Physiological Response of Indoor Plants according to Formaldehyde Concentrations

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포름알데히드 농도에 따른 실내식물의 생리적 반응

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Abstract

Physiological response of indoor plants was investigated according to formaldehyde concentrations. *Epipremnum aureum*, *Fatsia japonica*, and *Rhapis exelsa* were exposed 0, 2(2.5), 10(12.3), 100(123) ppm($\text{mg} \cdot \text{m}^{-3}$) of formaldehyde. Necrosis was a visible injury symptom in leaves and stem with exposure to 100 ppm formaldehyde for 5 h. Apparent injury appeared in young and old leaves of every plant, but displayed in stems of only *Fatsia japonica*. Cells in tissue of injury leaves were altered or demolished, and resulted in destruction of palisade and spongy parenchyma. Peroxidase and catalase content were highest in *R. exelsa* but lowest in *E. aureum*. Catalase activity increased with increasing formaldehyde concentrations. Stomata of *E. aureum* that was severely damaged in leaves closed up to 80% with exposing 100 ppm of formaldehyde, and catalase activity of *E. aureum* increased highest. As a result, stomata were closed and enzyme activity were changed in order to become tolerance when indoor plants were exposed in high concentration of formaldehyde, while it was not showed in low concentration of formaldehyde.

Key words : *Fliage plant*, *Phytoremediation*, *Sick building syndrome*, *Volatile organic compounds*

I. Introduction

The U.S. Environmental Protection Agency (EPA) reported detection of more than 900 volatile organic compounds (VOCs) in the air of public buildings (EPA, 1989) and in a Finnish study (Kostiainen, 1995), over 200 VOCs were identified in each of 26 homes. Formaldehyde is a major contaminant in indoor air that originates from particle board, plywood, carpet, curtain, paper products, certain adhesives, and other sources (Salthammer, 1999; Spengler and Sexton, 1983). Indoor VOCs such as formaldehyde can result in physical symptoms such as allergies, asthma, and headaches. (Jones, 1999; Kostiainen 1995). Due to its undesirable effect on health, $0.17 \mu\text{L} \cdot \text{L}^{-1}$ has been established as the upper limit for the concentration of formaldehyde in the

indoor air of new houses in Korea (Ministry of Environmental, Republic of Korea, 2006).

Indoor plants are known to absorb and metabolize formaldehyde in air. Indoor VOCs enter into the leaves via stomata and cuticle, and are more readily absorbed by abaxial surface and younger leaves (Giese et al., 1994; Ugrehelidze et al., 1997). Once absorbed by the leaves, they generally enter the Calvin cycle after a two-step enzymatic oxidation to CO_2 (Schmitz, 1995). Some of the formaldehyde is converted to S-methyl-methionine and translocated in the phloem to various organs such as seed and roots (Hanson and Roje, 2001).

Stomata are a major factor controlling air pollution injury to plants (Mansfield, 1973). Opening or closing of stomata during exposure has also varied with pollutant concentration, length of exposure, and different plant species (Noland and Kozłowski, 1979; Rosen et al., 1978). Visible symptoms such as necrosis, chlorosis, and cell death were generally observed in plants leaves

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exposed to pollutants (Cheng et al., 2010; Krupa et al., 2001; Lee et al., 2005; Novak et al., 2003). It has also reported that anti-oxidant enzyme such as catalase and peroxidase is activated in plants leaves exposed to pollutants (Cheng et al., 2010, Woo et al., 2004).

Thus, we observed visible symptoms and demonstrated catalase and peroxidase activity at plants leaves exposed to formaldehyde low and high concentrations.

II. Materials and Methods

1. Plant materials

Epipremnum aureum, *Fatsia japonica*, and *Rhapis exelsa* were obtained from a commercial market, and were about 18, 27, and 35 in plants height, respectively. The plants were transplanted into 19-cm-diameter pots containing a uniform growing medium comprised of Mix #4 (Sun Gro Horticulture, Bellevue, WA), bark-humus (Biocom. Co., Seoul, Korea), and sand at 5:1:1, v/v/v. Mix #4 contained Canadian sphagnum peatmoss (55% to 65% by volume), perlite, dolomitic lime, gypsum, and a wetting agent. The plants were acclimated within simulated living rooms designed to create a typical indoor environment (Kim et al., 2008, 2010) for more than one month ($23\pm 2^{\circ}\text{C}$, $40\%\pm 5\%$ relative humidity). The plants were acclimated at a light intensity of $20\pm 2\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using fluorescent lights and the photoperiod for all species was 12/12 h (day/night). The plants were watered every 3 day with the excess water allowed to drain. All plants were watered the day before the gas treatments.

2. Gas exposure and quantification

A gas generator and treatment system were the same as using Kim et al. (2008 and 2010). A gas generator converted a 35% formalin solution (Katayama Chemical Co., Hygro, Japan) to gaseous formaldehyde. The formaldehyde was collected in a sealed Teflon bag and was injected into the test chambers. The injected formaldehyde was mixed for 30 min using the chamber air circulation system. The initial concentration was determined and adjusted as needed to 0, 2 (2.5), 10 (12.3), 100 (123) ppm ($\text{mg}\cdot\text{m}^{-3}$), and plants were exposed for 5 h. The concentration

of formaldehyde was measured using a formaldehyde and data logging system (Z300-XP; Environmental Sensors Co., Boca Raton, FL).

3. Enzyme preparation and assays

Sampling leaf for assays was conducted 15h after gas exposure was done. The 0.5g of leave was weight from sampling each treatment, and was frozen immediately in liquid nitrogen, and then grounded in a mortar and pestle with T-buffer 2mL. The 1.5mL of suspensions was moved into 2mL-tube, and centrifuged at 15,000 rpm and 4°C for 30 min. The clear supernatant was used as the enzyme source for quantitative assays of peroxidase, and catalase activity (Kruger and Laberge, 1974). Enzyme activity was measured at 500 nm by spectrophotometer (UV-1601; Shimadzu Co., JA). To determine peroxidase activity, 0.02mL of supernatant was mixed with 3.27mL of 0.01mM Tris - HCl buffer (pH 7.3) adding 0.1ml of 3.2M guaiacol, and 0.05mL of 649mM H_2O_2 . To determine catalase activity, 0.1mL of supernatant was mixed with 2.8mL of 0.1M phosphate buffer (pH 7.0) adding 0.1mL of 1% H_2O_2 . The 0.1M phosphate buffer was obtained to mix 61.5mL of 0.1 M K_2HPO_4 , and 38.5mL of 0.1M KH_2PO_4 .

4. Light microscopic observations

Sampling leaf for observation was conducted 15h after gas exposure was done. Cross sections of specimens were observed under light microscope. Samples were fixed with 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH7.2) for 2h at 4°C , and were rinsed, post-fixed with 1% osmium tetroxide for 2 h, at 4°C , and then held overnight in phosphate buffer. After fixation, specimens were dehydrated in a graded series of ethyl alcohol to ensure complete dehydration; 40, 60, 80, 90, 95 and 100% in distilled water [v/v], processed tissue through 3 changes of propylene oxide and gradually infiltrated for 3 h each at 30, 50, and 100% embedding media in propylene oxide with embedding media, Epon. Specimens were held overnight in 100% Epon before polymerization at 60°C for 72 h. Specimens were sectioned (1500 nm), stained with periodic acid staining (P.A.S), and viewed with Axioskop 2 light microscope (Carl Zeiss, Germany).

III. Results and Discussion

Necrosis was a visible injury symptom in leaves, and stem with exposure to 100 ppm formaldehyde for 5h (Fig. 1). The degree of apparent injury differently appeared depending on plant species; it was severely damaged in *E. aureum* followed by *F. japonica*, and then *R. exelsa*. Previous studies have shown that air pollutants like ozone, sulfur dioxide cause necrotic, chlorotic, and colored symptoms in leaves and stems (Gravano et al. 2004; Bussotti et al. 2005). Formaldehyde of high concentration produced the similar symptoms with other

Fig. 1. Visible injury of leaves and stem when pot plants were exposed to 100 ppm of high concentration for 5 h in sealed chambers. Arrows indicate injury. A; *Epipremnum aureum*, B; stem of *Fatsia japonica*, C; *Rhaps exelsa*, D; leaf of *Fatsia japonica*.

Fig. 2. Cross sections of leaves and stem observed by scanning electronic microscopy (SEM) when pot plants were exposed to 100 ppm of high concentration for 5 h in sealed chambers. Arrows indicate damage. A; control, B; leaf of damage.

air pollutants, but that of low concentration did not cause any injury symptoms. It has known that formaldehyde and volatile organic compounds of low concentration were absorbed and metabolized by plants (Kil et al., 2008; Kim et al., 2008, 2010; Kim and Lee, 2008; Kim and Kim, 2008). Cells in tissue of injury leaves were altered or demolished, and it resulted in destruction of palisade and spongy parenchyma (Fig. 2). The injury leaves of *E. aureum* were severely destroyed and even width of them in cross section was reduced. Catalase and peroxidase content of *R. exelsa* were highest among test species, and were about two and twenty times more than those

Fig. 3. Catalase content in leaves according to deferent formaldehyde concentrations. Pot plants were exposed for 5 h in sealed chambers. Letters indicate mean separation within species by Duncan's multiple range test at $P = 0.05$. Vertical bars denote the SE.

Fig. 4. Peroxidase content in leaves according to deferent formaldehyde concentrations. Pot plants were exposed for 5 h in sealed chambers. Letters indicate mean separation within species by Duncan's multiple range test at $P = 0.05$. Vertical bars denote the SE.

Fig. 5. Percent rate of closed stomata when pot plants were exposed to 100 ppm of high concentration for 5 h in sealed chambers. Vertical bars denote the SE.

Table 1. Number and size of stomata in plant leaves tested.

Species	No. of stomata (No./mm ²)	Size of stomata (mm)
<i>Epipremnum aureum</i>	41.4±33.8	33.8±3.5
<i>Fatsia japonica</i>	204.3±13.9	13.9±3.6
<i>Rhaps exelsa</i>	142.3±23.2	23.2±3.3

of *E. aureum* in control without exposing formaldehyde, respectively (Fig. 3). It might cause that *R. exelsa* produced a little visible injury symptoms in leaves, whereas *E. aureum* was badly injured. Catalase activity increased with increasing formaldehyde concentration, while peroxidase activity was not shown on difference with formaldehyde concentrations. Therefore, it seemed that anti-oxidant enzyme for stress of formaldehyde was catalase. It has reported that anti-oxidant enzyme such as catalase and peroxidase responded differently according to plant species, and thus main anti-oxidant enzyme for stress was different according to plant species or exposed pollutants (Cheng et al., 2010). Catalase activity of *R. exelsa* started off in exposing more than 2 ppm of formaldehyde concentrations. *R. exelsa* contented sufficient anti-oxidant enzyme in control (Fig. 3 and 4) had sensibility for formaldehyde concentration.

However, the catalase activity of *E. aureum* tha twas badly damaged was increased at 100 ppm formaldehyde, and catalase of *F. japonica* was activated more than 10 ppm of formaldehyde. Stomata of *E. aureum* were closed up to 80% with exposing 100 ppm of formaldehyde, but the rate of closed stomata in *R. exelsa* and *F. japonica* was increased a little (Fig. 5). Stomata of *E. aureum* were big in size but small in number, while those of *F. japonica* reversely were small size and large number

(Table 1).

Air pollutants enter into plants via stomata mainly, and plant leaves also close stomata for defense (Noland and Kozlowski, 1979; Rosen et al., 1978). However, organic air pollutants, formaldehyde and volatile organic compounds, were taken up by cuticles as well as stomata, due to strong permeation into leaf tissue (Giese et al., 1994; Ugrekhelidze et al., 1997). Thus, *E. aureum* would be severely damaged even though most stomata were closed. As a result, stomata were closed and enzyme activity were changed in order to become tolerance when indoor plants were exposed in high concentration of formaldehyde, while it was not showed in low concentration of formaldehyde.

IV. References

- Bussotti, F., G. Agati, R. Desotgiu, P. Matteini, and C. Tani. 2005. Ozone foliar symptoms in woody plant species assessed with ultrastructural and fluorescence analysis. *New Phytologist* 166:941- 955.
- Cheng, H.C., S.Y. Woo, S.H. Lee, and S.G. Baek. 2010. Photosynthesis, antioxidant enzyme, and anatomical difference of *Sedum kamtschaticum* and *Hosta longipes* to ozone. *Kor. J. Hort. Sci. Technol.* 28:394-402.
- EPA. 1989. Report to Congress on indoor air quality. Vol. II: Assessment and control of indoor air pollution. EPA/400/1-89/001C, p.250.
- Giese, M., U. Bauer-Doranth, C. Langebartels, and H. Sandermann. 1994. Detoxification of formaldehyde by the spider plant (*Chlorophytum comosum* L.) and by soybean (*Glycine max* L.) cell-suspension cultures. *Plant Physiol.* 104:1301-1309.
- Gravano, E., F. Bussotti, R.J. Strasser, M. Schaub, K. Novak, and K. Skelly. 2004. Ozone symptoms in leaves of woody plants in open-top chambers: Ultrastructural and physiological characteristics. *Physiologia Plantarum*. 121:620-633.
- Hanson, A.D. and S. Roje. 2001. One-carbon metabolism in higher plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 52:119-137.
- Jones, A.P. 1999. Indoor air quality and health. *Atmos. Environ.* 33:4535-4564.
- Kanemasu, E.T., G.W. Thurtell, and C.B. Tanner. 1969. Design, calibration and field use of a stomatal diffusion porometer. *Plant Physiol.* 44: 881-885.
- Kil, M.J., K.J. Kim, J.K. Gho, and C.H. Pak. 2008. Formaldehyde gas removal effects and physiological responses of *Fatsia japonica* and *Epipremnum aureum* according to various light intensity. *Kor. J. Hort. Sci. Technol.* 26:189-196.
- Kim, K.J. and D.W. Lee. 2008. Efficiency of volatile formaldehyde removal of orchids as affected by species and crassulacean acid metabolism (CAM) nature. *Hort. Environ. Biotechnol.* 49:123-137.

- Kim, K.J. and H.D. Kim. 2008. Development of model and calculating equation for rate of volatile formaldehyde removal of indoor plants. *Hort. Environ. Biotechnol.* 49:155-161.
- Kim, K.J., M.I. Jeong, D.W. Lee, J.S. Song, H.D. Kim, E.H. Yoo, S.J. Jeong, S.Y. Lee, S.J. Kays, Y.W. Lim and H.H. Kim. 2010. Variation in formaldehyde removal efficiency among indoor plant species. *HortScience* 45:1489-1495.
- Kim, K.J., M.J. Kil, J.S. Song, E.H. Yoo, K.C. Son and S.J. Kays. 2008. Efficiency of volatile formaldehyde removal by indoor plants: Contribution of aerial plant parts versus the root zone. *J. Amer. Soc. Hort. Sci.* 133:521-526.
- Kostiainen, R. 1995. Volatile organic compounds in the indoor air of normal and sick houses. *Atmos. Environ.* 29:693-702.
- Kruger, J.E. and D.E. LaBerge. 1974. Changes in peroxidase activity and peroxidase isozyme patterns of wheat during kernel growth and maturation. *Cereal Chem.* 51:345.
- Krupa, S., M.T. McGrath, C.P. Andersen, F.L. Booker, K.O. Burkey, A.H. Chappelka, B.I. Chevone, E.J. Pell, and B.A. Zilimskas. 2001. Ambient ozone and plant health. *Plant Dis.* 85:4-12.
- Lee, J.C., C.Y. Oh, S.H. Han, and P.G. Kim. 2005. Changes on photosynthesis and SOD activity in *Platanus orientalis* and *Liriodendron tulipifera* according to ozone exposing period. *Kor. J. Agr. For. Meteorol.* 7:156-163.
- Mansfield, T.A. 1973. The role of stomata in determining the responses of plants to air pollutants. *Commun Plant Sci.* 2:11-20.
- Ministry of Environment, Republic of Korea, 2006. Republic of Korea's enforcement ordinance on indoor air quality management. Ministry of Environment, Seoul, Korea.
- Noland, T.L. and T.T. Kozlowski. 1979. Effect of SO₂ on stomatal aperture and sulfur uptake of woody angiosperm seedlings. *Can. J. For. Res.* 9:57-62.
- Novak, K., J.M. Skelly, M. Schaub, N. Krauchi, C. Hug, W. Landolt, and P. Bleuler. 2003. Ozone air pollution and foliar injury development on native plants of Switzerland. *Environ. Pollut.* 125:41-52.
- Rosen, P.M., R.C. Musselmawn, and W.J. Kender. 1978. Relationship of stomatal resistance to sulfur dioxide and ozone injury in grapevines. *Sci. Hort.* 8:137-142.
- Salthammer, T. 1999. Organic indoor air pollutant: Occurrence-measurement-evaluation. New York: Wiley.
- Schmitz, H. 1995. Bakterielle und pflanzliche entgiftungs mechainismen fuer formaldehyde und nikotin under rhizosphere von *Epipremnum aureum* und *Ficus benjamina*. PhD Dissertation, University of Köln, Köln, Germany.
- Spengler, J.D. and K. Sexton. 1983. Indoor air pollution: A public health perspective. *Science* 221:9-16.
- Ugrekheldze, D., F. Korte, and G. Kvesitadze. 1997. Uptake and transformation of benzene and toluene by plant leaves. *Ecotoxicol. Environ. Saf.* 6:24-29.
- Woo, S.Y., S.H. Lee, K.W. Kwon, J.C. Lee, and J.H. Choi. 2004. Growth, photosynthesis, and ascorbate peroxidase activity of several species to the ozone exposure. *J. Kor. For. Soc.* 93:409-414.

V. 적요

포름알데히드의 고농도와 저농도에 따른 식물의 생리적 반응을 보기 위하여 실험을 실시하였다. 실내식물은 관음죽, 팔손이나무, 스킨답서스를 이용하였으며, 포름알데히드는 0, 2(2.5), 10(12.3), 100(123) ppm($\text{mg} \cdot \text{m}^{-3}$) 농도로 처리하였다. 그 결과 포름알데히드 처리에 대한 잎 피해증상은 100 ppm의 고농도에서 5시간 처리 후 검은 반점으로 나타났다. 외관상 피해증상은 어린잎과 노화된 모두에서 나타났으며, 팔손이나무의 경우에는 줄기에서도 일부 나타났다. 피해 잎의 책상조직과 해면조직이 파괴되거나 변형되었다. 전체적으로 Peroxidase와 Catalase 함량은 관음죽>팔손이나무>스킨답서스 순서였으며, 처리농도가 높아질수록 함량도 대체로 증가하였다. 피해가 심한 스킨답서스의 경우 고농도 처리시에 기공이 닫히는 비율이 약 80% 가장 높았으며, Catalase 증가량도 가장 컸다. 결과적으로 100 ppm의 고농도에서 포름알데히드 흡수를 저해하기 위해 기공이 닫히거나 효소 함량의 변화가 일부 나타났으나, 저농도에서 적극적 흡수를 위한 반응은 보이지 않았다.