A lack of consensus in the literature findings on the removal of airborne benzene by houseplants:
effect of bacterial enrichment

Wararat Sriprapat¹, and Stuart E. Strand^{2*}

¹School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand.

²Department of Civil and Environmental Engineering, University of Washington, Seattle, WA, USA.

*Corresponding author address: Department of Civil and Environmental Engineering, P.O. Box 355014, University of Washington, Seattle, WA 98195-5014; phone: 206-543-5350; Fax: 206-685-9996; e-mail: sstrand@u.washington.edu.

Abstract

Removal rates of benzene and formaldehyde gas by houseplants reported by several laboratories varied by several orders of magnitude. We hypothesized that these variations were caused by differential responses of soil microbial populations to the high levels of pollutant used in the studies, and tested responses to benzene by plants and soils separately. Five houseplant species and tobacco were exposed to benzene under hydroponic conditions and the uptake rates compared. Among the test plants, *Syngonium podophyllum* and *Chlorophytum comosum* and *Epipremnum aureum* had the highest benzene removal rates. The effects of benzene addition on populations of soil bacteria were determined using reverse transcription quantitative PCR (RT-qPCR) assays targeting microbial genes involved in benzene degradation. The total bacterial population increased as shown by increases in the levels of eubacteria 16S rRNA, which was significantly higher in the high benzene incubations than in the low benzene incubations. Transcripts (mRNA) of genes encoding phenol monooxygenases, catechol-2,3-dioxygenase and

the housekeeping gene *rpoB* increased in all soils incubated with high benzene concentrations. Therefore the enrichment of soils with benzene gas levels typical of experiments with houseplants in the literature artificially increased the levels of total soil bacterial populations, and especially the levels and activities of benzene-degrading bacteria.

Key words: Benzene; hydroponic; phytoremediation; soil microbial; volatile organic compounds; formaldehyde.

Highlights

- Reported houseplant benzene removal rates vary over several orders of magnitude.
- The variations were likely caused by high concentrations of benzene in the assays.
- High levels of benzene increased soil bacterial populations and activities.
- Standard procedures for houseplant pollutant removal experiments are proposed.

1. Introduction

Household plants have been widely promoted as having the ability to remove air pollutants from indoor air. This approach is known as the “green liver” concept and is a central idea of the field of phytoremediation, the use of plants to remove xenobiotic pollutants from the environment (Sandermann, 1994). Several previous reports have proposed treating air pollutants using plants (Wolverton et al. 1984; Wolverton et al. 1989; Jen et al. 1995; Conrejo et al. 1999; Liu et al. 2007; Nelson and Wolverton 2011). Total VOCs were reported to be reduced 50 to 75 % in enclosures by potted plants (Wood et al., 2006). Early studies of benzene detoxification by

household plants found that plants can absorb and transform benzene, and use it as a carbon source (Ugrekheldze et al., 1997; Kvesitadze et al., 2009). We compared the reported rates of benzene removal from the air by seven plant species growing in potting soils in studies by five different laboratories (Orwell et al., 2004; Yoo et al., 2006; Liu et al., 2007; Yang et al., 2009; Mosaddegh et al., 2014) (Table 1). All of these studies were conducted as batch tests with plants potted in soil contained in closed vessels, dosed with benzene or formaldehyde injected into the headspace of the vessels, and with analysis of air samples to provide a time course of pollutant removal. In some studies the removal rates were corrected for leak rates and for losses with the potting media only. We also compared the reported rates of formaldehyde removal by seven plant species in studies by six different laboratories (Kil et al., 2008; Kim et al., 2009; Kim et al., 2010; Lim et al., 2009; Aydogan and Montoya, 2011; Jin et al., 2013; Khoa et al., 2013) (Table S1 in the supplement data). Due to limitations on the analytical techniques applied to small sampling volumes, the concentrations used in these tests were several orders of magnitude greater than those representative of home air, approximately $2 \mu\text{g benzene m}^{-3}$ and $22 \mu\text{g formaldehyde m}^{-3}$ (Logue et al., 2011).

Although they were several orders of magnitude higher than typical home air concentrations, the airborne concentrations of benzene that were used in the reported experiments were equivalent to less than 0.2 mg L^{-1} in an equilibrated aqueous solution. This concentration is about 10 times lower than reported Michaelis-Menten half saturation constants for benzene (Chang et al., 1993). Therefore it is likely that the reported removal kinetics in the test vessels was controlled by pseudo first-order kinetics. Accordingly, we approximated the pseudo first-order rate constants by dividing the reported removal rate by the reported initial pollutant concentration. The estimate rate constants (Table 1 for benzene and Table S1 for formaldehyde) also show a high degree of variation over several orders of magnitude, showing that the

variability between studies cannot be explained by first-order effects alone. Based on these reported results it is clear that no indoor plant genus has been shown to be superior to any other, and that there is no consensus in the literature among laboratories on rates of removal of benzene.

What accounts for these huge variations in plant efficacy for pollutant removal? A likely explanation is that soil bacteria responded to the increase in benzene availability by inducing increased expression of the benzene degradation pathway and by increased growth of the benzene degrading populations. Degradation of VOCs e.g. benzene, toluene, ethylbenzene or xylene in soil has been linked to bacterial activities by several studies (Thomas et al., 1990; Morgan et al., 1993). It is also possible that variations in apparatus, materials, procedures, and soil adsorption corrections may have caused the wide variation in removals, but without experimental controls in common between studies, one cannot compare or understand the results of these disparate studies.

In our studies we analyzed plant uptake of benzene in hydroponics and separately analyzed the effects of benzene exposure on soil bacterial populations. We compared the uptake rates of benzene by five houseplant species and tobacco exposed to high levels of benzene under hydroponic, soil-free conditions. We also report the effects of benzene addition on populations of benzene-degrading bacteria as reflected in the levels and expression of several genes in the benzene degrading pathway. In this experiment, phenol monooxygenase, toluene monooxygenase, toluene dioxygenase and catechol 2, 3 dioxygenase genes which are known to occur in bacteria that catabolize benzene and other aromatic compounds (Eltis and Bolin, 1996; Baldwin et al., 2003) were used to monitor benzene degradation in soil. Previous studies of houseplant soil populations (Russell et al., 2014) did not measure the effects of VOC enrichment on microbial populations quantitatively, which we designed our experiments to determine.

2. Materials and methods

2.1 Plant material and micropropagation

Six plant species – *Epipremnum aureum*, *Chlorophytum comosum*, *Syngonium podophyllum*, *Hedera helix*, *Dracaena godseffiana* and *Nicotiana tabacum* – were purchased from plant shops in Seattle WA. Nodal, bud, stem, shoot tip segments or seed of plant were excised from the healthy plants. In order to produce sterilized plants, all the collected explants were first washed thoroughly in running tap water for 15-20 min to remove the dust particles. These were then surface sterilized by immersing in 70% (v/v) ethanol for 30 s and then soaking in 10% (v/v) sodium hypochlorite containing 4–5 drops of Tween 20 emulsifier for 30 min with vigorous shaking, and again washed with sterile deionized (DI) water to remove any traces of detergent for 10 min.

After three rinses with sterile DI water and removal of the damaged ends, the explants were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with Gamborg vitamin (Gamborg et al., 1968) and 20 g L⁻¹ sucrose and 3.6 g L⁻¹ phytoigel. The pH of the medium was adjusted to 5.8 prior to autoclave. Cultures were maintained at a temperature of 25 °C under fluorescent light with a 16 h photoperiod. The outgrowths of the plant cuttings were used for the subsequent experiments.

2.2 Benzene treatment of sterilized plants, sampling and analysis

After growing plants for 4-8 weeks, sterilized plants from each species (4-6 leaves, 8-9 cm in height) were placed in clear glass volatile organic analysis (VOA) vials, 40 mL nominal volume, containing 10 mL of liquid MS medium mixture with 20 g L⁻¹ sucrose (pH 5.8).

In this experiment, two treatments in triplicate were used to study benzene removal efficiency of each plant species; sterilized plants and negative controls (MS medium only, i.e., –

no plant). The vials were capped with septum valves (Mininert), then 120 μg of benzene from a stock solution in water was injected into each VOA vial. The mass of benzene in the stock solution and in the experimental vials was calculated based on volume, solubility in water and temperature assuming equilibrium using a dimensionless Henry's law constant at 25 °C of 0.228 (US EPA, 1996). Plants were maintained at a temperature of 25 °C under fluorescent light with a 16 h photoperiod.

To determine the rate of removal of benzene gas, 50 μL of air in each VOA vial was manually injected into a gas chromatograph—with flame ionization detector (GC–FID) (Perkin–Elmer, Autosystem gas chromatograph) using a Restek Rtx–1 column (15 m $\times$ 0.32 mm, 0.25 μm I.D.). The analytical conditions for GC–FID were 250 °C injector temperature, 60 °C oven temperature and 250 °C detector temperature. Sample injections were performed in triplicate with 50 μL of gas sample taken from each vial. Initial headspace samples were taken 1–2 h after the introduction of benzene into the vials to allow for air-liquid equilibrium. Headspace samples were analyzed every 24 h for 144 h. At the End of the incubation all of the plants appeared healthy.

2.3 Microcosm experiment

Microcosm experiments were conducted to determine the effects of varying concentrations of benzene gas on the soil bacterial community and especially the benzene degrading population. A commercial garden soil mix (Miracle–Gro lawn products, Inc., Ohio) was purchased and aliquots of 100 g of soil were added to a 950 mL glass chamber capped with polyethylene lids fitted with brass tubes sealed with rubber septa. All experimental soil chambers were pretreated with 0.05 $\mu\text{g m}^{-3}$ of benzene gas for 2 weeks in order to acclimate the soil bacterial populations to levels of benzene typical of home air (de Bruin et al., 2008). Then

experimental soils were assigned to one of the following treatments: 0.05, 10, 100, 1,000, 10,000 and 100,000 $\mu\text{g m}^{-3}$ of benzene gas for 144 h. In addition, 50 μL of benzene solution was injected into the experimental soil chamber to produce $1.91 \times 10^9 \mu\text{g m}^{-3}$ of benzene gas and used as a positive control for this experiment. Each treatment level was replicated with triplicate soil chambers. After 7 days of exposure, 3 g of soil samples were taken from each of the three replicate of each treatment and placed into sterile polypropylene tubes, which were immersed in liquid nitrogen for 3–5 min and stored immediately at -80°C for subsequent molecular analysis of DNA and RNA.

2.4 Extraction of DNA and RNA from soil

Total DNA and RNA were extracted from 0.25 g (for DNA) or 2 g (for RNA) of soil using a PowerSoil DNA isolation Kit and RNA PowerSoil total RNA isolation Kit (Mo Bio Laboratories, Inc.), respectively. Then DNA and RNA were quantified using a Nanodrop spectrophotometer (ThermoFisher Scientific). All DNA and RNA were stored at -80°C until further analysis by PCR.

2.5 Primer selection and PCR amplification

PCR was used to amplify and detect the following genes: bacterial 16S rRNA, rpoB, phenol monooxygenase, catechol-2,3-dioxygenase (C23O), toluene monooxygenase and toluene dioxygenase genes. The sequences of the primers used and the genes targeted are shown in Table 2.

PCR reactions were performed in 20 μL volumes containing 10–20 ng DNA, 4 μL of $5\times$ green GoTaq Flexi buffer, 10 mM each dNTP (Thermo Scientific), 50 mM MgCl_2 , 0.5 U GoTaq Flexi DNA polymerase, 5 μM of each primer, 4% of DMSO and molecular grade water. The

PCR temperature program began with initial 2 min denaturation step at 94 °C (one cycle); 35 cycles of 94 °C for 30 s, annealing temperature as detailed in Table 2 for 30 s, 72 °C for 30 s, and a final 5 extension step 72 °C. PCR products were analyzed by electrophoresis on 1% (w/v) agarose gel.

Positive control DNA for the 16S rRNA gene was obtained from *Escherichia coli* carrying plasmid TOPO-pCR2.1 (Invitrogen) with the cloned 16S rRNA gene of *Dehalococcoides* sp. strain BAV1 (Ritalahti et al., 2006). DNA for phenol monooxygenase was obtained from *E. coli* carrying plasmid pVI261 with phenol hydroxylase genes (*dmpKLMNOP*) of *Pseudomonas* CF600 (Bartilson et al., 1990). DNA for C23O was obtained from *E. coli* carrying the plasmid pCM168 with *Methylobacterium extorquens* strain AM1 catechol-2,3-dioxygenase (Marx and Lidstrom, 2004). DNA for toluene monooxygenase was obtained from *E. coli* carrying the plasmid pSE380 with *Burkholderia* sp. strain JS150 toluene-4-monooxygenase (Johnson and Olsen, 1997). DNA for toluene dioxygenase was obtained from *E. coli* carrying the plasmid pDTG601 with toluene dioxygenase genes *todC1C2BADE* from *Pseudomonas putida* F1 (Zylstra and Gibson, 1989). DNA for *rpoB* was obtained from *E. coli* carrying plasmid pGEM-T with *rpoB* gene of *E. coli*. Clones were grown overnight and the plasmids were extracted with QIAprep Spin MiniPrep kit (Qiagen) according to the kit protocol.

2.6 Reverse transcription

Reverse transcription was performed to convert mRNA to cDNA using the First-Strand cDNA synthesis kit for RT-PCR, (AMV, Roche) according to the manufacturer's protocol. cDNA synthesis reactions were carried out at 42 °C with 70–80 ng of total RNA and random hexamer primers. A negative control containing all reagents except for the RNA template was also prepared. After reverse transcription process of mRNA, the concentration and quality of the

cDNA was determined using a Nanodrop spectrophotometer (ThermoFisher Scientific), and 2 μ L of the sample was PCR-amplified and analyzed by electrophoresis on 1% (w/v) agarose gel to check size and integrity of the synthesized cDNA.

2.7 Quantitative PCR (qPCR) and reverse transcription quantitative PCR (RT-qPCR)

Analyses by qPCR and RT-qPCR were performed on DNA and RNA extracted from soil samples that had been incubated at each concentrations of benzene gas. After DNA and RNA extraction and reverse transcription process of mRNA, the DNA and cDNA were stored at -20 °C until their use as a template in qPCR assays.

qPCR was performed with a LightCycler (Roche Diagnostics, Mannheim, Germany) with LightCycler DNA Master SYBR green I (Roche Diagnostics). Each reactions was conducted in a 10 μ L volume containing 1 μ L of LightCycler DNA Master SYBR green I, 3.5 mM of $MgCl_2$, 5 μ M of each primers, 4 μ L of cDNA. The PCR primer sets in Table 2 were used to quantify 16s rRNA, rpoB, phenol monooxygenases and C23O coding gene copies. Amplification conditions used were as follows: 95°C 5 min, then 45 cycles of 95°C for 15 s and annealing temperature as detailed in Table 2 for 15, 72 °C for 30 s.

2.8 Statistical Analysis

The benzene removal data are presented as the mean $\pm$ SEM. Standard analysis comparing treatments was performed using two-way repeated measures (RM) analysis of variance (ANOVA) (SPSS, version 20.0) and followed by Tukey's HSD tests. For qPCR and RT-qPCR, to detect differential expression, one-way ANOVA was employed and followed by Tukey's HSD tests. Results with a $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1 Benzene removal by plants

As shown in Figure 1, *N. tabacum*, *E. aureum*, *H. helix*, and *C. comosum* removed a greater percentage of benzene from hydroponic experimental vessels than *D. godseffina* and *S. podophyllum* after 144 h (the statistical analysis of the time course of benzene removal (%) in hydroponic incubations of five houseplants and *N. tabacum* over time is shown in Table S2). After correction for variation in leaf area for each plant, the best benzene removal rates were *S. podophyllum*, *C. comosum*, and *E. aureum* (Figure 2). These results show that plant species differed significantly in their ability to remove indoor air pollutant in near sterile conditions. Furthermore, the first-order rate constants of benzene removal from the air of the sterile plants including *S. podophyllum*, *E. aureum* and *H. helix* were in line with the only previously reported experiment (Table 1; Yang et al., 2009) that used plants with roots washed of soil and repotted in pine bark, perlite and peat moss, indicating that removing the rooting soil may have value in the determination of the intrinsic ability of plants to remove benzene without the added effect of bacterial activity. These results suggest that both plants and soil microorganisms play roles in benzene removal from the air as reported in laboratory experiments using plants in closed vessels with soils and high levels of benzene.

3.2 Response of rhizosphere bacteria to benzene gas

Microbial DNA from soil samples before (D_0) and after (D_7) exposure to benzene was extracted and amplified using primers designed for the 16S rRNA gene in eubacteria, phenol monooxygenase, C23O, toluene monooxygenases and toluene dioxygenase genes.

The 16S rRNA gene for eubacteria was detected in soil samples at all of benzene concentrations, showing that bacteria were present in all experimental soils (Figure 3).

Amplicons of C23O, toluene monooxygenase and toluene dioxygenase genes were detected only in the high concentration positive control chambers (data not show), suggesting that lesser concentrations of 0.05 to 100,000 $\mu\text{g m}^{-3}$ benzene gas either were not sufficient to enrich for bacteria containing those genes or that those genes were enriched at levels below the agarose gel detection limits. However, several previous reports have proposed that an extradiol ring-cleavage by C23O is the predominant reaction in the meta cleavage of catechol-like metabolites from a wide range of aromatic compounds (Smith, 1990). Especially for the subfamily I.2.A, this group seems to be important for the degradation of monocyclic aromatic compounds (Eltis & Bolin, 1996). Therefore, the C23O gene was selected for further study with qPCR and RT-qPCR. In contrast, the gel electrophoresis results for the phenol monooxygenase amplicon shows that the phenol monooxygenase gene was present in the bacteria population (Figure 4). After exposure to additional benzene for seven days (D_7) the amplicon bands were stronger than prior to benzene exposure (D_0). Soil bacteria are known to be capable of degrading benzene, using it as a source of carbon and energy (Kim et al., 2008a). Phenol is produced in several bacterial pathways for mineralization of benzene (Gibson and Parales, 2000; Yerushalmi et al., 2001; Tao et al., 2004; Vogt et al., 2011). The increase in the levels of the phenol monooxygenase gene in our experimental soil in response to additional benzene supports the hypothesis that benzene levels typical of experiments in the houseplant air pollution literature support the growth of benzene degrading bacteria. .

3.3 Quantification of gene levels and expression by qPCR and RT-qPCR

Analysis of DNA extracted from the soils exposed to benzene gas showed that the total bacterial population as measured by the eubacterial 16S rRNA primers was significantly higher ($p < 0.05$) in the soils incubated with high concentrations of benzene gas compared to those incubated with

low concentration (Figure 5). We found a similar increase in the copy numbers of the phenol monooxygenases and C23O genes, which indicated that the soils incubated with high concentrations of benzene gas were significantly enriched in bacteria harboring these genes associated with benzene degradation compared to the soils incubated with low concentrations of benzene gas ($p < 0.05$) (Figure 5B and C). These results support the hypothesis that exposure to increased levels of benzene results in an increase in the total number of bacteria and in the number of bacteria containing genes for enzymes involved in the degradation of benzene.

The total bacterial transcriptional activity as measured by RT-qPCR of the eubacterial 16S rRNA gene and transcripts of a housekeeping gene, the beta subunit of DNA polymerase, *rpoB*, were significantly higher ($p < 0.05$) in the soils incubated with high concentrations of benzene gas compared to those incubated with low benzene concentration (Figure 6A and B). This result suggests that the presence of benzene gas promoted significant growth and activity of bacteria in the soil. Furthermore, the transcriptional activity of genes encoding the benzene-degrading enzymes, phenol monooxygenase and C23O increased with increasing concentration of benzene. The mRNA copy number of the transcript of those genes in soil that was exposed to high concentration of benzene gas was significantly more abundant than in the low concentration of benzene exposed soil ($p < 0.05$) (Figure 6C and D).

The significant increase of the 16S rRNA, *rpoB*, phenol monooxygenase and C23O genes with increasing enrichments of benzene gas suggest that exposure to benzene changed the number of soil bacteria and especially enhanced the activities of benzene degrading bacteria. Our findings are consistent with the significant shift in the 16S phylogeny of root associated microbial populations when houseplants were exposed to high levels of VOCs (Russell et al., 2014). Measurements by qPCR and RT-qPCR of DNA and mRNA, respectively, from soil exposed to high levels of benzene revealed that the copy number and expression levels of the genes encoding

phenol monooxygenases and C23O increased significantly in soils incubated with high concentrations of benzene that were typical of conditions in house plant studies. Although we tested only one soil source in this study it is likely that different sources of soils in other laboratory conditions would have responded differently to benzene exposure with their benzene degrading populations growing to different levels and activities, providing a possible explanation for the wide range of benzene uptake observed in houseplant studies. These speculations need confirmation by experimentation. Future studies should also examine the effects of increased levels of formaldehyde used in studies on the formaldehyde degrading microbial population as reflected in number and expression of genes in the formaldehyde degradation pathway.

Based on these results it is clear that the levels of benzene used in houseplant experiments can be expected to induce increased expression and abundance of benzene degrading populations in the soil, resulting in invalid estimates of benzene degrading activity that are not representative of their levels in potting soils in homes.

4. Conclusions

A comparative analysis of the house plant literature revealed huge discrepancies in the observed removal rates of benzene as reported by several laboratories for similar houseplant species. We hypothesized that these differences were due to the response of the soil microbial community to the high levels of pollutants that were used in the experimental design. We tested five houseplant species and tobacco by exposing them to high levels of benzene under hydroponic conditions with minimal microbial populations and comparing the uptake rates to correct for variations in the indoor pollutant removal. Among the test plants, *S. podophyllum*, *E. aureum* and *C. comosum* showed the highest benzene removal efficiency and first order removal rate.

The effects of benzene on populations of benzene degrading bacteria were determined and found that the total bacterial population as measured by the eubacterial 16S rRNA gene and by the activity of the rpoB gene was significantly ($p < 0.05$) higher in soils exposed to high concentrations of benzene compared to those incubated with low benzene concentrations. Thus the increase in the levels of the phenol monooxygenase gene in our experimental soil in response to additional benzene supports the hypothesis that benzene levels typical of experiments in the houseplant air pollution literature support the growth of benzene degrading bacteria.

To avoid these errors and to promote consistency in the houseplant phytoremediation literature, we propose that future comparative studies of abilities of indoor plants to remove pollutants from the air be conducted under the following conditions:

1. Hydroponic conditions with plants treated to remove epiphytic bacteria. Such conditions would minimize the activities of bacteria, which are likely to be induced by the high pollutant concentrations used in small scale lab tests.
2. Include in every study a common plant such as tobacco, *Nicotiana tabacum*, so that studies from various labs can be normalized to a common standard. *N. tabacum* has several advantages as a standard plant: large leaf area per plant, complete genome sequence, extensive botanical and physiological literature, general availability, and clonal propagation.
3. All controls for leaks and for soil only experiments should be completely discussed and data shown.

Acknowledgements

We thank Quyen Nguyen, Kelley A. Meinhardt, Long Zhang and Manmeet Waria Pannu for their valuable help in the laboratory. Ms. Wararat Sriprapat would like to thank Dr. Paitip

Thiravetyan, and a financial support from Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program and King Mongkut's University of Technology Thonburi (Grant No. PHD/0284/2552). This work was partly supported by NSF grant CBET-1438266.

References

- Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A., 1990. Combination of 16s ribosomal-rna-targeted oligonucleotide probes with flow-cytometry for analyzing mixed microbial-populations. *Appl. Environ. Microbiol.* 56, 1919–1925.
- Aydogan, A., Montoya, L.D., 2011. Formaldehyde removal by common indoor plant species and various growing media. *Atmos. Environ.* 45, 2675–2682.
- Baldwin, B.R., Nakatsu, C.H., Nies, L., 2003. Detection and enumeration of aromatic oxygenase genes by multiplex and real-time PCR. *Appl. Environ. Microbiol.* 69, 3350–3358.
- Bartilson, M., Nordlund, I., Shingler, V., 1990. Location and organization of the dimethylphenol catabolic genes of *pseudomonas cf600*. *Mol. Gen. Genet.* 220, 294–300.
- Chang, M.K., Voice, T.C., Criddle, C.S., 1993. Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and p-xylene by two *Pseudomonas* isolates. *Biotechnol. Bioeng.* 41, 1057–1065.
- Cornejo, J.J., Munoz, G. F., Ma, Y. C., Stewart, J. A., 1999. Studies on the decontamination of air by plants. *Ecotoxicology*, 8, 311–320.
- de Bruin, Y.B., Koistinen, K., Kephelopoulos, S., Geiss, O., Tirendi, S., Kotzias, D., 2008. Characterisation of urban inhalation exposures to benzene, formaldehyde and acetaldehyde in the European Union. *Environ. Sci. Pollut. Res.* 15, 417–430.
- Eltis, L.D., Bolin, J.T., 1996. Evolutionary relationships among extradiol dioxygenases. *J Bacteriol.* 178, 5930–5937.

- 360 Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutrient requirements of suspension cultures of
361 soybean root cells. *Exp. Cell. Res.* 50, 151–158.
- 362 Gibson, D.T., Parales, R.E., 2000. Aromatic hydrocarbon dioxygenases in environmental
363 biotechnology. *Curr. Opin. Biotechnol.* 11, 236–243.
- 364 Jen, M. S., Hoylman, M.A., Edwards, T.N., Walton, T. B., 1995. Gaseous deposition of ^{14}C -
365 toluene to soybean (*Glycine max*) foliage. *Environ. Exp. Bot.*, 35, 389–398.
- 366 Jin, C., Zhou, X., Zhao, H., Liu, X., Feng, K., 2013. Comparison of removal of formaldehyde
367 capacity between *Hedera helix* and *Melissa officinalis*. *Asian J. Chem.* 25, 3823–3826.
- 368 Johnson, G.R., Olsen, R.H., 1997. Multiple pathways for toluene degradation in *Burkholderia* sp.
369 strain JS150. *Appl. Environ. Microbiol.* 63, 4047–4052.
- 370 Khoa, P.V., Nang, B.V., Hao, N.T.B., 2013. Study on gaseous formaldehyde removal capability
371 of some native plant species in Vietnam. *Inter. J. Chem. Environ. Pharm. Res.* 4, 1–7.
- 372 Kil, M.J., Kim, K.J., Gho, J.K., Pak, C.H., 2008. Formaldehyde gas removal effects and
373 physiological responses of *Fatsia japonica* and *Epipremnum aureum* according to various
374 light intensity. *Korean J. Hort. Sci. Technol.* 26, 189–196.
- 375 Kim, J.M., Le, N.T., Chung, B.S., Park, J.H., Bae, J.W., Madsen, E.L., Jeon, C.O., 2008a.
376 Influence of soil components on the biodegradation of benzene, toluene, ethylbenzene, and
377 o-, m-, and p-xylenes by the newly isolated bacterium *Pseudoxanthomonas spadix* BD-a59.
378 *Appl. Environ. Microbiol.* 74, 7313–7320.
- 379 Kim, K.J., Kil, M.J., Song, J.S., Yoo, E.H., 2008b. Efficiency of volatile formaldehyde removal
380 by indoor plants: contribution of aerial plant parts versus the root zone. *J. American. Soc.*
381 *Hort. Sci.* 133, 521–526.

- 382 Kim, K.J., Kil, M.J., Jeong, M.I., Kim, H.D., Yoo, E.H., Jeong, S.J. Pak, C.H., Son, K.C., 2009.
383 Determination of the efficiency of formaldehyde removal according to the percentage
384 volume of pot plants occupying a room. *Korean J. Hort. Sci. Technol.* 27, 305–311.
- 385 Kim, K.J., Jeong, M.I., Lee, D.W., Song, J.S., Kim, H.D., Yoo, E.H., Jeong, S.J., Han, S.W.,
386 2010. Variation in formaldehyde removal efficiency among indoor plant species.
387 *Hortscience* 45, 1489–1495.
- 388 Korczak, B., Christensen, H., Emler, S., Frey, J., Kuhnert, P., 2004. Phylogeny of the family
389 Pasteurellaceae based on *rpoB* sequences. *Int. J. Syst. Evol. Microbiol.* 54, 1393–1399.
- 390 Kvesitadze, E., Sadunishvili, T., Kvesitadze, G., 2009. Mechanisms of organic contaminants
391 uptake and degradation in plants. *World Acad. Sci. Eng. Technol.* 55, 458–468.
- 392 Lillis, L., Clipson, N., Doyle, E., 2010. Quantification of catechol dioxygenase gene expression
393 in soil during degradation of 2,4-dichlorophenol. *FEMS Microbiol. Ecol.* 73, 363–369.
- 394 Lim, Y.W., Kim, H.H., Yang, J.Y., Kim, K.J., Lee, J.Y., Shin, D.C., 2009. Improvement of
395 indoor air quality by houseplants in new-built apartment buildings. *J. Japan. Soc. Hort. Sci.*
396 78, 456–462.
- 397 Liu, Y.J., Mu, Y.J., Zhu, Y.G., Ding, H., Arens, N.C., 2007. Which ornamental plant species
398 effectively remove benzene from indoor air? *Atmos. Environ.* 41, 650–654.
- 399 Logue, J. M., McKone, T.E., Sherman, M.H., Singer, B.C., 2011. Hazard assessment of chemical
400 air contaminants measured in residences. *Indoor Air* 21(2), 92–109.
- 401 Marx, C.J., Lidstrom, M. E., 2004. Development of an insertional expression vector system for
402 *Methylobacterium extorquens* AM1 and generation of null mutants lacking *mtdA* and/or
403 *fch*. *Microbiology* 150, 9–19.

- 404 Morgan, P., Lewis, S.T., Watkinson, R.J., 1993. Biodegradation of benzene, toluene,
405 ethylbenzene and xylenes in gas-condensate-contaminated ground-water, *Environ Pollut.*
406 82(2),181–90.
- 407 Mosaddegh, M.H., Jafarian, A., Ghasemi, A., Mosaddegh, A., 2014. Phytoremediation of
408 benzene, toluene, ethylbenzene and xylene contaminated air by *D. deremensis* and plants. *J.*
409 *Environ. Health Sci. Eng.* 12, 39.
- 410 Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco
411 tissue cultures, *Physiol. Plantarum* 15, 473–497.
- 412 Nelson, M., Wolverton, B.C., 2011. Plants + soil/wetland microbes: Food crop systems that also
413 clean air and water. *Adv. Space Res.*, 47, 582–590.
- 414 Orwell, R.L., Wood, R.L., Tarran, J., Torpy, F., Burchett, M.D., 2004. Removal of benzene by
415 the indoor plant/substrate microcosm and implications for air quality. *Water Air Soil Pollut.*
416 157, 193–207.
- 417 Orwell, R.L., Wood, R.A., Burchett, M.D., Tarran, J., Torpy, F., 2006. The potted-plant
418 microcosm substantially reduces indoor air VOC pollution: II. Laboratory study. *Water Air*
419 *Soil Pollut.* 177, 59–80.
- 420 Ritalahti, K.M., Amos, B.K. Sung, Y., Wu, Q., Koenigsberg, S.S., Löffler, F.E., 2006.
421 Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously
422 monitors multiple Dehalococcoides strains. *Appl. Environ. Microbiol.* 72, 2765–2774.
- 423 Russell J.A., Hu, Y., Chau, L., Pauliushchyk, M., Anastopoulos, I., Anandan, S., Waring, M.S.,
424 2014. Indoor-biofilter growth and exposure to airborne chemicals drive similar changes in
425 plant root bacterial communities. *Appl. Environ. Microbiol.* 80, 4805–4813.
- 426 Sandermann, H. Jr., 1994. Higher plant metabolism of xenobiotics: the ‘green liver’ concept.
427 *Pharmacogenetics* 4, 225–241.

- 428 Sei, K., Asano, K., Tateishi, N., Mori, K., Ike, M., Fujita, M., 1999. Design of PCR primers and
429 gene probes for the general detection of bacterial populations capable of degrading aromatic
430 compounds via catechol cleavage pathways. J. Biosci. Bioeng. 88, 542–550.
- 431 Smith, M. R., 1990. The biodegradation of aromatic hydrocarbons by bacteria. Biodegradation 1,
432 191–206.
- 433 Tao, Y., Fishman, A., Bentley, W.E., Wood, T.K., 2004. Oxidation of benzene to phenol,
434 catechol, and 1,2,3-trihydroxybenzene by toluene 4-monooxygenase of *Pseudomonas*
435 *mendocina* KR1 and toluene 3-monooxygenase of *Ralstonia pickettii* PKO1. Appl. Environ.
436 Microbiol. 70, 3814–3820.
- 437 Thomas, J.M., Gordy, V.R., Fiorenza, S., Ward, C.H., 1990. Biodegradation of BTEX in
438 subsurface materials contaminated with gasoline: Granger, Indiana. Water Sci. Technol. 22,
439 53–62.
- 440 Ugrekhelidze, D., Korte, F., Kvesitadz, G., 1997. Uptake and transformation of benzene and
441 toluene by plant leaves. Ecotoxicol. Environ. Saf. 37, 24–29
- 442 U.S. EPA, 1996. Soil Screening Guidance: User's Guide, EPA/540/R-96/018, Office of
443 Emergency and Remedial Response, Washington, DC. (NTIS PB96.9634501).
- 444 Vogt, C., Kleinstuber, S., Richnow, H.H., 2011. Anaerobic benzene degradation by bacteria.
445 Microb. Biotechnol. 4, 710–724.
- 446 Weisburg, W.G., Barns, S.M., Pelletier, D.A. Lane, D. J., 1991. 16s ribosomal DNA
447 amplification for phylogenetic study. J. Bacteriol. 173, 697–703.
- 448 Wolverton, B.C., Douglas, W.L., Bounds, K., 1989. A study of interior landscape plants for
449 indoor air pollution abatement, NASA John C. Stennis Space Center.
- 450 Wolverton, B.C., McDonald, R.C., Watkins, E.A. Jr., 1984. Foliage plants for removing indoor
451 air-pollutants from energy-efficient homes. Econ. Bot. 38, 224–228.

- Wood, R.A., Burchett, M.D., Alquezar, R., Orwell, R.L., Tarran, J., Torpy, F., 2006. The Potted-Plant Microcosm Substantially Reduces Indoor Air VOC Pollution: I. Office Field-Study. *Water Air Soil Pollut.* 175, 163–180.
- Yang, D.S., Pennisi, S.V., Son, K.C., Kays, S.J., 2009. Screening indoor plants for volatile organic pollutant removal efficiency. *Hortscience* 44(5), 1377–1381.
- Yerushalmi, L., Lascourreges, J.F., Rhofir, C., Guiot, S.R., 2001. Detection of intermediate metabolites of benzene biodegradation under microaerophilic conditions. *Biodegradation* 12, 379–391.
- Yoo, M.H., Kwon, Y.J., Son, K.C., Kays, S.J., 2006. Efficacy of indoor plants for the removal of single and mixed volatile organic pollutants and physiological effects of the volatiles on the plants. *J. American Soc. Hort. Sci.* 131, 452–458.
- Zylstra, G.J., Gibson, D.T., 1989. Toluene degradation by *Pseudomonas putida* F1 – nucleotide sequence of the *todC1c2bade* genes and their expression in *Escherichia coli*. *J. Biol. Chem.* 264, 14940–14946.

Figure captions

Figure 1. The time course of benzene removal in hydroponic incubations of five houseplants and *N. tabacum* over time. Averages +/- standard error of triplicates.

Figure 2. Comparison of benzene removal rate normalized to leaf area of five houseplants and *N. tabacum* in hydroponic culture. Averages +/- standard error of triplicates.

Figure 3. PCR amplicons of the eubacterial 16S gene obtained from soil DNA extracted before (D_0) and after (D_7) exposure to benzene. Exposures were as follows: lanes 1-2: 0.05 $\mu\text{g m}^{-3}$, lanes 3-4: 10 $\mu\text{g m}^{-3}$, lanes 5-6: 100 $\mu\text{g m}^{-3}$, lanes 7-8: 1,000 $\mu\text{g m}^{-3}$, lanes 9-10: 10,000 $\mu\text{g m}^{-3}$, lanes 11-12: 100,000 $\mu\text{g m}^{-3}$, lanes 13-14: Soil + 5 μL benzene solution, lanes 15: the negative control or H_2O , lanes 16: the positive control for the 16S rRNA gene. The expected amplicon size was 312 bp. The results confirm the presence of eubacterial 16S rRNA gene in the soil.

Figure 4. PCR amplicons for the phenol monooxygenase gene from soil DNA extract before (D_0) and after (D_7) exposed to benzene. Lanes 1-2: 0.05 $\mu\text{g benzene m}^{-3}$, lanes 3-4: 10 $\mu\text{g m}^{-3}$, lanes 5-6: 100 $\mu\text{g m}^{-3}$, lanes 7-8: 1,000 $\mu\text{g m}^{-3}$, lanes 9-10: 10,000 $\mu\text{g m}^{-3}$, lanes 11-12: 100,000 $\mu\text{g m}^{-3}$, lanes 13-14: Soil + 5 μL benzene solution, lanes 15: the negative control or H_2O , lanes 16: the positive control or phenol monooxygenase gene. The expected size was 206 bp. The top and bottom panels show the presence of the amplicon before and after exposure to additional benzene, respectively. The results confirm the presence of the phenol monooxygenase gene in the soil and show increased detection of the amplicon following exposure to additional benzene.

Figure 5. The copy numbers of 16S rRNA (A), phenol monooxygenase (B) and C23O (C) genes expressed as counts per gram of soil increased significantly with exposure to increasing levels of benzene at 0, 10, 100, 1,000, 10,000, and 100,000 $\mu\text{g m}^{-3}$. The bars show the standard errors of the means. Values followed by different letters were significantly different using Tukey HSD test ($p < 0.05$).

Figure 6. Expression of 16S rRNA (A), rpoB (B), phenol monooxygenase (C) and C23O (D) genes counts per gram of soil increased significantly when exposed to airborne benzene at 0, 10, 100, 1,000, 10,000, and 100,000 $\mu\text{g m}^{-3}$. Bars show the standard errors of the means. Values followed by different letters were significantly different using Tukey HSD test ($p < 0.05$).

524 **Table captions**

525

526 **Table 1.** Pollutant concentration, rates of benzene removal and pseudo-first order rate constant of
527 indoor plants as measured by five labs. The discrepancies are over several orders of magnitude.

528

529 **Table 2.** PCR primers, protein targeted and annealing temperature.

Table 1. Pollutant concentration, rates of benzene removal and pseudo-first order rate constant of indoor plants as measured by six labs. The discrepancies were over several orders of magnitude.

Plant	Pollutant concentration ($\mu\text{g m}^{-3}$)	Removal rate ($\mu\text{g m}^{-2}\text{leaf h}^{-1}$)	Pseudo-first order rate constant (m h^{-1})	References
<i>Dracaena deremensis</i> cv. Variegata	523	2460	4.70	Liu et al., 2007
<i>Dracaena fragrans</i>	30900	0.006	1.87×10^{-7}	Yang et al., 2009
<i>Dracaena</i> Janet Craig	80000	27.3	3.41×10^{-4}	Orwell et al., 2004
<i>Dracaena marginata</i>	80000	49.0	6.12×10^{-4}	Orwell et al., 2004
<i>Dracaena deremensis</i>	6970	21.7	3.10×10^{-3}	Mosaddegh et al., 2014
<i>Epipremnum aureum</i>	30900	0.005	1.50×10^{-7}	Yang et al., 2009
" "	80000	12.9	1.61×10^{-4}	Orwell et al., 2004
<i>Hedera helix</i>	1744	165	9.46×10^{-2}	Yoo et al., 2006*
" "	3487	295	8.46×10^{-2}	Yoo et al., 2006
" "	30900	0.038	1.23×10^{-6}	Yang et al., 2009
<i>Howea belmoreana</i>	30900	0.008	2.72×10^{-7}	Yang et al., 2009
<i>Howea forsteriana</i>	80000	24.3	3.03×10^{-4}	Orwell et al., 2004
<i>Schefflera Amate</i>	80000	17.9	2.23×10^{-4}	Orwell et al., 2004
<i>Schefflera arboricola</i>	30900	0.005	1.5×10^{-7}	Yang et al., 2009
<i>Shefflera elegantissima</i>	30900	0.007	2.24×10^{-7}	Yang et al., 2009
<i>Spathiphyllum</i> Petite	80000	30.8	3.85×10^{-4}	Orwell et al., 2004
<i>Spathiphyllum</i> Sensation	80000	8.57	1.07×10^{-4}	Orwell et al., 2004
<i>Spathiphyllum</i> Supreme	523	445	8.51	Liu et al., 2007
<i>Spathiphyllum wallisii</i>	1740	106	6.09×10^{-2}	Yoo et al., 2006
" "	3490	501	1.43×10^{-1}	Yoo et al., 2006
" "	30900	0.008	2.55×10^{-7}	Yang et al., 2009
<i>Syngonium podophyllum</i>	1740	81	4.64×10^{-2}	Yoo et al., 2006
" "	3490	297	8.51×10^{-2}	Yoo et al., 2006
" "	30900	0.00032	1.02×10^{-8}	Yang et al., 2009

* The pots were covered with Teflon.

Table 2. PCR primers, protein targeted and annealing temperature.

Primer	Target	Sequence	Annealing temperature (°C)	Reference
27F 338R	Eubacterial 16S rRNA	5'-AGAGTTTGATCMTGGCTCAG-3' 5'-GCTGCCTCCCGTAGGAGT-3'	50	(Weisburg et al., 1991) (Amann et al., 1990)
PHE-F PHE-R	Phenol monooxygenase	5'-GTGCTGAC(C/G)AA (C/T)CTG(C/T)TGTTTC-3' 5'-CGCCAGAACCA(C/T)TT(A/G)TC-3'	49	(Baldwin et al., 2003)
C23O-F C23O-R	Catechol 2, 3 dioxygenase	5'-AAGAGGCATGGGGGCGCACCGGTTTCGATCA -3' 5'- AACAAA(AGT)GCGC(GC)GTCATGCGG -3'	60	(Sei et al., 1999) (Lillis ert al., 2010)
RDEG-F RDEG-R	Toluene monooxygenase	5'-T(C/T)TC(A/C/G)AGCAT(A/C/T)CA(A/G)AC(A/C/G)GA(C/T)GA-3' 5'-TT(A/G/T)TCG(A/G)T(A/G)AT(C/G/T)AC(A/G)TCCCA-3'	52	(Baldwin et al., 2003)
TOD-F TOD-R	Toluene dioxygenase	5'-ACCGATGA(A/G) GA(C/T)CTGTACC-3' 5'-CTTCGGTC(A/C)AGTAGCTGGTG-3'	53	(Baldwin et al., 2003)
Pasrpob-L Pasrpob-R	Bacterial RNA polymerase beta-subunit	5'-GCAGTGAAAGARTTCTTTGGTTC-3' 5'-GTTGCATGTTNGNACCCAT-3'	54	(Korczak et al., 2004)

Figure 1. The time course of benzene removal (%) in hydroponic incubations of five houseplants and *N. tabacum* over time. Averages $\pm$ standard error of triplicates.

Figure 2. Comparison of benzene removal rate normalized to leaf area of five houseplants and *N. tabacum* in hydroponic culture. Averages +/- standard error of triplicates.

Figure 3. PCR amplicons of the eubacterial 16S gene obtained from soil DNA extracted before (D_0) and after (D_7) exposure to benzene. Exposures were as follows: lanes 1-2: $0.05 \mu\text{g benzene m}^{-3}$, lanes 3-4: $10 \mu\text{g m}^{-3}$, lanes 5-6: $100 \mu\text{g m}^{-3}$, lanes 7-8: $1,000 \mu\text{g m}^{-3}$, lanes 9-10: $10,000 \mu\text{g m}^{-3}$, lanes 11-12: $100,000 \mu\text{g m}^{-3}$, lanes 13-14: Soil + $5 \mu\text{L}$ benzene solution, lanes 15: the negative control or H_2O , lanes 16: the positive control for the 16S rRNA gene. The expected amplicon size was 312 bp. The results confirm the presence of eubacterial 16S rRNA gene in the soil.

Figure 4. PCR amplicons for the phenol monooxygenase gene from soil DNA extract before (D_0) and after (D_7) exposed to benzene. Lanes 1-2: $0.05 \mu\text{g benzene m}^{-3}$, lanes 3-4: $10 \mu\text{g m}^{-3}$, lanes 5-6: $100 \mu\text{g m}^{-3}$, lanes 7-8: $1,000 \mu\text{g m}^{-3}$, lanes 9-10: $10,000 \mu\text{g m}^{-3}$, lanes 11-12: $100,000 \mu\text{g m}^{-3}$, lanes 13-14: Soil + $5 \mu\text{L}$ benzene solution, lanes 15: the negative control or H_2O , lanes 16: the positive control or phenol monooxygenase gene. The expected size was 206 bp. The top and bottom panels show the presence of the amplicon before and after exposure to additional benzene, respectively. The results confirm the presence of the phenol monooxygenase gene in the soil and show increased detection of the amplicon following exposure to additional benzene.

Figure 5. The copy numbers of 16S rRNA (A), phenol monooxygenase (B) and C23O (C) genes expressed as counts per gram of soil increased significantly with exposure to increasing levels benzene at 0, 10, 100, 1,000, 10,000, and 100,000 $\mu\text{g m}^{-3}$. The bars show the standard errors of the means. Values followed by different letters were significantly different using Tukey's HSD test ($p < 0.05$).

Figure 6. Expression of 16S rRNA (A), rpoB (B), phenol monooxygenase (C) and C23O (D) genes counts per gram of soil increased significantly when exposed to airborne benzene at 0, 10, 100, 1,000, 10,000, and 100,000 µg m⁻³. Bars show the standard errors of the means. Values followed by different letters were significantly different using Tukey's HSD test ($p < 0.05$).