22.7	12.5	10.2	0.5	8.1	8.7	10.9	85.6	93.4	17.3
T13	B	N	Bot	0.65	0.3	1.2	7.9	1.4	29.5	29.3	13.1	28.7	55.7	31.4	16.5	21.3	0.7	11.6	12	11.45	100.6	124.9	24.3
T13	R	F	Bot	0.65	0.3	0.35	2.9	0.65	19.8	18.9	12.6	31.6	35	17.9	9.2	12.2	0.5	5.9	11.1	4.85	82.9	74.3	17.5
T13	R	N	Bot	0.65	0.3	0.35	5.3	1.9	24.8	28.6	15	34.3	53.7	38.6	19.9	34.1	7.1	7.7	18.8	8.5	102.7	146.3	33.6
T13	B	F	Mid	0.65	0.3	2	3.9	3.7	33.9	27.7	11.2	29.5	30.4	12.6	11.1	14.9	7.5	4.5	7.9	10.55	102.3	69	19.9
T13	B	N	Mid	0.65	0.3	0.9	3.6	2	16.2	15.7	6.2	17.8	35.8	22.2	15	12.3	6.3	4	6.4	7.45	55.9	85.3	16.7
T13	R	F	Mid	0.65	0.3	0.35	0.5	2.7	14.7	12	4.9	15.7	13.7	3	4.7	4.9	5.8	0.05	0.4	4.5	47.3	26.3	6.25
T13	R	N	Mid	0.65	0.3	3.2	11.7	3.5	25.9	29.7	13	28	36.1	14.2	13.2	8	9.3	7.3	10.8	19.35	96.6	71.5	27.4
T13	B	F	Top	0.65	0.3	0.7	2.2	0.65	13.1	13.2	3.7	11.7	19.8	11.6	7.4	10.6	4.8	2.8	0.6	4.5	41.7	49.4	8.2
T13	B	N	Top	0.65	0.3	0.35	4.4	0.65	20.8	19.8	6.1	16.3	19.5	8.6	4.8	4.1	4.5	2.8	5.1	6.35	63	37	12.4
T13	R	F	Top	0.65	0.3	0.35	1.8	1.6	16.4	13.1	3.3	15	15	4.2	5	7.3	5	1.7	3.2	4.7	47.8	31.5	9.9
T13	R	N	Top	0.65	0.3	0.35	1.8	0.65	8.8	9.8	4	11.6	15.3	4.8	3.8	3.5	5.4	3	4.5	3.75	34.2	27.4	12.9
T14	B	F	Bot	0.65	0.3	1.4	6.3	0.65	20.4	22.2	13.8	31.4	38.1	17.4	11.2	6.5	5.1	5.2	6.1	9.3	87.8	73.2	16.4
T14	B	N	Bot	0.65	0.3	1.7	8.8	0.65	32.8	32.6	17.8	45	45.5	15.8	13.2	7.9	5.6	4.4	9.3	12.1	128.2	82.4	19.3
T14	R	F	Bot	0.65	0.3	1.8	5.5	0.65	19.7	22.9	8.6	24.3	38.5	21.4	10.5	15.5	2.9	5.5	7.8	8.9	75.5	85.9	16.2
T14	R	N	Bot	0.65	0.3	4	15.8	0.65	50.6	45	13.2	32.4	40.8	21.7	12.7	15.2	5.1	6.6	11.7	21.4	141.2	90.4	23.4
T14	B	F	Mid	0.65	0.3	0.8	2.3	0.65	29.1	20.4	14.8	37	41.1	17	10.5	6.5	4.3	3.6	3.4	4.7	101.3	75.1	11.3
T14	B	N	Mid	0.65	0.3	2.4	9.9	0.65	34.6	31.9	11.7	27.1	29.2	9.6	8.3	2	5.7	4.2	5.5	13.9	105.3	49.1	15.4
T14	R	F	Mid	0.65	0.3	0.35	0.5	0.65	15.5	14.4	3.3	14.6	14.6	2.1	4.3	2.4	6.3	2.7	3.2	2.45	47.8	23.4	12.2

T.	O.	D.T.	C.H.	Acy	Ace	Fl	Phe	Ant	Ft	Pyr	BaA	Chry	B(b+j)F	BkF	BeP	BaP	DBahA	IP	BghiP	3rings	4rings	5rings	6rings
T14	R	N	Mid	0.65	0.3	1.2	5.3	0.65	21.4	19.4	9.4	25.1	31.2	14.8	9.8	7.7	6.1	4.8	5.7	8.1	75.3	63.5	16.6
T14	B	F	Top	0.65	0.3	0.35	2.8	0.65	13.4	12	5.8	16.3	15.9	3.3	4.2	2.1	6.1	1.5	2.8	4.75	47.5	25.5	10.4
T14	B	N	Top	0.65	0.3	1	4.2	0.65	13.9	13.9	6.7	19.5	20	4.7	3.9	1.4	6.8	1.8	3	6.8	54	30	11.6
T14	R	F	Top	0.65	0.3	1	4.2	0.65	18.4	12	4.1	13.8	13.8	3.7	4.1	4.7	5	2.1	3.9	6.8	48.3	26.3	11
T14	R	N	Top	0.65	0.3	1	5.7	0.65	13.9	12.3	5	14.6	14.9	4.2	4.1	3.7	6.2	1.8	4.3	8.3	45.8	26.9	12.3
T15	B	F	Bot	0.65	0.3	1.9	8.1	0.65	25.5	25.1	10.3	26.3	27.9	11.9	9	6	6.5	4.9	8.5	11.6	87.2	54.8	19.9
T15	B	N	Bot	0.65	0.3	2.2	7.8	0.65	24.3	25.6	8.2	23.1	25.4	10.8	7.2	3.7	6.2	4.8	6.5	11.6	81.2	47.1	17.5
T15	R	F	Bot	0.65	0.3	0.35	11.1	0.65	36.5	30.2	7	20.8	21	5.6	5.8	4.5	6.1	3.3	7.2	13.05	94.5	36.9	16.6
T15	R	N	Bot	0.65	0.3	2	7.4	0.65	24.9	25.5	7.8	20.9	25	12.3	9	8.5	6.8	7.4	6.2	11	79.1	54.8	20.4
T15	B	F	Mid	0.65	0.3	1.7	5.7	0.65	26.4	20.2	6.8	18.7	19.6	5.8	5.7	4.1	6.4	0.05	5.4	9	72.1	35.2	11.85
T15	B	N	Mid	0.65	0.3	0.8	5.5	0.65	23.2	21.5	8	21.6	23.4	3.6	6.2	2.2	6	3.1	5.5	7.9	74.3	35.4	14.6
T15	R	F	Mid	0.65	0.3	0.35	4	0.65	16.1	15.6	3.8	12.1	16.9	5.9	4.9	7.1	7.5	2.7	4.3	5.95	47.6	34.8	14.5
T15	R	N	Mid	0.65	0.3	0.35	5.5	0.65	30.3	25.6	7.6	21.3	21.3	6.3	6.7	6	7.2	3.8	6.6	7.45	84.8	40.3	17.6
T15	B	F	Top	0.65	0.3	0.35	1.3	0.65	14.8	12.3	5.2	12.3	11.4	3.2	3.1	3.6	5.5	2.1	3.2	3.25	44.6	21.3	10.8
T15	B	N	Top	0.65	0.3	0.35	3.8	0.65	13.9	10.8	5.4	14.7	19	5.3	6.3	3.7	5.9	2.9	2.9	5.75	44.8	34.3	11.7
T15	R	F	Top	0.65	0.3	2	10.2	0.65	24	27.9	6.3	17	19.9	3.8	6.3	6.4	5.4	4.1	4.8	13.8	75.2	36.4	14.3
T15	R	N	Top	0.65	0.3	2.5	12.1	0.65	18.6	15.6	4.8	14.7	20.4	6.2	6.2	7.9	7	3.6	4.8	16.2	53.7	40.7	15.4

Table A.2. Statistical analysis results on the differences in PAHs concentration in the leaves according to their position in the canopy (n=120, i.e., the samples of group 1 trees: trees numbered 1 to 6, 10 and 13 to 15). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height -C.H.-, Trunk Distance -T.D.- and Orientation -O.-) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R^2": Adjusted R^2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^2
Phe	A	-	C.H. T.D.	Bot>Mid, Bot>Top N>F	0.12
Ft	A	-	C.H.	Bot>Mid, Bot>Top	0.14
Pyr	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.37
BaA	A	T.D. O.	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.34
Chry	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.40
B(b+j)F	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.34
BkF	A	-	C.H. T.D.	Bot>Mid, Mid>Top N>F	0.14
BeP	A	-	C.H. T.D.	Bot>Mid, Mid>Top N>F	0.22
IP	A	-	C.H. T.D.	Bot>Mid, Mid>Top N>F	0.22
BghiP	A	-	C.H. T.D. O.	Bot>Mid, Bot>Top, Mid>Top N>F R>B	0.45
3rings	P	-	C.H.	Bot>Top	-
4rings	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.36
5rings	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.28
6rings	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.30
Total	A	-	C.H. T.D.	Bot>Mid, Bot>Top, Mid>Top N>F	0.44

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Table A.3. Statistical analysis results on the differences in PAHs concentration in the leaves according to their position in the canopy (n=60, i.e., the samples of group 2 trees: trees numbered 7 to 9, 11 and 12). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height -C.H.-, Trunk Distance -T.D.- and Orientation -O.-) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R2": Adjusted R2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^2
Phe	A	-	C.H. T.D.	Bot>Mid, Bot>Top N>F	0.36
Ft	A	-	C.H.	Bot>Mid, Bot>Top	0.32
Pyr	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.45
BaA	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.58
Chry	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.59
B(b+j)F	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.57
BkF	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.59
BeP	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.56
BaP	A	-	C.H.	Bot>Mid, Bot>Top	0.35
IP	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.57
BghiP	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.54
3rings	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.42
4rings	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.50
5rings	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.59
6rings	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.57
Total	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.62

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Table A.4. Statistical analysis results on differences in PM2.5 and PM10 concentration in air according to their position in the tree (trees numbered 1, 2, 9, 10, 11, 12 and 14). “Analised part”: Samples that have been considered in the analysis, Intracanopy (samples from within the canopy) or Total tree (samples from both the canopy and samples taken at 1.70 meters, below the canopy). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height, Trunk Distance and Orientation) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R2: Adjusted R2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PM	Analised part	Test	Interaction	Main effect	Post hoc	Adjusted R^2
2.5	Intracanopy	A	-	-	-	
	Total tree	A	-	-	-	
10	Intracanopy	P	-	-	-	
	Total tree	P	-	-	-	

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Table A.5. Statistical analysis results on differences in leaf traits (SLA, δ13C, stomatal density and different fatty acids) according to their position in the canopy (samples from trees in group 1-trees numbered 1 to 6, 10 and 13 to 15- were used). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height -C.H.-, Trunk Distance -T.D.- and Orientation -O.-) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R2": Adjusted R2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^2
SLA	A	-	C.H. T.D. O.	Bot>Mid, Bot>Top, Mid>Top N>F R<B	0.55
$\delta^{13}C$	A	T.D. O.	C.H.	Bot<Mid, Bot<Top, Mid<Top	0.50
Stomata Density	A	-	C.H. T.D.	Bot<Top N<F	0.09
Heptadecanoic Ac.	A	-	-	-	-
Decanoic Ac.	A	-	-	-	-
Oleic Ac.	A	-	-	-	-
Linoleic Ac.	P	-	-	-	-
Linolenic Ac.	P	-	-	-	-
Myristic Ac.	A	-	T.D.	N>F	0.18
Stearic Ac.	A	-	-	-	-
Palmitic Ac.	A	-	-	-	-

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Table A.6. Statistical analysis results on differences in leaf traits (SLA, δ13C and stomatal density) according to their position in the canopy (n=60, i.e., the samples of group 2 trees: trees numbered 7, 8, 9, 11 and 12). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height -C.H.-, Trunk Distance -T.D.- and Orientation -O.-) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R2": Adjusted R2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^2
SLA	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.56
			T.D.	N>F	
			O.	R<B	
$\delta^{13}C$	P	-	C.H.	Bot<Top	-
Stomata Density	A	-	C.H.	Mid<Top	0.19
			T.D.	N<F	

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Table A.7. Statistical analysis results on differences in SLA-corrected PAH concentration (PAH concentration/SLA) in leaves according to their position in the canopy (n=120, i.e., the samples of group 1 trees: trees numbered 1 to 6, 10 and 13 to 15). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height -C.H.-, Trunk Distance -T.D.- and Orientation -O.-) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R2": Adjusted R2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^2
Phe	A	-	O.	R>B	0.03
Ft	A	-	O.	R>B	0.03
Pyr	A	C.H. T.D.	O.	R>B	0.18
BaA	A	-	C.H.	Bot>Top	0.10
Chry	A	-	C.H.	Bot>Top	0.10
B(b+j)F	A	-	C.H.	Bot>Top	0.10
BkF	A	-	C.H.	Bot>Top	0.06
BeP	A	-	C.H.	Bot>Top	0.07
IP	A	-	C.H.	Bot>Top	0.05
BghiP	A	-	C.H. T.D. O.	Bot>Mid, Bot>Top, Mid>Top N>F R>B	0.29
3rings	A	-	-	-	-
4rings	A	C.H. T.D.	-	-	0.10
5rings	A	-	C.H.	Bot>Top	0.09
6rings	A	-	C.H. O.	Bot>Top R>B	0.09
Total	A	-	C.H.	Bot>Mid, Bot>Top	0.14

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Table A.8. Statistical analysis results on differences in SLA-corrected PAH concentration (PAH concentration/SLA) in leaves according to their position in the canopy (n=60, i.e., the samples of group 2 trees: trees numbered 7, 8, 9, 11 and 12). "Test": test used, ANOVA (A) or permutation (P). "Interaction": which variables (Canopy Height -C.H.-, Trunk Distance -T.D.- and Orientation -O.-) have a significant interaction ("-" indicates no significant interaction). "Main effect": variables with significant differences between their levels; "Post hoc": levels of each variable between which there are significant differences (Canopy Height: Bottom -Bot-, Mid and Top; Trunk Distance: Near -N- and Far -F- from de trunk; and Orientation: Road -R- and Building -B-). "Adjusted R2": Adjusted R2 of the regression model constructed with the significant variables. The significance level (α) selected was 0.05 for all analyses.

PAH	Test	Interaction	Main effect	Post hoc	Adjusted R^2
Phe	A	-	C.H.	Bot>Mid, Bot>Top	0.16
Ft	A	-	C.H.	Bot>Mid, Bot>Top	0.12
Pyr	A	-	C.H.	Bot>Mid, Bot>Top	0.20
BaA	A	-	C.H.	Bot>Mid, Bot>Top	0.37
Chry	A	-	C.H.	Bot>Mid, Bot>Top	0.32
B(b+j)F	A	-	C.H.	Bot>Mid, Bot>Top	0.35
BkF	A	-	C.H.	Bot>Mid, Bot>Top	0.48
BeP	A	-	C.H.	Bot>Mid, Bot>Top	0.36
BaP	A	-	C.H.	Bot>Mid, Bot>Top	0.23
IP	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.37
BghiP	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.36
3rings	A	-	C.H.	Bot>Mid, Bot>Top	0.23
4rings	A	-	C.H.	Bot>Mid, Bot>Top	0.25
5rings	A	-	C.H.	Bot>Mid, Bot>Top	0.41
6rings	A	-	C.H.	Bot>Mid, Bot>Top, Mid>Top	0.39
Total	A	-	C.H.	Bot>Mid, Bot>Top	0.40

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Appendix III: Chapter III Supplementary Material

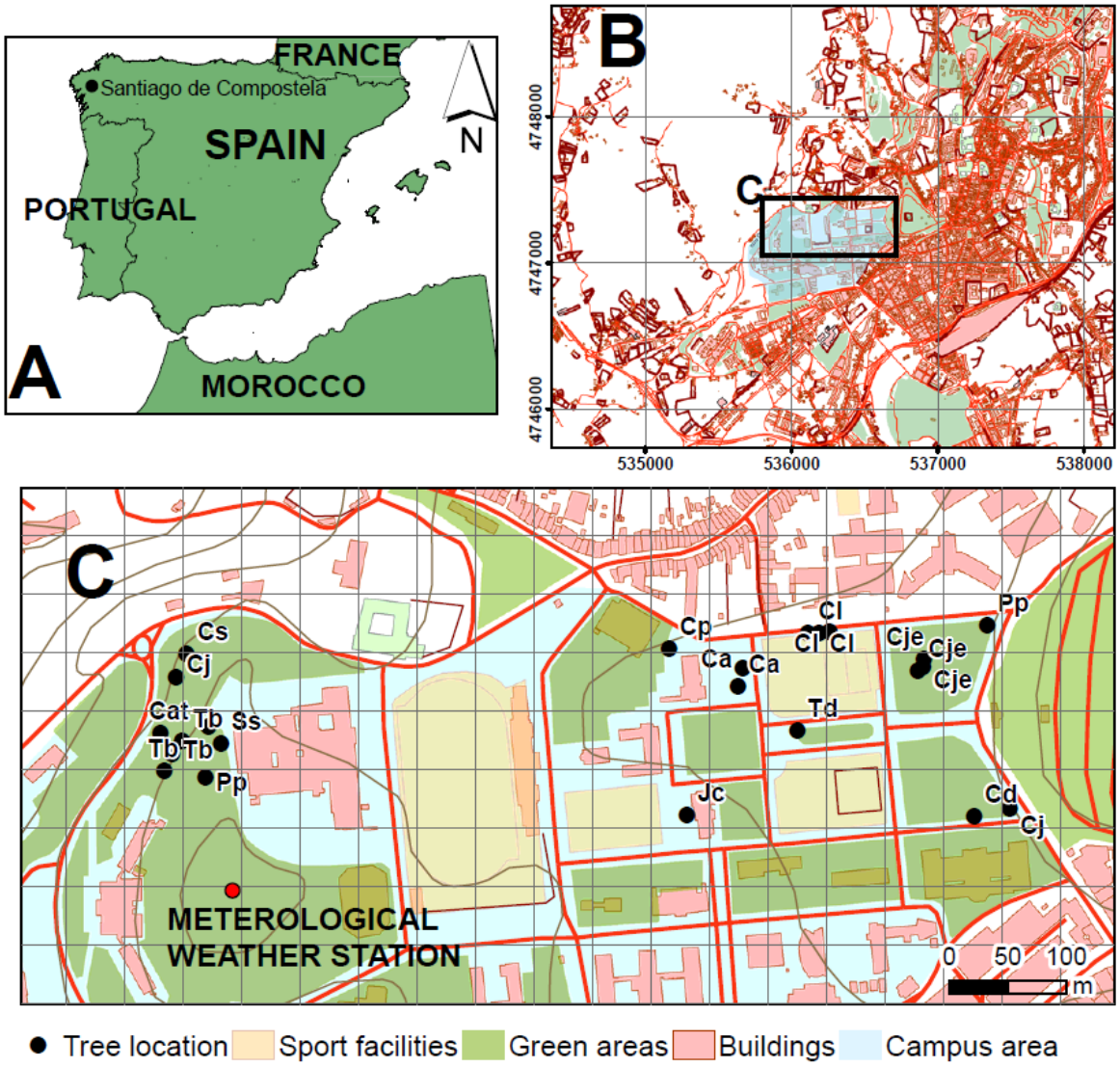


Fig. 1SM. Maps showing (A) the location of Santiago de Compostela in Spain; (B) the location of the USC Campus Vida in Santiago de Compostela; (C) the location of sampled trees is showed in a 50 x 50 m grid. Cp: C*hamaecyparis pisifera*; Cj: *Cryptomeria japonica*; Cje: *Cryptomeria japonica f. elegans*; Ca: *Cupressus arizonica*; Cl: *Cupressus lusitanica*; Cs: *Cupressus sempervirens*; Jc: *Juniperus chinensis*; Ss: *Sequoia sempervirens*; Td: *Thujopsis dolabrata*; Tb: *Taxus baccata*; Cat: *Cedrus atlantica*; Cd: *Cedrus deodara*; Pa: *Picea abies (L.)*; Pp: *Pinus pinaster*.

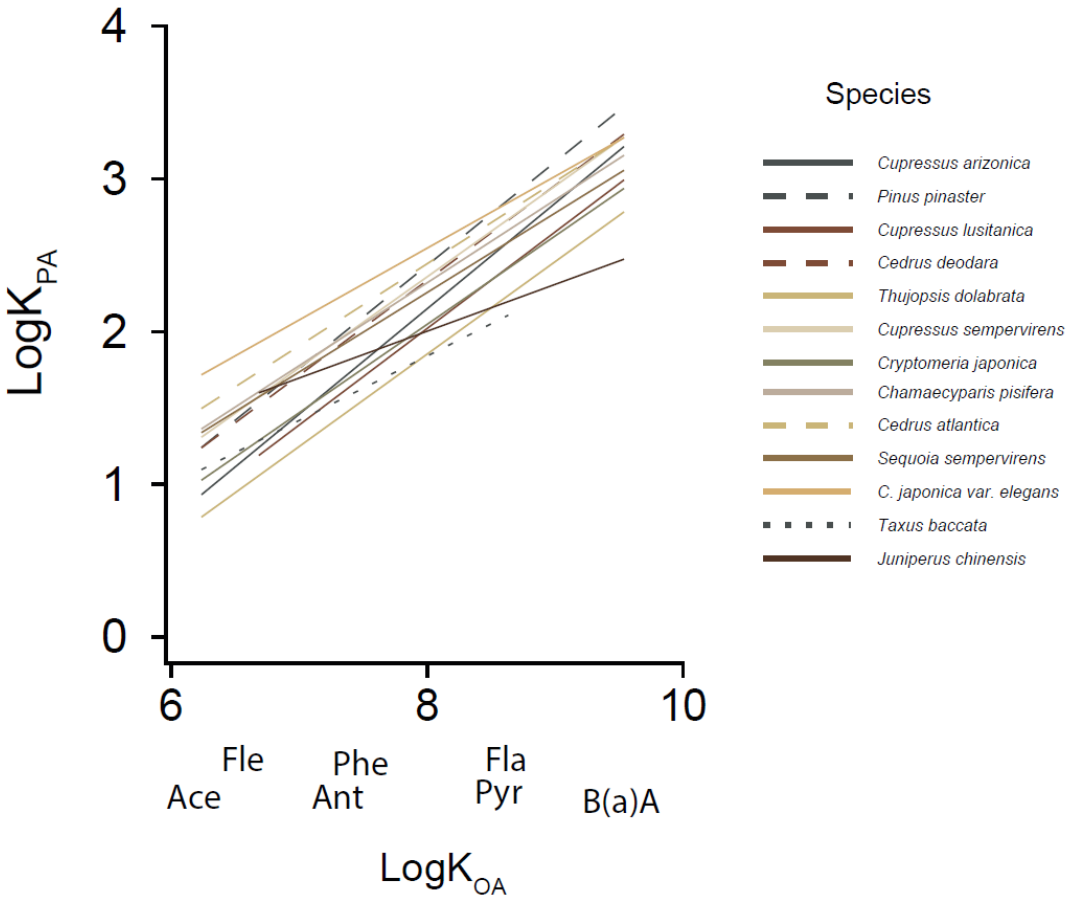


Fig. 2SM. Linear regression models relating Log K_{PA} (at sampling temperature 9 ºC) and Log K_{OA} of the gas phase associated PAHs. Cupressaceae family are represented by a solid line, those of the Pinaceae family by a dashed line and the Taxaceae family by a dotted line

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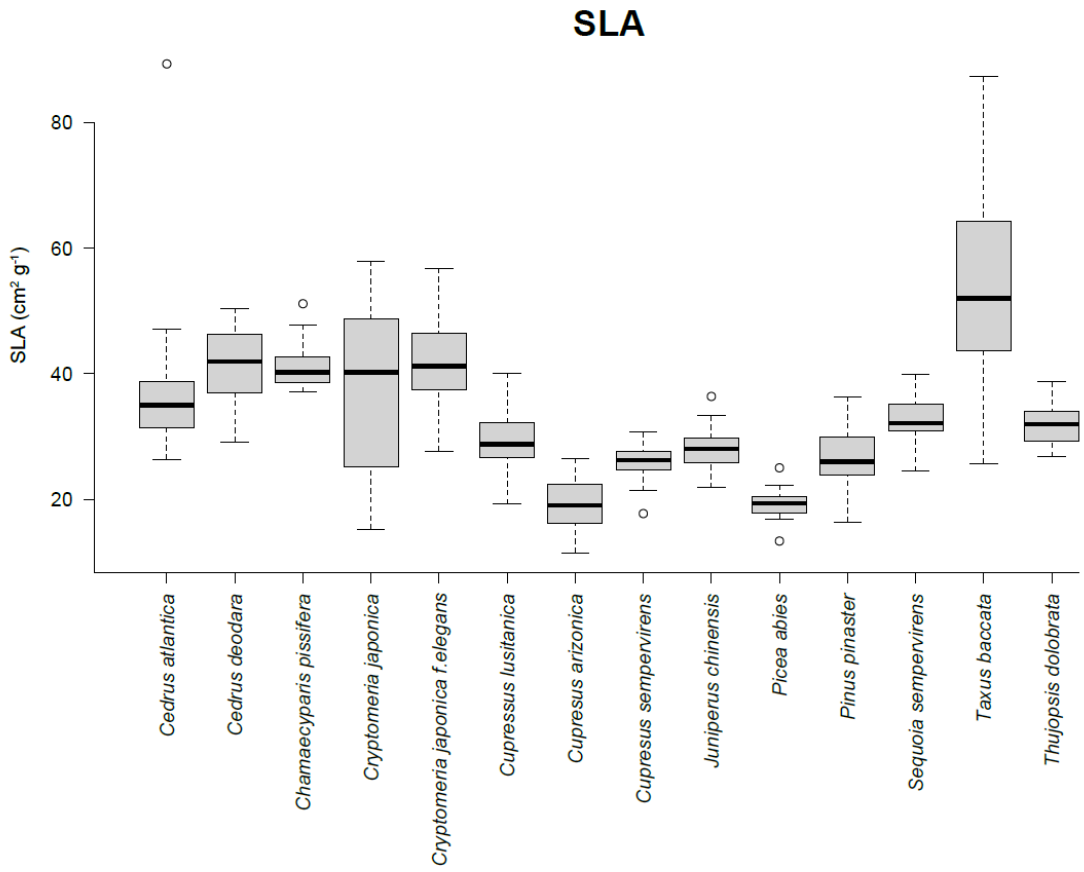


Fig. 3SM. Boxplot of SLA (cm^2 g^{-1}) measurement for each of the studied species.

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Table 1SM. Physico-chemical properties of the analyzed PAHs. n Rings: number of rings. Abbreviations of the properties: MW (molecular weight), VP (vapor pressure), WS (water solubility), Log K_{OW} (octanol-water partition coefficient), VapE (vaporization enthalpy). Abbreviations of PAHs: Acy (acenaphthylene), Ace (acenaphthene), Fle (fluorene), Phe (phenanthrene), Ant (anthracene), Fla (fluoranthene), Pyr (pyrene), B(a)A (benz(a)anthracene), Chr (chrysene), B(b+j)F (benzo(b+j)fluoranthene), B(k)F (benzo(k)fluoranthene), B(e)P (benzo(e)pyrene), B(a)P (benzo(a)pyrene), D(ah)A (dibenz(a,h)anthracene), Ind (indeno(1,2,3-cd)pyrene), B(ghi)P (benzo(g,h,i)perylene).

PAH	Formula	n Rings	MW* (g mol^{-1})	VP* (Pa)	WS* (mol m^{-3})	LogK_{OW}*	VapE** (kJ mol^{-1})	Log K_{OA}***
Acy	$C_{12}H_8$	3	150.19	9.00E-01	1.07E-01	4.00	66.90	6.5
Ace	$C_{12}H10$	3	154.21	3.00E-01	2.46E-02	3.92	66.50	6.2
Fle	$C_{13}H_{10}$	3	166.22	9.00E-02	1.14E-02	4.18	72.10	6.7
Ant	$C_{14}H_{10}$	3	178.23	1.00E-03	2.52E-04	4.54	79.90	7.3
Phe	$C_{14}H_{10}$	3	178.23	2.00E-02	6.17E-03	4.57	78.30	7.5
Fla	$C_{16}H_{10}$	4	202.25	1.23E-03	1.29E-03	5.22	87.10	8.6
Pyr	$C_{16}H_{10}$	4	202.25	6.00E-04	6.53E-04	5.18	89.40	8.6
B(a)A	$C_{18}H_{12}$	4	228.29	2.80E-05	4.82E-05	5.91	105.80	9.5
Chr	$C_{18}H_{12}$	4	228.29	5.70E-07	8.76E-06	5.60	106.20	10.2
B(e)P	$C_{20}H_{12}$	5	252.31	7.40E-07	1.59E-05	6.04	118.20	10.8
B(a)P•	$C_{20}H_{12}$	5	252.31	7.00E-07	1.51E-05	6.04	116.70	10.8
B(b+j)F••	$C_{20}H_{12}$	5	252.31	5.00E-08	3.17E-06	6.00	117.40	11.2
B(k)F	$C_{20}H_{12}$	5	252.31	5.00E-08	3.17E-06	6.00	117.40	11.2
Ind•••	$C_{22}H_{12}$	6	276.33	1.00E-08	6.88E-07	6.50	128.90	11.7

NOTES: * from (Mackay et al., 2006); ** from (Roux et al., 2008); *** $K_{OA}=K_{OW}/K_{AW}$, $K_{AW}=H/RT$, $H=VP/WS$; • the same Log K_{OW} of B(a)P was assumed; •• physico-chemical properties of B(k)F; ••• the same Log K_{OW} and VapE of B(ghi)P were assumed

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Table 2SM. Parameters of the equation Log K_{PA} = a + b log K_{OA} (model 2, gas + particle phase PAHs linear) (a: intercept, b: slope, r^2: coefficient of determination)

Species	a	b	r^2
Cupressus lusitanica	-2.20	0.53	0.87
Thujopsis dolabrata	-2.25	0.51	0.85
Cupressus arizonica	-1.78	0.48	0.89
Cryptomeria japonica	-1.69	0.46	0.88
Cedrus deodara	-1.35	0.45	0.91
Cupressus sempervirens	-1.29	0.45	0.89
Cedrus atlantica	-1.14	0.44	0.95
Picea abies	-1.77	0.44	1.00
Chamaecyparis pisifera	-0.89	0.39	0.87
Juniperus chinensis	-1.03	0.38	0.95
C. japonica var. *elegans*	-0.49	0.38	0.86
Sequoia sempervirens	-0.69	0.36	0.78
Pinus pinaster	-0.37	0.33	0.48
Taxus baccata	-0.77	0.32	0.88

NOTES: K_{PA} values refer to the sampling temperature (9°C); the species are shown in decreasing slope order; K_{PA} is expressed in ng g^{-1} of dry leaf/ ng m^{-3} of air; for *Picea abies* equation parameters reported in Nizzetto et al., 2008 are shown.

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Table 3SM. Parameters of the equation Log K_{PA} = a + b log K_{OA} + c (log K_{OA})^2 (model 3, gas + particle phase PAHs polynomial 2nd degree) (a, b, c; regression parameters, r^2: adjust coefficient of determination)

Species	a	b	c	r^2 adjust
Pinus pinaster	-12.52	3.11	-0.15	0.88
Cupressus arizonica	-8.75	2.10	-0.09	0.90
Cedrus deodara	-7.30	1.85	-0.08	0.90
Cupressus sempervirens	-7.08	1.79	-0.07	0.89
Sequoia sempervirens	-6.80	1.77	-0.08	0.90
Cupressus lusitanica	-6.95	1.59	-0.06	0.78
Cryptomeria japonica	-5.54	1.35	-0.05	0.76
Chamaecyparis pisifera	-4.54	1.24	-0.05	0.80
Thujopsis dolabrata	-5.40	1.24	-0.04	0.73
Cedrus atlantica	-4.22	1.17	-0.04	0.91
C. japonica var. *elegans*	-3.78	1.14	-0.04	0.77
Taxus baccata	-2.63	0.75	-0.02	0.74
Picea abies	-1.54	0.39	0.00	1.00
Juniperus chinensis	0.77	-0.03	0.02	0.99

NOTES: K_{PA} values refer to the sampling temperature (9°C); the species are shown in decreasing slope order; K_{PA} is expressed in ng g^{-1} of dry leaf/ ng m^{-3} of air; for *Picea abies* equation parameters reported in Nizzetto et al., 2008 are shown.

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References

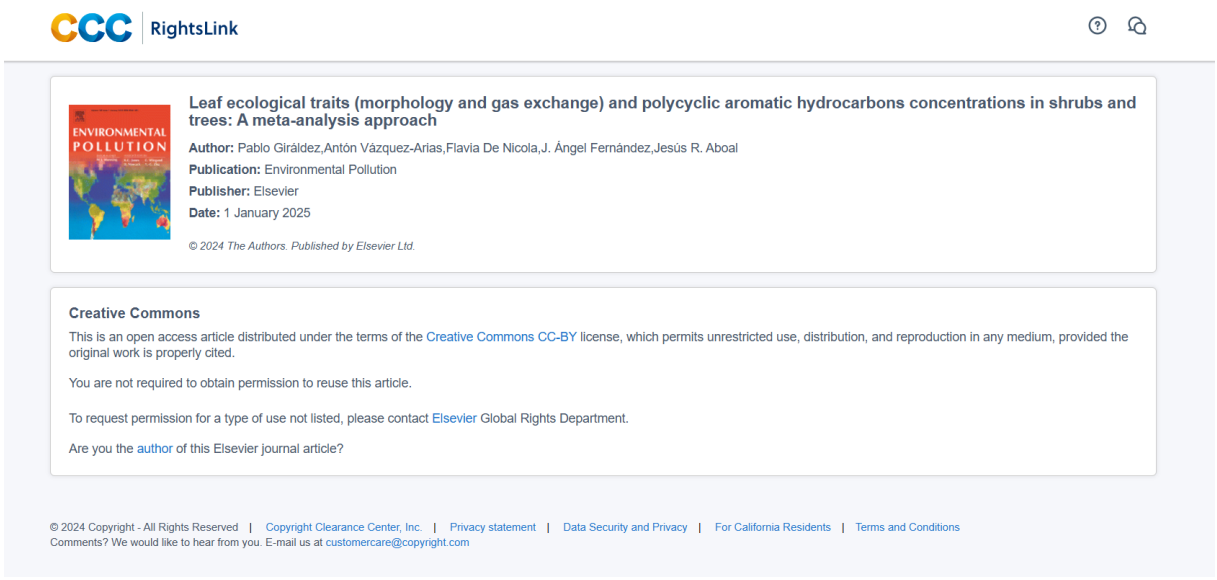
Roux, M.V., Temprado, M., Chickos, J.S., Nagano, Y., 2008. Critically Evaluated Thermochemical Properties of Polycyclic Aromatic Hydrocarbons. J. Phys. Chem. Ref. Data 37, 1855. https://doi.org/10.1063/1.2955570

Mackay, D., Shiu, W.Y., Ma, Kuo-Ching, Lee, S.C., 2006. Handbook of physical-chemical properties and environmental fate for organic chemicals, 2nd ed. ed. CRC/Taylor & Francis, Boca Raton, FL.

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Appendix IV: Authorizations of journals

Chapter I



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Leaf ecological traits (morphology and gas exchange) and polycyclic aromatic hydrocarbons concentrations in shrubs and trees: A meta-analysis approach

Author: Pablo Giráldez,Antón Vázquez-Arias,Flavia De Nicola,J. Ángel Fernández,Jesús R. Aboal

Publication: Environmental Pollution

Publisher: Elsevier

Date: 1 January 2025

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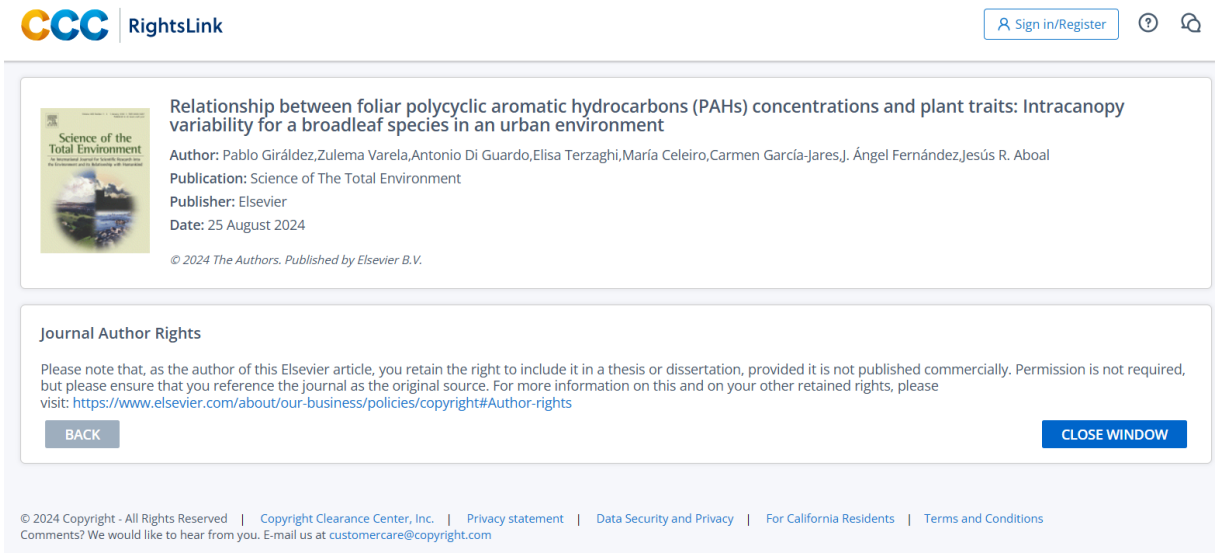
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Chapter II



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Relationship between foliar polycyclic aromatic hydrocarbons (PAHs) concentrations and plant traits: Intracanopy variability for a broadleaf species in an urban environment

Author: Pablo Giráldez,Zulema Varela,Antonio Di Guardo,Elisa Terzaghi,María Celeiro,Carmen García-Jares,J. Ángel Fernández,Jesús R. Aboal

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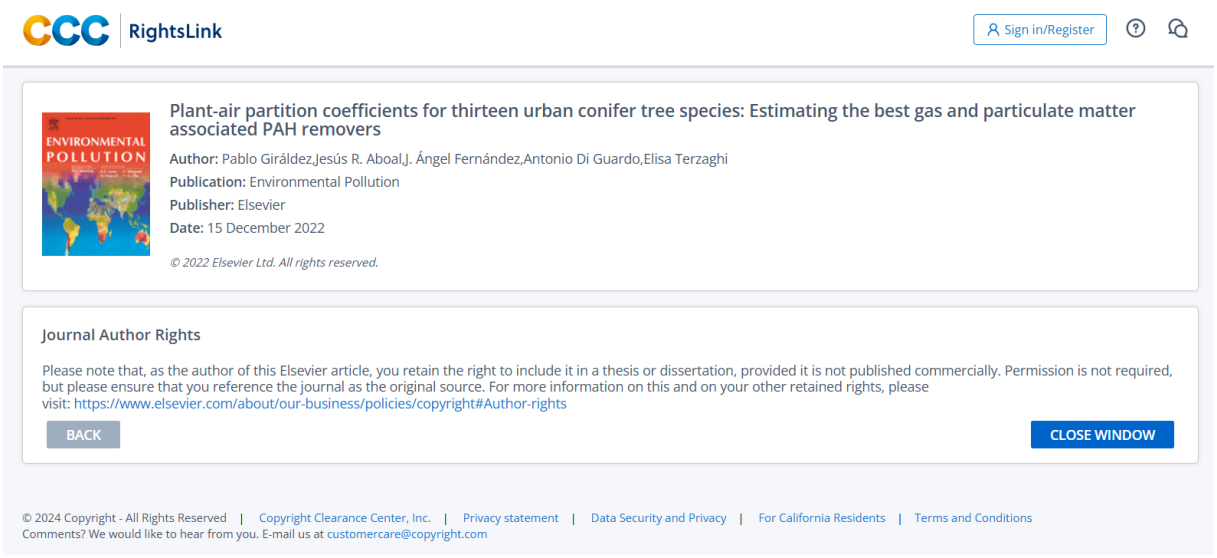
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Chapter III



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Sign in/Register

Plant-air partition coefficients for thirteen urban conifer tree species: Estimating the best gas and particulate matter associated PAH removers

Author: Pablo Giráldez,Jesús R. Aboal,J. Ángel Fernández,Antonio Di Guardo,Elisa Terzaghi
Publication: Environmental Pollution
Publisher: Elsevier
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Nowadays we are facing a serious air pollution problem, especially in urban areas. Polycyclic aromatic hydrocarbons (PAHs) are a group of toxic pollutants produced by the partial combustion of organic matter that are present in urban air due to emissions from various sources. Planting trees can reduce their concentration in the air by acting as sinks. However, there are still significant gaps in knowledge about their ability to capture PAHs, the relationship between leaf PAH concentrations and foliar traits, the intra-individual (within-tree) variability of PAH concentrations, and the interspecific variation of trees in the capture and removal of PAHs from air. All these issues will be addressed in this thesis.