NEO PX
WHITE PAPER

neoplants

Summary

Background

VOCs And Indoor Air Quality

Indoor air pollution can be categorized in two major groups: **Particulate Matter** (PM) and **Gaseous contaminants**, including **Volatile Organic Compounds** (VOCs).

Particulate matter are further categorized according to size. **Coarse particles**, between 10 µm to 2.5 µm in diameter are called PM_{10}, **fine particles** ($PM_{2.5}$) are 2.5 µm or smaller, and **ultrafine particles** (PM_1) are 1 µm or smaller. These are particles of various nature and origin that cause many adverse effects on human health. Fortunately, many studies have shown that fibrous media portable air purifiers, using technologies like High-Efficiency Particulate Air (HEPA) filters, can drastically reduce PM exposure, with efficiencies depending on particle size and filter type. Ref.

Gas-phase pollutants can be divided into two groups: **Inorganic** like SO2, NO, NO2, CO, O3 and **organic**. These volatile organic compounds (VOCs) are among the most harmful indoor air pollutants, and include Formaldehyde (HCHO), Benzene, Toluene, Ethylbenzene, Xylene (BTX) and Polycyclic Aromatic Hydrocarbon (PAHs).

These VOCs can come from **outdoor pollution**, like car exhaust or industrial activities and are found in higher concentration in cities. Ref. But their main sources are located **inside our homes**. They are continuously emitted by building materials, engineered wood products, architectural coatings, solvents, paints, household products like cleaning chemicals or deodorisers, and human activities like cooking or smoking. Ref. **This causes indoor air to be on average 5 times more polluted than outdoor.**

Chronic exposure to these VOCs, even at low concentrations found typically indoors, can cause a variety of **adverse health effects**. They are known to cause allergies, eye and throat irritation Ref., aggravated asthma symptoms Ref., and even cancers in some cases. Ref. Ref. These effects are especially pronounced in vulnerable groups like children Ref. and the elderly. Ref.

The impact of indoor VOCs is not limited to physical health, but also impairs **cognitive function**, significantly reducing problem-solving abilities at work, with drastic effect of information usage and strategy. Ref. This impacts both individuals and organizations and illustrates the extent of the negative effects of VOCs in our lives.

At the heart of the VOCs problem, in contrast with PM, is our inability to effectively purify them using traditional air purifiers. More on that later. The only two ways to partially mitigate them today is to **(i) limit the presence** of VOC emitting substances, by buying low VOC versions of some products and **(ii) ventilate living spaces** by opening windows. Ref. Ref. Ref. The first solution is rarely an option, as many VOC emitting products do not have low VOC alternatives widely available. The second solution remains unsatisfactory, as opening windows is not always practical in many climates and some buildings. It would also require a dozen ventilation sessions per day, as these VOCs are often continuously and constantly emitted from various indoor sources. Ref.

The limits of air purifiers

As knowledge and awareness of **the negative impact of low air quality** has increased over the past decades, so has the efforts to find practical solutions. One of these solutions is using portable air purifiers, evidenced by the drastic increase in public demand over the past few years and the expected market growth of this segment. Ref.

The technologies behind portable air purifiers vary according to the type of pollutants addressed, with **HEPA filters** being the most successful at targeting PMs. But **this technology cannot filter out VOCs**, as Background 03 these compounds usually do not exceed 1 nanometer in diameter, a thousand times smaller than PM1 HEPA filters operating at the micrometer scale.
To address VOCs, many technologies have been developed, powering new generations of portable air purifiers. Some of these technologies have interesting properties, all have significant drawbacks, and in some cases can lead to more pollution being generated than remediated, as stated in the Environmental Protection Agency (EPA) technical summary (Table 1).

Air-cleaning technology	Targeted pollutants(s)	Mechanism(s) of action	Advantages	Disadvantages
Fibrous filter media	Particles	Collection: Filter fibers capture particles • Mechanical filtration media rely on mechanical forces alone. • Electrostatically-charged (i.e., «electret») media use mechanical fibers with an electrostatic charge applied to collect oppositely charged particles, enhancing removal efficiency	• If rated efficiency is high, they can have excellent removal capabilities for many particle sizes • Mechanical media filters see improved efficiency with loading	• Regular replacement is required • Used particle filters can be a source of sensory pollution/ odors • High pressure drops on some fibrous media filters can negatively impact HVAC systems • Electric media filters see reduced efficiency with loading • Confusing number of test standards and rating metrics
Electrostatic precipitation (ESP)	Particles	Collection: Corona discharge wire charges incoming particles, which collect on oppositely charged plates	• Can have high removal efficiency for a wide range of particle sizes • Low pressure drop and minimal impacts on HVAC systems • Low maintenance requirements	• Sometimes ESPs have high ozone and nitrogen oxide generation rates • Efficiency typically decreases with loading and plates require cleaning • High electric power draw requirements
Ionizers (i.e., ion generators)	Particles	Collection: Like ESP, ionizers employ a high-voltage wire or carbon fiber brush to charge air molecules electrically, generating negative ions that cling to airborne particles. These charged particles are then gathered on oppositely charged plates within the air cleaner or become attracted to and deposited on other room surfaces.	• Typically low power draw requirements • Quiet • Low maintenance	• Generates ozone • <Typically low effectiveness because of very low airflow rates and clean air delivery rates (CADRs)

Table 1: Summary of air-cleaning technologies. Adapted from table 1 of the EPA residential air cleaners technical summary. EPA 402-F-09-002 | July 2018 | EPA Indoor Environments Division.

Ultraviolet germicidal irradiation (UVGI)	Microbes	Destruction: UV light kills/ inactivates airborne microbes	• Can be effective at high intensity with sufficient contact time • Can be used to inactive microbes on cooling coils and other surfaces	• Uncoated lamps can generate ozone Potential for eye injury • Effectiveness increases with lamp intensity, which is typically low in residential UVGI air cleaners • High electrical power draw requirements • Inactivates but does not remove microbes
Adsorbent media	Gases	Collection: Gases physically adsorb onto high-surface-area media (typically activated carbon)	• Potential for high removal efficiency for many gaseous pollutants in air cleaners with a sufficient amount of media for the application • No byproduct formation	• Regular replacement is necessary as its adsorption capacity diminishes and physical adsorption is reversible, indicating pollutants might not be permanently trapped. • The efficacy of consumer-grade systems with minimal activated carbon is uncertain. • High pressure drops on some sorbent media filters can negatively impact HVAC systems • Different removal efficiency for different gases at different concentrations • Standard test methods are not widely used
Chemisorbent media	Gases	Collection: Gases chemically adsorb onto media coated or impregnated with reactive compounds	Potential for high removal efficiency for many gaseous pollutants Chemisorption is an irreversible process, meaning pollutants are permanently captured	• Regular replacement is required because its chemisorption capacity is exhausted • Effectiveness of many consumer-grade systems is unknown • High pressure drops on some sorbent media filters can negatively impact HVAC systems • Different removal efficiency for different gases at different concentrations
Catalytic oxidation	Gases	Conversion: Most utilize photocatalytic oxidation (PCO) in which a high-surface-area medium is coated with titanium dioxide as a catalyst; incoming gases adsorb onto the media and UV lamps irradiate and activate the titanium dioxide, which reacts with the adsorbed gases to chemically transform them	• Can degrade a wide arrayof gaseous pollutants (e.g., aldehydes, aromatics, alkanes, olefins, halogenated hydrocarbons) • Can be combined with adsorbent media to improve effectiveness	• Can generate harmful byproduct such as formaldehyde, and acetaldehyde, and ozone • No standard test methods • Often relatively low removal efficiency for many indoor gases, but high variability in removal for different gases • Lack of field studies to validate performance • Catalyst often has a finite lifespan

Table 1: Summary of air-cleaning technologies. Adapted from table 1 of the EPA residential air cleaners technical summary. EPA 402-F-09-002 | July 2018 | EPA Indoor Environments Division.

Plasma	Microbes	Conversion: Electric current is applied to create an electric arc; incoming gases are ionized and bonds are broken to chemically transform the gaseous pollutants	• Can have high removal efficiency • Can be combined with other air-cleaning technologies (e.g., PCO) to improve performance and minimize byproduct formation	• Wide variety of plasma generation types yields confusion on how a product actually works • Byproducts are formed from many plasma technologies, including particles, ozone, formaldehyde, carbon monoxide, chloroform, nitrogen oxides, and a large number of other organic gases • Most studies have investigated gaseous removal while fewer have evaluated particle removal
Intentional ozone generation	Gases	Conversion: Intentional generation of ozone using corona discharge, UV, or other method to oxidize odorous compounds and other gases	• Reacts with many indoor gases • Can be combined with other less harmful technologies such as adsorbent media	• High ozone generation rates • High amounts of byproduct formation • Can cause degradation to indoor materials

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Air purifiers claimling to remediate VOCs use one, or more, of the 4 main technologies addressing gasses: Catalytic oxidation, plasma, ozone generation and adsorbent media.

Catalytic oxidation

Also called **photocatalytic oxidation** (PCO), this technology is used in many recent air purifiers and is based on the activation of compounds such as titanium dioxide by photons, usually in the UV range. These now activated catalysts are able to oxidize air molecules they come in contact with. The most concerning drawback of PCO is its lack of specificity: in some cases, **air cleaners based on these technologies can generate very harmful byproducts, including formaldehyde**. For example, the oxidation of ethanol produces acetaldehyde, a type 2B carcinogen. Ref.

Plasma

Plasma air purifiers apply high-voltage discharges to ionize incoming gasses, breaking their chemical bonds. They can have high removal efficiency, specifically against VOCs, but **may form a number of harmful byproducts**, such as carbon monoxide, ozone and formaldehyde.

Ozone generation

The generation of ozone indoors is harmful, as it is a strong lung irritant. The EPA report states "Do not use ozone generators sold as air cleaners in occupied spaces. No federal agency has approved ozone generators for use in occupied spaces."

Adsorbent media

More often than not, the media in question is **activated carbon**, aka activated charcoal. This material has the interesting property of possessing a large amount of micropores or cavities, highly increasing its surface area. These cavities trap various chemical compounds, which makes it a popular solution for water and air purification for various pollutants. Its efficiency depends on many factors, including the type of VOC and the air cleaner design. But in all cases, the biggest drawback comes from the fact that **the VOCs are only adsorbed on the surface of activated carbon**. This leads to relatively quick saturation of the media, and can cause the release of these pollutants back into the indoor air in certain conditions, like heat, or if filters are not changed.

Plants as natural air purifier

In 1989, **NASA** launched a research project, led by Wolverton, to study biological life support systems for space travel. Ref. The results of this study suggests that various plants, and their associated microorganisms, have interesting removal properties for some VOCs. This sparked widespread public perception that potted plants positively impact indoor air quality, fueled by scores of articles about the **"best plants to purify the air in your home".** But, contrary to popular belief, while the plant's ability to take up some VOCs is well documented in laboratory studies, the effect of regular potted plants on indoor air in complex environments, like a bedroom, is not as clear cut. Ref. **Dozens of potted houseplants would be needed to have an impact in a single room, which is not practical.**

Synthetic Biology and Neoplants

Faced with the inadequacies of traditional mechanical air purifiers, and the inefficiency of potted plants, people that would like to live in a healthy indoor environment do not have a real solution. At Neoplants, we believe in leveraging the power of Nature to create sustainable solutions to difficult problems. We do this through a groundbreaking type of technology, merging engineering and biology, called Synthetic Biology, or SynBio.

Using a combination of molecular biology, plant physiology and microbiome engineering, we created a bioengineered plant system that can effectively fight air pollution. Giving Nature the ability to remediate the pollution caused by petro-chemical industries that ends up in our lungs in the form of VOCs.

Our first product, Neo Px, is designed to fight against the most prevalent, harmful and difficult to target indoor air VOCs: Benzene, Toluene, Ethylbenzene and Xylene (BTEX).

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BTEX is a class of VOCs that have in common a very stable 6 carbon benzene ring. These compounds are constantly present in indoor air and the chronic exposure to them can cause a wide range of health issues, from fatigue, headaches, nausea and skin irritation, to more serious blood, central nervous system and upper respiratory tract problems. Ref. Ref. Ref. Ref.

General Approach

Nature as a depollution powerhouse

At its core, the problem we are tackling is simple: **how can we maximize the VOC depollution capacity of houseplants** so they have a positive impact on the indoor air quality of a room. However, not relying on electricity to power our technology is a major design constraint we have chosen to give ourselves. It's an engineering problem, but one that should use biology and not traditional technology as a solution, turning it into a bioengineering problem.

We realized this breakthrough by masterfully integrating three unique components: a bioengineered microbiome, an exceptionally effective air-purifying houseplant, and a meticulously optimized planter, which we have aptly named "the Shell."

Bioengineered microbiome

Every plant exists not in isolation but within a vibrant community comprising billions of beneficial fungi and bacteria. This complex network forms a microbiome that plays a pivotal role in many of the plant's traits, such as nutrient absorption, resistance to harmful pathogens and resilience to stress. Of particular importance to our application is the potential of plant-associated microorganisms to **increase Volatile Organic Compounds (VOCs) phytoremediation**. Ref. Ref. Ref.

In light of this, we have meticulously engineered the microbial community of our plant through directed evolution techniques. Our goal was to amplify the depollution prowess of this biological system, thereby optimizing its effectiveness in cleansing the environment.

Air-purifying houseplant

Choosing the right initial plant to create Neo Px, was crucial. We chose Epipremnum aureum, commonly known as pothos, because of its physiological characteristics:

- This plant boasts remarkable phytoremediation abilities, thanks to its large, waxy leaves and rapid growth rate, enabling it to absorb significant amounts of Volatile Organic Compounds (VOCs) per unit mass. Intrinsically, it also harbors genes that encode for endogenous enzymes capable of mitigating certain VOCs.
- It's a good houseplant. Being very robust in various indoor conditions, requiring low maintenance, and being a perennial plant, can survive and grow continuously for decades.

The Shell

The "Shell," the designated planter for Neo Px, is ingeniously crafted with dual purposes:

- To optimize airflow exposure to Neo Px, thereby significantly improving the plant system's capacity for environmental depollution.
- To greatly ease plant maintenance through the incorporation of a water tank and a water level indicator, which grants the system up to a month of autonomy.

The air flow between the outer layer and the inner basket is optimized thanks to openings located at the top and bottom of the planter. The basket holds the plant and keeps the soil hydrated thanks to capillary transfer of water through two small ropes running from the water tank to the soil.

Figure 1: Front and Sectional Views of The Shell

Crafted from PLA-Flax, a biocomposite derived from sustainable crops like beetroot and sugarcane, and reinforced with up to 30% flax fibers, this ten-piece structure offers unmatched durability and a unique, speckled look that showcases its eco-friendly origins. It merges technological advancement with sustainable design, making it a sophisticated, eco-conscious addition to any indoor space. Importantly, it's free from VOC emissions, lacks electronic components, and operates without electricity, negating the need for filters or batteries. This approach significantly reduces its environmental impact while enhancing indoor air quality.

Microbiome Engineering

General concept

The objective of our **microbiome engineering efforts** is to customize the composition of the Neo Px's microbial ecosystem to maximize its VOC depollution efficiency. To choose the right microbial strain, we tested dozens of carefully selected bacteria and fungi that can use different VOCs as carbon sources. In our series of experiments, one strain demonstrated exceptionally intriguing characteristics, notably its ability to utilize the BTX class of pollutants as its sole carbon source with remarkable efficiency. This strain is not only harmless to humans and animals but also proves to be beneficial for plant health.

The microbe's innate capability to metabolize VOCs as a carbon source is rooted in its genetic makeup. Specific genes encode for **enzymes** that catalyze biochemical reactions, establishing metabolic pathways that convert VOCs into benign microbial byproducts, such as amino acids. These byproducts serve as vital carbon sources, enabling the bacteria not only to flourish but also to support plant growth and health.

To enhance this natural process, we employed lab-assisted artificial evolution, or directed evolution, to refine the microbe's VOC metabolism efficiency.

Furthermore, we optimized the microbe's capacity for establishing enduring associations with plants. This advancement led to the formation of a stable microbiome that maintains its VOC degradation efficiency at an optimal level for extended periods, ensuring a consistent and lasting benefit.

Directed Evolution

Directed evolution is a step by step process that selects for, and enhances, the VOC degrading properties of microbes. A few milliliters of culture contains hundreds of billions of bacteria, some harboring tiny differences in genetic sequences, a subset of which will, by random chance, code for a slightly **increased efficiency in VOC metabolism.** We introduce in this culture a concentration of liquid VOC that is slightly higher than the initial tolerance level of these bacteria. The very few cells that survive are those that harbor the genetic code for this slightly increased VOC remediation efficiency. We then multiply those bacteria to recreate a population size of hundreds of billions of slightly enhanced organisms.

We repeated this cycle dozens of times over 5 years to create organisms highly evolved for bioremediation of VOCs.

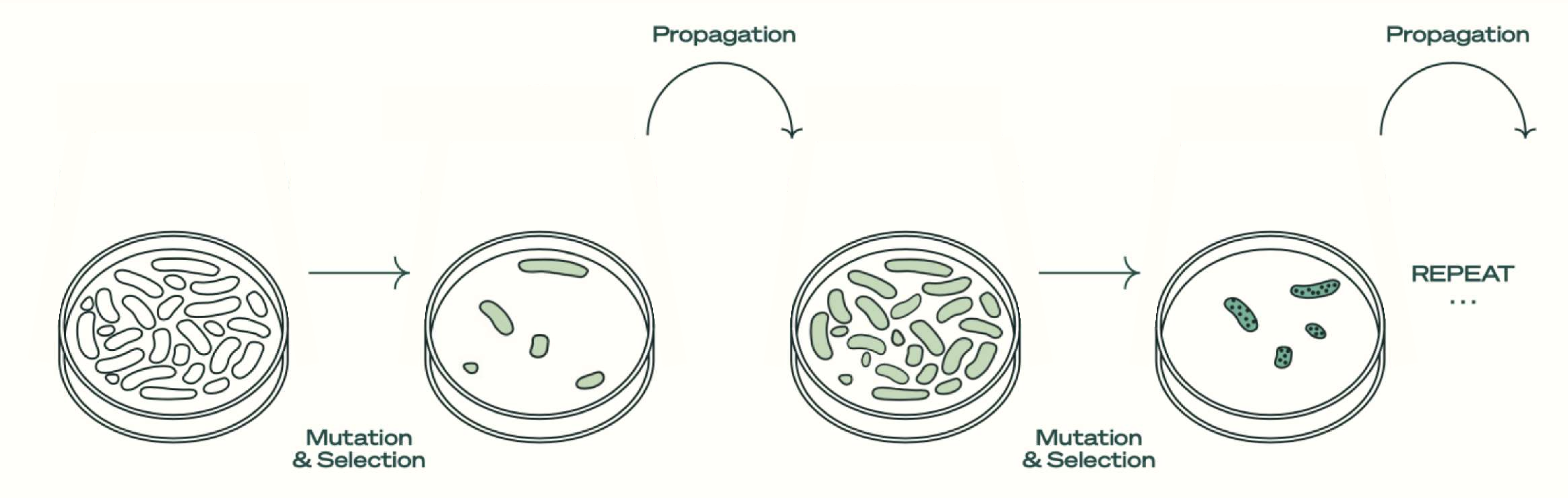

Figure 2: General directed evolution scheme Ref.

Targeting BTX

To address the challenge of metabolizing BTX pollutants, we screened a variety of microbes and identified a strain of **Pseudomonas putida** with exceptional bioremediation capabilities. This particular strain, when inoculated on Epipremnum aureum, exhibited unparalleled efficiency in degrading BTX pollutants. Among the various microbes tested, this Pseudomonas putida strain stood out for its high bioremediation performance.

This strain is classified as a rhizosphere bacterium, thriving in the soil around and within plant roots, creating a symbiotic relationship with its host plant. To enhance its bioremediation capacity, **the strain was subjected to numerous cycles of directed evolution in our laboratory.** This process involved exposing the bacteria to gradually increasing concentrations of toluene and benzene, simulating challenging environmental conditions. Through automated processes, we meticulously advanced the strain's ability to break down these pollutants, specifically when paired with Epipremnum aureum.

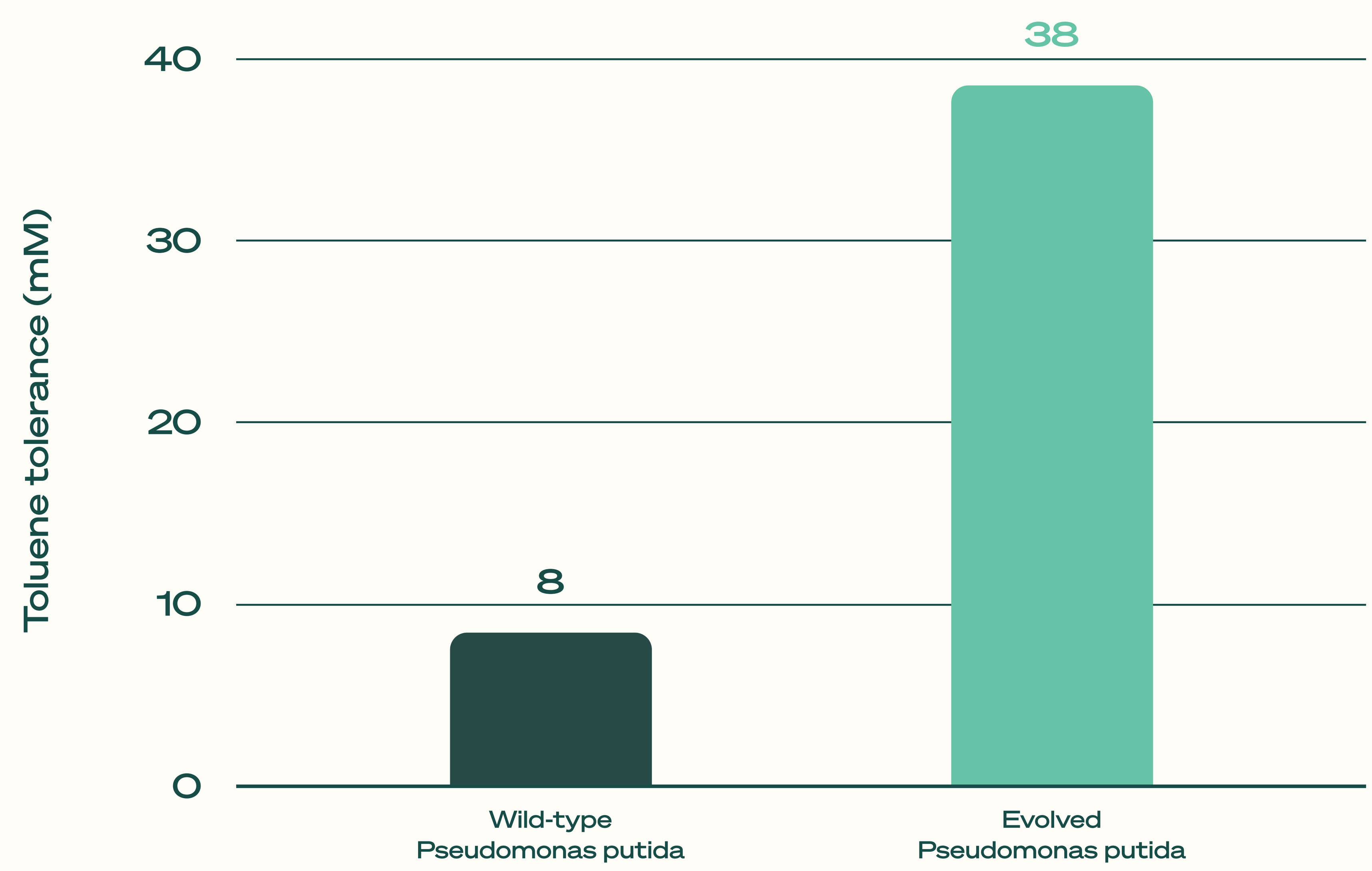

Figure 3: Increase of Pseudomonas putida tolerance to liquid toluene (mM) before and after directed evolution rounds

Enhancing VOCs degradation using the Powerdrops

Our Powerdrops contain l**yophilized cells of our Pseudomonas putida engineered strains, as well as everything they need to thrive** in the soil of Neo Px and keep their populations high. These bacteria are also known to interact closely with the plant, mostly within its rhizosphere, where they exchange chemical compounds including sugars, minerals, nitrogen, and some pollutants. Hence, both the plant and its microbiome participate in the degradation of VOCs.

Acknowledging our bacteria's specific metabolic needs, we crafted a tailored formulation for Powerdrops to: (1) support bacterial growth, (2) enhance BTX metabolism, and (3) extend shelf life, ensuring an efficient, durable bioremediation solution.

To optimize the Power Drops' impact, **monthly inoculations** are advised for sustained bioremediation and pollutant breakdown. For potency and extended shelf life, refrigeration is crucial, preserving bacterial viability for lasting effectiveness.

For application, dissolve a pouch's contents in water, mixing to a uniform solution, and evenly apply over Neo Px's soil for thorough integration. This streamlined approach ensures Power Drops' benefits are maximized, supporting consistent and effective pollutant degradation.

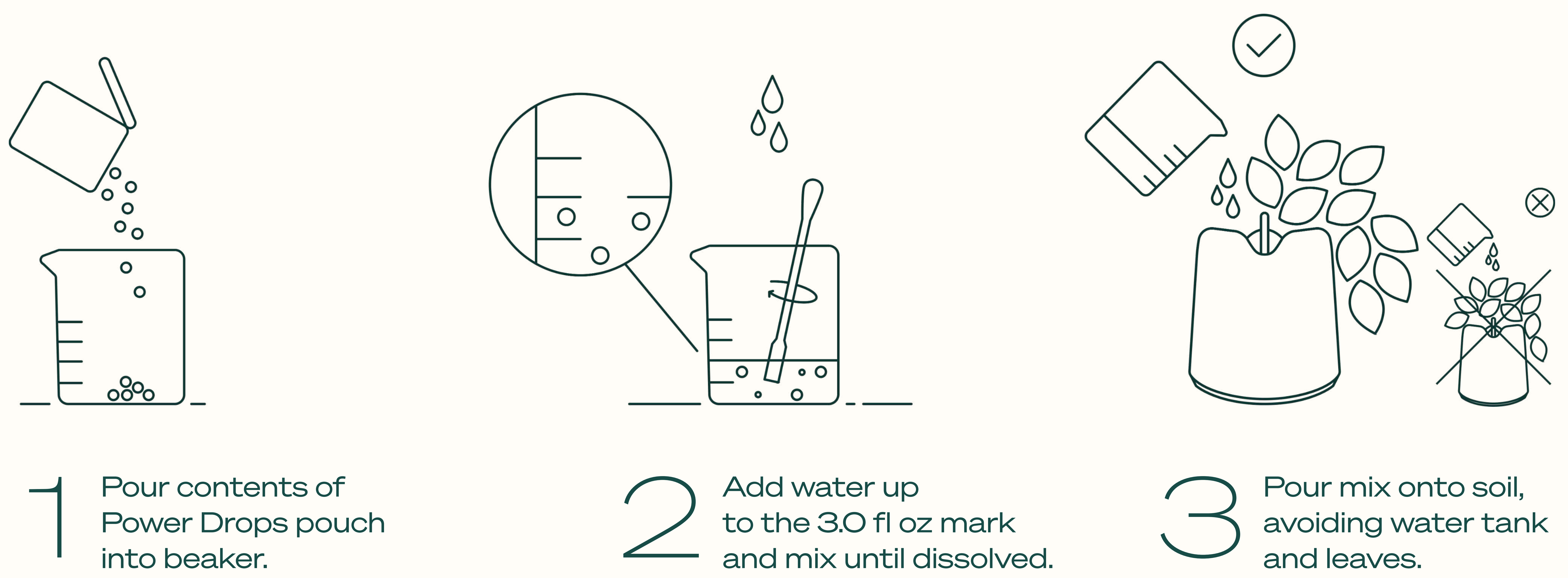

Figure 4: Process Summary for Inoculating Neo Px with Power Drops

Experimental Procedure

In order to assess the capacity of Neo Px to capture VOCs, we designed an experiment in which Neo Pxs were placed in a **35L glass chamber with a continuous flow of Toluene at around 750 ppb** (parts per billion).

The output gas is measured by a specific analyser per gas (HC51M Ref.). The tests were designed and run in collaboration with *IMT Nord Europe*, CERI EE, one of the leading institutes on indoor air quality in France. (Figure 5).

The system was tested to ensure it was leak-proof, and the air exchange rate of the empty chamber was 0.65 L/h. The Neo Pxs were tested over a period of 3 days to calculate the Clean Air Delivery Rate (CADR) normalized by plant weight. CADR being a common metric used by traditional mechanical air purifiers.

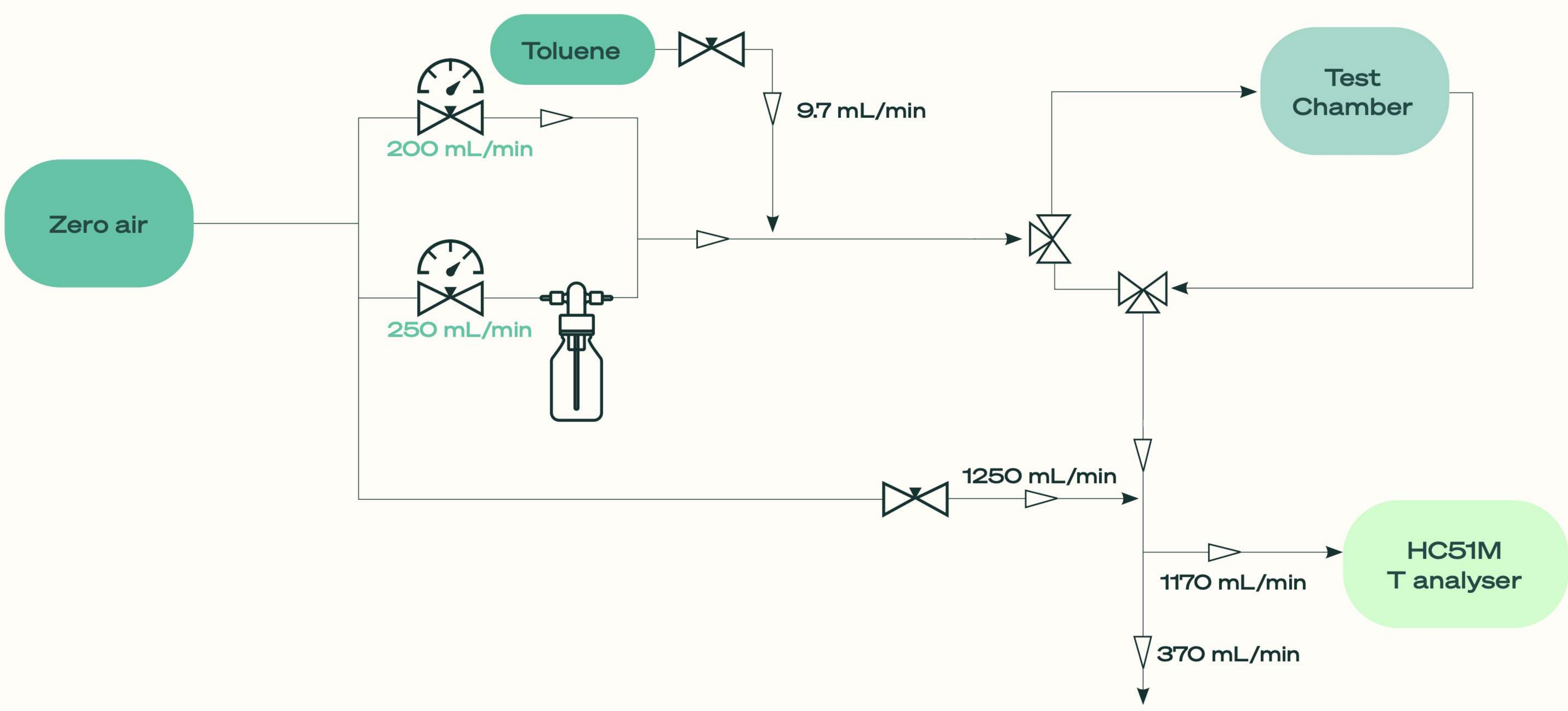

Figure 5: Visual representation of the experimental setup used to test the performance of Neo Px

Neo Px compared to Epipremnum aureum

By continuously measuring the concentration of Toluene of the outlet gas after a single passage through the test chamber, **we observed a stabilization around 110 ppb for the non-enhanced Epipremnum aureum and at 90 ppb for Neo Px** (Figure 6-A).

In this context, we calculated the CADR for both Neo Px and the normal plant, and normalized these values by their weight to avoid bias due to plant size. **The CADR/weight for Neo Px is 1.33 for Neo Px and 0.14 for Epipremnum aureum** (Figure 6-B).

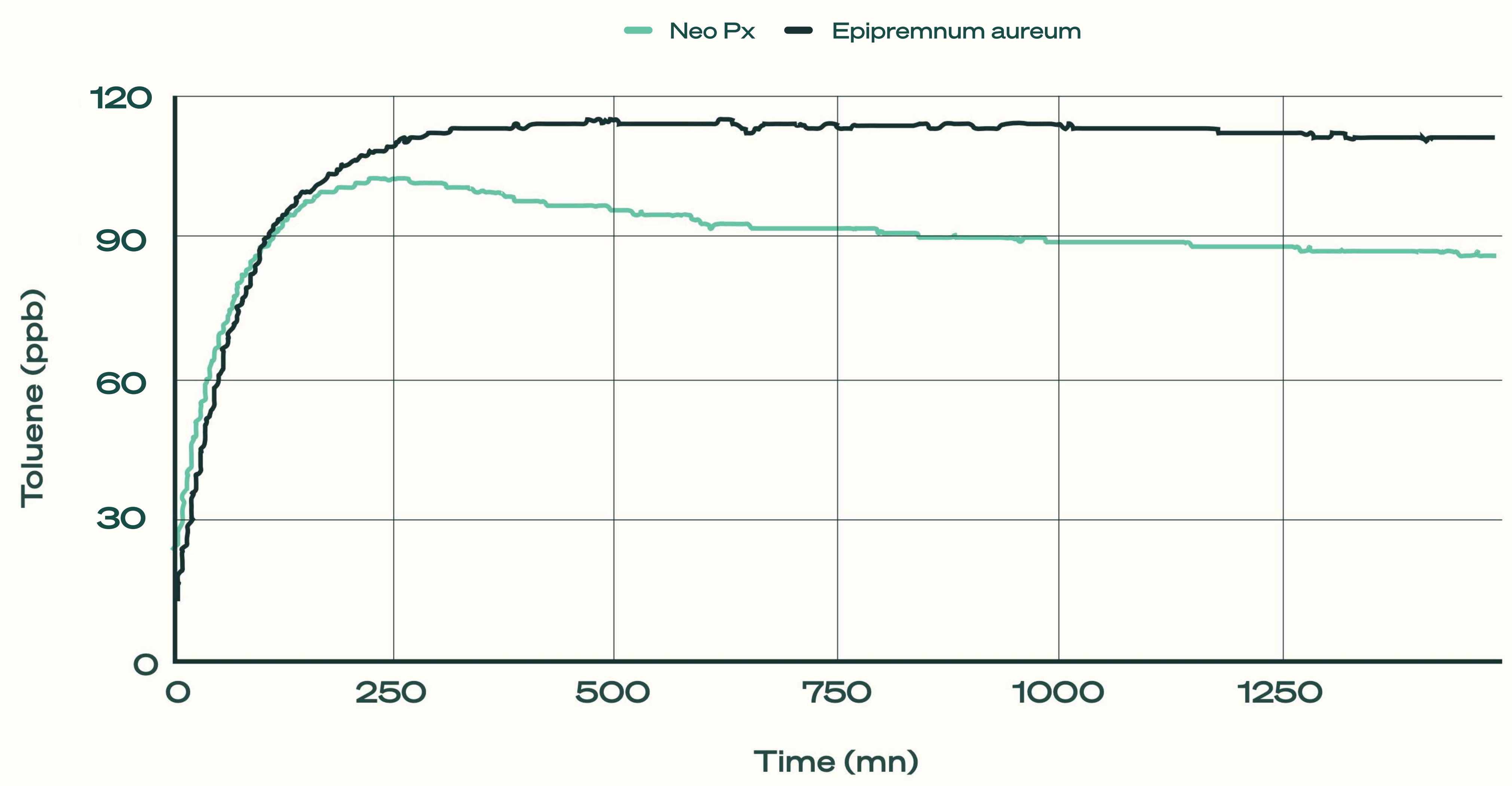

Figure 6-A: Toluene outlet concentration after single passage through the test chamber containing either Neo Px or non-enhanced Epipremnum aureum

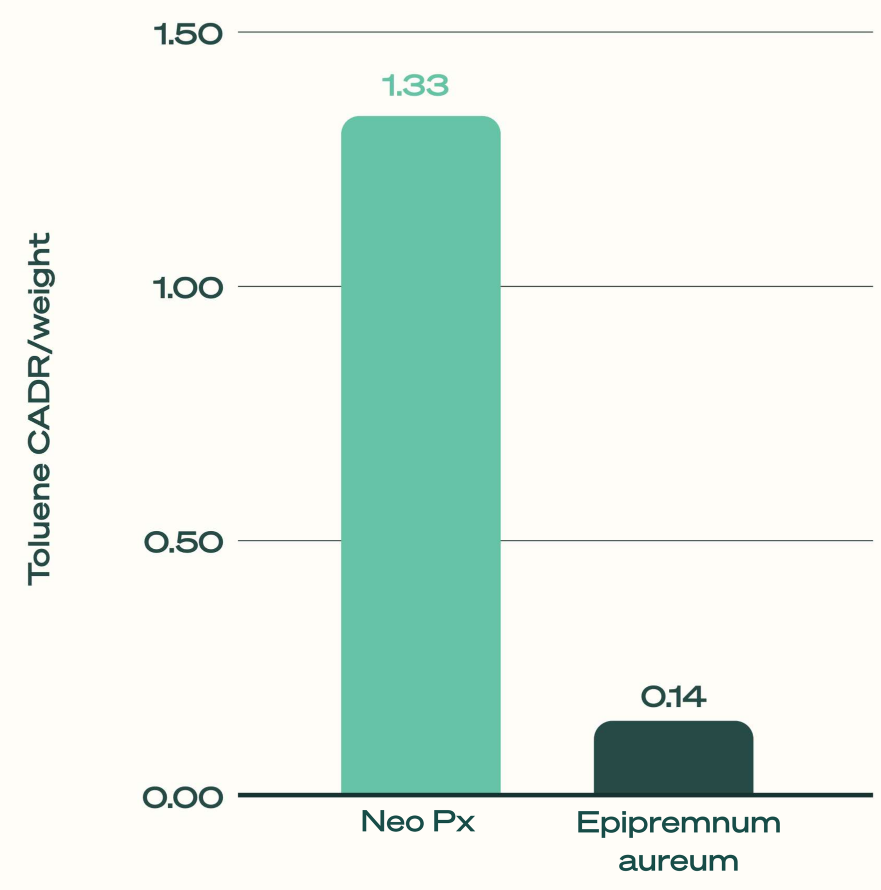

Figure 6-B: Toluene CADR of Neo Px & non-enhanced Epipremnum aureum normalized by plant weight

Neo Px compared to other regular houseplants

The ability of regular indoor houseplants to clean the air has been extensively discussed in popular articles and studied in scientific literature. The most frequently cited "air cleaning" houseplants include Epipremnum aureum (Pothos), Spathiphyllum (Peace Lily), Chlorophytum comosum (Spider Plant) and Dracaena trifasciata (Snake Plant).

Using the same approach as described above, we compared the bioremediation efficiencies of these plants forToluene (Figures 7).

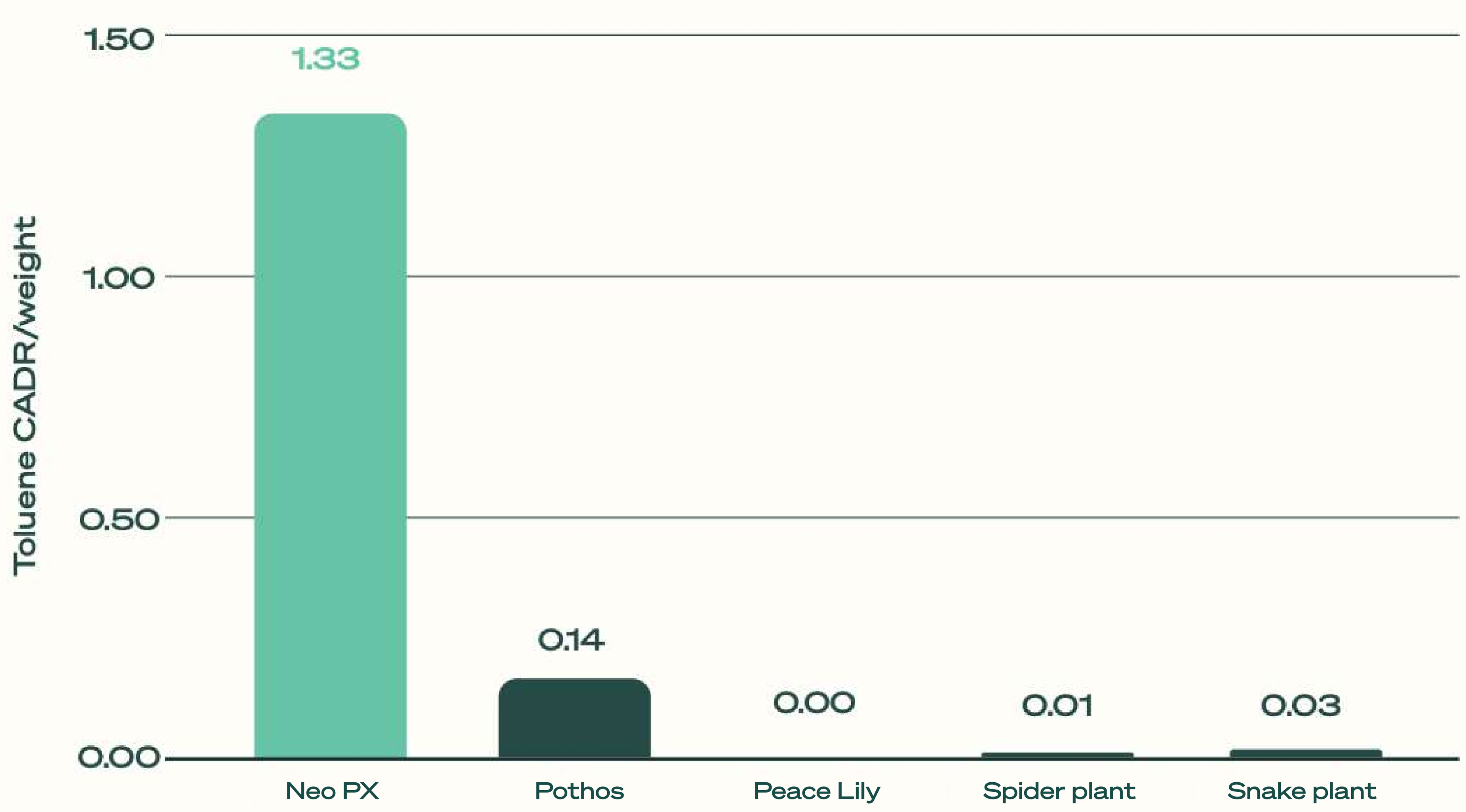

Figure 7: Comparison of the bioremediation efficiencies of a selection of indoor houseplants for Toluene

Results And Conclusion

The performance results presented in the previous section demonstrate a significant disparity in efficacy between Neo Px and conventional houseplants. This disparity is particularly evident in the Clean Air Delivery Rate (CADR), where Neo Px exhibits markedly superior capabilities.

Specifically, in the context of Toluene bioremediation, the CADR for Neo Px is thirty times higher than the average CADR of other houseplants commonly recognized for their air-purifying properties.

Neo Px is the fruit of several years of synthetic biology efforts, using the latest technologies of microbiome engineering combined with custom designed hardware. It has drastically improved phytoremediation efficiencies on the most harmful and ubiquitous indoor VOCs compared to regular houseplants. It has the potential to improve the health and wellbeing of millions of people worldwide, while being a sustainable solution that has drastically less negative impact on the environment and the planet (figure 8).

	Traditional Air Purifiers	House Plants	Neo Px
Benzene	✗	✗	✓
Toluene	✗	✗	✓
Xylene	✗	✗	✓
Particulate Matter	✓	✗	✗
Sustainability	✗	✓	✓

Figure 8: Value proposition of Neo Px

Consequently, embracing Neo Px for indoor air purification not only highlights a significant leap in synthetic biology and environmental engineering but also sets a new benchmark for sustainable living spaces, marrying technological innovation with ecological harmony.