THE POTTED-PLANT MICROCOSM SUBSTANTIALLY REDUCES INDOOR AIR VOC POLLUTION: II. LABORATORY STUDY

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Abstract. Indoor air-borne loads of volatile organic compounds (VOCs) are usually significantly higher than those outdoors, and chronic exposures can cause health problems. Our previous laboratory studies have shown that the potted-plant microcosm, induced by an initial dose, can eliminate high air-borne VOC concentrations, the primary removal agents being potting-mix microorganisms, selected and maintained in the plant/root-zone microcosm. Our office field-study, reported in the preceding paper, showed that, when total VOC (TVOC) loads in reference offices (0 plants) rose above about 100 ppb, levels were generally reduced by up to 75% (to <100 ppb) in offices with any one of three planting regimes. The results indicate the induction of the VOC removal mechanism at TVOC levels above a threshold of about 100 ppb. The aims of this laboratory dose-response study were to explore and analyse this response. Over from 5 to 9 days, doses of 0.2, 1.0, 10 and 100 ppm toluene and *m*-xylene were applied and replenished, singly and as mixtures, to potted-plants of the same two species used in the office study. The results confirmed the induction of the VOC removal response at the lowest test dosage, i.e in the middle of the TVOC range found in the offices, and showed that, with subsequent dosage increments, further stepwise induction occurred, with rate increases of several orders of magnitude. At each dosage, with induction, VOC concentrations could be reduced to below GC detection limits (<20 ppb) within 24 h. A synergistic interaction was found with the binary mixtures, toluene accelerating *m*-xylene removal, at least at lower dosages. The results of these two studies together demonstrate that the potted-plant microcosm can provide an effective, self-regulating, sustainable bioremediation or phytoremediation system for VOC pollution in indoor air.

Keywords: indoor air pollution, VOC, TVOC, toluene, *m*-xylene, "sick building syndrome", "building related illness", environmental biotechnology, bioremediation, phytoremediation, potted-plant

1. Introduction

As discussed in the preceding paper (Wood *et al*., DOI: 10.1007/s11270-006-9092-3) the possible effects of indoor air pollution on human health are an issue of international concern, since urban dwellers spend about 90% of their lives indoors (Environment Australia [EA], 2003; Mølhave and Krzyzanowski, 2003; Wolkoff, 2003). Average indoor levels of volatile organic compounds (VOCs), which are derived from a combination of outdoor and indoor sources, are generally higher,

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sometimes several times higher, than those outdoors (Brown *et al*., 1994; Brown, 1997; Rehwagen *et al*., 2003). Over 350 VOCs have been identified in indoor air, and the resulting chemical mixtures, although usually in very low concentrations (TVOC loads of 100–500 ppb; Brown *et al*, 1994; EA, 2003), are recognized as causative agents of "sick building syndrome" or "building-related illness" (Carpenter, 1998; Brasche *et al*., 1999; Carrer *et al*., 1999; Sullivan Jr *et al*., 2001). Symptoms include irritated eyes, nose or throat, headache, drowsiness or breathing problems. Even where symptoms are not overt, chronic exposure may lead to reduced concentration and performance, and the onset of other health problems such as asthma and heart disease (Bascom, 1997; Brasche *et al*., 1999).

The findings of a number of northern hemisphere studies have demonstrated that the potted-plant microcosm can substantially reduce air-borne contaminants from indoor air (Wolverton, 1985; Wolverton *et al*., 1989; Wolverton and Wolverton, 1993; Coward *et al*., 1996; Lohr and Pearson-Mims, 1996). Our previous laboratory studies, using seven indoor potted-plant species, have shown that they can eliminate high air-borne concentrations of model VOCs (benzene and *n*-hexane), within 24 hours, once the system has been stimulated (induced) by initial exposure to the compound (Tarran *et al*., 2002; Wood *et al*., 2002; Orwell *et al*., 2004). These studies included numerous trials in which VOC removal activity was sustained for up to seven days after the plants had been removed and the potting mix returned to the test chambers. Similarly, when unused ('virgin') potting mix was watered normally, and then challenged, moderate levels of VOC removal activity were induced by the initial dose, but in this case, after six days, activity began to decline. No absorption was recorded in the presence of empty pots alone, or a tray of water to provide a possible VOC sink. The findings strongly indicated that it was the microorganisms of the potting mix which are the primary agents of VOC removal, the role of the plants being mainly via the establishment and maintenance of their species-specific root-zone microbial communities. The role of substrate microorganisms in the potted-plant VOC removal process was first suggested by Wolverton and colleagues (1985, 1989, 1993). It was also implicated by Godish and Guindon (1989) who concluded that the removal of formaldehyde emitted from particleboard (ie a continuous source in their dynamic test chambers) in the presence of spider-plants (which were successively defoliated to 50%, 25% and zero), was not primarily via plant leaves (as had also been suggested by Wolverton). It must therefore, as Godish and Guindon put it, presumably be rather 'due to factors of the potting soil (such as soil moisture, plant roots, soil surface, microorganisms or a combination of all) and with a moisture-related source phenomenon'. Our studies have since shown that their unknown, unspecified, 'moisture-related source phenomenon', is in fact the phenomenon of the induction and operation of microbiological enzymic VOC removal, augmented by any associated population shifts in the microorganism community concerned in favour of the VOC degrading species.

Despite all these previous laboratory studies, our own and others, prior to our office field-study, reported in the preceding paper (Wood *et al.*, DOI: 10.1007/s11270-

006-9092-3) no field-based experimental study had ever been made to test the real-world effectiveness of the potted-plant microcosm in reducing indoor VOC pollution. The office field-study comprised two experimental investigations, over 14 to 18 weeks each, comparing the possible effects of three potted-plant regimes, using two plant species, on levels of TVOCs in office air. In each investigation, weekly TVOC samplings were conducted in offices in an air-conditioned and a non-air-conditioned building (total of 60 offices). TVOC levels in the offices ranged from 60–350 ppb over the experimental period (June–October, 2003, ie winter/spring). In particular, it was also found that in weeks in which TVOC concentrations in reference offices (0 plants) rose above about 100 ppb, those in offices with any of the three plantings, were reduced by up to 75%, to below 100 ppb once more. In addition, the potted-plants appeared equally effective under air-conditioned and non-air-conditioned circumstances.

These results indicated that (i) there was an induction of the metabolic VOC removal response in the potted-plant microcosm at TVOC levels above ∼100 ppb, and (ii) the smallest planting regime, comprising 6 'table-sized' potted-plants, was as effective as either 3 or 6 larger, floor specimens. In other words, there was evidently an abundant VOC removal capacity even in the smallest planting regime. The experimental aims of this laboratory test-chamber study, therefore, were to conduct controlled dose-response investigations of this VOC removal mechanism, using as model VOCs two of those detected in the office air. Specifically, the experiments were designed to test:

(a) patterns of induction and functioning of the VOC-removal response mechanism in the potted-plant microcosm across a wide range of VOC concentrations, including the low levels encountered in the office study;
(b) possible interactions between the two VOCs, using a set of binary mixtures, which might affect induction or rates of functioning of the VOC removal mechanism.

The same two plant species were used as in the office study, namely *Spathiphyllum* 'Sweet Chico' and *Dracaena deremensis* 'Janet Craig', which are commonly used, 'international' indoor-plant species. In our earlier test-chamber studies, cited above, these species had also been found to be effective in removing high air-borne concentrations of benzene and *n*-hexane.

Toluene (a methylbenzene) and xylene (dimethylbenzenes) were chosen as the test VOCs, since they were among the most prevalent of the 14 VOCs identified in the air of the offices sampled. These compounds are among the four comprising the 'BTEX' group (benzene, toluene, ethylbenzene and xylenes), a quartet of hazardous pollutants arising from vehicle fuel emissions (Sullivan Jr. *et al*., 2001; EA, 2003). Short-term exposure to any of these compounds may produce symptoms of dizziness, loss of concentration, nausea or respiratory difficulties. The first and last are known carcinogens. Toluene is ranked internationally

as a high priority indoor air pollutant for action, because of its prevalence and potential health effects (Mosqueron *et al*., 1993; Greenberg, 1997; Environment Australia [EA], 2003). Symptoms of exposure to any of the BTEX compounds can occur in susceptible individuals at very low concentrations, particularly when present in mixtures with other VOCs in indoor air (Prah *et al.*, 1998; EA, 2003; MSDS, 2005). The effects of BTEX contribute to sick-leave absences and lowered productivity in the workplace (Brasche *et al*., 1999; Carrer *et al*., 1999; American Lung Association, 2001). Thus, both health and economic considerations highlight the need to obtain reliable data on the dose-response removal capacities of the potted-plant microcosm over a range of concentrations of these contaminants.

Possible interactions between VOCs, antagonistic or synergistic, could affect their rates of removal by the potted-plant microcosm, particularly, in this case, since toluene and xylene structurally belong to the same class of organic compounds (methyl-substituted aromatics). Furthermore, in this microcosm, such interactions can result not only from competition for processing sites in microbial enzymic pathways, but also from population shifts within the root-zone microorganism community resulting from exposure to a particular VOC (Pucci *et al*., 2000; Siciliano *et al.*, 2003; Paralesi and Haddock, 2004).

Four dosage concentrations (0.20, 1.0, 10, 100 ppm) were selected for study, for the following reasons. The lowest concentration (0.20 ppm; 200 ppb) was in middle of the range of concentrations encountered in the office study when a VOC removal response was in evidence; it also corresponded with TVOC levels in what is regarded as 'good quality' indoor air (Sullivan Jr. *et al*., 2001; EA, 2003); and it was the lowest dosage at which we considered that removal rates could be monitored with maximum accuracy through the whole induction and removal process, using our sampling and gas chromatographic assay system (detection limit for each compound $\sim$ 20 ppb). The intermediate VOC concentrations (1.0 and 10 ppm), encompassed TVOC levels likely to provoke complaints from building occupants about air quality (Carpenter, 1998; Carrer *et al*., 1999). The highest concentration (100 ppm) encompassed and exceeded exposure levels known to compromise occupational health and safety (eg the Worksafe Australia 8-h time-weighted average exposure limits are, for toluene, 100 ppm (378.8 mg m^{-3}) and for xylene, 80 ppm (349.2 mg m^{-3}); NOHSC, Australia, 1995). In these experiments, doses were administered as a single initial and ensuing daily (or more frequent, as necessary) top-up doses to the original concentration in the test chambers.

To assess possible interactions between toluene and xylene during removal, a comparison was made of the removal rates for each compound when applied singly (toluene *or* xylene), with those obtained in the binary mixtures (toluene + xylene). Since in industrial-grade xylene (a mixture of *o*-, *m*- and *p*- isomers) the *m*-isomer is the major component (Merck, 1989), *m*-xylene was used throughout the study.

TABLE I
Characteristics of potted-plants used; leaf areas and dry weights

Plant characteristic (per plant)	*S*. 'Sweet Chico' ($n = 8$)	*D*. 'Janet Craig' ($n = 12$)
Leaf area (m^2)	0.442 ± 0.020	0.117 ± 0.013
Leaf dry weight (g)	18.4 ± 0.62	8.9 ± 0.84
Root dry weight (g)	32.7 ± 4.61	7.2 ± 2.05
Potting mix dry weight (g)	410 ± 6.6	1014 ± 20.4
Root :Shoot Ratio	1.76 ± 0.20	0.80 ± 0.17

Values are means± S.E.

2. Materials and Methods

2.1. Materials

2.1.1. *Potted-plants*

Well-established 12-month old specimens of *S*. 'Sweet Chico' and *D*. 'Janet Craig', were used, 4 replicates per treatment. Heights were 30–40 cm, in pots 15 cm diameter, in a standard potting mix (∼0.7 L per pot with *S*. 'Sweet Chico'; 1.2 L with *D*. 'Janet Craig'; refer also to Table I). The mixture was composed of composted hardwood sawdust, composted bark fines and coarse river sand (2:2:1) (bulk density ∼0.6 g mL^{-1}; air-filled porosity ∼30%) with Macrocote "green-plus" 9-month fertilizer (12:4.6:10 N:P:K, with trace elements; Langley Chemicals, Welshpool, WA). At the end of the experimental sequence for every experiment, plants were harvested and measurements made of leaf area, and plant and potting mix dry weights (oven-dried at 70 °C for 24 h), to provide alternative bases for comparing the effectiveness of VOC removal by the microcosm, both between species and between different VOC dosages.

2.1.2. *Chemicals*

Toluene was 99.8% HPLC grade and *m*-xylene 99+% anhydrous (Aldrich Chemical Co, Milwaukee, USA).

2.2. Apparatus and sampling procedures

The test apparatus and analytical methods were the same as those used in our previous laboratory studies (Wood *et al*., 2002; Tarran *et al*., 2002; Orwell *et al*., 2004). Four replicate Perspex bench-top test chambers were used, $0.6 \times 0.6 \times 0.6$ m (internal volume 0.216 m^3), with removable lids on stainless steel frames, sealed with adhesive foam-rubber tape and adjustable metal clips. Each chamber had rubber silicone septa for VOC injections and air sampling, a 0.5 m coil of copper tubing (i.d. 4 mm) circulating water from a thermostat bath at 21.0 ± 0.1 °C; a

suspended min-max thermometer; a 2.4 W fan to accelerate atmospheric equilibration; an overhead light box (with air gap of 50 mm) with five 18 W fluorescent tubes designed for optimum plant growth (Wotan L 18/11 Maxilux daylight, Ozram, Germany) ($\sim$120 μmol quanta $m^{-2}s^{-1}$). High-precision plunger-in-needle syringes were used for all VOC injections of 10 μL or less, and conventional syringes of similar precision for larger volumes (SGE Australia).

VOC estimations were carried out using a Shimadzu GC-17A gas chromatograph (GC), equipped with a 15 m DB5 Megabore column (0.34 mm i.d; Alltech Australia), FID detector and Class-VP 4.2 integration software (Shimadzu, Sydney, Australia). Chromatography was performed isothermally at 70 °C (toluene alone), 95 °C (*m*-xylene alone), or 85 °C (toluene+*m*-xylene mixture). Toluene and *m*-xylene retention times under these conditions varied from 1.8 to 3.5 min. When mixtures of toluene and *m*-xylene were used, baseline separation of the two compounds was achieved and each VOC was estimated separately. Calibrations were based on initial peak areas (after 1 h of equilibration) derived from at least 8 replicate injections into test chambers at each concentration of VOC.

At the start of each experimental trial, the four replicate potted-plants were watered to saturation and allowed to drain for 1 h before being placed, one per chamber, with lids sealed and lights on. An initial dose of VOC (toluene and/or *m*-xylene) was injected onto suspended absorbent paper in each chamber, the volume calculated to achieve the required concentration in the chamber air after equilibration. For every sampling, 1.0 mL of chamber air was withdrawn in a gas-tight syringe. Chambers were sampled in triplicate and VOC concentrations estimated over time, using the GC. VOC injections were found to equilibrate in chamber air in approximately 1 h. As mentioned earlier, the lower limit of detection of this system was 0.020 ppm (20 ppb) of either VOC (0.076 mg m^{-3} toluene; 0.087 mg m^{-3} *m*-xylene). Samplings were carried out at intervals of several hours, or daily, as required. Additional 'top-up' injections to restore the original concentration were performed daily. Lighting was maintained continuously throughout all experiment (as is commonly found in office blocks and other commercial buildings). The same four potted-plant replicates were exposed to the stepwise dosage increments over the course of each experiment, with the plants being given a 3-day 'rest' period in normal ambient air outside the test chambers between stages. A new set of replicate plants was used for each of the five experiments. When working with binary mixtures, doses were administered separately for the toluene and *m*-xylene, to ensure that each was restored to the appropriate repeated-dose concentration.

2.3. TEST PROTOCOL

The test protocol was initially set up as follows:

Stage 1. The potted-plants were dosed with an initial injection of 0.20 ppm VOC on to a suspended paper tissue, and the resultant chamber concentration measured

over a 1–2 h period (to check equilibration times). Subsequent samplings and top-up doses were performed at daily intervals over 5 days (i.e. 5 doses in all). The potted-plants were then removed from the chambers and rested for 3 days, by watering them and placing them in ambient air in a well ventilated area within the laboratory, under normal room lighting.

Stage 2. The same plants were then replaced in the chambers and dosed with 1.0 ppm VOC. Daily sampling and the application of top-up doses to a total of 5 × 1.0 ppm doses, were performed as in Stage 1, followed by a 3 day rest period.

Stage 3. The same plants were treated as described in Stage 2, using 5 × 10 ppm VOC doses, followed by a 3 day rest period.

Stage 4. The same plants were treated in the same way, using 5 × 100 ppm VOC doses, and then harvested.

An extension was made to the protocol early in the study, when it was noted that in some cases progressive acceleration of VOC removal activity (ie induction of the removal response) occurred following each of the 5 daily top-up doses given within any one Stage of the original protocol. This response indicated that, in such cases, induction might well be incomplete after five doses. Therefore, if indicated by the responses, additional VOC doses, up to a total of 9, were administered, to obtain an estimate of the number of doses needed to elicit maximal induction of activity at that particular dosage.

In the first four experiments, batches of *S*. 'Sweet Chico' and *D*. 'Janet Craig' potted-plants, respectively, were exposed to toluene alone, and *m*-xylene alone. In a fifth experiment, using *D*. 'Janet Craig' only, potted-plants was exposed to binary mixtures (toluene + *m*-xylene) over the same range of concentrations, using the same 4-Stage protocol. Thus the fifth experiment generated two data sets.

2.4. Leak tests

During each 3-day plant rest period, after each stage of each experiment, leak tests were performed on the empty chambers, applying the VOC dose used in the preceding Stage. Thus, for each experiment, four plant test periods and four rest periods with chamber leak tests were conducted. During the leak tests a beaker containing 500 mL water was placed in each chamber, to simulate pot-plant evapotranspiration.

2.5. Data analysis

From the results of each set of leak tests, exponential VOC decay constants were estimated for each chamber, using the curve fit facility of Cricketgraph 1.5.1 (Microsoft Australia Corp.), and corrections appropriate to each chamber were applied to the corresponding test data. VOC losses in blank chambers were 4–10% per day. During each experiment, VOC removal activity was assessed by estimating daily potted-plant removal rates, exponential decay constants and VOC half-lives for each dose,

using the curve fit menu of Cricketgraph 1.5.1 (Microsoft). Results were also calculated on the basis of alternative plant and potting mix parameters. Statistical comparisons were performed using one-factor ANOVA (Excel 2001, Microsoft, Australia Corp.) and pair-wise Tukey's HSD test. Differences between treatments are reported as statistically significant where $p \leq 0.05$.

3. Results

3.1. Plant and potting mix characteristics

Table I presents the leaf area and dry weight characteristics of the potted-plants used. As expected, within each species plants were closely matched, being cloned specimens of identical provenance, age and general size. The biomass of the *D*. 'Janet Craig' specimens (ie leaf and root dry weights) was only about half that of the *S*. 'Sweet Chico' plants. It can also be seen that the smaller root mass of *D*. 'Janet Craig' plants permitted more than twice the amount of substrate (potting mix) per pot, compared with the *S*. 'Sweet Chico' specimens.

3.2. General patterns of VOC removal

Figures 1A–B and 2A–B present the results for toluene and *m*-xylene levels during the four experiments in which each VOC was administered singly, to either *S*. 'Sweet Chico' or *D*. 'Janet Craig'. Within each figure, plots a–d show the four Stages of the experimental protocol, and they demonstrate a general pattern of induction of a removal response, for every dosage applied, for each VOC, with both plant species. Thus, at each succeeding dosage level, VOC removal rates were initially low, but increased further with each subsequent top-up dose, until, on about Day 5, most of the VOC was usually eliminated within 24 h. It is clear that an induction occurred at the lowest dose concentration, and that further incremental induction occurred with every increase in dose concentration thereafter. The 'saw-tooth' patterns seen in Figures 1 and 2 closely resemble those obtained in our previous test-chamber studies, using benzene and *n*-hexane as model VOCs (Tarran *et al*., 2002; Wood *et al*., 2002; Orwell *et al*., 2004). Figure 3A–B presents the daily VOC concentrations measured during the final experiment with binary mixtures of toluene and *m*-xylene. Once again, the patterns were very similar to those shown in Figures 1 and 2, for each compound in the mixtures.

Exponential rate constants (λ) were estimated for the daily decreases in each VOC in test-chamber air, and are presented in Figures 4 and 5. In most cases induction took place progressively over Days 1–5 of each Stage (dosage). However, when, in some later experiments, additional daily top-up doses were applied, further increases in activity were seen, indicating that, in these cases, full induction took more than 5 days to accomplish. Figures 1–5 demonstrate that an adaptive response

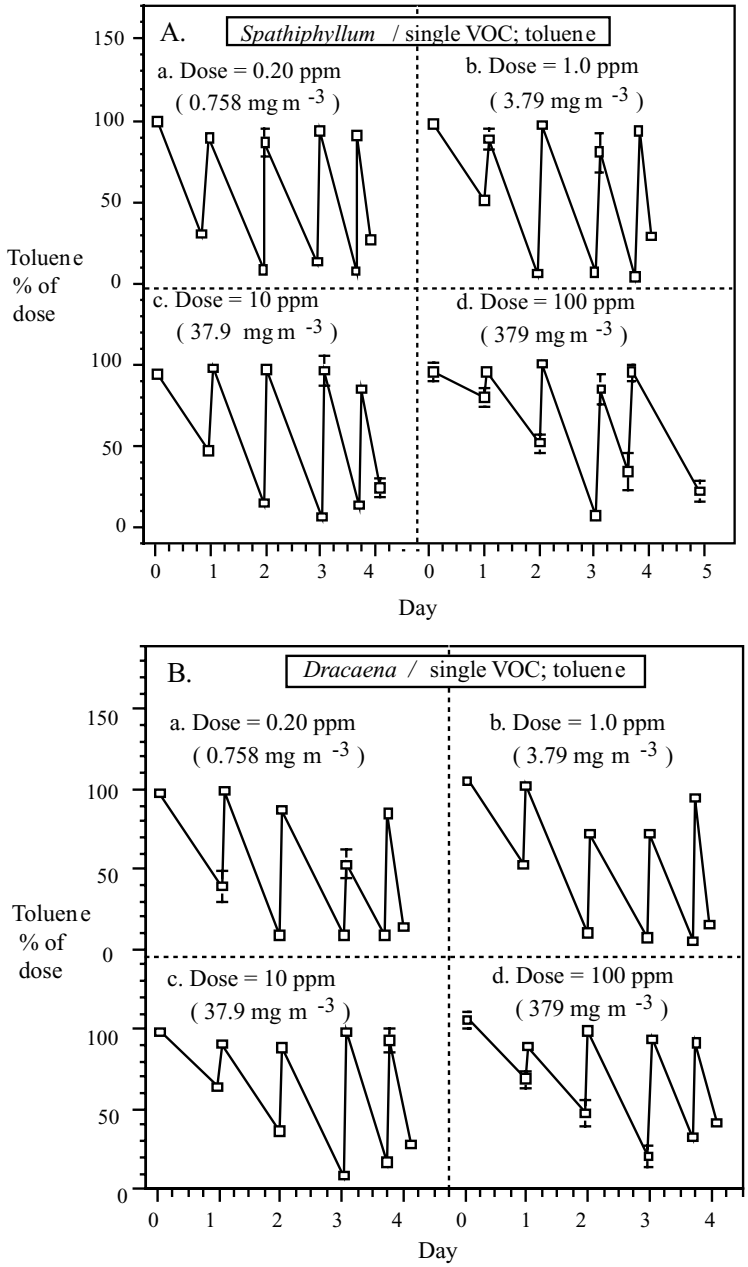

Figure 1. Removal of toluene from test-chamber air by (A) *Spathiphyllum* 'Sweet Chico' and (B) *Draceana* 'Janet Craig', the potted-plants challenged with daily doses at the concentrations indicated. Values are means ± S.E. ($n = 4$).

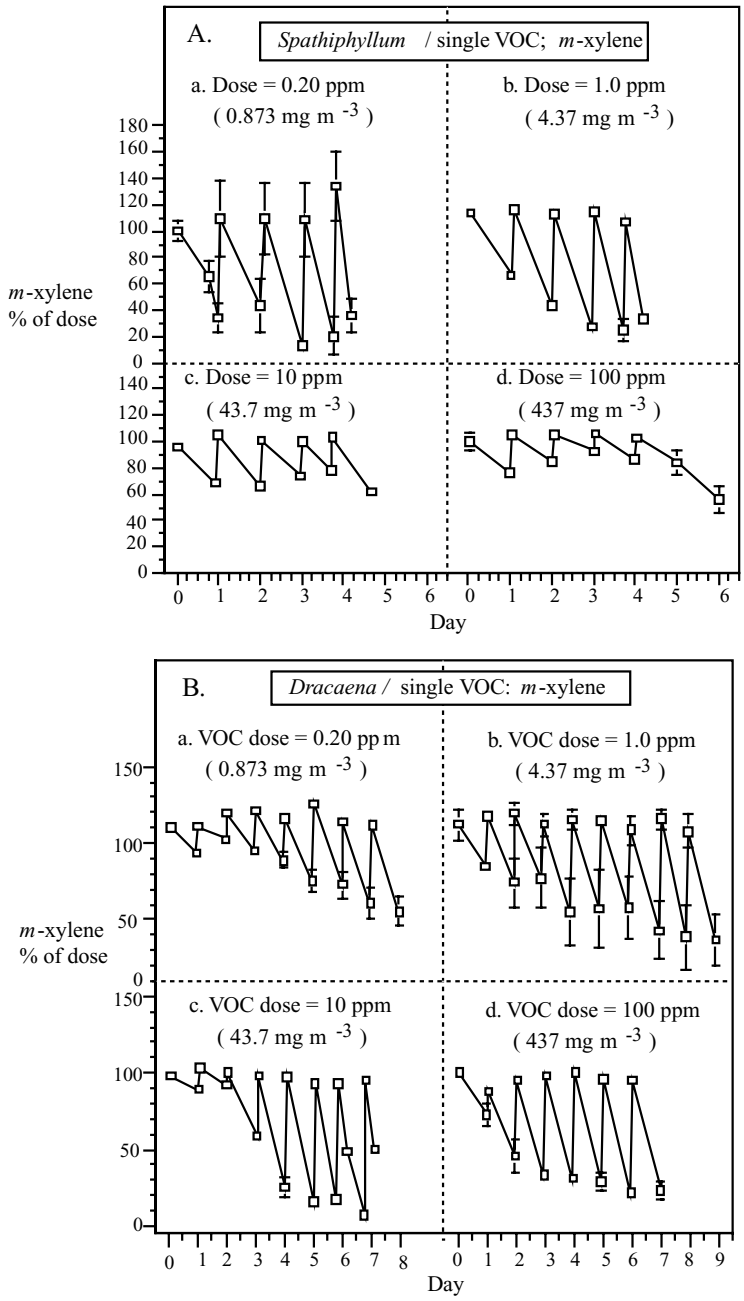

Figure 2. Removal of *m*-xylene from test-chamber air by (A) *Spathiphyllum* 'Sweet Chico' and (B) *Draceana* 'Janet Craig', the potted-plants challenged with daily doses at the concentrations indicated. Values are means ± S.E. ($n = 4$).

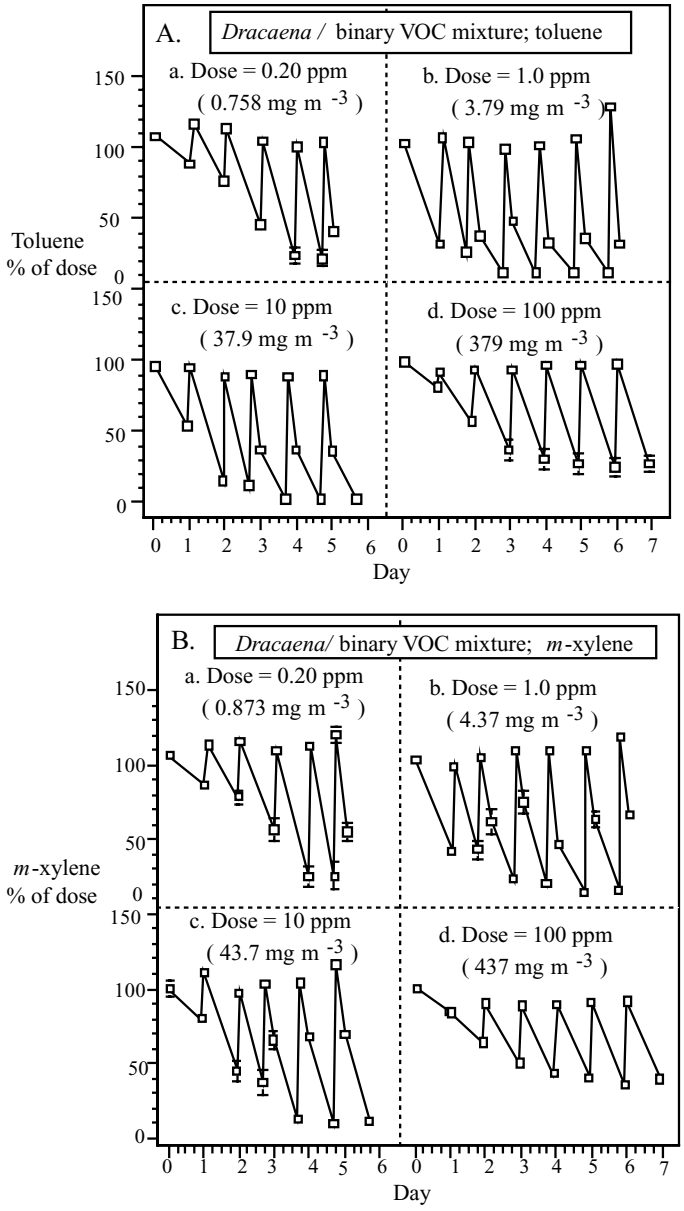

Figure 3. Removal of (A.) toluene and (B.) *m*-xylene from test-chamber air by *Draceana* 'Janet Craig', the potted-plants challenged with daily doses of (toluene + *m*-xylene) binary mixtures in which both VOCs were present at the concentrations indicated. Values are means ± S.E. ($n = 4$).

occurred in the potted-plant system following exposure to each of these two VOC, and to mixtures of the two, at every VOC dosage concentration, even though dosage levels were increased by more than three orders of magnitude. The dimensions of the overall responses are summarised in Table II, where the activities achieved on

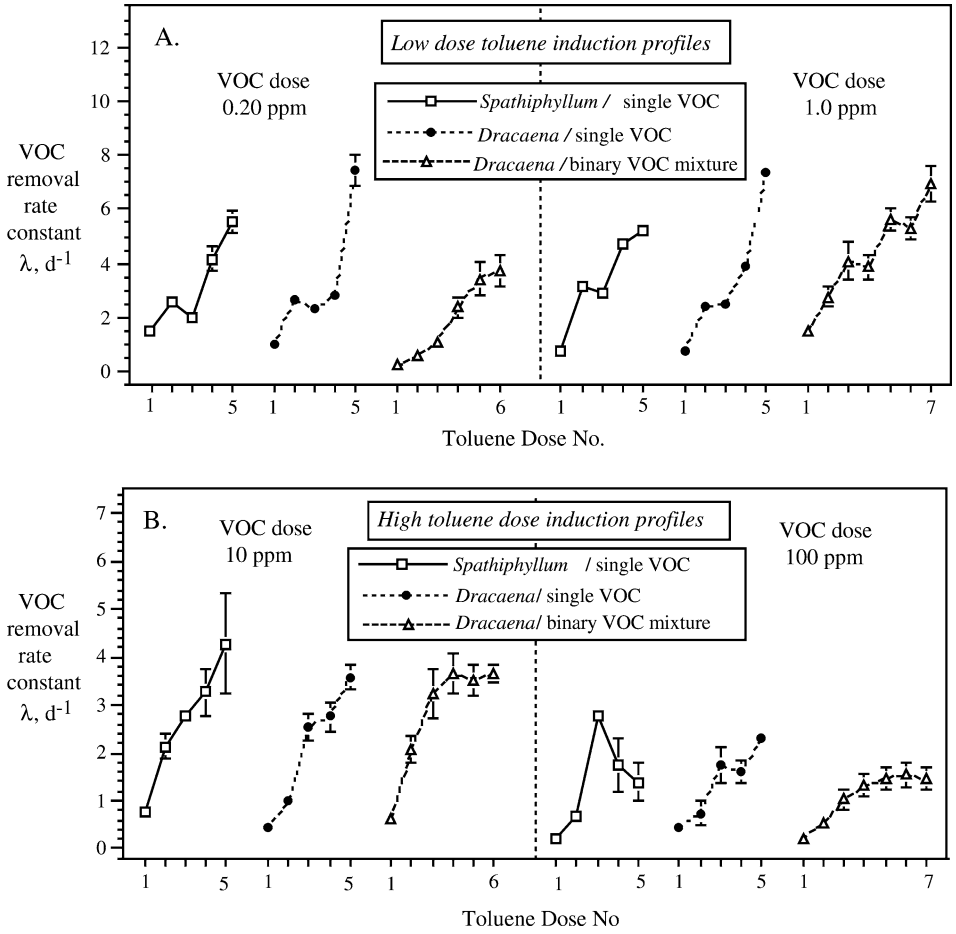

Figure 4. Exponential rate constants (λ) for toluene removal by *Spathiphyllum*'Sweet Chico' and *Dracaena* 'Janet Craig' plants challenged with daily doses of toluene, at (A) 0.20 and 1.0 ppm, and (B), 10 and 100 ppm alone, and in binary mixtures (toluene + *m*-xylene) in which each VOC was present at the concentrations indicated. Values are means ± S.E. ($n = 4$).

Day 5 in each experimental Stage are listed, in terms of % dose removed per day (% d^{-1}); mg VOC removed per m^3 air; exponential rate constants (λ); VOC half-lives ($t_{1/2}$). Table III presents the VOC removal rates based on alternative plant and potting mix parameters.

3.3. Removal of Toluene

With either plant species, and toluene dosages of 0.20, 1.0, 10 ppm, applied either singly or in binary mixtures, removal rates of ~180% dose d^{-1} or more were attained by Day 5 (Table II). Daily top-up doses for 4 or 5 days were generally required for

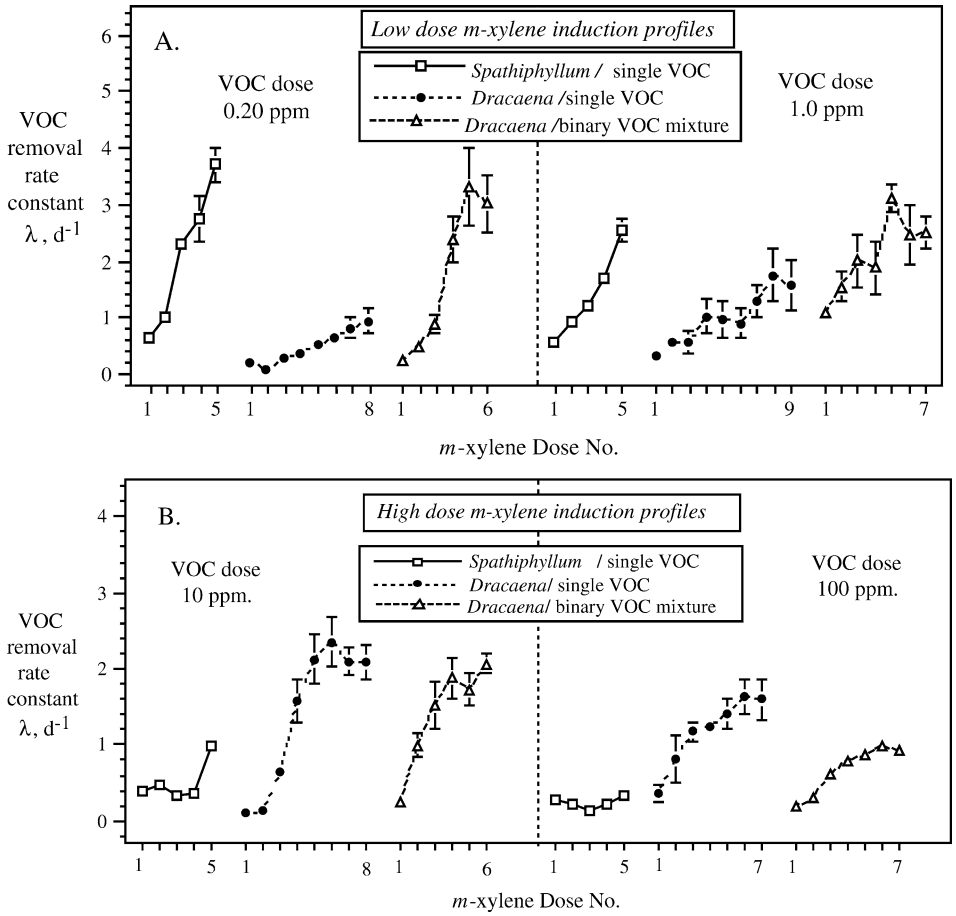

Figure 5. Exponential rate constants (λ) for *m*-xylene removal by *Spathiphyllum* 'Sweet Chico' and *Dracaena* 'Janet Craig' plants challenged with daily doses of *m*-xylene, at (A) 0.20 and 1.0 ppm, and (B) 10 and 100 ppm alone, and in binary mixtures (toluene + *m*-xylene) in which each VOC was present at the concentrations indicated. Values are means ± S.E. ($n = 4$).

full induction at any one concentration, with greatest accelerations occurring on Day 4 or 5 (Figure 4). However, with 100 ppm toluene, % d^{-1} removal rates were only about half of those seen at the lower doses, and when *S*.'Sweet Chico' was challenged with 100 ppm toluene alone, the increases which occurred over Days 1–3 were followed by falls on Days 4 and 5. However, this was an isolated result within this study (Figure 4), and the absolute rate (ppm d^{-1} or mg d^{-1} $plant^{-1}$) for *S*. 'Sweet Chico' exposed to 100 ppm toluene was still substantial, at 50 mg d^{-1} (61% d^{-1}) on Day 5 (Tables II, III). This pattern of induction followed by a drop in removal rate at 100 ppm toluene was not found with *D*. 'Janet Craig' (Figure 4). The loss of the newly induced activity observed with *S*. 'Sweet Chico', therefore, may

TABLE II

Rate parameters for daily removal of toluene and/or *m*-xylene from air in test-chambers by potted-plants at Day 5 (ie after 5 doses), at the VOC concentrations indicated

Plant species	VOC	VOC dose ppm	% of dose per day (% d^{-1})	mg m^{-3} d^{-1}	Exponential rate constant (λ, d^{-1})	VOC half-life ($t_{1/2}$, h)
S. 'Sweet Chico'	toluene as single VOC	0.20	288 ± 10.8	2.2 ± 0.08	5.56 ± 0.42	3.0 ± 0.23
		1.0	286 ± 17.4	10.8 ± 0.66	5.18 ± 0.18	3.2 ± 0.11
		10	177 ± 23.4	67 ± 8.9	4.27 ± 1.05	3.9 ± 1.0
		100	61 ± 11.0	231 ± 41.7	1.39 ± 0.40	12.0 ± 3.4
S. 'Sweet Chico'	*m*-xylene as single VOC	0.20	268 ± 20.7	2.3 ± 0.18	3.71 ± 0.30	4.5 ± 0.37
		1.0	176 ± 10.5	7.7 ± 0.46	2.55 ± 0.19	6.5 ± 0.48
		10	87 ± 6.7	38.0 ± 2.92	0.99 ± 0.09	16.8 ± 1.5
		100	24 ± 2.3	105 ± 10.0	0.32 ± 0.04	51.4 ± 6.9
D. 'Janet Craig'	Toluene as single VOC	0.20	286 ± 19.0	2.2 ± 0.14	7.44 ± 0.58	2.2 ± 0.17
		1.0	317 ± 7.1	12.0 ± 0.27	7.39 ± 0.24	2.3 ± 0.07
		10	195 ± 21.1	74 ± 8.0	3.58 ± 0.26	04.6 ± 0.34
		100	145 ± 8.4	549 ± 31.8	2.31 ± 0.17	7.2 ± 0.52
D. 'Janet Craig'	*m*-xylene as single VOC	0.20	42 ± 5.7	0.37 ± 0.05	0.52 ± 0.09	32.2 ± 5.7
		1.0	60 ± 10.1	2.6 ± 0.44	0.98 ± 0.33	16.9 ± 5.6
		10	89 ± 5.3	39 ± 2.3	2.13 ± 0.32	7.8 ± 1.12
		100	77 ± 5.0	336 ± 21.8	1.40 ± 0.20	11.9 ± 1.66
D. 'Janet Craig'	Toluene in binary mixture	0.20	115 ± 5.0	0.87 ± 0.04	3.43 ± 0.59	4.8 ± 0.83
		1.0	274 ± 3.3	10.4 ± 0.13	5.63 ± 0.42	3.0 ± 0.22
		10	207 ± 13.1	78 ± 5.0	3.54 ± 0.33	4.7 ± 0.43
		100	75 ± 7.2	284 ± 27.3	1.46 ± 0.25	11.4 ± 1.92
D. 'Janet Craig'	*m*-xylene in binary mixture	0.20	126 ± 11.5	1.10 ± 0.10	3.33 ± 0.69	5.0 ± 1.03
		1.0	250 ± 13.1	10.9 ± 0.57	3.11 ± 0.25	5.3 ± 0.42
		10	145 ± 15.3	63 ± 6.7	1.73 ± 0.21	9.6 ± 1.17
		100	52.5 ± 2.6	229 ± 11.4	086 ± 0.07	19.3 ± 1.54

Values are means ± S.E ($n = 4$).

have been the result of toxicity to the plant and/or to its substrate microorganism community, or it may have signalled the onset of VOC saturation of the relevant microbial enzymic degradation pathways within this community.

3.4. Removal of *m*-xylene

S. 'Sweet Chico' pots challenged with the two lower dosages of *m*-xylene (0.20 and 1.0 ppm) achieved removal rates exceeding 170% d^{-1} by Day 5, while at 10 ppm the corresponding rate was 87% d^{-1} (Table II). At 100 ppm, however, this species performed somewhat slowly, removing only 24% d^{-1} after little or no induction over 5 days (Table II; Figure 5). However, in terms of absolute activity (ppm d^{-1} or mg d^{-1} $plant^{-1}$) 24% d^{-1} removal at 100 ppm represents a greater metabolic

TABLE III

Removal rates (mg d^{-1}) of toluene and/or *m*-xylene from air in test-chambers, after 5 daily doses at the concentrations indicated, expressed on the basis of alternative plant and potting-mix parameters

Plant species	VOC	VOC dose (ppm)	mg $^{*}pl^{-1}$ d^{-1}	mg kg^{-1} d^{-1} (pot-mix)	mg $m^{-2}d^{-1}$ (leaf area)
S. 'Sweet Chico'	Toluene as single VOC	0.20	0.47 ± 0.02	1.15 ± 0.05	1.07 ± 0.06
		1.0	2.34 ± 0.14	5.7 ± 0.36	5.29 ± 0.40
		0.20	14.5 ± 1.92	35.3 ± 4.7	32.8 ± 4.6
		100	50 ± 9.0	122 ± 22.0	113 ± 21.0
S. 'Sweet Chico'	*M*-xylene as single VOC	0.20	0.51 ± 0.04	1.23 ± 0.10	1.14 ± 0.10
		1.0	1.66 ± 0.10	4.05 ± 0.25	3.8 ± 0.28
		10	8.2 ± 0.63	20.0 ± 1.57	18.6 ± 1.66
		100	22.6 ± 2.17	55.2 ± 5.4	51.2 ± 5.4
D. 'Janet Craig'	Toluene as single VOC	0.20	0.47 ± 0.03	0.46 ± 0.03	4.0 ± 0.52
		1.0	2.59 ± 0.06	2.56 ± 0.08	22.2 ± 2.51
		10	16.0 ± 1.73	15.7 ± 1.73	136 ± 21.2
		100	119 ± 6.9	117 ± 7.2	1014 ± 127
D. 'Janet Craig'	*M*-xylene as single VOC	0.20	0.08 ± 0.01	0.08 ± 0.01	0.68 ± 0.12
		1.0	0.57 ± 0.10	0.56 ± 0.09	4.84 ± 0.98
		10	8.4 ± 0.50	8.3 ± 0.05	72 ± 9.0
		100	73 ± 4.7	71.6 ± 4.9	621 ± 80
D. 'Janet Craig'	Toluene in binary mixture	0.20	0.19 ± 0.01	0.19 ± 0.01	1.6 ± 0.19
		1.0	2.24 ± 0.03	2.21 ± 0.05	19.2 ± 2.14
		10	16.9 ± 1.07	16.7 ± 1.11	145 ± 18.5
		100	61.4 ± 5.9	60.5 ± 5.94	524 ± 77.0
D. 'Janet Craig'	*M*-xylene in binary mixture	0.20	0.24 ± 0.02	0.23 ± 0.02	2.0 ± 0.29
		1.0	2.36 ± 0.12	2.32 ± 0.13	20.2 ± 2.47
		10	13.7 ± 1.44	13.5 ± 1.48	117 ± 17.9
		100	50 ± 2.5	48.8 ± 2.6	423 ± 51.5

Values are means ± S.E. ($n = 4$); *pl = the potted-plant, i.e. the microcosm.

activity level than 87% d^{-1} at 10 ppm (22.9 versus 8.2 mg d^{-1} $plant^{-1}$), although the increase is modest (Tables II, III). The observations suggest that, with this plant species, the onset of enzyme saturation in the microorganisms of its root-zone community for this VOC may occur at about 100 ppm *m*-xylene. That is, it seems either that the level of induction tends to match, but not exceed, the capacity needed to metabolise the concentration levels encountered, or that incipient toxicity might follow a further concentration increase of this substance.

With 0.20 or 1.0 ppm *m*-xylene alone, *D*. 'Janet Craig' performed more slowly than *S*. 'Sweet Chico', achieving 42–60% d^{-1} removal by Day 5, and increasing by only a further 10–20% per day up to 9 days. However, at 10 ppm, removal rates reached 89% d^{-1} at 5 days, rising to 150% d^{-1} on Day 6 (Table II, Figure 5). At 100 ppm, activity was again relatively low in this species, yielding a Day 5 rate of

77% d^{-1}, ie, similar to those seen at 0.20 an 1.0 ppm *m*-xylene. Nevertheless, with 100 ppm *m*-xylene, *D*. 'Janet Craig' displayed markedly higher removal rates than *S*. 'Sweet Chico' (77 versus 24% d^{-1} on Day 5; Figure 5; Table II).

In summary, in response to *m*-xylene applied as a single VOC, *S*. 'Sweet Chico' exhibited about twice the activity of *D*. 'Janet Craig' at the two lower doses (0.20 and 1.0 ppm), but only about half that of *D*. 'Janet Craig' at the two higher doses (10 and 100 ppm). The slower performance of *D*. 'Janet Craig' at the lower doses seems to reflect a delay in the adaptation of this species-microcosm to *m*-xylene, ie, a slower induction process compared with that for toluene, especially when the stimulus is weak. The slower performance of *S*. 'Sweet Chico' at the higher doses, and especially the lack of further induction at the highest dose, suggest that 100 ppm *m*-xylene may be sufficient to bring about either saturation of this plant/substrate system for this VOC, or the onset of toxicity at some point in the microcosm of this species. *D*. 'Janet Craig', in contrast, performed well and underwent further induction in response to high *m*-xylene levels, indicating that saturation and/or toxicity had not occurred with this species, which may have been helped by having over twice as much potting mix per pot as S. 'Sweet Chico'; (Tables I, III). Overall, however, the differences in response with the two plant species at each of the various dosage levels of the same VOC, point once again to plant species-specific differences in composition and/or population balance and dynamics of the microbial community, in addition to any differences in the amount of potting mix present.

3.5. Removal from binary mixtures

With *D*. 'Janet Craig', when the toluene removal rates (λ or % d^{-1}) from pots exposed to the mixtures (toluene + *m*-xylene) are compared with those exposed to toluene alone, the recorded rates were similar with dosages of 1.0, 10 and 100 ppm, but lower from the mixed dosage of 0.2 ppm (Figure 4; Table II). However, these differences in rates from the mixture cf. from toluene alone were very small. There thus appeared to be no significant interaction between the two VOCs affecting toluene removal from the mixtures. In these experiments, additional daily doses were applied beyond the five used in the original protocol, and, with the two lower dosages, there was evidence of further induction of toluene removal on Days 6–7, but with no additional induction beyond Day 5 at the two highest dosages (Figure 4; Tables II, III). In seems, then, that, depending on dosage, the removal induction process for toluene required 4–9 days, being shorter with higher dosages (greater stimulation).

Comparing *m*-xylene removal rates from the binary mixtures with those from *m*-xylene alone, for the two lower dosages (0.2, 1.0 ppm), the removal rates (λ or % d^{-1}) from the mixtures were much higher than with the single VOC. However, at the two higher doses (10 and 100 ppm) rates from the mixtures and *m*-xylene alone were similar (Figure 5; Table II). These results suggest that, at least at lower

dosages, a synergistic reaction was occurring between the two VOCs, the presence of toluene accelerating the removal of *m*-xylene and thereby overcoming the slow induction responses to low dosages of *m*-xylene alone. The converse does not appear to hold: ie. the presence of *m*-xylene had little or no effect on toluene removal rates in the *D*. 'Janet Craig' microcosm.

4. Discussion

4.1. General patterns of response

Overall, the results of the dose-response experiments demonstrate that in these potted-plant microcosms:

- induction of the VOC removal response does indeed occur at concentrations of 200 ppb, ie in the mid-range of TVOC concentrations found in the office study to be high enough to induce removal responses of up to 75% (see preceding paper)
- the system having been stimulated, test-chamber concentrations can be reduced to below detection limits of the GC (ie <20 ppb) within 24 h
- further stepwise induction occurs, by up to ten orders of magnitude in activity, as VOC dose concentrations are increased (and to orders of magnitude above allowable Australian occupational 8-hour averaged exposure concentrations of the two VOCs tested)
- synergistic interactions can be found in removal responses when the potted-plant microcosm is exposed to mixtures of VOCs

In addition to the rate parameters considered above, Table III presents, for all five experiments, removal activities after five daily dosages (Day 5), expressed in absolute terms (mg VOC d^{-1} pot-$plant^{-1}$, and alternative bases of calculation). This measure of plant activity permits direct calculations of the expected air purification impact of one or more potted-plants in room-sized spaces. Corresponding Day-5 estimates of the exponential constants for VOC decay and VOC half-lives are also presented (Table II). It is noteworthy that, for 12 of the 24 half-lives reported values were less than 6 h, with only five of the remainder exceeding 12 h. As implied by the % dose removed per day data, the shortest half-lives occurred at the lower VOC dosage levels. These findings point to VOC removal activity (after 4–5 days of exposure for induction) being capable of making a very substantial contribution to indoor air quality in real world situations.

At the lower end of the concentration range examined, the study demonstrates that the potted-plant/substrate microcosm can and does mount a metabolic induction response to airborne toluene and/or *m*-xylene at levels of TVOCs generally associated with 'good quality indoor air' (<0.20 ppm). At the intermediate concentrations

(1.0 and 10 ppm), the system responded in an equally effective manner. Yeom *et al.* (1997) found that, in laboratory microbial cultures, cells of an *Alcaligines* species isolated from a BTEX-contaminated soil required 5–10 h of exposure ('pre-adaption') to benzene, toluene or *m*-xylene before removal activity commenced. This finding is consistent with the 4–5 days required to induce full activity in response to the air-borne toluene and/or *m*-xylene used in the present study.

At the upper end of the concentration range tested, only with *m*-xylene at the highest concentration (100 ppm), and only with one of the two plant species (*S*. 'Sweet Chico'), was a lack of any further induction response observed. Alagappan and Cowan (2003) found that when benzene and toluene were used as sole carbon sources (ie sole nutriment) in bacterial cell cultures, substrate inhibition of cell growth could occur at high concentrations of these VOCs, and that toxic effects could be encountered with toluene. In the current study, incipient toxicity may have contributed to the lack of further responsiveness with *S*. 'Sweet Chico' in the face of 100 ppm *m*-xylene, along with possible saturation of the particular enzyme(s) of the degradation pathway, in accordance with Michaelis-Menten enzyme kinetics. No evidence of possible saturation was found in any of our previous studies (cf. Tarran *et al*., 2002; Wood *et al*., 2002; Orwell *et al*., 2004).

The evidence of a synergistic reaction in the binary mixtures, at least at the lower end of the concentration range, is in line with the findings of Yeom *et al*. (1997), who reported that *Alcaligines* species, after pre-adaption to benzene, degraded toluene and *m*-xylene much faster than toluene-adapted cells, but that the presence of toluene was required for the cells to sustain *m*-xylene removal rates. The authors suggested that the induction of activity of the enzyme catechol 1,2 dioxygenase (the catechol ring-splitting step in the microbial degradation of benzene and toluene) was the site of the mechanism responsible for these interactions. The results are consistent with the one-way synergy observed in the present investigation, ie., stimulation of *m*-xylene removal by toluene, but the lack of stimulation of toluene removal by *m*-xylene. Figure 6 summarises alternative degradative path-

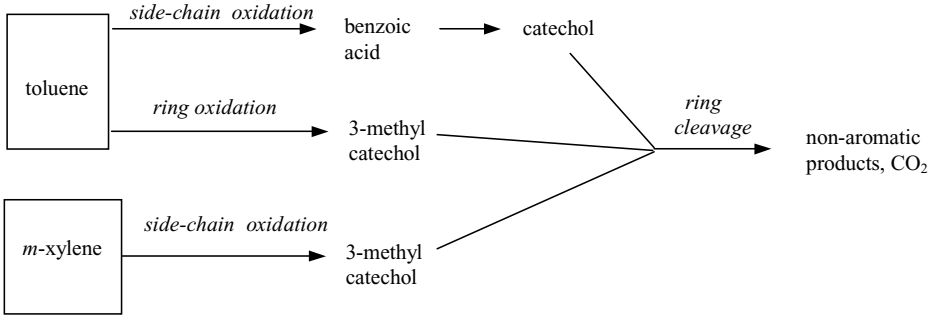

Figure 6. Outline scheme for bacterial degradation of toluene and *m*-xylene (adapted from: Wrenn, 1998; Hyatt and Oh, 2004; Zeng, 2004).

ways that could be involved in the observed removal (with carbon dioxide as the end-product).

4.2. Removal rates expressed on alternative bases

Very different impressions of rates and hence of potted-plant performance are to be gained by expressing rates on different bases (Table III), eg, as a function of 'per pot-plant' microcosm (i.e. per chamber); per kg dry weight of potting mix; or per square metre of leaf area (as a measure of plant material supporting VOC removal activity). Such comparisons are of relevance to research on optimising potting mix components of the system, on inter-species potted-plant performance, intra-species performance with different VOCs, and investigating plant/rhizosphere microorganism interactions. From an applied horticultural perspective, the 'per pot-plant' basis provides a direct measure of the VOC(s) removed by the potted-plant microcosm per day, since the 'pot of specified diameter' is the standard unit of plant material in the horticultural industry and among building owners/managers. For this purpose, the measure is the most practical basis of comparisons among plant species with respect to removal of different VOCs (see also Orwell *et al*., 2004).

5. Implications of Findings

Over 350 VOCs have been identified in indoor air (Sullivan Jr. *et al*., 2001). The number of possible subsets and mixtures in any one building, or room, is thus almost infinite, although similar sets of predominant VOC constituents are often found at any one time in any one building and/or locality (see preceding paper; Brown *et al*., 1994; Brown, 1997; Sullivan Jr. *et al*., 2001; EA, 2003) The plant/potting-mix microcosm is also complex, the balance of species presence and relative abundance of the root-zone community being specific to the plant species in question, and responding to environmental variations such as pH, media composition and nutrient factors (Atwell *et al*., 1999). We isolated over fifty species of potting mix bacteria in previous studies (Wood *et al*., 2002). In response to different TVOC mixtures, it is to be expected that, in addition to the induction of biochemical enzyme processes within the cells, there will be population shifts within the bacterial community as part of the induction response (Pucci *et al*., 2000; Siciliano *et al.*, 2003; Sharma *et al*., 2003; Paralesi and Haddock, 2004). The degree of versatility of response exhibited in this and the office study (and with benzene and *n*-hexane in our previous laboratory studies), strongly implicates both biochemical induction and substrate population changes as being involved in the induction and operation of VOC removal processes of the potted-plant microcosm. The findings confirm and elucidate the findings of the office study, and add to an understanding of the microbial ecophysiological basis of the VOC removal response.

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