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Comparison of Removal of Formaldehyde Capacity Between *Hedera helix* and *Melissa officinalis*

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In order to clarify the different ability to remove formaldehyde, *Melissa officinalis* and *Hedera helix* were treated in sealed chamber over a 5 h period. *M. officinalis* showed 12 times higher removal efficiency of formaldehyde (25.06 mg m^{-2} h^{-1}) than *H. helix*. The chlorophyll a content, membrane permeability, respiratory rate and catalase activity of each plant were also measured to investigate the plant responses to formaldehyde exposure. The increased membrane permeability and decreased respiratory rate were observed in both plants after the formaldehyde exposure. Interestingly, chlorophyll a content and catalase activity showed opposite patern in two tested plants. Leaf surface appearance and chloroplast ultrastructure of two plants were then further examined by SEM and TEM. The results of which indicated *M. officinalis* had more gas adsorbing surface and higher stomata density than *H. helix*, making the former superior in formaldehyde adsorbing and diffusion. In addition, the cell structure of *M. officinalis* kept intact after formaldehyde treatment, possibly or partly due to the induced catalase activity.

Key Words: *Melissa officinalis*, *Hedera helix*, Formaldehyde removal capability.

INTRODUCTION

As the largest formaldehyde producer and consumer in the world, China has also increased formaldehyde pollution considerably[1]. Formaldehyde has become the second order on the list of priory control toxic chemicals in China. Because formaldehyde is generally used to make construction materials, during the period of 2002-2004, more than 69 % newly built or remodeled house had indoor formaldehyde levels exceeding the national standard of 0.1 mg m^{-3} [1]. Formaldehyde is highly reactive and can denature nucleic acid and protein. The exposure to formaldehyde is known to cause irritation, allergic asthma and neurasthenia and to induce genotoxicity and carcinogenesis[2,3]. Therefore, the removal of formaldehyde is important and necessary to improve indoor air quality and to reduce public health risk, especially in China.

To date, several approaches have been studied for formaldehyde removal including chemisorption, photo catalytic oxidization, thermal catalytic oxidization, plasma, adsorption by modified activated carbon and biological process[4]. Traditional technologies based on physico-chemical ones are relative expensive and may produce undesirable side-effects. Over the past decades, biological process has been developed and quickly improved. Unlike traditional technologies, biofiltration is mainly based on biological principles. A number of studies prove that biofiltration minimizes the problems mentioned above for traditional technologies and is believed to be a cost-save and environmental friendly way to remove formaldehyde.

As formaldehyde biofiltration materials, many plants have shown ability to remove formaldehyde, such as *Rhapis humilis*, *Spathiphyllum patinii*, *Pachira aquatica*, *Nephrolepis exaltata*, *Ficus benjamina*, *Schefflera arboricola*, *Hedera helix*, *Chrysanthemum morifolium*, *Dieffenbachia compacta*, *Epipremnum aureum*, *Chlorophytum comosum*, *Aloe vera* and so on[5-7]. The difference of formaldehyde removal capacity between plants was significant. In terms of formaldehyde absorption abilities, *S. patinii*, *P. aquatica* and *R. humilis* had higher efficiencies than those of *N. exaltata* and *S. arboricola*[5]. The turn of formaldehyde removal efficiency was found in another study as *C. morifolium* > *E. aureum* > *D. compacta* > *H. helix*[6]. Among above plants, *C. comosum* had the higher formaldehyde removal capacity than those of *A. vera* and *E.aureum*[7]. Moreover, many researchers have pointed out that there was a threshold in plant formaldehyde removal process, that is, the increasing removal capacity of plant along with the formaldehyde concentration would switch to decrease when being exposed to a concentration beyond the threshold. The biodegradation process by plants would be inhibited at the concentration over thus threshold[8,9]. But what makes different plants have different

formaldehyde removal capacity and what is the reason for the threshold are still unknown, especially on molecular mechanism or ultrastructure level.

Our preliminary experiments have found some common indoor plants had different capacity to remove formaldehyde, among them, *Melissa officinalis* and *H.helix* showed the remarkable difference. The purpose of this paper is to compare different reactions of these two plants submitted to formaldehyde treatment, including physiological and ultrastructural changes and try to discuss the factors accounting for their different formaldehyde removal capacity.

EXPERIMENTAL

Test chamber: The test chamber was as described by Aydogan[6] with minor modifications. Briefly, a clear glass chamber with dimensions of 90 cm × 70 cm × 60 cm was used in these experiments with a removable top cover with a diameter of 5 cm and adhesive foam-rubber insulation tape was used to provide airtight seal on the top. A small fan inside the chamber promoted complete mixing. Room temperature was kept at 25 ºC. Artificial lighting was provided by two fluorescent bulbs placed outside the chamber, provided in 12 h cycles (day/night). The lighting provided *ca.* 2000-5000 luxes of illumination intensity to the plant leaves.

Plant materials: Two plant species were used in these experiments: (1) *Hedera helix*, average leaf area of 0.132 m^2; (2) *Melissa officinalis*, average leaf area of 0.036 m^2. Plants were obtained from commercial distributors. The plants were acclimated to the indoor environment conditions used in these experiments for more than 2 weeks at 25 ºC and 12 h cycles (day/night). Prior to entering testing chamber, the leaves of plants were washed by distilled water and dried naturally.

Experimental setup: 40 % formaldehyde solution was added into a 2 L beaker, sealed with adhesive foam-rubber insulation tape. The beaker was kept at 25 ºC for 12 h to make formaldehyde to evaporate into gas completely.

The formaldehyde gas was introduced into the chamber through the top hole, with a pulse injection of 50 mL to generate an initial concentration of 2.5 mg m^{-3} inside the chamber. The formaldehyde levels were measured with an HCHO monitor (INTERSCAN 4160, USA). Treat duration was 5 h with triplicates.

To facilitate comparisons, plant leaf surface area was determined using Image J, a Java-based image processing program (http://rsbweb.nih. gov/ij/). The individual leaf area was determined by tracing the leaf on paper and then analyzing the traces with Image J. The amount of formaldehyde gas removed per surface area of plant leaf was calculated to describe the formaldehyde removal capacity.

Physiological parameters investigated: In order to investigate the plant responses induced by formaldehyde treatment, the chlorophyll a content, membrane permeability, respiratory rate and CAT (catalase activity) were measured[10].

Sample preparation for scanning electron microscope (SEM) and transmission electron microscopy (TEM): Leaves of plants were collected, cut into 5 mm × 5 mm pieces, fixed by FAA (Formalin-alcohol-acetic acid with mixed stationary phase), pasted on the cylindrical copper table, placed in IB-5 ion plating apparatus for low-vacuum drying and sputtered gold film, observed and photographed in JSM-T300 scanning electron microscope.

The plants were taken out of the testing chamber after formaldehyde treatment for 5 h. Leave samples from similar part of plants were cut into 1 mm × 1 mm pieces, fixed in 2-4 % pre-cooled glutaraldehyde for 1-2 h. Then, samples were rinsed three times for 15 min each with 0.1 mol/L phosphate buffer (pH = 7.2-7.4) before the dehydration in a sequence of reagents: 50 % alcohol, 70 % alcohol, 80 % alcohol, 90 % alcohol, 95 % alcohol, 100 % alcohol (twice), mixture of alcohol and acetone (1:1), 100 % acetone (twice), mixture of acetone and anhydrous sodium sulfate. Finally, the samples were preserved in Epon812 until being gilded and Uranium- lead stained and submitted to transmission electron microscope of the type of Tecnai 12.

RESULTS AND DISCUSSION

Formaldehyde removal capability of two plants: *M. officinalis* and *H. helix* were treated by formaldehyde for 5 h in the testing chamber. The initial concentration of 2.5 mg m^{-3} formaldehyde inside the chamber was reduced by both plants. *M. officinalis* showed high formaldehyde removal capability (Fig. 1), with removal efficiency of 25.06 mg m^{-2} h^{-1}, while *H. helix* removal efficiency was only 2.22 mg m^{-2} h^{-1}. The former removal efficiency was 12 time higher than the latter.

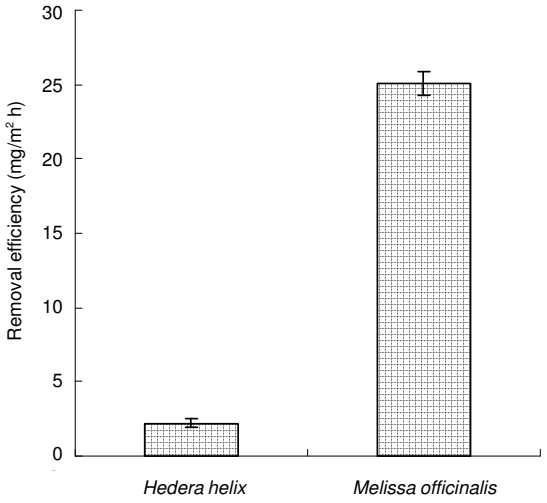


Fig. 1. Formaldehyde removal efficiency of two plants

Physiological effects of formaldehyde on two plants: Several physiological characteristics of two plants submitted to formaldehyde treatment were examined, the results were shown in Table-1 and the relative changes were calculated in Fig. 2. Two plants experienced a series of physiological changes. Physiological characteristics of two plants changed at different extent even in different directions for some parameter due to formaldehyde treatment.

Among the four physiological characteristics, Chl-a, MP and RR of control samples ranged in adjacent values for two plants, while CAT showed remarkable difference with 0.57 mg (H_2O_2) g^{-1} (FW) min^{-1} in *H. helix* and 0.07 mg (H_2O_2) g^{-1} (FW) min^{-1} in *M. officinalis*.

TABLE-1
EFFECTS OF FORMALDEHYDE ON PHYSIOLOGICAL CHARACTERISTICS OF TWO PLANTS

Parameter	*Hedera helix*		*Melissa officinalis*	
	Control	Treatment	Control	Treatment
Chl-a [mg g^{-1}(FW)]	1.81 ± 0.09	2.52 ± 0.11	1.58 ± 0.05	1.44 ± 0.07
MP (%)	3.83 ± 0.05	11.71 ± 0.11	7.12 ± 0.08	13.84 ± 0.13
RR (mg g^{-1} h^{-1})	7.71 ± 0.31	5.68 ± 0.10	2.55 ± 0.22	1.72 ± 0.09
CAT [mg (H_2O_2) g^{-1} (FW) min^{-1}]	0.57 ± 0.02	0.21 ± 0.01	0.07 ± 0.01	0.16 ± 0.03

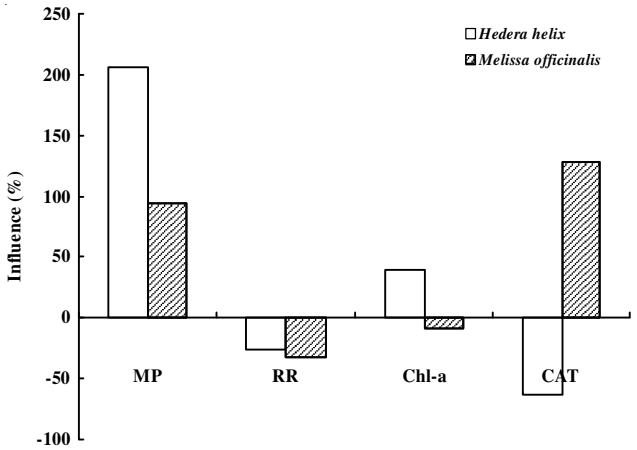


Fig. 2. Physiological characteristics changes of two plants caused by formaldehyde treatment, Note:The abbreviations of MP, RR, Chl-a and catalase activity in the figure present for membrane permeability, respiratory rate, chlorophyll a content and catalase activity, repectively

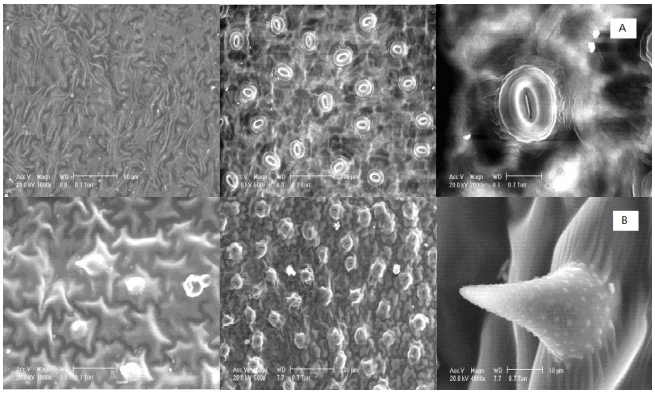
For both plants, membrane permeability increased greatly after formaldehyde treatment. Membrane permeability of *H. helix* increased 206 %, while that of *M. officinalis* increased-94 %. *H. helix* was more sensitive to formaldehyde treatment than *M. officinalis* in terms of membrane permeability.

According to Table-1 and Fig. 2, respiratory activity of both plants was depressed by formaldehyde. Comparing with controls, it caused respiration slowed down 26 % in *H. helix* and 33 % in *M. officinalis*. Formaldehyde suppressed respiratory activity a little bit more fiercely in *M. officinalis* than that in *H. helix*.

Formaldehyde had different effects on chlorophyll a content of two plants. The chlorophyll a content of *H. helix* increased, becoming 1.4 times of control by the end of the treatment. On the contrary, for *M. officinalis*, it decreased by around 10 % comparing with the control. In general, the influence of formaldehyde treatment was weak for this piont.

Catalase activity of *M. officinalis* was induced by formaldehyde treatment, becoming twice as high as control. Catalase activity of *H. helix* was inhibited, as low as 36 % of control after treatment. For the catalase activity, formaldehyde showed inducing effect for *M. officinalis*, inhibiting effect for *H. helix*.

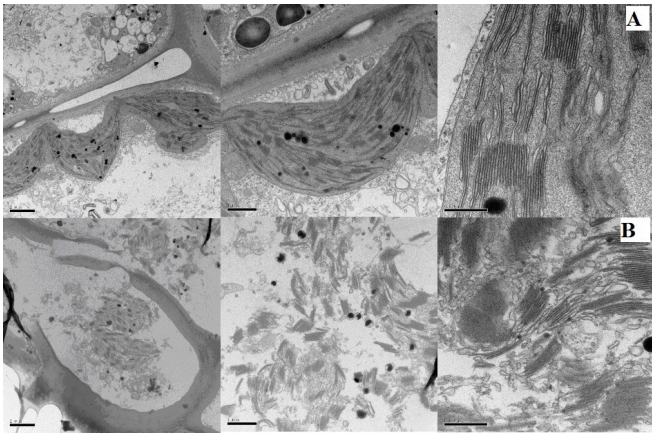
Surface appearance of leaves of two plants: Leaves surface appearance of two plants were examined by SEM and the pictures were shown in Fig. 3. The leaf of *H. helix* had flat positive side except for some wrinkles, while the reserve side was full of wrinkles and stomata but without fluff (Fig. 3A). The leaf surface of *M. officinalis* was quite different. It is full of fluff, stomata and pits on both positive and reverse sides (Fig. 3B).



Positive side ×1000 Rreverse side × 500 Stomata (A) and pit (B) ×2000

Fig. 3. Leaf surface appearance of *H. helix* (A) and *M. officinalis* (B)

Effects of formaldehyde on chloroplast ultrastructure of two plants: Furthermore,chloroplast ultrastructure of two plants were also examined by TEM. As shown in Fig. 4A, the unaffected chloroplast of *H. helix* was in shape of oval, covered with two-layer membrane. They distributed at the edge of leaf cell with high density. The thylakoid lamellaes were clear, covered with one-layer membrane. There was a unity membrane system in the chloroplast. Treatment of formaldehyde made the shape of chloroplast changed with loose and disordered structure (Fig. 4B). Two-layer membrane of chloroplast was damaged or disappeared. The disintegrated chloroplasts were observed. The one-layer membrane of thylakoid was damaged and the thylakoid itself was broken. The lamellae were dispersed in matrix.



× 5800 × 13500 × 37000

Fig. 4. Effects of formaldehyde on chloroplast ultrastructure of *H. helix* (A: Control; B: treated by formaldehyde)

The chloroplast ultrastructure of *M. officinalis* did not show significant difference between the control (Fig. 5A) and

the treatment by formaldehyde (Fig. 5B). The membrane layer for chloroplast and thylakoid were clear. The lamellae were still observed in matrix. Not only chloroplast but also thylakoid kept normal shape and structural integrity.

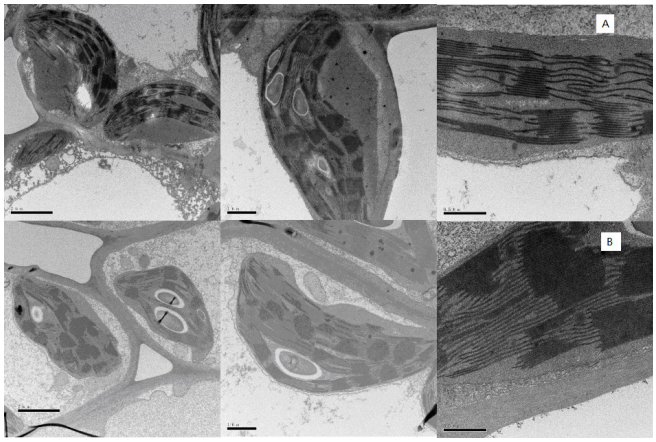


Fig. 5. Effects of formaldehyde on chloroplast ultrastructure of *M. officinalis* (A: Control; B: treated by formaldehyde)

Formaldehyde removal process includes three steps, which are adsorbing, diffusion and reaction. We have no exact proof what are responsible for the different effects in two plants, but we are sure their formaldehyde adsorbing and diffusion efficiency are quite different. The leaf surface of *M. officinalis* is full of fluff and pits while that of *H. helix* is smooth, which indicates *M. officinalis* has larger effective surface to adsorb formaldehyde than *H. helix* in the same leaf area. In fact, higher formaldehyde removal capacity can be achieved by increasing adsorbing leaf area in a formaldehyde removal process by plant.

Plants uptake air pollutants through their stomata during normal gas exchange process for various plant species[11]. The gas amount (Q) entering plant obeys Darcy's law: Q = KAJ, where K, A and J mean diffusion index, channel area and pollutants concentration difference between stomata and air, respectively[12]. For a certain plant species, the higher A value indicates the larger amount of gas diffusion. The stomata density of *M. officinalis* was 2.5 times higher than that of *H. helix*. Therefore *M. officinali* can absorb and diffuse more formaldehyde gas when compared to *H. helix*. Here we are sure *M. officinalis* has better gas adsorbing and diffusion characteristics than *H. helix*.

Since the saturation point of formaldehyde adsorption on the surface of plant easily reach in a short time, formaldehyde removal is due to plant metabolism in long period. Formaldehyde removal is highly related to plant metabolism because formaldehyde is an intermediate of photosynthetic carbon dioxide fixation in green plants, which can be removed by forming S-formylglutathione[13]. For this reason, high metabolism rate becomes the foundation of formaldehyde removal. To keep normal metabolism, integrated cell structure is necessary. In this study, it is no doubt that formaldehyde is poisonous to plants. It destroyed membrane structure of *H. helix* heavily. Accordingly, *H. helix* removed smaller amount of formaldehyde than *M. officinalis*, which kept integrated cell structure in the whole treatment process.

The factor of catalase activity attracted much attention because it showed different change after formaldehyde treatment for the tested two plants. It was depressed for *H. helix*, while it was induced to a high level for *M. officinalis*. As an important protective enzyme, catalase activity becomes higher under stress in order to remove more H_2O_2 to protect plant from damage. This kind of protective function attributes to protect cell structure, which make normal metabolism possible. But there is a limitation or threshold for this protective function. When stress beyond the limitation or threshold, catalase activity can not protect the cells any more, resulting in the damaged cell structure, which will depress catalase activity in return[14]. The background values of catalase activity in these two tested plants vary, which may also indicate they possess different ability to survive under stress conditions. Associated with the threshold of formaldehyde removal, catalase activity might act as one of reasons for threshold existing. In this study, the threshold for *H. helix* should have been exceeded by experimental concentration, which resulted in a dramatic decrease in catalase activity. Meanwhile, the experimental concentration should be still under the threshold for *M. officinalis*, with catalase activity responsed efficiently to protect the cells.

Conclusion

M. officinalis has much better formaldehyde removal capability than *H. helix*. *M. officinalis* has more gas adsorbing surface and higher stomata density making it superior in formaldehyde adsorbing and diffusion. *M. officinalis* not only removed more formaldehyde than *H. helix*, but also kept its cell structure intact after formaldehyde treatment which partly owing to induced catalase activity. These factors count for *M. officinalis*'s high formaldehyde removal capability.

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