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# Elucidation of the mechanisms of VOC removal in botanical filters during indoor air treatment in a test chamber

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#### ABSTRACT

The removal of volatile organic compounds (VOCs) from indoor environments using plants has attracted increasing attention as an effective natural mitigation strategy. In this study, five indoor plant species (*Epipremnum aureum, Syngonium podophyllum, Spathiphyllum wallisii, Dieffenbachia* and *Monstera adansonii*) grown hydroponically under controlled conditions were used to systematically quantify VOC removal by the leaves, whole cuttings and roots. Acetone, toluene, α-pinene, o-xylene, and limonene were selected as model indoor air pollutants. The results showed a marked variability in the leaf-based VOC removal efficiency among the plant species and pollutant, but complete VOC removal was never obtained. However, the whole plant cuttings supported complete and rapid (20–115 h) removals of all VOCs. Finally, the root-associated microorganisms were shown to significantly contribute to VOC removal, mainly through rhizodegradation. Overall, this study suggest that VOC removal by plant cuttings is due to the combined effects of physical adsorption and metabolic degradation mediated by plants and microorganisms, highlighting the synergistic role of plant morphological traits and rhizospheric microbial communities in phytoremediation.

#### 1. Introduction

Volatile organic compounds (VOCs) are responsible for a significant share of air pollution, with indoor concentrations often exceeding those in ambient air [1]. VOCs are a diverse group of carbon-based chemicals characterized by their high vapor pressure at room temperature, which allows them to easily evaporate into the air, making them common in indoor environments [1]. Major sources of VOCs include building materials, furniture, office equipment, cleaning products and personal care items. Indoor VOCs include benzene, formaldehyde, toluene and xylene, which are known to have potential adverse health effects, ranging from respiratory irritation to carcinogenicity [2,3]. Indoor air quality (IAQ) is a critical aspect of environmental health, especially as individuals in modern societies spend approximately 90 % of their time indoors [4,5]. Poor IAQ has been linked to various health issues, including sick building syndrome (SBS), asthma, and other respiratory conditions, with VOCs contributing significantly to indoor air pollution. Therefore, a reduction in the concentration of VOCs is essential for improving IAQ and safeguarding human health.

Botanical filters can be implemented in various configurations, including potted plants, active systems with forced airflow, and vertical green walls. Although green walls are a prominent application, they are not the only configuration, as demonstrated by camera-based systems and pot experiments widely used in controlled studies. This concept, generally referred to as phytoremediation, is based on the ability of plants to absorb, sequester, and metabolize pollutants from the environment [6,7]. Several studies have shown that certain indoor plants can effectively reduce VOC concentrations through their leaves, roots, and associated microorganisms [8].

Plants have the ability to remove pollutants by multiple remediation mechanisms. These mechanisms are classified according to the processes involved and their association with the different parts of the plant, particularly the phyllosphere (leaf tissue) and rhizosphere (root zone). The main mechanisms of VOC removal by plants include: i) phytoextraction, which absorbs and concentrates soil contaminants into plant biomass; ii) phytodegradation, which involves the enzymatic transformation of hazardous substances, within plant tissues, into less toxic forms; iii) phytostabilisation, which immobilizes contaminants into the

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soil, reducing their bioavailability; iv) phytovolatilization, where the organic and inorganic pollutants absorbed by plants are metabolically transformed and subsequently released into the atmosphere through stomatal emission. Transpired pollutants can be degraded by hydroxyl radicals in the atmosphere and may remain as atmospheric pollutants but with lower toxicity than the parent contaminant [9]; v) rhizodegradation, which increases rhizospheric microbial activity to break down organic contaminants, and vi) rhizofiltration, which targets aquatic contaminants, allowing plant roots to adsorb and metabolize them [9]. In this context, microorganisms that naturally coexist with plants play a crucial role in these processes, as certain bacteria absorb and metabolize pollutants, using them as carbon and energy sources.

Although the mechanisms mentioned above have been studied individually, the relative contributions of plant foliage, whole plants, and root-associated microbial communities in the most commonly used indoor plant species remain poorly quantified. This lack of integrated evidence hinders the optimization of botanical filters for practical applications. Therefore, the objective of this study was to elucidate and quantify the removal mechanisms of acetone, toluene,  $\alpha$ -pinene, oxylene, and limonene by foliar tissues, whole plants (cuttings), and root-associated microorganisms, considering the potential roles of adsorption onto surfaces, absorption into aqueous medium and plant tissues, and biotransformation mediated by metabolic activity. The plant species most commonly used in botanical filters, namely *Epipremnum aureum*, *Syngonium podophyllum, Dieffenbachia, Spathiphyllum wallisii*, and *Monstera adansonii*, were used as model plants [3].

On the other hand, model VOCs were selected based on their high prevalence in indoor environments and their toxicological or environmental relevance [3]. Acetone and toluene are frequently detected in offices and homes due to solvents and cleaning products, while xylenes are associated with paints and adhesives. In contrast,  $\alpha$ -pinene and limonene, although sometimes associated with positive sensory effects, are recognized as important indoor terpene pollutants and precursors of secondary organic aerosols at high concentrations [10]. These monoterpenes are commonly emitted from household cleaning products, air fresheners, scented candles, and personal care products, as well as from wooden furniture and building materials [10]. This combination of compounds also encompasses a wide range of physicochemical properties, allowing for the evaluation of hydrophilic *versus* hydrophobic elimination pathways.

#### 2. Materials and methods

#### 2.1. Chemicals

Acetone (CAS-67–64–1), toluene (CAS-108–88–3),  $\alpha$ -pinene (CAS-80–56–8), o-xylene (CAS-95–47–6) and limonene (CAS-138–86–3) were selected as representative indoor air pollutants. These compounds were purchased from Sigma-Aldrich (Madrid, Spain). The mineral medium used was Murashige and Skoog (MS) basal medium, commonly used for in vitro micropropagation [11]. This medium consisted of both macronutrients and micronutrients (Table S1), which were also purchased to Sigma-Aldrich [12].

#### 2.2. Plants preparation

The adaptability of the selected plant species to hydroponic cultivation was evaluated in a previous study [13], and *Epipremnum aureum, Syngonium podophyllum, Spathiphyllum wallisii, Dieffenbachia*, and *Monstera adansonii* were selected for the present study. These plants were obtained from local nurseries in Valladolid (Spain). Cuttings were taken from the aerial parts of the plants and placed in beakers with deionized water. The cuttings were acclimatized in the laboratory for approximately six weeks at 25  $\pm$  2 °C. Once rooted, they were transferred to a 15 % diluted MS mineral medium, selected based on previous experiments that demonstrated optimal root growth and leaf formation at this

concentration.

The number of cuttings used in the experiments was determined based on their total leaf area, with a reference value of  $\sim 280~\rm cm^2$  per experiment. The number of leaves per cutting varied between species according to their natural morphology (between 2 and 6 leaves, which were not cut or modified). The leaf area of each species was calculated using the free software ImageJ, uploading photographs of each cutting. A scale was set in centimeters, and image parameters were adjusted to optimize definition (Fig. S1). The leaf area of individual leaves was measured with the Analyze > Measure Area tool and the resulting data were exported to Excel for calculation of the total leaf area per replicate. A selection of leaves from each plant was conducted in each experiment to start with 280 cm² of leaves.

#### 2.3. Experimental setup

The study was conducted in three tests series: (i) evaluation of VOC removal by plant foliage, (ii) assessment of VOC removal by plant cuttings comprising leaves, stem and roots, and (iii) evaluation of VOC degradation by microorganisms associated with plant roots.

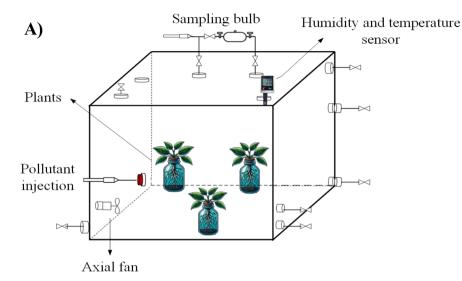
### Test series 1-Assessment of the removal of VOCs by the foliar part of plants

The experimental setup consisted of an external polyethylene terephthalate glycol (PETG) chamber (1  $m \times 0.80 m \times 0.65 m$ ) simulating an indoor environment. To ensure a rapid homogenization of air inside the chamber, an axial fan (115.2 m<sup>3</sup> h<sup>-1</sup>) was used; while an external LED light panel provided 2000 lx of illumination under a 12/12 h day/night photoperiod. A humidity and temperature sensor (Testo 605-H1), and a 250 mL glass bulb (Supelco, Sigma Aldrich, USA) functioning as a VOC sampling port, were installed on the top of the chamber. Borosilicate glass bottles (1.2 L) containing 1.2 L of 15 % diluted MS mineral medium and cuttings of the selected plant species were placed inside the chamber (Fig. 1, Fig. S2). Bromobutyl septa (DWK DURAN™) were placed on top of the bottles to prevent that the contaminated air from the chamber would interact with the root zone and rhizosphere of the plants. The total leaf area was standardized to approximately 280 cm<sup>2</sup>, with actual measured values of 284.3, 280.6, 284.7, 287.0, and 286.6 cm<sup>2</sup> for E. aureum, S. podophyllum, Dieffenbachia, Sp. wallisii, and M. adansonii, respectively (Table S2). Aliquots of 2  $\mu L$  of acetone, toluene,  $\alpha$ -pinene and o-xylene, and of 1.5  $\mu L$  of limonene, were injected into the chamber, resulting in initial concentrations of  $\sim$ 3 mg m $^{-3}$  for each VOC at the beginning of each experiment. The chamber was operated in batch mode. Each biotic test contained three bottles with the same plant and lasted 6 days. Before starting the biotic experiments, a batch abiotic test (without plants) was also performed for 6 days to rule out VOC adsorption and photolysis inside the chamber (Fig. S3).

VOC concentrations were measured every day using GC-FID coupled with solid phase microextraction (SPME). A 100 mL gas-tight syringe was used to pump the air from the chamber into the glass bulb to obtain a representative sample.

### Test series 2-Assessment of the removal of VOCs by plant cuttings

Three groups of 1.2 L borosilicate bottles were prepared in triplicate. The first group (control) contained 150 mL of 15 % diluted MS mineral medium, the second group contained 150 mL of 15 % diluted MS mineral medium and the roots of the cuttings, while the third group contained 150 mL of 15 % diluted MS mineral medium with the entire cutting of the selected plant species (Fig. 2). The bottles were sealed with DWK DURANTM bromobutyl septa and plastic caps. Each bottle was dosed with 20 mL of a gas VOC mixture, resulting in average initial concentrations of 64.7  $\pm$  0.0 mg m $^{-3}$  for acetone, 68.4  $\pm$  4.8 mg m $^{-3}$  for toluene, 80.3  $\pm$  6.2 mg m $^{-3}$  for limonene. The gaseous VOC mixture was prepared in a 500 mL glass bulb by injecting 2 µL of each VOC (acetone, toluene,  $\alpha$ -pinene, o-xylene, and limonene) in liquid phase, and allowing it to volatilize for 4 h. Prior to the experiment, the 15 % diluted MS



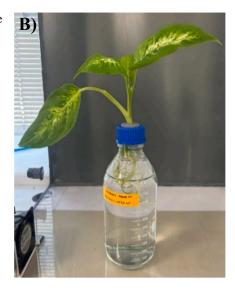


Fig. 1. A) Experimental chamber and B) 1.2 L glass bottle with Dieffenbachia plant cutting immersed in 15% MS nutrient medium.



**Fig. 2.** Experimental setup to assess VOC removal by cuttings in gastight bottles.

mineral medium, septa and bottles were autoclaved, and the insertion of roots and cuttings was conducted under sterile conditions in a laminar flow hood to avoid contamination. The concentration of VOCs in the bottles was measured every day by GC-FID.

## Test series 3-Assessment of the removal of VOCs by root-associated microorganisms

Three groups of  $1.2\,L$  borosilicate bottles were prepared in triplicate. The first group (control) contained 150 mL of 15 % diluted MS mineral medium, the second group contained 150 mL of 15 % diluted MS mineral medium with the roots of the cuttings, and the third group contained 150 mL of 15 % diluted MS mineral medium with microorganisms extracted from the roots of the selected plant species. To extract microorganisms, the roots of the cuttings were immersed in  $1.2\,L$  glass bottles and incubated in 150 mL of 15 % diluted MS mineral medium under mild magnetic agitation at 100 rpm and 25 °C for 24 h. After incubation, the roots were removed, leaving the 15 % diluted MS mineral medium containing the microorganisms detached from the roots (Fig. 3). The glass bottles were sealed with DWK DURANTM bromobutyl septa and plastic caps. The 15 % diluted MS mineral medium, septa and bottles were autoclaved before use. Root insertion and removal were performed under sterile conditions in a laminar flow hood to avoid

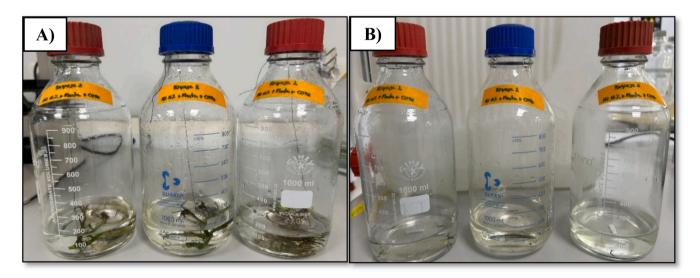


Fig. 3. A) Bottles containing plant roots prior to incubation. B) Bottles containing the microorganisms extracted from the plant roots in 15% diluted MS mineral medium.

contamination. Aliquots of 20 mL of a gas phase VOC mixture, prepared as described in the previous section, were injected in each gas-tight bottle to obtain average initial headspace concentrations of 64.7  $\pm$  0.0 mg m $^{\text{-}3}$  for acetone, 71.8  $\pm$  5.3 mg m $^{\text{-}3}$  for toluene, 97.2  $\pm$  8.7 mg m $^{\text{-}3}$  for  $\alpha$ -pinene, 68.6  $\pm$  7.1 mg m $^{\text{-}3}$  for o-xylene and 91.5  $\pm$  12.7 mg m $^{\text{-}3}$  for limonene. The concentration of the VOCs in the bottles was measured every day by GC-FID.

#### 2.4. Analytical procedures

The concentration of VOCs was determined using SPME-GC-FID. Gas samples were pre-concentrated for 10 min using 85  $\mu m$  CAR/PDMS SPME fibers (Supelco, Bellefonte, USA) in 250 mL glass bulbs (Supelco, Sigma-Aldrich). The SPME fibers were then injected in a GC-FID (Agilent 8860) equipped with an HP-5 column (30  $m\times320~\mu m\times0.25~\mu m$ ). The injector and detector temperatures were set at 150 and 250 °C, respectively. The oven temperature was set at 50 °C for 7.5 min, increased at 25 °C min $^{-1}$  up to 80 °C (held for 2.5 min), and finally increased at 40 °C min $^{-1}$  to 150 °C (held for 1 min). Helium was used as the carrier gas (3.2 mL min $^{-1}$ ) and nitrogen was used as make-up gas (25 mL min $^{-1}$ ). Hydrogen and air flowrates were set at 30 and 400 mL min $^{-1}$ , respectively. SPME fibers were initially conditioned at 300 °C for 1 h before calibration. External standards of each VOC prepared in 250 ml glass bulbs were used for SPME calibration (Fig. S4).

Due to the destructive nature of the SPME method, which prevents repeated measurements within the headspace of the bottles, an alternative method was employed to determine VOC concentrations in tests series 2 and 3. Gas samples were taken from the bottles using a 500  $\mu L$  gas-tight syringe (Hamilton, USA), followed by direct injection into the same Agilent 8860 GC-FID system, with the same column and method specifications. Calibration was performed using external standards of each compound (Fig. S5), and the VOC standard mixture was prepared by volatilizing 1.5  $\mu L$  of each liquid VOC in a 500 mL bulb for 3 h.

#### 2.5. Data processing

The removal efficiencies (REs) of the target VOCs were calculated using Eq. (1), based on the initial ( $C_{\rm in}$ ) and final ( $C_{\rm fin}$ ) concentrations. The initial concentration corresponded to the VOC concentration measured after complete volatilization of the contaminants in the chamber, while the final concentration was defined as the VOC concentration obtained at the end of the experiment.

$$\%RE = 100 \times \frac{C_{in} - C_{fin}}{C_{in}}$$
 (1)

Average concentrations (in mg m<sup>-3</sup>) obtained from triplicate measurements of the bottles headspace were herein reported. To evaluate the significance between these treatments, a one-way ANOVA was performed (GraphPad Prism 9, USA).

#### 3. Results and discussion

#### 3.1. Assessment of the removal of VOCs by the foliar part of plants

VOC removal by the foliar part of five houseplant species was evaluated and compared to an abiotic control (without plants) (Fig. S3). The results of the biotic tests revealed variability in VOC removal compared to the abiotic test.

Acetone was the VOC with the highest removal efficiency regardless of the plant. This observation can be explained by the combination of adsorption, absorption, and subsequent biotransformation processes occurring in plant foliage. Although our study was not designed to quantify each mechanism separately, similar patterns have been reported in previous works. For instance, Widhalm et al. [14] described the enzymatic biotransformation of volatile pollutants once absorbed into plant cells, Yamane & Tani [15] developed an absorption model

identifying stomata as the most influential site for VOC uptake, and Matheson et al. [8] summarized evidence of both adsorption to leaf cuticles and microbial-assisted degradation. These comparative findings support our interpretation that VOC removal cannot be solely attributed to physical processes, but also involves plant metabolic activity. *Dieffenbachia* supported the highest removal efficiency, removing more than 55 % of the initial acetone in 5 days, outperforming all other species and the abiotic test, which showed a 16 % removal in 6 days (Fig. 4). This superior performance suggests that *Dieffenbachia* possesses a higher foliar uptake capacity. Other species, including *S. podophyllum*, and *M. adansonii*, supported REs of around 40 % in 6 days, which indicates that foliar processes, potentially including both adsorption on leaf surfaces and metabolic transformation within the foliage, played a significant role in acetone removal.

All plant species tested showed low toluene REs. The highest removal efficiencies were observed for E. aureum and Dieffenbachia, both achieving approximately 25 % removal (Fig. 4). At this point it should be stressed that the abiotic control carried out in the experiment (Fig. 4) showed a toluene removal efficiency of around 20 %, suggesting that non-biological processes, such as adsorption to the chamber walls, contributed significantly to toluene removal. The relatively low biotic efficiency confirmed that foliar adsorption or degradation of toluene was limited in the selected plant species. This observation can be explained by the physicochemical properties of toluene. Toluene is a non-polar, hydrophobic compound with a log Kow of 2.7, maximum water solubility of 0.5 g L<sup>-1</sup> at 25 °C, and a relatively high vapor pressure of 28.4 mmHg at 20  $^{\circ}$ C, which makes it highly volatile. Its Henry's law constant of  $6.6\times 10^{-3} \mbox{ atm m}^{3} \mbox{ mol}^{-1}$  entails a strong tendency to partition into the gas phase rather than to remain dissolved in aqueous media [16]. These characteristics likely limited the affinity of toluene for foliar surfaces, thus restricting the potential for diffusion through the cuticle or stomata.

On the other hand, E. aureum showed a slightly higher  $\alpha$ -pinene RE (~33 %) than the abiotic control (~31 %) (Fig. 4), suggesting a limited but measurable contribution of foliar biological processes. This slightly higher removal performance may be attributed to specific foliar traits of E. aureum, such as a relatively high stomatal conductance or a favorable cuticular composition, which could facilitate the partial diffusion of volatile monoterpenes into the leaf interior. In contrast, the other plant species showed  $\alpha$ -pinene REs equal to or lower than those of the abiotic control, indicating a negligible foliar uptake. This limited biological removal was probably due to the physicochemical properties of  $\alpha\text{-pinene:}$  it is a highly hydrophobic monoterpene (log  $K_{ow}\approx 4.8),$  with low water solubility (2.5 mg L-1 at 25 °C) and high vapor pressure (4.7 mmHg at 25 °C), which favors its partitioning into the gas phase and reduces its water solubility [17]. Its relatively large molecular size and low polarity may also hinder its diffusion through the cuticle and stomata, especially in the absence of root-mediated transport pathways or microbial degradation. These results suggest that non-biological processes such as adsorption to the chamber surfaces and passive volatilization played a more dominant role in the removal of  $\alpha$ -pinene than foliar mediated mechanisms. The slightly superior performance of E. aureum underscores the importance of plant-specific anatomical and physiological traits in VOC adsorption, even in scenarios of low pinene removal. In addition, these findings also reinforce the fact that effective removal of hydrophobic VOCs such as α-pinene likely requires the combined action of roots, microbes, and leaves.

The REs for o-xylene were generally low for all plant species tested, with *E. aureum* showing the highest efficiency of  $\sim 25$  % (Fig. 4). The abiotic control showed a removal of  $\sim 15$  %, which suggests that physical adsorption played an important role in the removal of o-xylene. The minimal differences between biotic and abiotic conditions confirmed that o-xylene is not efficiently absorbed or metabolized by plant foliage, possibly due to its chemical structure limiting diffusion through the leaf cuticles or interaction with metabolic enzymes. O-xylene is a monocyclic aromatic hydrocarbon with a log  $K_{\rm ow}$  of 3.1, low

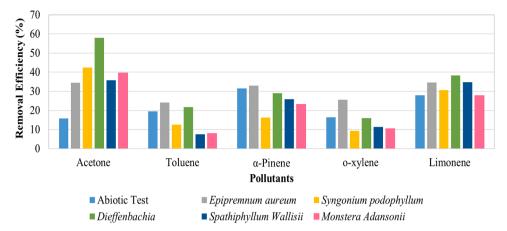


Fig. 4. VOC removal efficiency through the foliar components of the plant species tested. Each bar represents a single experimental measurement; therefore, no error bars are shown.

water solubility (178 mgL $^{-1}$  at 25 °C), and a vapor pressure of 6.6 mmHg at 25 °C, which makes it moderately hydrophobic and volatile [18]. These properties favor its adsorption to the chamber walls and leaf cuticles, but may limit aqueous diffusion across plant surfaces.

In the case of limonene, E. aureum, S. podophyllum, Dieffenbachia, Sp. wallisii and M. adansonii supported REs above 30 %, outperforming the abiotic control, which showed a removal efficiency of 28 % (Fig. 4). This indicates that, while physical adsorption on chamber surfaces contributes to limonene attenuation, biological processes associated with plant foliage also play a significant role. Limonene is a hydrophobic monoterpene characterized by a high octanol-water partition coefficient (log  $K_{ow} \approx 4.6$ ), low water solubility (~13.8 mg L<sup>-1</sup> at 25 °C) and moderate vapor pressure (~1.5 mmHg at 25 °C). These physicochemical properties facilitate its partitioning into lipid-rich environments such as plant cuticles and cell membranes. Preliminary research has shown that leaf uptake of limonene correlates positively with leaf lipid content, suggesting that species with higher concentrations of lipids in their leaves may absorb greater amounts of limonene. Once absorbed, limonene may undergo enzymatic transformations or be sequestered within plant tissues, contributing to its removal from the surrounding environment [19, 20].

Plants-VOC interactions typically occur through multiple mechanisms, including adsorption, absorption and enzymatic degradation. Stomatal uptake is considered the primary pathway for VOC removal in leaves, where VOCs can be adsorbed onto leaf surface or absorbed into plant tissues [21]. Once inside the plant, VOCs can be metabolized by enzymes or stored in compartments such as vacuoles [22]. The efficiency of VOC removal through leaves is modulated by several physiological and morphological characteristics of plants. The leaf surface area determines the total interface available for interaction with VOCs. Hence, plant species with larger or more numerous leaves provide greater contact with airborne VOCs, thereby enhancing passive and active uptake. Stomatal conductance, which reflects the degree of stomata opening, regulates the rate of gas exchange between the leaf interior and the atmosphere, which directly influences VOC diffusion in leaf tissue. A high conductance allows for a greater uptake of VOCs, but is also sensitive to diurnal and environmental regulation. Plant metabolic activity, including photosynthetic and respiratory rates, affects the ability of leaves to energetically support VOC detoxification processes, including enzymatic degradation, sequestration, and transport [15,23]. However, it should be noted that no direct analysis of metabolites or degradation by-products (organic or inorganic) was performed in this study. The evidence of degradation is therefore indirect and relies on the consistently higher VOC removal observed in the presence of roots and whole cuttings compared to abiotic controls. Similar strategies have been employed in previous phytoremediation studies, where reductions in parent VOC concentrations were interpreted as indicative of biological activity [8,24,25]. Furthermore, earlier research has demonstrated that both plants and their associated microorganisms can metabolize VOCs into less harmful compounds, including alcohols, organic acids, and  $CO_2$  [14,19,26,27]. In addition, leaves can contribute to VOC removal through phytovolatilization, where certain pollutants are absorbed by the plant and released back into the atmosphere in a less harmful form [9].

Although the experiments were designed with leaf areas close to 280 cm<sup>2</sup>, the actual measured values ranged from 280.6 to 287.0 cm<sup>2</sup>, with a mean of 284.6  $\pm$  2.5 cm<sup>2</sup>, corresponding to a relative variation of less than 1 %. This small variation is unlikely to influence the overall interpretation of the results. The exact leaf area values for each species are provided in Table S2. Despite the standardized leaf area and identical exposure conditions across the five plant species, E. aureum consistently exhibited higher VOC removal efficiency than the others. Previous studies have also reported the superior performance of E. aureum in removing indoor pollutants, with removals exceeding 70 % for compounds such as  $PM_{2.5}$ ,  $PM_{10}$ ,  $CO_2$ , acetone, toluene, and  $\alpha$ -pinene under controlled conditions [13,28]. This indicates that factors beyond leaf surface area, such as higher stomatal density, greater cuticular permeability, or increased enzymatic detoxification capacity, may contribute to its superior performance in VOC degradation. Furthermore, cuticle composition, including wax content and microstructural characteristics, is known to influence VOC absorption and retention, particularly for hydrophobic compounds [29-31]. These findings are consistent with previous reports emphasizing the role of species-specific anatomical and physiological traits in determining VOC removal capacity [32].

#### 3.2. Assessment of the removal of VOCs by plant cuttings

The time required for complete removal of acetone, toluene,  $\alpha$ -pinene, o-xylene, and limonene varied with plant species, but was approximately 4 days, reflecting the different affinities for VOC uptake, except for acetone. Overall, the removal efficiency trends for these VOCs were consistent across the experiments (Figs. 5,6,7, and 8) (Tables S3-S6).

Acetone was not detected in any of the assay as a result of its high solubility in aqueous media. Thus, due to its high hydrophilicity (log  $K_{ow} \approx -0.24$ ), acetone was rapidly absorbed into the mineral medium, thus masking any plant-mediated sorption or biodegradation. Consequently, the initial decrease in concentration was identical in the control and in assays containing whole roots or cuttings, confirming that the initial acetone removal recorded was driven by sorption into the 15 % diluted MS medium and not by biological processes. This finding highlights the

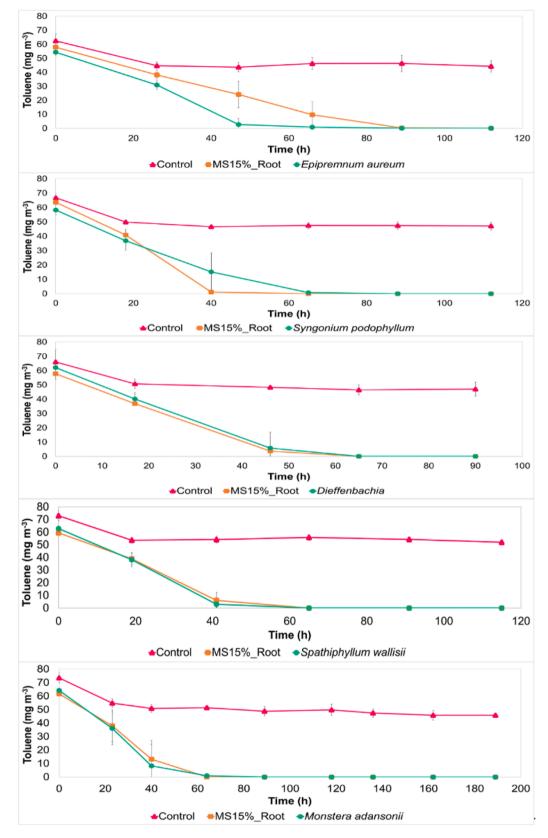


Fig. 5. Time course of the headspace toluene concentrations in the assays conducted with plant cuttings ( ), with roots ( ) and control ( ). Vertical bars represent the standard deviation of three independent replicates.

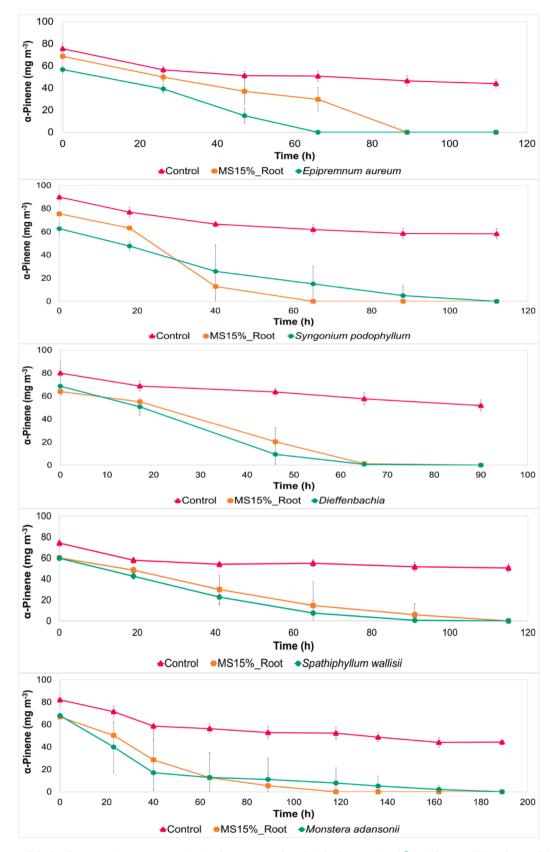


Fig. 6. Time course of the headspace α-pinene concentrations in the assays conducted with plant cuttings ( $\clubsuit$ ), with roots ( $\clubsuit$ ) and control ( $\spadesuit$ ). Vertical bars represent the standard deviation of three independent replicates.

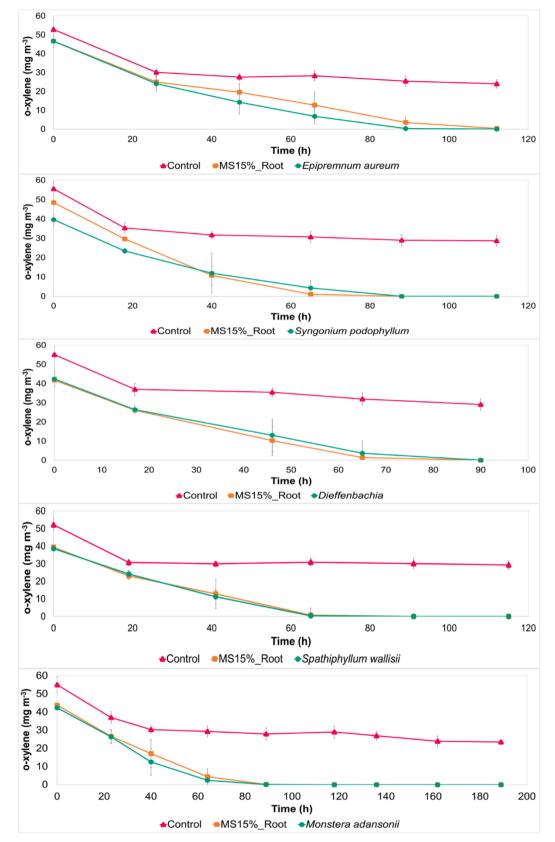


Fig. 7. Time course of the headspace o-xylene concentrations in the assays conducted with plant cuttings ( ), with roots ( ) and control ( ). Vertical bars represent the standard deviation of three independent replicates.

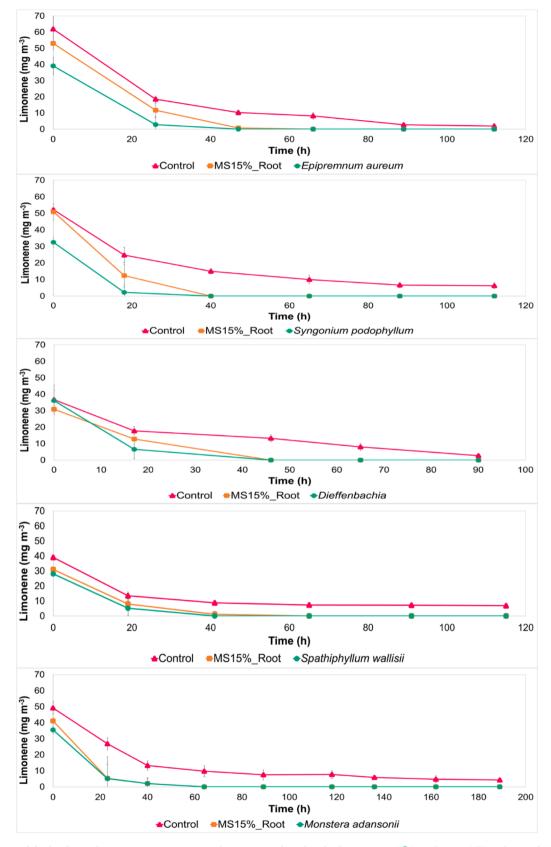


Fig. 8. Time course of the headspace limonene concentrations in the assays conducted with plant cuttings ( ), with roots ( ) and control ( ). Vertical bars represent the standard deviation of three independent replicates.

challenge of evaluating highly hydrophilic VOCs in closed-system experiments, where solvent partitioning dominates the removal kinetics. Studies on VOC phytoremediation have highlighted that hydrophobicity and experimental design critically influence the interpretation of removal mechanisms, and recommend distinguishing physical absorption from true biotransformation using non-aqueous substrates, monitoring liquid phase concentrations and CO<sub>2</sub> release, or through mass balances [33,34].

Recent advances in the field of phytoremediation revealed that plant root is dominant in VOC removal, accounting for approximately 90 % of the pollutant disappearance, with the foliage contributing around 10 %[8,35,36]. The experiments conducted in this work confirmed these findings. Bottles containing whole plant cuttings showed apparently faster VOC removal rates in the gas phase compared to controls. However, these results should be interpreted with caution, as only the gas phase was quantified. The observed decreases may reflect not only biological activity but also abiotic processes such as partitioning into the aqueous medium and adsorption to chamber surfaces. As clearly demonstrated in the case of acetone, absorption into the medium can mask plant- or microbe-mediated effects, and similar phenomena may contribute to the apparent disappearance of other VOCs. For this reason, the present results show VOC gas phase removal, but there is no conclusive evidence of biodegradation. Future research should incorporate gas-liquid mass balances, metabolite identification, and CO2 or TOC monitoring to confirm degradation pathways.

It is important to note that bottles containing whole plant cuttings showed consistently faster VOC removal rates than those containing only roots, regardless of the VOC analyzed. Statistical analysis confirmed that these differences were significant (p < 0.05), thereby supporting the efficiency of the combined contribution of the phyllosphere and roots. In contrast, systems containing only roots showed slower removal rates, indicating that although roots actively contributed to VOC absorption and degradation, their effectiveness was significantly enhanced by the presence of leaves. The leaf compartment usually provides the first barrier of interception: epicuticular waxes adsorb hydrophobic molecules, while open stomata allow gas-phase diffusion into the mesophyll, where VOCs can be oxidized or conjugated before being translocated to other tissues [15,37]. Roots, in turn, absorb VOCs from the surrounding medium and supply exudates that support a metabolically versatile rhizomicrobial community capable of rapid rhizodegradation [38]. When both plant organs operate simultaneously, foliar adsorption and stomatal uptake rapidly reduce headspace concentrations, while root uptake and rhizospheric catabolism prevent the reemission and complete mineralization of the translocated VOCs. This dual pathway integration likely explains the complete and accelerated VOC removal observed with the whole cuttings in the experiment of Test series 2.

## 3.3. Assessment of the removal of VOCs by root-associated microorganisms

As previously reported in Test Series 2, acetone could not be quantified in any experimental condition because its high solubility in the mineral medium caused a rapid absorption. Thus, acetone concentrations remained essentially unchanged in the control and in assays with roots and microorganisms detached from roots, indicating that the high absorption capacity of the mineral medium played a dominant role in acetone retention, masking any potential effects of the roots and microorganisms.

In contrast, toluene,  $\alpha$ -pinene, o-xylene, and limonene experience an effective and rapid removal, with similar patterns observed between the assays containing plant roots and those with extracted microorganisms. Statistical analysis revealed no significant differences (p>0.05) in removal rates between the two systems, indicating that both compartments displayed similar degradation capacities. The presence of the entire root system alone supported a slightly faster removal rates

compared to the extracted microorganisms, suggesting that synergy between plant roots and their associated microorganisms enhances VOC removal (Figs. 9,10,11 and 12) (Tables S7-S10). The microorganisms associated with plant roots likely contributed to the observed VOC attenuation. While our data suggest an active microbial role, the absence of abiotic and surface-sterilized root controls, as well as the lack of microbial characterization, prevents us from conclusively demonstrating this mechanism. Nevertheless, previous studies have identified rhizosphere-associated bacteria capable of degrading VOCs, supporting the plausibility of this pathway [7,13,24,25]. Within the rhizosphere, the dynamic interaction between plant roots and the surrounding microbial community facilitate the uptake and degradation of VOCs [9]. Rhizodegradation, based on the bacterial break down of VOCs in the root zone, enhances the removal of contaminants that are otherwise difficult for plants to directly metabolize. Plant roots provide a favorable environment for these microorganisms by continuously supplying root exudates that serve as a nutrient, thereby promoting the degradation of indoor air pollutants transfer to the aqueous phase. Thus, root-associated microbial communities are essential for breaking down VOCs into less harmful compounds, thereby improving the overall efficiency of the phytoremediation process. Moreover, studies have shown that biostimulating the microbial community in the rhizosphere, for example, by increasing the population of VOC-degrading bacteria, can further enhance VOC removal efficiency [24]. For example, microorganisms commonly present in botanical filters, including members of the Actinobacteria phylum (e.g., Corynebacterium, Rhodococcus, Nocardia, Gordonae, and Mycobacterium), have been shown to degrade various environmental pollutants, including aromatic hydrocarbons [25,26]. These genera not only exhibit intrinsic resistance to stress conditions but also possess the catabolic machinery to degrade a wide range of VOCs, underscoring their suitability for use in phytoremediation systems [13]. Additionally, rhizofiltration relies on the direct adsorption and absorption of VOCs by the root biomass, which can play an important role in VOC removal. The large surface area of the roots allows for the efficient adsorption of VOCs, which are either stored in the plant or transported to other tissues for further processing. Therefore, the efficiency of rhizofiltration for VOC mitigation depends on the root surface area, root biomass and the specific plant species used, as these parameters govern the extent of VOC absorption and accumulation.

The results obtained in this study are consistent with previous research showing species-specific variability in VOC removal efficiency [8,32]. However, unlike most previous studies, which mainly quantified overall removal efficiency, our study systematically distinguishes the contribution of leaf tissues, whole cuttings, and root-associated microbial suspensions. Similar to our observations, Irga et al. [7] and Torpy et al. [24] highlighted the importance of microbial activity in the rhizosphere. However, the present results provide direct experimental evidence for such microbial contributions. This mechanical approach reinforces the current understanding of phytoremediation and underscores the synergistic role of plant traits and microbial metabolism in reducing indoor air pollutants. However, it is important to acknowledge certain limitations of the study: the experiments were conducted under controlled conditions, in sealed chambers and bottles, which do not accurately reproduce real indoor environments; only a limited set of representative VOCs were analyzed; lack of liquid-phase measurements; and associated microorganisms were not identified or quantified. These limitations may restrict the direct generalization of the results to complex real-world environments, but they provide a solid experimental basis for future field studies under environmentally relevant conditions.

#### 4. Conclusions

This experiment demonstrated the potential of plant cuttings to remove VOCs typically found in indoor environments, systematically elucidating the different contribution of the foliar and root components of different plants on VOC removal. The results showed that the removal

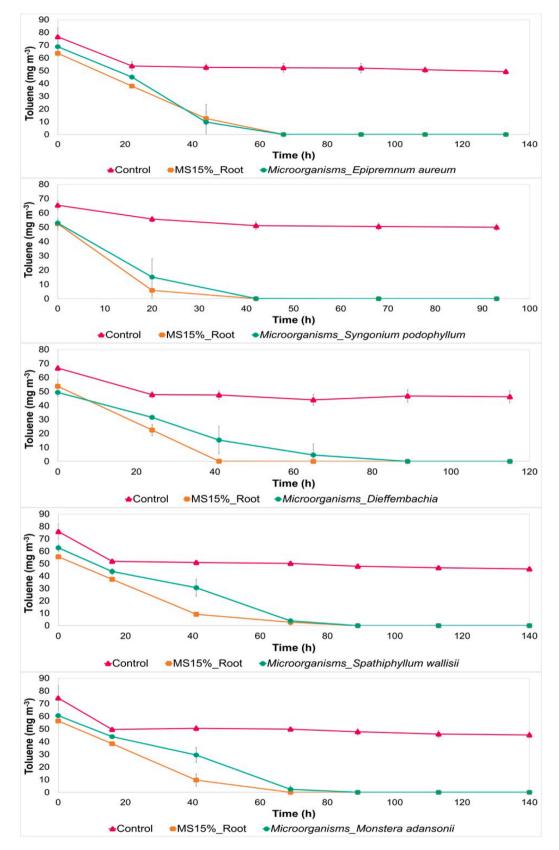


Fig. 9. Time course of the headspace toluene concentrations in assays conduced with the entire root system (), the microorganisms extracted () and control (). Vertical bars represent the standard deviation of three independent replicates.

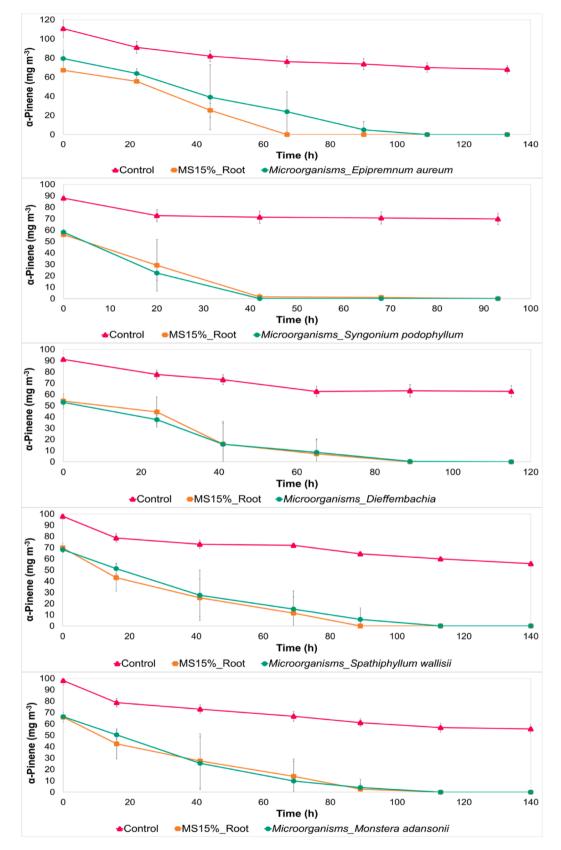


Fig. 10. Time course of the headspace  $\alpha$ -pinene concentrations in assays conduced with the entire root system ( $\clubsuit$ ), the microorganisms extracted ( $\blacksquare$ ) and control ( $\clubsuit$ ). Vertical bars represent the standard deviation of three independent replicates.

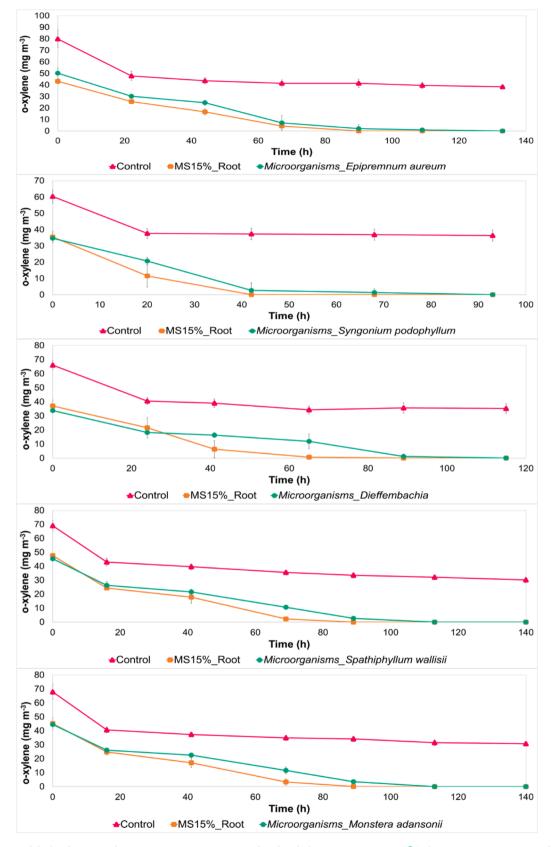


Fig. 11. Time course of the headspace o-xylene concentrations in assays conduced with the entire root system (), the microorganisms extracted () and control (). Vertical bars represent the standard deviation of three independent replicates.

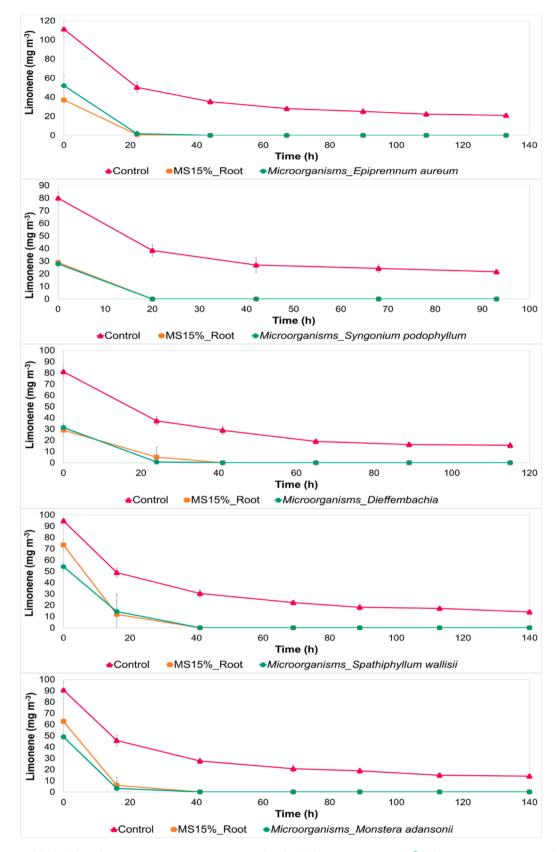


Fig. 12. Time course of the headspace limonene concentrations in assays conduced with the entire root system (), the microorganisms extracted () and control (). Vertical bars represent the standard deviation of three independent replicates.

efficiency of acetone, toluene, α-pinene, o-xylene, and limonene was a function of the plant species and the specific VOC tested. The high aqueous solubility of acetone did not allow to elucidate differences among plants and plant components. Toluene, α-pinene, o-xylene and limonene experienced efficient removals, with Epipremnum aureum and Dieffenbachia supporting the fastest degradation. Our results suggest that VOC removal was more effective when both the foliar and root systems were involved, as observed with whole plant cuttings compared to assays involving only the roots. The microorganisms associated with plant roots played a key role in the degradation of VOCs, which underlined the importance of rhizodegradation in the removal process. In brief, the results of this work highlighted the importance of selecting plant species with larger leaf area and more extensive root systems to boost VOC removal. The synergistic interaction among plant roots, leaves and associated microorganisms represents a promising platform to mitigate indoor air pollutants.

#### CRediT authorship contribution statement

María Sol Montaluisa-Mantilla: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. Raquel Lebrero: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. Pedro A. García-Encina: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition. Raúl Muñoz: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition.

#### 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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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.buildenv.2025.113775.

#### Data availability

Data will be made available on request.

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