

Phytoremediation of BTEX from indoor air by Hyrcanian plants

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Abstract

Background: Phytoremediation is one of the available and simple techniques for removing benzene, toluene, ethylbenzene, and xylene (BTEX) from indoor air. This study aimed to evaluate phytoremediation of low concentrations of BTEX by Hyrcanian plants including *Ruscus hyrcanus* and *Danae racemosa*.

Methods: The test chamber was used to evaluate the removal of BTEX. Benzene, toluene, ethylbenzene, and xylene were injected into the chamber using Gastight syringes (Hamilton) to generate the concentration of 10 (benzene), 20 (toluene), 20 (ethylbenzene), and 50 (xylene) $\mu\text{L/L}$.

Results: *Ruscus hyrcanus* was able to remove BTEX (10, 20, 20, and 50 $\mu\text{L/L}$) from air after 3 days. *D. racemosa* could uptake BTEX (10, 20, 20, and 50 $\mu\text{L/L}$) from air after 4 days. Removal efficiency was calculated based on leaf area and volume of the chamber. *R. hyrcanus* showed the highest removal efficiency ranged from 8.5075 $\text{mg/m}^3/\text{h}\cdot\text{cm}^2$ for benzene to 86.66 $\text{mg/m}^3/\text{h}\cdot\text{cm}^2$ for xylene. The increase in BTEX phytoremediation was assessed after repeated exposures. A significant phytoremediation efficiency was obtained after the third injection of BTEX to the chamber. Afterwards, the effects of BTEX on anatomical and morphological structure of plants were studied. The results of Photomicrography showed that tissue structures of leaves and stems changed. Study of *D. racemosa* and *R. hyrcanus* stems showed that vascular bundles also changed. The development of crystal in vacuole of spongy parenchyma was the main anatomical change of *R. hyrcanus* and *D. racemosa* compared to the control samples.

Conclusion: It can be concluded that *R. hyrcanus* and *D. racemosa* can be used for phytoremediation of indoor air pollution.

Keywords: Volatile organic compounds, Air pollution, Indoor, Plant leaves, Sick building syndrome

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Introduction

Indoor air pollution is generally more than the ambient air pollution that can provide potential risk to human health (1,2). People spend 90% of their time in indoor spaces such as homes, offices, hospitals, and schools (1,3,4). Volatile organic compounds (VOCs) are present in the indoor spaces. Benzene, toluene, ethylbenzene, and xylene (BTEX) are common VOCs present in both outdoor and indoor air, and also indoor air is a significant source of human exposure to BTEX (5). Indoor sources of benzene are typically newspapers, school books, liquid waxes, fiberglass, adhesives, paints, wooden paneling, paint remover, and nylon carpets (3,4). Toluene and ethylbenzene are found in gasoline, paints, fingernail polish, lacquers, and insecticides (6). The level of BTEX

that a person is exposed to in a day depends on the person's personal activities and indoor sources (7).

Benzene is carcinogenic for human (8). Although toluene, ethylbenzene, and xylene are not considered as carcinogenic, these compounds can create problems for nervous system, liver kidney, and respiratory system (6,9). The American Conference of Governmental Industrial Hygienists (ACGIH) has proposed threshold limit value for benzene, toluene, ethylbenzene, and xylene as 0.5, 20, 20, and 100 ppm TWA (9). The threshold limit values are not set by the Iranian government for BTEX in homes. High concentrations of benzene and toluene, which were found in some schools of Italy, were 1.405 and 2.83 $\mu\text{g}/\text{m}^3$, respectively (10). The indoor concentrations of these compounds, which were measured in the United States,



was 2.6–5.8 $\mu\text{g}/\text{m}^3$. On the other hand, the indoor level of benzene was reported between 2 and 12 $\mu\text{g}/\text{m}^3$ in central European cities, and it was between 0.7 and 7.2 $\mu\text{g}/\text{m}^3$ in Japan, which is similar to those reported in Europe and the United States (11).

Low indoor air quality can result in sick building syndrome and physical problems such as headache, asthma, fatigue, irritation of eyes, nose, and throat, and dry/itchy skin (12,13).

Many studies have demonstrated that plants have the ability to remove VOCs despite the fact that the physiology of these plants has not been completely understood. Microorganisms in the root area can also remove pollutants (7,14–23). Cuticle and stomata of plants have been proven to be efficient in treating polluted indoor air. In plants' tissues, benzene is converted to nonvolatile organic acids with the aromatic ring cleavage in leaves (24).

Several indoor plants have been found which have the ability to reduce BTEX such as *Hemigraphis alternata*, *Hedera helix*, *Tradescantia pallida*, *Asparagus densiflorus*, *Zamioculcas zamiifolia*, *Sansevieria trifasciata*, *Epipremnum aureum*, *Philodendron domesticum*, *Hemigraphis alternata*, *Tradescantia pallida*, *Spathiphyllum wallisii*, and *Syngonium podophyllum* (5,13,20). The Hyrcanian (Caspian) region, which extends throughout the south coast of the Caspian Sea in the northern part of Iran, covers an area of 1925 125 ha, which has been investigated in many research (25–28). *Ruscus hyrcanus* Woron and *Danae racemosa* are perennial plants, which cultivate in this region. They are monocot plants that belong to the Asparagaceae family (29). The present study aimed to evaluate the potential of *R. hyrcanus* and *D. racemosa* for phytoremediation of BTEX from indoor air. Also, the effects of BTEX on morphological and anatomical characteristics of *R. hyrcanus* and *D. racemosa* were investigated. For this purpose, 10 $\mu\text{L}/\text{L}$ benzene was injected into the chamber, which was twenty times more than the threshold limit value (TWA) of benzene proposed by the ACGIH. The concentrations of toluene and ethylbenzene in chamber was 20 $\mu\text{L}/\text{L}$, which was equal to TWA of the ACGIH. Also, 50 $\mu\text{L}/\text{L}$ xylene was injected into the chamber, which was half of the TWA proposed by the ACGIH.

Materials and Methods

Plant culture condition

Ruscus hyrcanus Woron and *D. racemosa* are native plants of Iran. There is no report of the use of Iranian native plants such as *R. hyrcanus* and *D. racemosa* for remediation of the indoor air pollution. The species were selected

by considering some characteristics such as evergreen, low/medium water requirement, and plant families with high absorption of pollutants. Three-year-old plants were collected from Noor, Sari, and the Hyrcania region in Iran. The plants were kept indoor for 6 months with $50\pm 10\%$ relative humidity, 21–25°C temperature, and 1000 lux light. They were watered every 2 days. The plants were examined for no pest. Before the experiments, plants' leaves were thoroughly cleaned with distilled water. Leaf areas of plants were measured by a graph paper. The characteristics of the plants are presented in Table 1.

Exposure condition

The test chamber was made of Plexiglas (based on the reviewed texts) with volume of 144 L. Three chambers were made, then, the best one was selected. Testing was done to make sure that the chambers had the quality required for this study such as sealing, without BTEX, and adsorption, and then, the best chamber was selected. Because of the presence of VOCs in adhesive, no BTEX adhesive was used for sealing the chamber. A 12 W fan was put in the chamber for the evaporation and circulation of BTEX. Benzene, toluene, ethylbenzene, and xylene were injected into the chamber using Gastight syringes (Hamilton) to generate the concentration of 10 (benzene), 20 (toluene), 20 (ethylbenzene), and 50 (xylene) $\mu\text{L}/\text{L}$. These concentrations were compared with TLV-TWA proposed by ACGIH.

BTEX remediation

To consider plant and soil evapotranspiration, a beaker with 250 mL water was placed in the test chamber. Prior to set up plants in the chamber for gas exposure, the plants were watered until saturation, and then, they were allowed to stay in the lab for 1 hour before testing. Due to adsorption, leakage, and chemical reaction of the chamber, the empty chamber was tested for 10, 20, 20, and 50 $\mu\text{L}/\text{L}$ concentrations of BTEX. The BTEX concentration decreased by the empty chamber about 5–7% per 24 hours. The plants were placed in the sealed chambers and the initial concentrations of 10, 20, 20, and 50 $\mu\text{L}/\text{L}$ of BTEX were injected into the chambers. For self-inspection and quality audit, some procedures such as calibration curve, blank chamber, repeated test, repeated sampling each time, and CO_2 testing were used. Sampling was performed at hourly or daily intervals as required. Three samples were taken each time.

At first, the daily remaining concentration of BTEX was calculated to observe the differences in reduction among 4 days (96 hours). Three replicates of each species were

Table 1. Characteristics of plants

Species	Leaf Area (cm^2)	Pot Diameter (cm)	Plant Height (cm)
<i>Ruscus hyrcanus</i> Woron	732.58 \pm 10	20	26 \pm 1.02
<i>Danae racemosa</i>	722.6 \pm 10	20	48 \pm 2.1

studied.

The removal efficiency was calculated as follows (13).

$$(\text{leaf area}) (\text{mg}/\text{m}^3/\text{h}.\text{cm}^2) = (P \times F \times CV) / (L \times T)$$

where P is the gas concentration removed in a chamber with plants ($\mu\text{L}/\text{L}$), F is the factor for converting ($\mu\text{L}/\text{L}$) to (mg/m^3), CV is the volume of the chamber (m^3), L is the total leaf area (cm^2), and T is the gas exposure time (72 hours).

The effect of subsequent treatments

Three replicates of *R. hyrcanus* and *D. racemosa* were tested. The initial concentrations of BTEX (10, 20, 20, and 50 $\mu\text{L}/\text{L}$) were injected into the chambers. To understand subsequent removal, every plant was assessed three times.

Gas analysis

Gas chromatography flame ionization detector (GC-FID) was used to analyze benzene concentration in the chamber. BTEX was analyzed by a gas chromatography flame ionization detector (Varian series CP3800). A capillary column CP-sil 13CB (25 m \times 0.25 mm \times ID 0.25 μm) was used. The operating conditions for GC-FID were injector and detector temperatures of 200 and 220 $^\circ\text{C}$, respectively. The GC oven was held at 40 $^\circ\text{C}$ for 1 minute, and then, ramped at 0.5 to 42 $^\circ\text{C}/\text{min}$ and held for 3 minutes.

Data analysis

The experiments were performed in triplicate and standard errors were calculated. The data were analyzed using independent t test by SPSS version 17.

Anatomical studies

Stem and leaf were laid in alcohol and glycerin at the same portions. The cutting sections were located in the hypochlorite sodium (5%) for 30 minutes, and then, placed in Carmen Zuji for 30 minutes to become purple. They were located in acid acetic (3%) for 3 minutes to be neutralized. The sections were placed in methylene blue for 2 seconds. Leaf and stem sections were studied through light photomicrography (Nikon, YS100).

Results

Benzene, toluene, ethylbenzene, and xylene remediation and removal efficiency

Plants were exposed to different BTEX concentrations, which could show the removing ability. Figure 1a presents the results for *R. hyrcanus* and *D. racemosa*, which were exposed to benzene. *Ruscus hyrcanus* was able to remove 10 $\mu\text{L}/\text{L}$ of benzene after 48 hours while *D. racemosa* could uptake 10 $\mu\text{L}/\text{L}$ after 96 hours. The removal efficiency of benzene after 72 hours by *R. hyrcanus* (8.5075 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$) was greater than that by *D. racemosa* (2.1418 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$). Both plants reduced the toluene concentration in the chamber. *R. hyrcanus* removed 20 $\mu\text{L}/\text{L}$ toluene

after 72 hours and *D. racemosa* removed 20 $\mu\text{L}/\text{L}$ toluene after 96 hours (Figure 1b). The removal efficiencies of toluene for *R. hyrcanus* and *D. racemosa* after 72 hours were 22.576 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$ and 10.11 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$ respectively. Patterns of ethylbenzene and xylene removal for plants after 96 hours are shown in Figures 1c and 1d. Remediation speed of ethylbenzene and xylene by *R. hyrcanus* was more than that by *D. racemosa*. Also, the phytoremediation rate of ethylbenzene by *R. hyrcanus* and *D. racemosa* were 17.33 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$ and 2.9 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$, respectively. The xylene removal efficiency by *R. hyrcanus* and *D. racemosa* was about 86.66 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$ and 29.14 $\text{mg}/\text{m}^3/\text{h}.\text{cm}^2$, respectively.

Change in remediation by the number of exposure

When plants were frequently exposed to BTEX concentrations (3 times), they showed increased phytoremediation. Figure 2.a1 shows *R. hyrcanus* which was exposed to benzene. Comparison of residual concentrations between the first and third exposure in the chamber showed no significant increase in the ability reduction of benzene. *Danae racemosa* showed a significant increase in phytoremediation between the first and third exposures (Figure 2.b1). There was significant changes in toluene, ethylbenzene, and xylene residual concentrations regarding the first and third exposure of *R. hyrcanus* (Figure 2.a2, a3, a4) and of *D. racemosa* (Figure 2.b2, b3, b4). A significant increase in phytoremediation was indicated between the first and third exposure of both plants to toluene, ethylbenzene, and xylene.

The effect of BTEX on leaf and stem

Ruscus hyrcanus and *D. racemosa* stems were similar to monocotyledonous plants, which include epidermis on

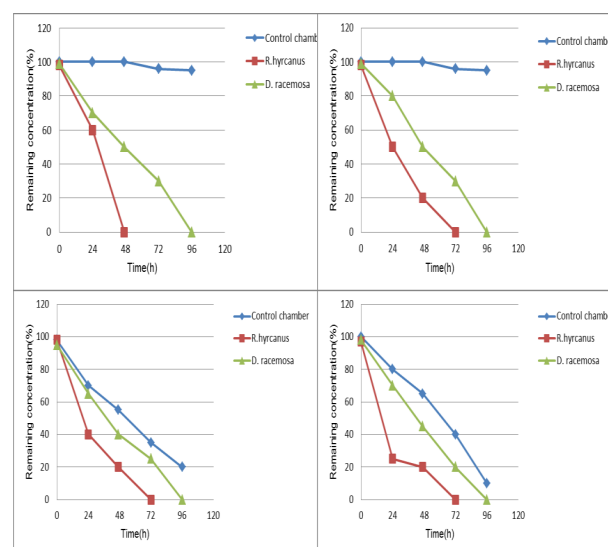


Figure 1. BTEX removal of *R. hyrcanus* and *D. racemosa*. Initial concentration of benzene, toluene, ethylbenzene, and xylene were 10, 20, 20, and 50 $\mu\text{L}/\text{L}$. Benzene (a), Toluene (b), Ethyl Benzene (c), and Xylene (d) (n=3)

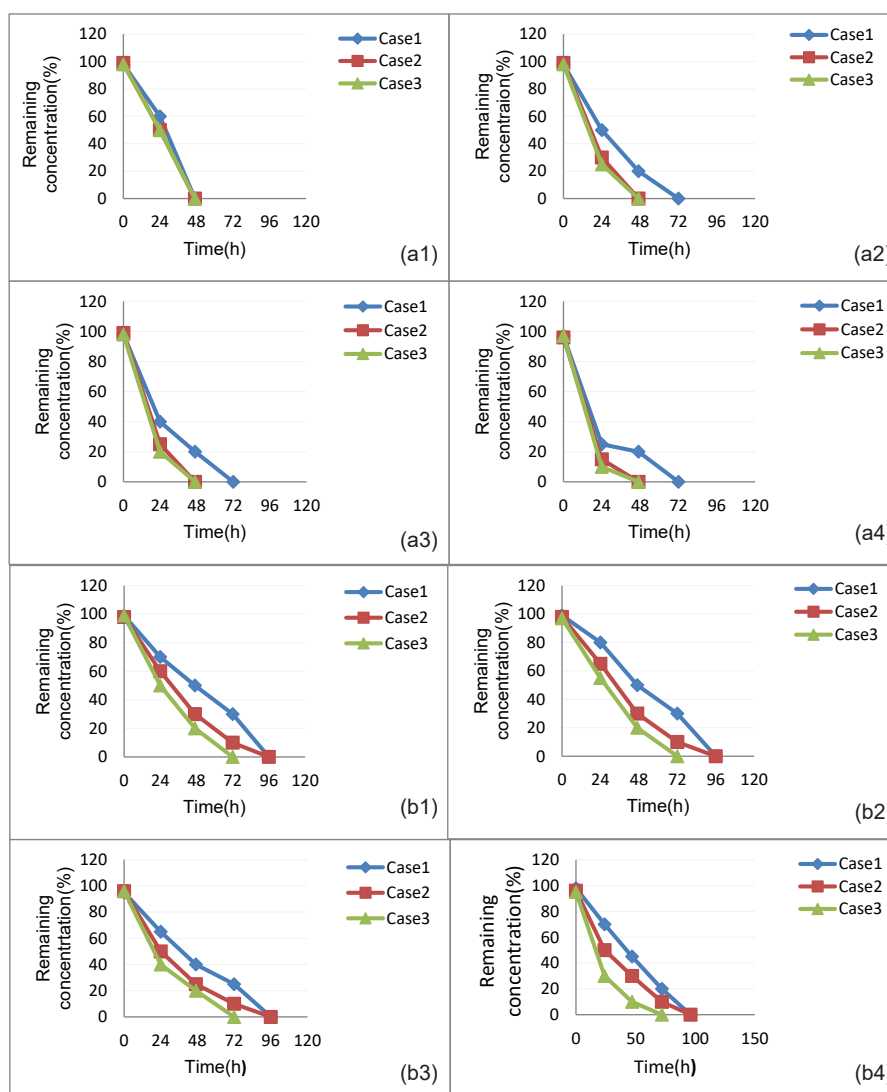


Figure 2. The decrease in BTEX concentration by the plants in consecutive frequent exposure. The decrease in benzene (a1), toluene (a2), ethylbenzene (a3), and xylene (a4) by *R. hyrcanus*. The decrease of benzene (b1), toluene (b2), ethylbenzene (b3), and xylene (b4) by *D. racemosa*. Case 1 (The first exposure to BTEX), Case 2 (The second exposure to BTEX), and Case 3 (The third exposure to BTEX).

the surface. The strong tissue (collenchyma cells) was found under the epidermis. The cortex was composed of cells with a thin and pectocellulosic wall, which had a relatively spherical shape. Ground tissue was in the central section which became a lignified cell by aging. Vascular bundles around the same central circles, were developed in the ground tissue (Figure 3a, Figure 4a). There was no significant change in *R. hyrcanus* and *D. racemosa* stems exposed to BTEX, indicating that plants were not resistant to BTEX exposure. Study of *D. racemosa* stems showed that the cortex cells increased and the vascular bundles reduced (Figure 4b, 2c). Of two species studied, *R. hyrcanus* had the highest reduction in the number of vascular bundles (Figure 3b, 3c).

Ruscus hyrcanus and *D. racemosa* leaves were similar to monocotyledonous plants. The outer layer of the leaf consisted of epidermis cells. The spongy parenchyma was

observed in the middle section of leaves, and parenchyma close to the outer sections was more compact than the middle sections. Among the cells of spongy parenchyma, vascular bundles were found (Figure 5.1a, 11a). Comparing stems and leaves of *R. hyrcanus* and *D. racemosa*, there were no significant changes in response to BTEX exposure. The differences observed between the control and treatment leaves of *R. hyrcanus* and *D. racemosa* were characterized mainly by the development of crystal in the vacuole of spongy parenchyma.

In the leaf of *D. racemosa*, more crystal accumulation was observed (Figure 5.1b, 11b).

R. hyrcanus and *D. racemosa* looked healthy and some of them produced new leaves and fruits (red berry).

Discussion

Of two species tested, *R. hyrcanus* had the highest ability

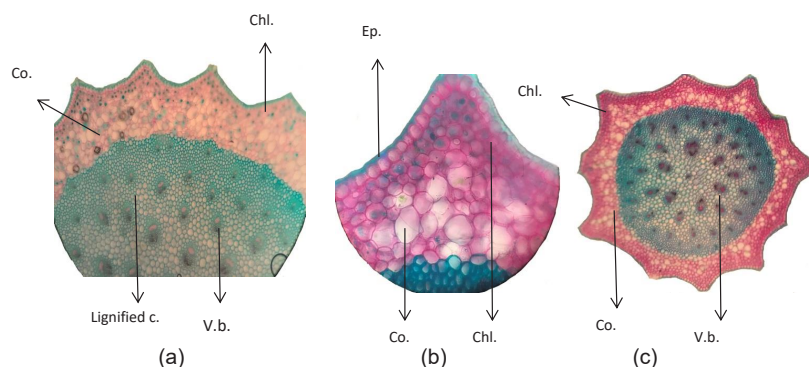


Figure 3. Stem anatomy. *Ruscus hyrcanus*. (a) Control (objective×40), (b) BTEX exposure (objective×40), and (c) BTEX exposure (objective×10). Collenchyma cell (Chl.), Cortex (Co.), Lignified cell (Lignified c.), Vascular bundles (V.b.), and Epidermis (Ep.).

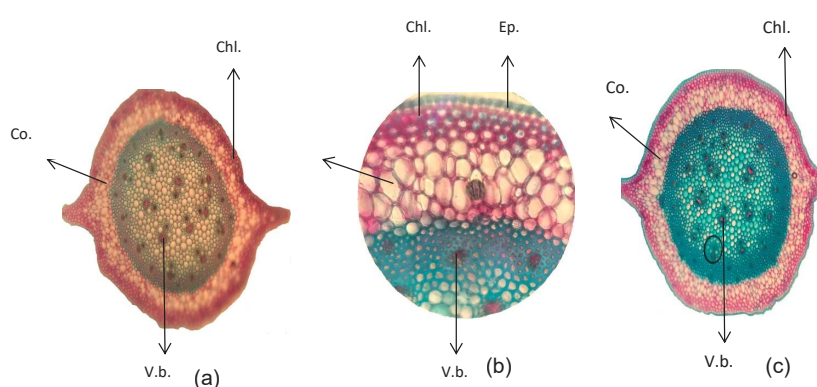


Figure 4. Stem anatomy. *Danae racemosa*. (a) Control (objective×10), (b) BTEX exposure (objective×40), and (c) BTEX exposure (objective×10). Collenchyma cell (Chl.), Cortex (Co.), Epidermis (Ep.), Lignified cell (Lignified c.), and Vascular bundles (V.b.).

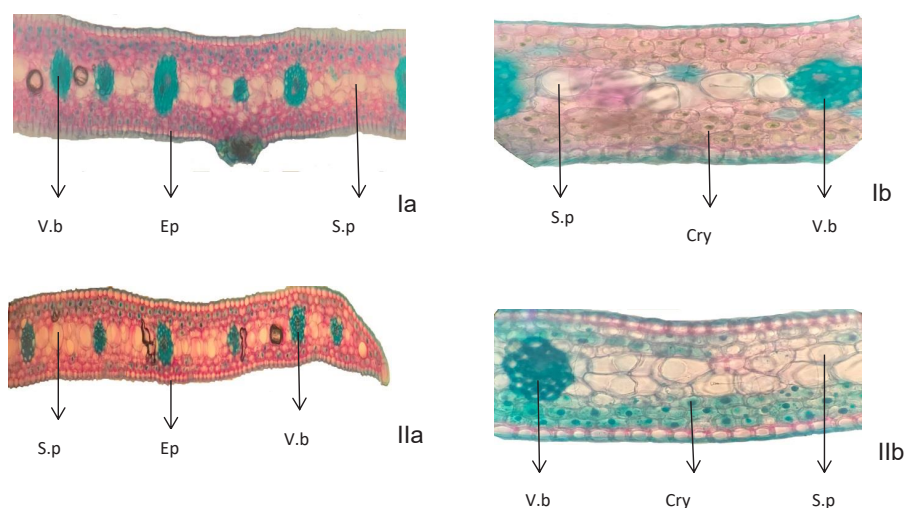


Figure 5. Leaf anatomy. I. *Ruscus hyrcanus*. (Ia) Control (objective×10), (Ib) BTEX exposure (objective×40). II. *Danae racemosa*. (IIa) Control (objective×10), (IIb) BTEX exposure (objective×40). Epidermis (Ep.), Spongy parenchyma (S.p.), Vascular bundles (V.b.), and Crystal (Cry).

in the phytoremediation of air polluted with BTEX. Benzene reduction pattern was totally different from that observed for toluene, ethylbenzene, and xylene. When *R. hyrcanus* was exposed to four gases simultaneously, benzene reduced faster than toluene, ethylbenzene,

and xylene. This may be due to the different doses of BTEX which were injected into the chamber. Reduction patterns of benzene, toluene, ethylbenzene, and xylene were similar to those of BTEX phytoremediation by *D. racemosa*. Different concentrations were injected into

the chamber and *D. racemosa* reduced all of them in four days. Various plants have shown different potentials in phytoremediation of BTEX. In a study by Mosaddegh et al, 2 $\mu\text{L/L}$ of benzene was completely removed by *Opuntia microdasys* after 48 hours (3). The findings of this study demonstrated that 10 $\mu\text{L/L}$ of benzene was reduced after 48 hours by *R. hyrcanus*. In addition, 2 $\mu\text{L/L}$ toluene and xylene were removed after 55 and 47 hours, respectively, by *O. microdasys* (3). However, xylene and toluene were removed after 72 and 96 hours, respectively, in the present study.

In the presence of four gases (total BTEX $\sim 100 \mu\text{L/L}$), toluene, ethylbenzene, and xylene were taken up at 72 hours by *R. hyrcanus* while toluene, ethylbenzene, and xylene were removed at 96 hours by *D. racemosa*. *R. hyrcanus*, and *D. racemosa* removed benzene after 48 and 72 hours, respectively. In contrast, *Zamioculcas zamiifolia* lowered the concentration of 80 $\mu\text{L/LBTEX}$ mixture within 14 days (5). It can be due to the effect of different plant species and different leaf areas (5). The removal efficiency varied among different plants. The results demonstrated that removal efficiency depends on BTEX concentration and plant species. Yang et al found that *H. alternata* plant had the highest removal efficiency for benzene and toluene (20). The removal efficiency of benzene by *R. hyrcanus* was greater than that by *D. racemosa*. The results showed that the removal efficiency of toluene, ethylbenzene, and xylene by *R. hyrcanus* was higher than that by *D. racemosa*. The results also indicated that *R. hyrcanus* and *D. racemosa* had the highest ability in phytoremediation of xylene. But, *R. hyrcanus* had more potential for phytoremediation of xylene. BTEX can be adsorbed by plant surfaces (e.g., fruits, stems, leaves) and can also be absorbed by stomata and microorganisms in the root zone. In this study, the removal efficiency of leaves by isolating the root system was assessed. In general, it was found that the BTEX removal efficiency by *R. hyrcanus* was significantly higher than that by *D. racemosa*. Yoo et al found that a synergistic effect can decrease the plant phytoremediation potential efficiency (13). The removal efficiency of benzene and toluene per unit area of leaf per hour for *H. helix* were 57.5 $\text{ng/m}^3/\text{h.cm}^2$ and 112.2 $\text{ng/m}^3/\text{h.cm}^2$, respectively, when both gases were injected into the chamber (13). In the present study, however, BTEX removal efficiency that was injected into the chamber, was tested. The removal efficiency can be increased if benzene, toluene, ethylbenzene, and xylene will be exposed singly to plants in the present study.

Comparing the toluene, ethylbenzene, and xylene removal, potted plant and soil showed a significant difference between the first (10, 20, 20, and 50 $\mu\text{L/L}$) and third (10, 20, 20, and 50 $\mu\text{L/L}$) concentrations exposures by *R. hyrcanus*. Similarly, there was a significant difference between potted plant and soil regarding exposure to *D. racemosa*. So, repeated injections can cause the greatest removal (24 hours) for both plants. However, it was demonstrated that

the repetition of injections can result in a great increase in removal, which is consistent with the results of a study by Kim et al. They investigated the effect of different plants on the remediation of toluene, and found that *Pittosporum tobira* and *Ilex cornuta* had the highest removal rate. Herb plants uptake toluene more than woody foliage plants and herbaceous plants (19).

It should be noted that BTEX is not toxic for plants. Visual symptoms of toxicity such as chlorosis or necrosis were not observed in *R. hyrcanus* and *D. racemosa*, and some of them produced new leaves and fruits (red berry).

In a study by Campos et al, *Impatiens walleriana* was affected by benzene exposure. Yellow coloration and white patches were observed on the leaves and flowers (30).

In this experiment, there was no significant change in *R. hyrcanus* and *D. racemosa* stems. The number of vascular bundles was reduced in both plants. It was observed that the number of *R. hyrcanus* vascular bundles was more reduced in BTEX exposure. It can be due to the effect of BTEX on *R. hyrcanus* vascular bundles. *R. hyrcanus* had the highest ability as well as the highest reaction stems in BTEX phytoremediation. The results indicate that cortex was increased in *D. racemosa* but there was no relationship between the cortex changes and BTEX exposure for *R. hyrcanus*. The results of comparative anatomical study *R. hyrcanus* and *D. racemosa* leaves showed crystal accumulation in spongy parenchyma. However, *D. racemosa* leaves indicated more crystalline accumulations. It can be due to the issue that BTEX mainly incorporated into organic acids in *R. hyrcanus* leaves (24).

The BTEX removal was assessed by the control chamber (5%-7% during a day) and the results obtained in this study are consistent with those reported by Orwell et al (12). Kim et al also reported 7.3% toluene removal during a day for the empty chamber (19). The findings of the present study showed that *R. hyrcanus* and *D. racemosa* can be commonly used in different buildings.

Conclusion

Ruscus hyrcanus and *D. racemosa* have the ability to remove different concentrations of benzene, toluene, ethyl, benzene, and xylene from contaminated indoor air. They can be used to improve the indoor air quality. Plants are considered to be a good candidate for air phytoremediation because they did not show specific symptoms of any toxicity damages. Different concentrations have different removal efficiency and different effects on plants. The removal efficiency of BTEX varied among different plants. *R. hyrcanus* showed the highest removal efficiency ranged from 8.5075 $\text{mg/m}^3/\text{h.cm}^2$ for benzene to 86.66 $\text{mg/m}^3/\text{h.cm}^2$ for xylene while *D. racemosa* showed the removal efficiency ranged from 2.14 $\text{mg/m}^3/\text{h.cm}^2$ for benzene to 29.14 $\text{mg/m}^3/\text{h.cm}^2$ to xylene. This index can be used to identify *R. hyrcanus* and *D. racemosa* for phytoremediation of indoor air.

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Ethical issues

The authors certify that all data collected during the study are as stated in the manuscript, and no data from the study has been or will be published separately elsewhere.

Competing interests

The authors declare that they have no conflict of interests.

Authors' contribution

All authors were equally involved in the collection, analysis, and interpretation of the data. All authors critically reviewed, refined, and approved the manuscript.

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