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Highlights

- Active botanical filters using Photos effectively removed indoor organic pollutants
- Plants and rhizospheric microbes contributed to VOC degradation
- Optimal irrigation and airflow boosted VOC removal in botanical filters

Harnessing the potential of *Epipremnum aureum* (Pothos) for indoor air purification in botanical filters

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Abstract

Air pollution and human exposure to poor air quality rank nowadays as the most serious environmental threats to public health worldwide. Botanical biofiltration using active green walls based on air-purifying plants can support an effective control of indoor air

pollution. This study focuses on the design and evaluation of the performance of an active botanical filter for the removal of acetone, toluene and α-pinene at low concentrations using the houseplant *Epipremnum aureum* (commonly known as golden Pothos). The botanical filter was constructed with a vertical polyurethane foam wall supporting Pothos, and internal mineral salt medium and air recirculation. Maximum steady state removal efficiencies of 99.8 ± 0.8%, 83.6 ± 7.3% and 71.1 ± 5.2% were recorded for acetone, α-pinene and toluene, respectively. The reduction in the gas recirculation flow rate through the wall from 36 to 0 L min^{-1} decreased the removal efficiency of α-pinene to 70.37 ± 1.72%, while acetone and toluene maintained their removal efficiency under these conditions. Reducing the internal recirculation rate of the nutrient medium from 1.50 to 0.95 L min^{-1} and the absence of Photos in the polyurethane wall of the biofilter also decreased the pollutant abatement efficiency of the botanical filter. In addition, the analysis of the microbial community composition revealed significant differences in microbial composition and differences in the relative abundances between liquid samples of the medium in which the plant species grew and the mineral salt medium recirculated across the biofilter, which might contribute to the pollutant removal mechanisms.

Keywords: biofiltration, indoor air pollution, microbial community, removal efficiency, VOCs.

1. Introduction

Humans spend about 90% of their time in indoor spaces (houses, offices, schools, public and private transportation, etc.), where the concentrations of pollutants are typically 2 to 5 times higher than outdoors. A direct relationship between indoor air quality, public

health, and well-being, has been consistently identified. In 2013, 5.5 million deaths were attributed to exposure to (ambient and indoors) air pollution, which represents the fourth largest health risk factor [1]. Indoor air pollution is a health hazard that causes numerous diseases such as reduced brain function, respiratory disorders, leukemia, effects on the central nervous system, and various types of cancers, and often leads to death [2]. Indoor air pollution represents a considerable economic burden and causes significant economic losses. These losses are materialized in the form of reduced labor productivity and increased healthcare costs, which are estimated to reach hundreds of billions of dollars worldwide [1], [3]. This economic burden severely impacts on the economic growth of developing countries.

Indoor air pollutants include particulate matter (PM), biological pollutants (spores, bacteria, fungi, etc.), physical agents (noise, electromagnetic waves, humidity, temperature) and hundreds of different volatile organic compounds (VOCs) and volatile inorganic compounds (VICs) [4], [5]. The sources of these pollutants are diverse and can be both occasional and permanent [6], [7]. Several organizations such as the World Health Organization (WHO), the Environmental Protection Agency (EPA) and the Occupational Safety and Health Administration (OSHA) have established Indoor Air Quality (IAQ) standards for some indoor air pollutants to safeguard health [8]. In this context, VOCs are regarded as a relevant type of indoor air pollutants. The indoor concentration of these VOCs depends on factors such as the total confined space volume, *in-situ* pollutant production and removal rates, air renewal rate and outdoor VOC concentrations. Thus, during the first few months after construction or renovation of indoor spaces, primary VOC emissions are responsible for the high levels of indoor pollutants recorded. These emissions originate primarily from building materials, furnishings and finishes that release VOCs as they cure or settle. However, physical degradation and chemical

breakdown of these materials over time becomes the primary release mechanism of VOCs [9], [10].

The first strategy recommended to improve IAQ include prevention, elimination, replacement or limitation of pollution sources indoors. Within the European Union (EU), these strategies are enforced by directives and regulations such as the EU Directive 99/77/EC that banned certain forms of asbestos to reduce exposure from building materials [11]. Similarly, EU Directive 2004/42/CE limits the amount of VOCs in certain products, aiming to reduce ozone formation and improve both indoor and outdoor air quality [12]. In addition, national laws across many EU countries restrict smoking in enclosed public places, which improves IAQ and safeguards health risk associated with passive smokers [13]. Finally, the Ecodesign Directive (2009/125/EC) ensures the energetic efficiency of the combustion appliances such as stoves and heaters, thus decreasing direct pollutants emissions indoors.

The second strategy is based on the use of ventilation to increase the mass flow rate of air entering the building and thus diluting indoor pollutants [14]. The third strategy relies on the use of physicochemical purification technologies such as adsorption, mechanical filtration, ozonation, ultraviolet photolysis, etc. to actively abate indoor air pollutants. Unfortunately, to date, there is no physicochemical technology capable of removing all pollutants from indoor air cost-effectively and sustainably, which requires the development of novel technologies. In the past decade, biofilters engineered as green walls, known as botanical filters, have attracted an increasing attention based on their high efficiency during the degradation of indoor air pollutants [15]–[17]. Indeed, the potential of plants to remove VOCs from indoor environments in sealed chambers was initially demonstrated in a study conducted by the National Aeronautics and Space Administration (NASA) [18]. More recently, a wide variety of passive and active

botanical filters have been developed and tested. Passive systems, such as potted-plants, rely on the direct diffusion of air pollutants through the plants [19]–[22], whereas active systems typically entail the forced mechanical flow of polluted air to the plants [23].

By mechanically directing the polluted air into the botanical filter, the treatment capacity of the system to remove contaminants is maximized. Furthermore, this process continuously exposes the microbial community of the plant rhizosphere to a constant flow of contaminants [23], [24]. Despite the higher energy consumption compared to passive botanical filters, active botanical filters typically support higher VOC removal efficiencies. A limited number of studies have demonstrated the VOC removal efficiency of active green walls, which can be engineering in multiple configurations [17], [25]–[29].

Activated carbon (AC) filters are among the most widely used technologies for indoor air purification due to their high VOC removal efficiency, typically ranging from 85% to 99% [30]. However, their finite adsorption capacity requires periodic replacement or regeneration of the adsorbent, leading to increased operational costs and environmental concerns associated with the disposal of spent carbon [31]. In contrast, botanical filters have demonstrated VOC removal efficiencies ranging between 70% and 99.8%, comparable to AC filters. Unlike AC systems, botanical filters do not require media replacement, as VOC degradation occurs continuously through biological processes, enabling long-term operation. Additionally, botanical filters offer multiple co-benefits, including aesthetic enhancement and improved indoor environmental quality, aspects not typically addressed by AC filtration [32]. Energy consumption is also a critical factor in air purification sustainability. AC filters require 30–60 W for operation due to forced air circulation and heating, with annual energy consumption reaching 550 kWh [33]. In contrast, botanical filters consume approximately 120 W per square meter, primarily for

water recirculation and lighting, with the main energy demand associated with sustaining microbial and plant-based VOC degradation [34].

This work aims at assessing the potential of novel active botanical filter constructed with the indoor plant *Epipremnum aureum* to improve IAQ. *Epipremnum aureum* was selected as model plant due to its well-documented potential to abate a variety of VOCs [17], [35], [36] under indoor conditions. Acetone, toluene and α-pinene were selected as model indoor air pollutants with low, moderate and high hydrophobicity. The influence of gas circulation and internal liquid recirculation across the filter medium supporting hydroponic plant growth was also investigated. Unlike previous studies, our research did not only confirms the ability of the plant to remove VOCs from indoor air, but also integrated a detailed analysis of the impact of the controlled airflow and irrigation on VOC removal efficiency. In addition, this study included a microbiological analysis of the biofilter system, highlighting the relationship between microbial communities and plants during VOC degradation.

To contextualize the experimental conditions used in this work, previous studies have reported that indoor acetone concentrations typically range from 5.7 to 11.6 µg m^{-3} (0.0057-0.0116 mg m^{-3}) [4], while toluene levels in residential environments range from 3-20 µg m^{-3} (0.003-0.02 mg m^{-3}) according to the final report of the European Commission on indoor air quality standards [37]. α-pinene has been detected in indoor environments at concentrations in the range 3.2-14.5 µg m-3 (0.032-0.145 mg m-3) [4].

2. Materials and methods

2.1. Chemicals

Acetone (CAS-67-64-1), toluene (CAS-108-88-3) and α-pinene (CAS-80-56-8) were selected as representative indoor air pollutants with low, moderate and high hydrophobicity, and purchased to Sigma-Aldrich (Madrid, Spain). The mineral medium used for plant growth was Murashige and Skoog (MS) Basal Medium, that is typically used for in vitro micropropagation [38]. This mineral medium was composed of the following macronutrients and micronutrients (purchased to Sigma-Aldrich): 1650.0 mg L^{-1}, NH_4NO_3, 6.20 mg L^{-1}, H_3BO_3, 332.20 mg L^{-1}, $CaCl_2 \cdot 2H_2O$, 0.02 mg L^{-1}, $CoCl_2 \cdot .6H_2O$, 0.02 mg L^{-1}, $CuSO_4 \cdot 5H_2O$, 37.26 mg L^{-1}, $Na_2EDTA \cdot 2\ H_2O$, 27.80 mg L^{-1}, $FeSO_4 \cdot 7H_2O$, 2.0 mg L^{-1}, $C_2H_5NO_2$, 180.70 mg L^{-1}, $MgSO_4 \cdot H_2O$, 16.90 mg L^{-1}, $MnSO_4 \cdot H_2O$, 100.0 mg L^{-1}, $C_6H_{12}O_6$, 0.50 mg L^{-1}, $C_6H_5NO_2$, 0.83 mg L^{-1}, KI, 1900.0 mg L^{-1}, KNO_3, 170.0 mg L^{-1}, KH_2PO_4, 0.50 mg L^{-1}, $C_8H_{12}ClNO_3$, 0.25 mg L^{-1}, $Na_2MoO_4 \cdot 2H_2O$, 0.10 mg L^{-1}, $C_{12}H_{17}ClN_4OS \cdot HCl$ and 8.60 mg L^{-1}, $ZnSO_4 \cdot 7H_2O$ [39].

2.2. Plants preparation

The adaptability to hydroponic cultivation of different species of indoor plants such *Epipremnum aureum, Hedera Helix, Syngonium podophyllum, Spathiphyllum wallisii*, *Dieffenbachia*, *Monstera Adansonii* and *Chlorophytum comosum*, was initially evaluated. The results showed that the species *Epipremnum aureum* and *Syngonium podophyllum* experienced a better development and root growth under hydroponic cultivation. *Epipremnum aureum*, commonly known as golden Pothos, was selected as model plant to carry out this research based on its reported ability to abate atmospheric pollutants such as acetone, toluene and α-pinene [40]. In this context, a NASA study also highlighted its ability to attenuate indoor air contaminants such as formaldehyde, benzene, and xylene [36]. The strength of Photos, combined with its aesthetic appeal, facilitates its acceptance in indoor environments [41]. In addition, its high transpiration rate enhances air purification by drawing greater volumes of air into the root system of Photos, which

ultimately entails pollutant absorption and neutralization [42]. Finally, the roots of *Epipremnum aureum* in hydroculture systems foster a diverse microbial community that contributes to the breakdown of airborne pollutants [37].

The plants of golden Photos used in this study were obtained from nurseries in Valladolid (Spain). Cuttings were taken from the aerial parts of the plant and placed in beakers with deionized water. Cuttings were acclimatized in the laboratory for approximately six weeks at a temperature of 25 ± 2 °C. After rooting, they were exposed to varying concentrations (100, 50, 25, and 15%) of MS mineral medium (described in section 2.1) to determine the optimal nutrient concentration for *Epipremnum aureum* growth. The results indicated that MS concentrations of 100% and 50% induced putrescence of cuttings, MS concentrations of 15% and water mediated a similar response, while MS concentration of 25% supported the greatest root growth and formation of new leaves.

2.3. Experimental set-up

The experimental set-up consisted of an external polyethylene terephthalate glycol (PETG) plastic chamber simulating a room (1 m × 0.80 m × 0.65 m), which contained a green-wall active biofilter (Fig. 1, Fig. S1). This chamber hosted an axial fan (115.2 m^3 h^{-1}) to aid in the internal homogenization of the chamber atmosphere, and an external LED light panel providing 2160 lux under a 12/12 h day/night illumination regime. An inlet flow rate of contaminated air of 2.8 L min^{-1} was introduced into the chamber, resulting in eight air removals per day. A liquid mixture of VOCs (acetone, toluene and α-pinene) was injected into the inlet air at a flow rate of 1.8 uL h^{-1} using a syringe pump (Chemyx Fusion 100, USA) and a 500 uL liquid syringe (Hamilton, USA), which resulted in average concentrations of acetone of 2.2 ± 0.5 mg m^{-3} (Fig. S2, Table S1), toluene of 3.5 ± 0.6 mg m^{-3} (Fig. S3, Table S1) and α-pinene of 3.8 ± 0.7 mg m^{-3} (Fig. S4, Table S1). A mixing chamber equipped with a magnetic stirrer (Selecta Agimatic-S, Spain) was

installed after VOC injection to facilitate the homogenization of the VOCs in the air stream. The chamber was constructed with quick-connect fittings for recirculation in the botanical filter of MS mineral medium at a flow rate of 1.50 L min^{-1} (unless otherwise specified). The internal air was pumped into the botanical filter at a flow rate of 36 L min^{-1} using an EAD Boxer E30 compressor. To cool down the air from the compressor a 1 m height jacketed condenser was connected to a thermostatic bath (Thermo Scientific AC150, USA) with a water recirculation at 4 ºC. A humidity and temperature sensor (Testo 605-H1) was installed at the top of the chamber, which was provided with two sampling bulbs at the air inlet and air outlet, which served as VOC sampling ports. The concentration of VOC at the inlet and outlet ports was daily determined along with the temperature and humidity. Before starting the biological experiments, a continuous abiotic test (without plants) was performed for 14 days to rule out VOC adsorption and photolysis.

The botanical filter consisted of a square plastic box made of PETG (0.60 m × 0.60 m × 0.11 m) supporting a polyurethane foam (PUF) packing medium (Filtren TM 25280, Recticel Ibérica S.L., Spain) that served as a substrate (Fig. S5). The PUF exhibited a density of 0.01 g mL^{-1}, a porosity of 96% and a water retention capacity of 0.12 L_{water} L_{PUF}^{-1} [44]. This PETG botanical filter was constructed with 25 circular holes for plant insertion. 48 cuttings with their roots were planted in the PUF packed bed. MS mineral medium was recirculated from a 4 L holding chamber located at the bottom of the botanical filter and irrigated over the PUF packed bed using a horizontal tube perforated with 10 holes of 0.005 m located at the top of the module. The indoor air flowing through the PUF packed bed hosting the plants was introduced homogeneously from the front-bottom section of the botanical filter.

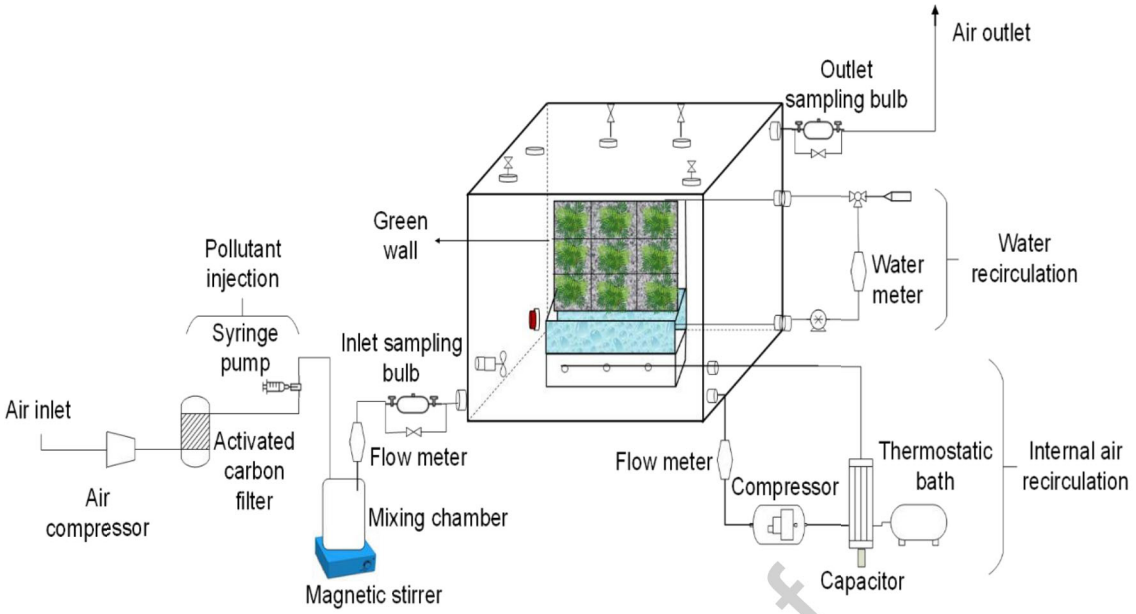

Fig. 1. Experimental setup including chamber and biofilter.

2.4. Influence of the internal air recirculation flow rate on VOC abatement

The experimental system was operated for 114 days, which accounted for eight operational conditions (Table 1). After 14 days of continuous operation under abiotic conditions (without plants) at 1.50 L min^{-1} of internal liquid recirculation and 36 L min^{-1} of air recirculation (stage 0), *Epipremnum aureum* was planted into the PUF medium and allowed to root for 31 days under the same air and liquid recirculation rates as in stage 0 (stage I). In the stage II the airflow rate pumped into the botanical filter was reduced to 20 L min^{-1} for 4 days (32 air removals) and then to 0 L min^{-1} for 5 days (stage III).

Table 1. Operational conditions tested during the optimization of the botanical filter.

Parameters	Stage 0	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII
Inlet air flow rate (L min^{-1})	2.8							
Air recirculation flow rate (L min^{-1})	36	36	20	0	36			

MS internal recirculation flow rate (L min^{-1})	1.50	1.50	0.95	1.50 for 15 min every 4h.	1.50
VOC liquid mixture injection rate (uL h^{-1})	1.8				
Average temperature inside the chamber (°C)	23.7 ± 1.4				
Average humidity insider the chamber (%)	98.3 ± 2.4				
Plant presence	✔				✖

2.5. Influence of mineral medium recirculation flow rate on VOC abatement

Once the influence of internal air recirculation had been evaluated, the experimental system was returned to the operating conditions established in stage I to allow stabilization of the process (stage IV). The internal liquid recirculation flow rate was reduced from 1.50 L min^{-1} (stage IV) to 0.95 L min^{-1} for 5 days (40 air removals) under an internal gas recirculation of 36 L min^{-1} (stage V). Then, the botanical filter was operated under intermittent liquid recirculation, i.e., a flow rate of 1.50 L min^{-1} for 15 min every 4 hours in stage VI, which lasted 4 days (32 air removals).

2.6. Assessment of the role of plants on VOC abatement

In stage VII, all plants in the botanical filter were removed to evaluate for 11 days the potential of the biofilm established in the PUF packed bed (88 air removals).

2.7. Analytical procedures

The concentration of VOCs was determined using SPME-GC-FID. Gas samples were pre-concentrated for 10 min in 250 mL glass bulbs (Sigma-Aldrich) using 85 µm

CAR/PDMS SPME fibers (Supelco, Bellefonte, USA). The SPME fibers were then injected in a GC-FID (Varian 3900) equipped with an Agilent HP-5MSI capillary column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were set at 150 and 200 °C, respectively. The oven temperature was set at 40 °C for 1.5 min, increased at 10 °C min^{-1} to 50 °C (held for 1 min), and finally increased at 40 °C min^{-1} to 250 °C (held for 5 min). Nitrogen was used as the carrier gas (2.5 mL min^{-1}) and as make-up gas (25 mL min^{-1}). Hydrogen and air flowrates were set at 30 and 300 mL min^{-1}, respectively. SPME fibers were initially conditioned at 300 °C for 1 h before calibration. VOCs were measured every day at the chamber inlet and outlet. External standards of each compound prepared in 250 ml glass bulbs were used for SPME calibration (Fig. S6 and S7).

2.8. Data processing

The VOC removal efficiencies (REs) of the botanical filter under each operational condition were calculated using Equation 1 based on the inlet (C_{in}) and outlet (C_{out}) concentrations of each VOC. Data are presented as average REs and standard deviations when steady state was reached. Subsequent statistical analyses, using *p*-values, were performed to elucidate differences in removal efficiencies for each VOC in the different experimental stages.

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

2.9. Shotgun Metagenomic Sequencing and Bioinformatic Analysis

Plant growth media (PGM) and biofilter water (BW) samples were collected and filtered using a 0.45 μm pore size filter until clogged, maximizing the bacterial content for subsequent DNA extraction (Merck Millipore, USA). A total volume of 186 mL of PGM and 200 mL of BW were used. Subsequently, DNA extraction was performed on filters using the DNeasy PowerWater Kit (Qiagen, Netherlands), following the manufacturer's

instructions. The elution step was carried out to a final volume of 50 µL, which was aliquoted and stored at -20°C. The amount of eluted DNA was determined with a Qubit fluorometer (Invitrogen, USA), while the length and completeness of the library DNA fragments were assessed using QIAxcel (Qiagen, Netherlands). An Illumina Hiseq sequencer (Illumina, USA) was used for whole genome sequencing. Libraries were prepared using the Nextera DNA Flex Library Prep Kit and MiSeq Reagent Kit v2 (Illumina, USA) following the manufacturer's instructions. Libraries were loaded at concentrations ranging from 10-12 pM [45]. Quality assessment of the raw reads was initially performed using FastQC v0.12.1. To improve the overall data quality, paired-end reads were trimmed using Trimmomatic v0.36, with a minimum read quality threshold set at 20. The trimmed reads were then reassessed for quality using FastQC v0.12.1. For metagenome assembly, metaSPAdes v3.15.3 was used, and the resulting assembled contigs were aligned against the trimmed reads using Bowtie2 v2.3.2. Taxonomy classification of the aligned reads was accomplished using Kaiju v1.9.0, which involved comparing the sequences to the RefSeq database [46].

3. Results and discussion

In the abiotic test (carried out with gas and liquid circulation in the absence of plants, stage 0), VOC removal occurred by adsorption onto the chamber material and the PUF, by photolysis, and by absorption in the recirculating medium. The removal efficiencies averaged 66.6 ± 13.3%, 10.5 ± 13.4% and 17.2 ± 12.7% for acetone, α-pinene and toluene, respectively, under steady state (Fig. S8).

In the biotic tests in which *Epipremnum aureum* was planted, removal efficiencies of 99.8 ± 0.8%, 83.6 ± 7.3% and 71.1 ± 5.2% were recorded for acetone, α-pinene and toluene, respectively, during stage I, when the system reached steady state operating with an

internal air recirculation flow rate of 36 L min^{-1} and a MS mineral medium recirculation of 1.50 L min^{-1} (Fig. 2). The abiotic control test exhibited significant differences in terms of VOC REs with the rest of the biotic experiments (Table S2). The *p*-values obtained from these comparisons were below the significance threshold, confirming that biotic processes contribute substantially to the overall capacity of the botanical filtering system. This indicates that VOC removal is not merely due to passive physical mechanisms such as adsorption or photolysis, but is actively driven by biological interactions. Both microbial metabolism and plant-associated enzymatic activity contribute to VOC degradation, as microorganisms and plant roots interact synergistically within the biofilter system to enhance VOC uptake and biotransformation.

The gas-liquid mass transfer coefficient, along with the Henry`s law constant of the contaminant, are key factors influencing the VOC removal efficiency of a botanical filter. Henry's law constant quantifies the solubility of a gas in a liquid at constant temperature, where higher values imply a higher compound solubility in the aqueous phase. The Henry's law constant for acetone is 3.3×10^{-1} mol $m^{-3}Pa^{-1}$ [47], 1.5×10^{-3} mol $m^{-3}Pa^{-1}$ for toluene [48] and 2.9×10^{-4} mol $m^{-3}Pa^{-1}$ for α-pinene [49], indicating that acetone is more hydrophilic than toluene and α-pinene. Thus, the higher polarity of acetone enhances its solubility in water compared to α-pinene and toluene. The relationship between Henry's law constant and hydrophobicity is essential for understanding the behavior of these VOCs in aqueous environments, such as in botanical filtration systems, where solubility influences their availability for microbial degradation. Hydrophobic compounds like α-pinene and toluene, due to their lower aqueous solubility, may require more effective mass transfer mechanisms to achieve removal efficiencies comparable to those of more hydrophilic compounds such as acetone. In this context, multiple mass transfer mechanisms are involved in their removal. Gas-liquid partitioning determined

the initial transfer of VOCs to the aqueous phase, while adsorption on organic surfaces, including plant roots and microbial biofilms, increased local concentration and bioavailability. In addition, the polyurethane foam matrix used in the biofilter provided a high surface area that facilitated microbial colonization, further enhancing the VOC degradation efficiency.

A higher solubility entails a more efficient VOCs transport from the indoor air into the plant tissues, thus facilitating their effective degradation or biotransformation. Despite the lower solubility of α-pinene, its degradation was higher than that of toluene during stage I. This discrepancy can be attributed to the fact that: i) the hydrophobic nature of α-pinene favors a strong adsorption onto organic matter and microbial biofilms in the rhizosphere, which increased its local concentration and bioavailability for microbial degradation; ii) the specific enzymes and microbial pathways that efficiently degrade α-pinene might be more prevalent or active in the filter compared to those for toluene [50]. Additionally, physical and chemical interactions between VOCs and plant roots vary, which influences degradation rates. Co-metabolic interactions and operating conditions, such as airflow and humidity, can also play a role by affecting microbial activity and the fate of the VOCs.

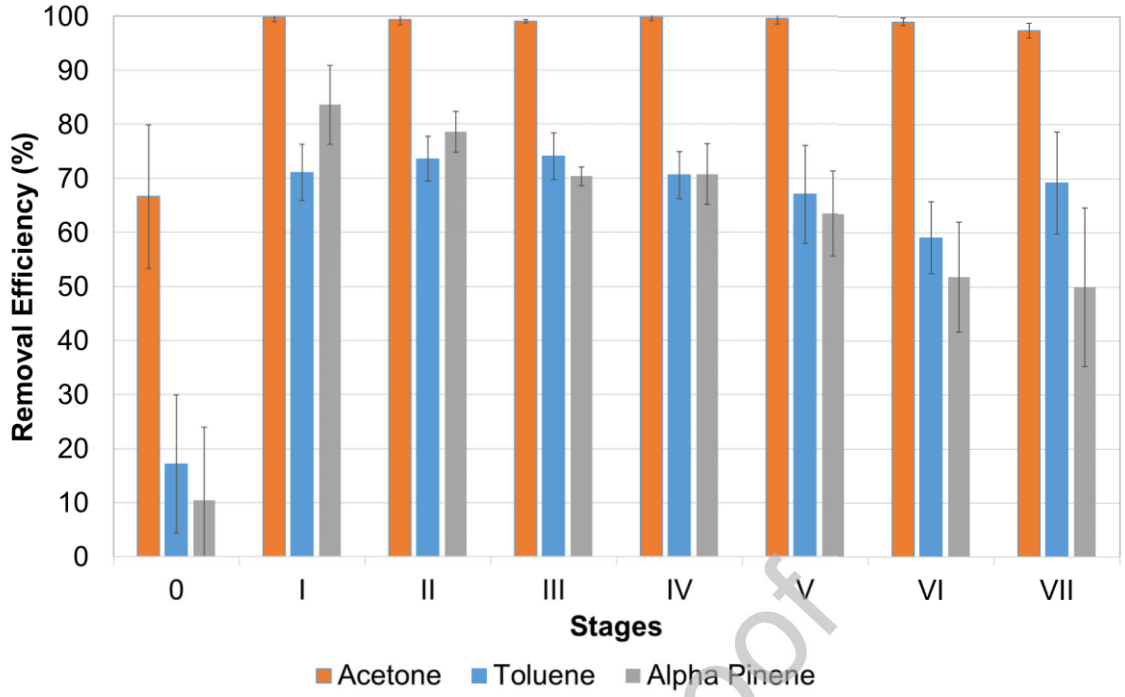

Fig. 2. Average VOC removals as a function of the operational stage. Vertical bars stand for the standard deviation during steady state.

The application of botanical filters in real indoor environments has been preliminarily explored, providing valuable insights into their scalability and effectiveness. Pettit et al. [26] conducted an situ pilot-scale study in a classroom setting, where an active green wall (AGW) system was installed, comprising a mix of plant species, including *Epipremnum aureum, Nephrolepis exaltata, Peperomia obtusifolia, Schefflera arboricola,* and *Spathiphyllum wallisii*. The classroom had a floor area of 40.07 m^2 and a volume of 120.2 m^3, with the biofilter operating at a volumetric airflow rate of 283.53 m^3 h^{-1}, corresponding to 2.36 air changes per hour. This configuration resulted in significant reductions in total volatile organic compounds (TVOCs) and PM concentrations, demonstrating the potential of the system for improving indoor air quality in larger spaces. Specifically, the AGW reduced the average TVOC concentration by approximately 28% within a 20-min testing period, highlighting its effectiveness as an indoor air purification strategy.

Furthermore, Mannan and Al-Ghamdi [25] reviewed the performance of active botanical biofiltration (ABB) systems in real indoor environments, reporting removal efficiencies ranging from 54% to 85% for total suspended particulates and up to 90% for gaseous pollutants such as formaldehyde.

Influence of the internal air recirculation flow rate on indoor air quality

A significant decrease in α-pinene removal was observed in stages II and III, when the internal recirculation airflow was reduced from 36 L min^{-1} to 20 L min^{-1} (stage II, *p*-value=0.04), followed by complete cessation of internal recirculation (stage III, *p*-value=5.33×10^{-8}), with efficiencies of 78.6 ± 3.8% and 70.4 ± 1.7%, respectively (Fig. 2). The observed reductions were statistically significant compared to the baseline established at stage I. For acetone, the removal efficiencies remained relatively constant at 99.3 ± 1.0% in stage II and 99.0 ± 0.3% in stage III. The variations in acetone removal was significant only in stage III compared to the steady state (stage I), as indicated by the *p*-value=0.003 (Table S2). Toluene removal efficiencies accounted for 73.5 ± 4.1% and 74.1 ± 4.3% in stages II and III, respectively, with no significant variation compared to the steady state in stage I (Table S2).

A comparison between stages II to III showed that decreasing and stopping internal air recirculation exerted no significant impact on the removal efficiencies of acetone and toluene. In contrast, for α-pinene, the *p*-value of 0.007 revealed a statistically significant decrease in its removal efficiency when internal recirculation was stopped (Table S2). This can be attributed to the different physicochemical properties and microbial interactions of each contaminant. The monoterpene α-pinene is the most structurally complex contaminant among those studied, characterized by a larger molecular size, lower volatility, and pronounced hydrophobicity compared to smaller size and more polar compounds, such as toluene and acetone. These physicochemical properties of α-pinene,

in particular its hydrophobic nature, make it less soluble in the aqueous phase, which may hinder its bioavailability and its interaction with the microbial community in the biofilter. In addition, microbial communities may exhibit compound-specific biodegradation preferences. The internal airflow within a botanical filter plays a critical role in VOC abatement performance because i) it ensures that the contaminated air is evenly distributed throughout the green wall, allowing plants and microorganisms to remove VOCs effectively, and ii) it provides plants with the CO_2 needed for photosynthesis, which activates plant metabolism and ultimately the activity of bacteria in the rhizosphere [23]. Interestingly, if the airflow through the packed bed of the filter is too high, the VOCs removal per pass is low as the contact time between the VOCs and the microorganisms is reduced, but the overall efficiency increases as a result of the increased internal recirculation. Conversely, if the airflow is too fast in a single pass without recirculation, the plants may not receive enough CO_2 for photosynthesis, which would affect their ability to remove VOCs.

Wu and Yu [51] demonstrated that higher airflow rates enhance pollutant loading and removal capacity up to an optimal threshold. Beyond this point, excessively high airflow rates can negatively affect plant health and photosynthetic activity, driven by rapid water loss, nutrient depletion, reduced CO_2 availability, and disruptions in stomatal regulation.

To optimize the performance of a botanical filter, it is crucial to maintain the airflow at an optimal velocity. Darlington et al. [46] demonstrated that the most effective VOC removal efficiency were achieved at faster velocities (0.200 m s^{-1}). Interestingly, the differences in removal efficiency between 0.100 and 0.200 m s^{-1} were marginal, showing only a 5% to 10% improvement in removal at higher velocities. This could be attributed to a more uniform distribution of VOC concentrations throughout the depth of the biofilter. Additionally, the study found that improvements in VOC removal efficiency

were associated with a reduction in operating temperature, which in their case was 19.13 °C. In contrast, the air velocity through the biofilter in the present experiment was 0.002 m s^{-1}, which is lower than that recorded in the research by Darlington et al. [46]. Despite this, high VOC removal efficiencies were achieved in the active botanical filter system in this study. Likewise, another study showed that air contaminated with $PM_{2.5}$, PM_{10} and VOCs was effectively removed at higher airflow rates through the filter media [53].

Influence of mineral medium recirculation flow rate on VOC abatement

A stabilization phase (stage IV) in the experimental system implemented to return the operating conditions to stage I. In the stage IV, the removal efficiencies recorded accounted for 99.7 ± 0.6% for acetone, 70.6 ± 4.4% for toluene and 70.7 ± 5.6% for α-pinene, which matched the performance observed in stage I.

In stages V and VI, the flow rate and recirculation frequency of the MS mineral medium were modified. In stage V, the flow rate was reduced from 1.50 L min^{-1} to 0.95 L min^{-1}, while in stage VI, the botanical filter operated under intermittent liquid recirculation, with a flow rate of 1.50 L min^{-1} for 15 minutes every 4 hours. This reduction in operating parameters significantly affected the removal efficiency of contaminants, particularly for α-pinene, with removal efficiencies decreasing of 63.5 ± 7.8% in stage V and 51.8 ± 10.2% in stage VI (Fig. 2). In stage V, the decrease in the removal efficiencies of acetone and toluene was not significant. The botanical filter removed acetone with a removal efficiency of 99.5 ± 1.0% and toluene at 67.0 ± 9.1%. In stage VI, removal efficiencies of 99.1 ± 0.7% for acetone and 59.1 ± 6.7% for toluene were recorded, with a significant variation for toluene when compared to stage I (*p*-value=0.015,Table S2). These results suggest that variations in the recirculation of the medium potentially influence the abatement of certain VOCs compared to the reference levels of stage I, where the system operated at a constant flow rate of 1.50 L min^{-1} and achieved the highest removal

efficiencies. However, when comparing the removal efficiencies between stages V and VI for the three pollutants, no significant variations were observed (Table S2).

VOC removal in biofiltration systems initiates with their solubilization in the aqueous phase, a critical step that makes these contaminants accessible for further processes such as substrate adsorption and microbial degradation [54]. The solubility of VOCs is governed by their respective Henry's law constants, which describe the equilibrium between the gas and dissolved phases of a compound. According to Pettit et al. [27], the saturation point of VOCs is a key factor in determining the extent to which these compounds remain in the aqueous phase. In this context, acetone, with the highest Henry's law constant among the 3 model VOCs ($H_{Acetone} > H_{Toluene} > H_{\alpha\text{-pinene}}$), is more hydrophilic than the other target pollutants and experienced a lower impact of the decreases in the recirculation of the medium. In contrast, α-pinene, characterized by its hydrophobic nature, demonstrated a more pronounced sensitivity to changes in medium recirculation. This disparity underscores the influence of hydrophobicity on VOC removal efficiency in botanical filters.

Mikkonen et al. [49] identified potentially VOC-degrading bacteria within the irrigation water in a green wall, confirming an additional mechanisms of VOC removal in indoor air. It is evident that modification of the flow rate and frequency of mineral medium irrigation (which includes water and micro/macronutrients) affects the removal of VOCs. It can also be interpreted that for plants to effectively degrade VOCs, they must be in optimal metabolic conditions, which requires adequate irrigation. In this series of tests, modifying irrigation probably affected both plant growth and the activity of the associated microbial communities. Thus, there is a direct relationship between the irrigation regime of the mineral medium and the removal efficiency of certain VOCs.

Assessment of the role of plants on VOC abatement

The withdrawal of plants in stage VII caused a decrease in the removal of acetone to 97.4 ± 1.4% and α-pinene to 50.0 ± 14.7%, while toluene was the only pollutant that maintained the removal efficiency (69.3 ± 9.4%). Statistical analysis revealed significant differences for acetone and α-pinene when comparing the removal efficiencies of stage VII with those of stage I, which represents the maximum steady-state performance of the botanical filter. More specifically, the *p*-values obtained were 0.0008 for acetone and 0.0001 for α-pinene (Table S2), underlining the marked variation between stages. These results suggest that the presence of plants significantly impacts VOC removal. The active botanical filters contain a unique microbial community, supported by the plant root system, which is capable of degrading a wide range of VOCs at low concentrations [10]. Plants and microorganisms collaborate to remediate air pollutants through a symbiotic process in which plants provide nutrients to microbial communities via root exudates [56]. This could explain why the degradation of contaminants did not completely decrease despite the absence of plants in the system, since part of the microbial community remained in the PUF used as substrate.

In this context, Zhang et al. [51] found that rhizospheric bacteria contained the toluene monooxygenase gene, confirming their genetic potential to metabolize toluene. Additionally, Chun et al. [52] analyzed the impact of the rhizosphere bacterial population of various plants on the removal of benzene, toluene and *m, p*-xylene. The study demonstrated a reduction in the concentration of these pollutants, which suggested that the rhizosphere microbial population of the potted plants played a significant role on the removal of VOCs from indoor environments, thus improving IAQ. Therefore, an enhancement in the VOC removal potential can be achieved by incorporating plants into biofilters [55], [59], selecting appropriate plant species [16] and adjusting planting densities [60]. Yang et al. [55] further confirmed that plant species influence the removal

of benzene, toluene, octane, trichloroethylene, and α-pinene at concentrations of approximately 10 ppm_v in a study conducted with 28 indoor plants. These findings highlight the potential for improved VOC biodegradation functionality of houseplants.

Bacterial community composition

Microbial communities inhabiting the PUF substrate and rhizosphere play a key role in the degradation of VOCs, as pollutants diffuse directly into the potting mix and are taking up by bacteria, which then metabolize them as a carbon and energy source [56], [62]. To evaluate the potential impact of microbial communities in the experimental setup, liquid samples of plant growth media (PGM) and biofilter recirculating water (BW) were collected and sequenced at the end of the experiment. The PGM provided information on the composition of the bacterial community composition in direct contact with the roots, serving as a proxy for the rhizosphere. Meanwhile, the BW provided a snapshot of the bacterial community composition in the experimental setup. Fig. 3 shows the total number of reads whose taxonomy was assigned and unassigned (a) and the bacterial community composition at the phylum level, as well as the DNA viruses, indicating their relative abundances (b). The sample obtained from PGM exhibited a significantly higher number of reads (253,306 reads) compared to the sample from the biofilter water (BW) (78,317 reads). The dominant phylum *Proteobacteria* exhibited a significantly higher relative abundance in PGM (86.7%) compared to BW (34.13%). In contrast, *Actinobacteria* accounted for a relative abundance of 1.75% in PGM, whereas it became the predominant phylum in BW, with a relative abundance of 61.2%. Members of *Actinobacteria* include several aromatic hydrocarbon-degrading bacteria [63], [64], such as the genera *Corynebacterium*, *Rhodococcus*, *Nocardia*, *Gordonae*, and *Mycobacterium*. These genera not only have intrinsic resistance to survive under stress conditions but also have the potential for degradation of several environmental pollutants. *Rhodococcus* has been

shown to degrade hydrocarbons, chlorophenols, polychlorinated biphenyls and sulfonated azo dyes [65]. *Mycobacteria* have demonstrated the capacity to remove polychlorophenols, heavy metals, and various polycyclic aromatic hydrocarbons (PAHs), or to transform them into less toxic forms [66]–[68]. Similarly, *Nocardia* species can decompose PAHs, polychlorinated biphenyls, chlorophenols, sulfonated azo dyes, and alkanes [66], [69]. In addition, *Gordoniae* are capable of degrading alkanes [70].

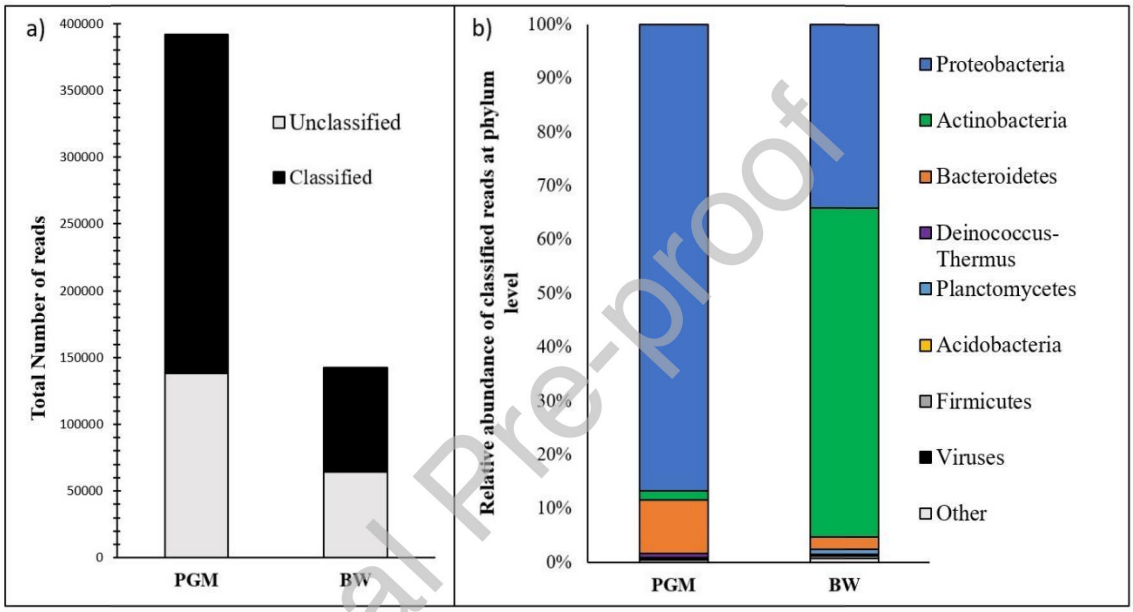

Fig. 3. Total number of reads with classified and unclassified taxonomy (a) and the composition of the bacterial community at the phylum level, along with DNA viruses and their relative abundances (b) are presented for PGM and BW samples. The taxonomy is arranged in descending order of abundance when combined.

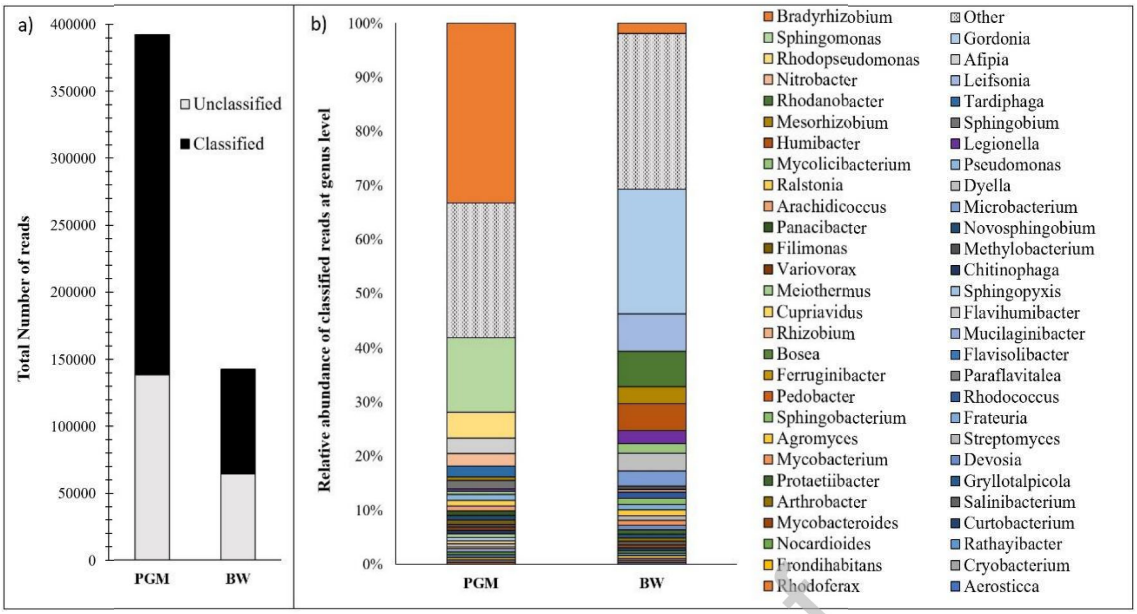

Fig. 4. Total number of reads with classified and unclassified taxonomy (a) and the composition of the bacterial community at the genus level, along with DNA viruses and their relative abundances (b) are presented in PGM and BW samples. The taxonomy is arranged in descending order of abundance when combined.

At the genus level (Fig. 4), a significant difference in the microbial community was also observed between the two samples. In PGM, the predominant genera were *Bradyrhizobium* (33.34%), *Sphingomonas* (13.80%), *Rhodopseudomonas* (4.77%), *Afipia* (2.80%), and *Tardiphaga* (2.03%). In contrast, the dominant genera in BW were *Gordonia* (22.99%), *Leifsonia* (6.92%), *Rhodanobacter* (6.62%), *Humibacter* (4.97%), Dyella (3.27%), *Mesorhizobium* (3.12%), *Microbacterium* (2.80%), and *Legionella* (2.42%). Analysis of the microbial community composition at both the phylum and genus levels revealed significant differences between PGM and BW. At the phylum level, the dominance of *Proteobacteria* in PGM was in agreement with previous studies highlighting its prevalence in soil and plant-associated environments [71]. *Proteobacteria* have been extensively studied and have been shown to play crucial roles in several important functions, including nitrogen fixation [72], [73], mineral phosphate

solubilization [74], synthesis of auxinic phytohormone indole acetic acid [75], copper nitrite reductase activity [76] and production of antimicrobial compounds for defense against plant infections [77]. Due to the crucial role of *Proteobacteria* in plant health, their reduced abundance in the biofilter, while still substantial, may raise concerns about the efficiency of the biofilter and could potentially increase the susceptibility of plants to infections and diseases within the system.

The shift to dominance of *Actinobacteria* in BW may suggest their potential role in the degradation of VOCs. *Actinobacteria* are well-known for their diverse metabolic capabilities, including the ability to degrade a wide range of organic compounds [63], [78]. The presence of *Gordonia*, *Leifsonia*, *Rhodanobacter*, *Humibacter*, *Dyella*, *Mesorhizobium*, *Microbacterium*, and *Legionella* suggests their tentative involvement in the degradation of VOCs within the botanical filter. *Gordonia* and *Leifsonia*, in particular, have previously been identified as important contributors to the degradation of various organic compounds, including toluene [79], [80]. *Rhodanobacter* and *Humibacter* bacteria, known for their ability to utilize organic pollutants as carbon and energy sources, are capable of degrading toluene and complex hydrocarbon mixtures [81]. This study confirmed the key role of the bacterial community in the degradation of VOCs in botanical filters. However, a better understanding of VOC degradation pathways requires more comprehensive studies with larger sample size and a focus on the functional profiles of metagenomes.

4. Conclusions

This study demonstrated the potential of a plant biofilter to successfully reduce VOC concentrations in indoor air over a period of ~ four months. Acetone, α-pinene, and toluene were removed at efficiencies of 99.77 ± 0.85%, 83.56 ± 7.29%, and 71.07 ± 5.19%, respectively. These results were observed under conditions where the system was

operated with an internal air recirculation flow rate of 36 L min^{-1} and a constant mineral medium recirculation flow rate of 1.5 L min^{-1} inside a 520 L gas-tight chamber. This study demonstrated that internal airflow and irrigation flow govern the performance of botanical indoor air filters. The microbial community within the substrate and the rhizosphere water could play a key role in the degradation of VOCs. Indeed, the biofilter exhibited a satisfactory VOC removal even after plants were withdrawn (the removal efficiencies of acetone and α-pinene decreased to 97.45 ± 1.39% and 49.97 ± 14.66%, respectively). The high abundance of *Actinobacteria* in the PUF support suggested the active role of bacteria in VOC degradation. Future studies in the field of botanical filters should focus on the selection and optimization of plant species based on their specific ability to remove indoor air pollutants. In addition, further studies are needed to evaluate the performance of botanical filters over extended periods, including plant growth, plant health, and pollutant removal efficiency.

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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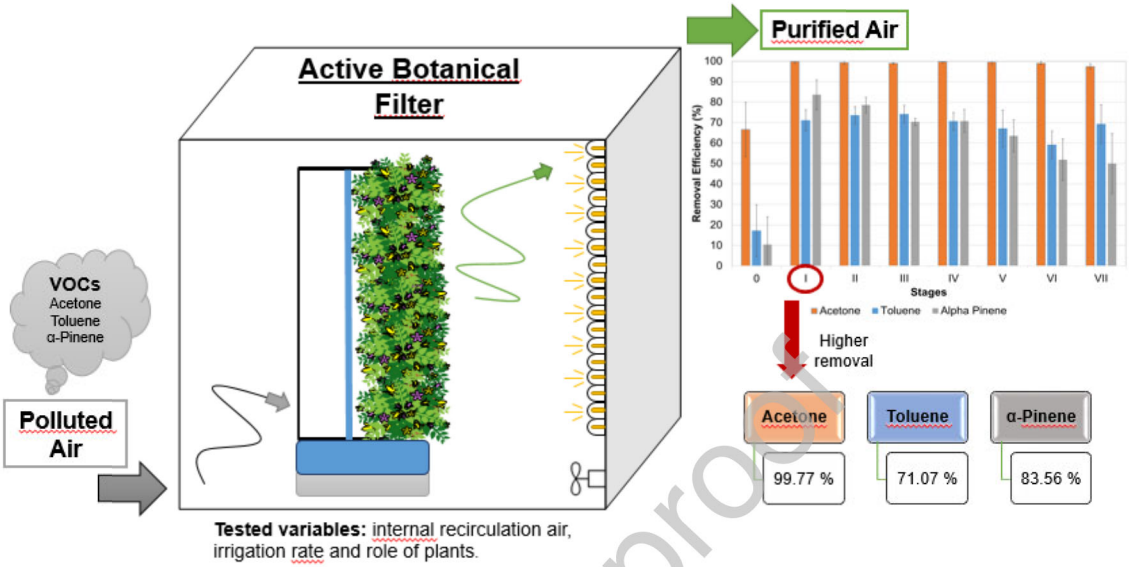
Graphical Abstract



Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: